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Solution and solid phase synthesis of *N,N'*-diacetyl chitotetraoses

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dedicated to my amma, appa, and anna

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Abstract - German

Synthese von *N,N'*-Diacetylchitotetraosen in Lösung und an Fester Phase

Die drei wichtigsten Biopolymere sind Proteine, Nukleinsäuren und Glykokonjugate. Sie sind von fundamentaler Bedeutung für lebenswichtige Prozesse, wie z.B. den Informationstransfer. Die biologische Bedeutung von Proteinen und Nukleinsäuren ist eingehend erforscht, während Oligosaccharide in Form von Glykokonjugaten erst in neuerer Zeit an Bedeutung gewonnen haben. Die β-(1→4) verknüpfte *N*-Acetylglucosamin (GlcNAc) Einheit kommt häufig als in vielen natürlichen und biologisch wichtigen Oligosacchariden und ihren Konjugaten vor. Chitin, ein Polymer von GlcNAc, ist in der Natur weit verbreitet, während das verwandte Polysaccharid Chitosan (Polymer of GlcN und GlcNAc) in gewissen Pilzen auftritt. Chitooligosaccharide gemischter Acetylierungsmuster sind von Bedeutung für die Bestimmung von Substratwirkungen und für den Mechanismus von Chitinasen. In dieser Arbeit beschreiben wir die chemische Synthese von drei Chitotetraosen, nämlich GlcNAc-GlcN-GlcNAc-GlcN, GlcN-GlcNAc-GlcNAc-GlcN and GlcN-GlcN-GlcNAc-GlcNAc.
Benzyloxycarbonyl (Z) und p-Nitrobenzyloxycarbonyl (PNZ) wurden aufgrund ihrer Fähigkeit, die β-Verknüpfung während der Glykosylierung durch die Nachbargruppenbeteiligung zu steuern, als Aminoschutzgruppen verwendet. Zur Aktivierung der Donoren wurde die Trichloracetamidat Methode angewendet. Monomere und dimere Akzeptoren und Donoren wurden unter Verwendung von Z und PNZ Gruppen hergestellt. Die Kupplung von geeigneten Donoren und Akzeptoren in Gegenwart einer Lewis Säure ergaben die Tetrasaccharide. Schließlich ergab die Entschützung von PNZ, gefolgt von der Reacetylierung der Aminogruppe und Abspalten der übrigen Schutzgruppen die *N,N'*-Diacetylchitotetraosen in guten Ausbeuten. Weiterhin wird die erfolgreiche Synthese der geschützten Diacetylchitotetraosen durch Festphasensynthese beschrieben.

Schlüsselwörter: Chitooligosaccaride, Chemische Synthese, Festphasensynthese, Glykosylierung.

Abstract

Solution and solid phase synthesis of *N,N'*-diacetyl chitotetraoses

The three major biopolymers, proteins, nucleic acids and glycoconjugates are mainly responsible for the information transfer, which is a fundamental process of life. The biological importance of proteins and nucleic acids are well explored and oligosaccharides in the form of glycoconjugates have gained importance recently. The β-(1→4) linked *N*-acetylglucosamine (GlcNAc) moiety is a frequently occurring structural unit in various naturally and biologically important oligosaccharides and related conjugates. Chitin which is the most abundant polymer of GlcNAc is widely distributed in nature whereas the related polysaccharide chitosan (polymer of GlcN and GlcNAc) occurs in certain fungi. Chitooligosaccharides of mixed acetylation patterns are of interest for the determination of the substrate specificities and mechanism of chitinases. In this report, we describe the chemical synthesis of three chitotetraoses namely GlcNAc-GlcN-GlcNAc-GlcN, GlcN-GlcNAc-GlcNAc-GlcN and GlcN-GlcN-GlcNAc-GlcNAc.

Benzyloxycarbonyl (Z) and p-nitrobenzyloxycarbonyl (PNZ) were used for the amino functionality due to their ability to form the β-linkage during the glycosylation reactions through neighboring group participation and the trichloroacetimidate approach was utilized for the donor. Monomeric, dimeric acceptors and donors have been prepared by utilizing the Z and PNZ groups and coupling between the appropriate donor and acceptors in the presence of Lewis acid yielded the protected tetrasaccharides. Finally cleavage of PNZ followed by reacetylation and the deblocking of other protecting groups afforded the *N,N'*-diacetyl chitotetraoses in good yield. Successful syntheses for the protected diacetyl chitotetraoses by solid phase synthesis have also been described.

Keywords: Chitooligosaccharides, Chemical synthesis, Solution phase synthesis, Solid phase synthesis, Glycosylation.

Contents

1. Introduction

The three major biopolymers, proteins, nucleic acids and glycoconjugates are responsible for the transfer of information which is a fundamental process of life and central to all cellular systems. The importance of proteins and nucleic acids has been studied for a long time and the role of glycoconjugates gained more importance recently. Oligosaccharides in the form of glycolipids and glycoproteins[1-3] play a major role in inflammation, immune response, fertilization and other biomedical processes[4,5]. The β-(1→4) linked *N*-acetylglucosamine moiety is a frequently occurring structural unit in various naturally and biologically important oligosaccharides and their conjugates.

Polysaccharides are generally derived from agricultural feed stock, e.g. starch, cellulose and pectin, or from crustacean shell wastes, e.g. chitin. Chitin and Chitosan are linear copolymers of GlcNAc (= **A**) and GlcN (= **D**) residues distributed randomly and are linked entirely in β-(1→4) configuration. Neither chitin, nor chitosan are homopolymers, as both contain varying fractions of GlcNAc and GlcN residues. The polymers may be distinguished by their solubility in 1% aqueous acetic acid. Chitin, containing (degree of acetylation: F_A) $F_A > 40$ **(Figure 1.1)** are insoluble whereas soluble polymers are named as chitosan ($F_A < 40$)[6].

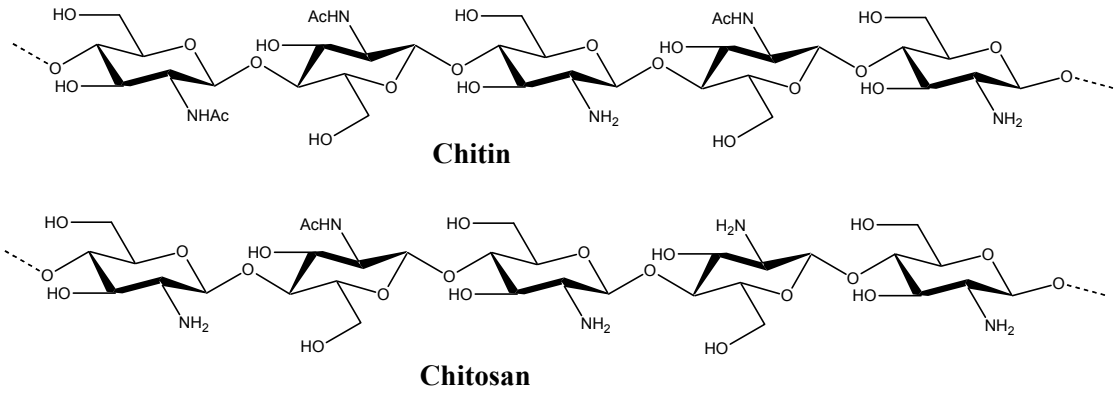


Figure 1.1 Structure of Chitin ($F_A > 40$) and Chitosan ($F_A < 40$)

1.1 Special features of Chitin and Chitosan

Both chitin and chitosan are used in wide range of applications in their native as well as in their modified forms. These include food, biotechnology, material science, drugs and pharmaceuticals and recently in gene therapy as well. The net cationicity as well as the presence of reactive functional groups makes chitosan an interesting biomolecule. The free

amino group present in each monomeric unit affords an ammonium group, due to protonation in aqueous acidic media. This offers scope for preparing broad spectrum of derivatives. Chitosan is prepared from suitable chitinous raw materials, mostly by a sequence of deproteinization, demineralization, and chemical deacetylation procedures. The molecular weight of chitosan depends on the source of the biological materials, as well as the conditions of deacetylation process.

The very high molecular weight and high viscosity of chitosan precluded its use in several biological applications. More than chitosan, its degradation products such as water soluble low molecular weight chitosan (LMWC), chitooligosaccharides (COs) and monomers were found to be more useful. A variety of degradation methods, namely chemical, physical and enzymatic are worked out to generate these degradation products. Both chitin and chitosan oligomers possess additional functional properties such as antitumour activity[7-9], immuno-enhancing effects in mice[10], antifungal[11,12] and antimicrobial activities[13].

Even though the chitin and chitosan oligomers can be prepared by chemical and physical methods, enzymatic methods are gaining importance because they allow regioselective depolymerization under mild conditions. In the case of enzymatic degradation of chitosan, LMWC with high water solubility were produced by chitinase, chitosanase, glucanase, lipase and some proteases[14-16]. Non specific enzymes[17] including lysozyme, cellulose, lipase, amylase, papain and pectinase[18,19] that are capable of depolymerizing chitosan are known. Among these papain is particularly attractive because of its plant origin, wide industrial use and its inhibition by human salivary cystatin.

D-glucosamine oligosaccharides attracted much attention, as they have physiological functions in a great variety of living organisms. Due to its beneficial plasma cholesterol level lowering effect, which plays an important role in the alleviation and treatment of cardiovascular diseases, chitosan has become a useful dietary ingredient[20]. Wound healing is a process for promoting rapid dermal regeneration and accelerated wound healing. The chitosan membrane showed controlled evaporative water loss, excellent oxygen permeability and promoted fluid drainage ability, at the same time effectively inhibiting invasion of exogenous microorganisms[21].

Chitosan is a versatile carrier for biologically active species such as drugs due to the presence of free amino groups as well as its low toxicity. The chitosan conjugated pentapeptide (Tyr-Ile-Gly-Ser-Arg – YIGSR) has enhanced activity against enzyme digestion in vivo than the native pentapeptide[22]. Recently chitosan which is a natural cationic polymer

has emerged as an alternative nonviral gene delivery system[23]. The LMWCs are neither toxic nor haemolytic and they are shown to form complexes with DNA and protect against nuclease degradation, thereby validating LMWC as components of a synthetic gene delivery system. The above feature shows the potential of the chitin and chitosan polymers in various biological processes.

1.2 Chitooligosaccharides

Recently chitooligosaccharides gained more importance due to their remarkable biological activity of fully or partially *N*-acetylated chitosans and chitooligosaccharides. These include immune stimulation through activation of macrophages[24,25], signaling[26], elicitor functions in plant cells[27-29]. In several cases chitooligosaccharides of mixed acetylated pattern show higher biological activities than homo oligomers of either GlcN or GlcNAc[30,31]. Chitooligosaccharides containing GlcN residues at the non-reducing end are strong inhibitors of chitobiases[32]. The mixed acetylation patterns of these oligosaccharides are of interest for the determination of the substrate specificities and mechanisms of chitinases[33] and lysozymes[19,34,35].

Chitosanase is a member of glycoside hydrolase family of enzymes and it is characterized by the ability to catalyze the hydrolytic cleavage of chitosan. Chitooligosaccharides have been used as a substrate in many enzymatic studies, not only with chitosanase but also with chitinases, lysozymes and several other classes of hydrolytic enzymes. It is apparent that the various categories of enzymes have different preferences with respect to the degree of acetylation of chitosan.

The elucidation of differences in the mechanisms of chitosan hydrolysis from various enzymes can be analyzed from the structure of hydrolysis products (sequences of oligosaccharides). From these sequences, the cleavage specificity of several enzymes could be deduced[36]. The proposed classification of chitosanases is based on enzymes that hydrolyze chitosan without splitting the linkage GlcNAc-GlcNAc (**A-A**). But chitosanases cleave the GlcNAc-GlcNAc linkage, but not the GlcN-GlcN (**D-D**) linkage. Based on this observation chitosanases are classified into three sub classes based on their specificity. Class I enzymes split both **D-D** and **A-A** linkages, class II enzymes split only **D-D** linkages and class III enzymes split both **D-D** and **D-A** linkages.

Subsequent work on chitinases[33,37] showed that they can be divided into at least two

more subclasses; the chitinases from *Bacillus circulans*, which can cleave both **A-A** and **A-D** linkages; the chitinases from *Streptomyces griseus* HUT 6037, which cleaves **A-A** and **D-A** linkages. The enzyme which belongs to class I chitosanases have been reviewed[38] to understand the substrate recognition mechanism, particularly the chitinases from *Streptomyces sp. N174*. $(GlcN)_6$ cleavage by N174 chitosanase led to the conclusion that the productive binding of $(GlcN)_6$ to the enzyme was better described by a symmetrical model including subsites, with cleavage occurring in the middle. A higher proportion of trimer $(GlcN)_3$ product was obtained from the hydrolysis of $(GlcN)_6$ together with $(GlcN)_4$ and $(GlcN)_2$ to a smaller extent by N174 chitosanase[39]. In order to find out these binding sites and comprehensive cleavage patterns of chitosanase, hetero chitooligosaccharides with different acetylation patterns are necessary.

The glycoprotein YKL-40, a chitin binding protein was identified in 1989, secreted *in vitro* in large amount by the human ostersarcoma cell line MG63. The protein was named YKL-40 based on its three N-terminal aminoacids Tyrosine (Y), Lysine (K) and Leucine (L) and its molecular mass was 40 kDa[40]. YKL-40 has high amino acid sequence homology with bacterial chitinases[41] and has strong binding affinity to chitin[42]. Chitooligosaccharides bind to YKL-40 with μM affinity[43] and oligomeric chitin could be a physiological ligand for YKL-40. It has been found in vertebrates in an embryonic stage and short chito-oligosaccharides are used as primers for the synthesis of hyaluronan[44-46].

Chitin oligosaccharides (COs) play an important role as signal molecules in plant and animal developmental processes, and defense mechanisms against pathogens. These oligosaccharides can elicit a number of direct responses in plant cells, such as enhanced ion flux across the plasma membrane resulting in a rapid alkalization of the medium, formation of reactive oxygen species, antimicrobial phytoalexin production, changes in protein phosphorylation, and lipid oxidation[47]. This polysaccharide together with hyaluronan (HA) has a structural function in the extracellular matrix, but also has a function in the signaling process that precedes cell proliferation and migration during the vertebrate embryogenesis[48].

The symbiotic relationship between bacteria of the genera *Rhizobium* and legumes results in the formation of a nitrogen-fixing root organ, the nodule[49]. The bacteria respond to specific compounds secreted by the plant roots (flavanoids) by production and excretion of lipochitooligosaccharides (modified chitooligosaccharides, also known as Nod factors)[50]. The back bone of the Nod factors consists of an oligomer of 3 to 5 residues of *N*-acetylglucosamine, *N*-acylated on the non reducing end. This backbone is synthesized by

three enzymes Nod A, B and C[51-53]. Polymeric chitin has been found in the epidermal cuticle of teleost fish[54] and chitin oligosaccharides have been isolated from *Xenopus laevis* and zebrafish embryos[55].

1.3 Enzymatic synthesis of chitooligosaccharides

In nature, partially *N*-deacetylated chitosan which are made up of GlcNAc residues randomly localized in the chitosan chain, was produced from chitin by chitin deacetylases[56]. But in the same time bacterial chitinases can also hydrolyze the β-(1→4) glycosidic linkage between the GlcNAc-GlcN of the *N*-deacetylated chitosan[33,57,58]. Drouillard and co workers established methods for the preparation of a series of monodeacetylated chitin oligomeric derivatives with GlcN residues at the reducing end[32,59].

Tokuyasu et al. reported that the chitin deacetylase can also acetylate substrates in the presence of 3M sodium acetate, and **DA** can be synthesized from chitosan dimer by a reverse hydrolysis reaction[60]. By this method chitotetraose (**AAAD**) has been synthesized from deacetylated chitosan tetramer (**DDDD**). The main strategies utilized for the preparation of partially deacetylated chitin oligomers with defined distribution of acetyl groups have been categorized into the following methods.

i) The enzymatic degradation of partially deacetylated chitin using lysozyme[61], chitinases or chitosanase[33,62],

ii) The synthesis of DAAAA using a recombinant *Escherichia coli* system harbouring *nod* B and *nod* C genes from *Rhizobium* sp[59],

iii) The enzymatic degradation of chitin oligomers using *N*-deacetylases[63,64],

iv) Reverse hydrolysis reaction by chitin deacetylases to form the partially deacetylated chitin oligomers[65].

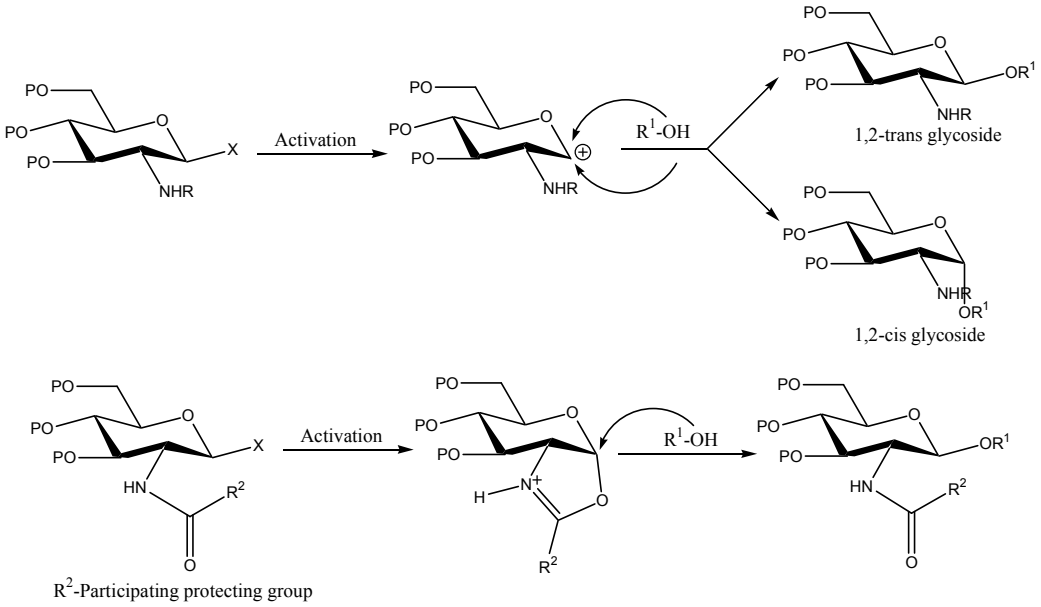
The enzymatic method has its own disadvantages in the form of limited substrate specificity, difficulty in purification of the products and characterization of the hetero deacetylated chitooligomers. Because of these drawbacks we decided to explore the chemical synthesis for the synthesis of partially acetylated chito oligosaccharides.

1.4 Chemical synthesis of oligosaccharides

The main conceptual difference between the proteins and DNA is the complexity of bond connecting the monomeric units. The glycosidic bond represents a new chiral center which paves way for the stereoselective synthesis. The necessity to form either 1,2-cis or 1,2-

trans glycosidic linkage with complete stereoselectivity and obtaining high yields are the major synthetic challenges in oligosaccharide synthesis. Glycosides of 2-amino-2-deoxysugars are present in the most important classes of glycoconjugates and naturally occurring oligosaccharides[66-68].

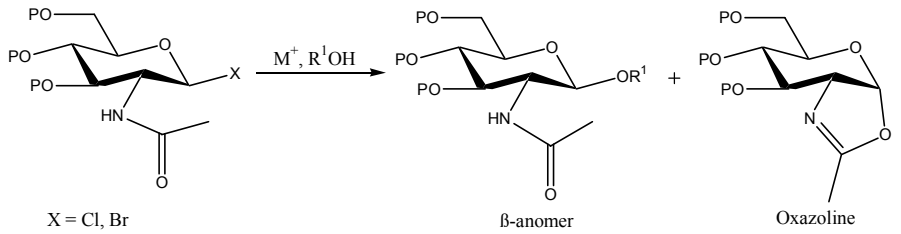
Generally, a promoter assisted departure of the leaving group of glycosyl donors resulted in the formation of glycosyl cation and the nucleophilic attack is almost equally possible from either top (trans, β) or bottom face (cis, α) of the ring. Even though the α-product is thermodynamically favored[69], a substantial amount of the kinetic β-linked product is often obtained. The use of participating protecting group at C-2 leads to the exclusive 1,2-trans glycosidic bond formation[70,71]. This glycosylation proceeds primarily via a reactive bicyclic oxazolinium intermediate directing the nucleophilic attack mainly to the top face of the ring and allowing stereoselective bond formation (**Scheme 1.1**).



Scheme 1.1 Stereochemical issues in the synthesis of carbohydrates

1.4.1 Koenigs-Knorr reactions

This reaction is one of the oldest methods for the preparation of 1,2-trans glycosides involving per-*O*-acetylated glucopyranosyl halides as donors and silver salts as promoters[72] (**Scheme 1.2**). The reactivity of the glycosyl donor can be varied over relatively wide ranges by the choice of halogen, the catalyst (promoter) and the protecting group patterns.



Scheme 1.2 Representation of Koenigs-Knorr reaction

But this method has been restricted to primary hydroxyl position at C-6 or in other words more complex oligosaccharides (secondary alcohol) were synthesized in moderate yields[73]. The main drawback of using the acetamido as amine protecting was the formation of oxazoline intermediate. This intermediate has been quiet stable under harsh Lewis acid conditions and did not exert higher glycosyl donor properties. Apart from glycosyl donors such as halides, thioglycosides[74], *O*-trichloroacetimidates[75] and dibutylphosphates[76] has been well utilized as donors in the formation of stereoselective glycosidic linkages. A variety of synthetic approaches towards the synthesis of 2-amino-2-deoxyglycosides have been developed[77,78]. Various glycosamine donors with modified functionalities have been investigated, in particular those bearing an *N*-2 substituent capable of either efficient participation via oxazolinium intermediate but not oxazoline intermediate to form 1,2-trans glycosides.

1.4.2 Synthesis of 2-Amino-2-deoxyglycosides from glycals

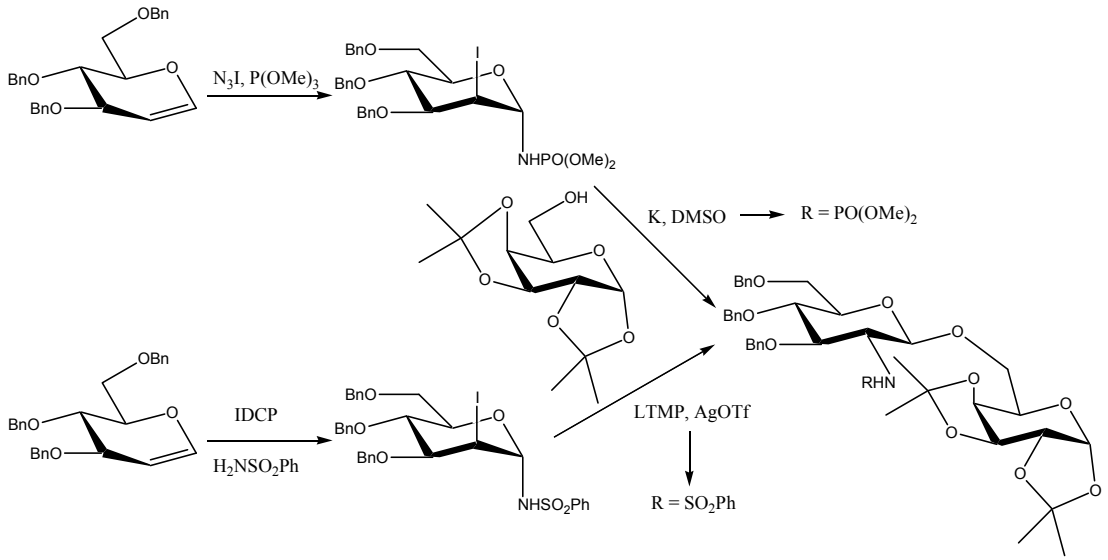
Glycals (1,2-dehydro sugar derivatives) are often employed as versatile building blocks in carbohydrate synthesis[79,80]. They are excellent starting materials for the synthesis of 2-amino-2-deoxysugars by *N*-functionalization at C-2 accompanied by C-1 bond formation. In the last few decades variety of methods have been developed for the nitrogen transfer to glycals (**Scheme 1.3**).

Scheme 1.3 Reaction with glycals

The advantage of employing azide as an amine protecting group in general synthetic applications include lower steric hindrance, greater solubility, lack of rotamer formation, and the absence of hydrogen as well as carbon nuclei in NMR spectra[81]. The common method employed for the formation of azide derivative was the reaction between alkene and sodium azide in the presence of ceric ammonium nitrate (CAN) in acetonitrile[82].

Transformation of the 2-azido-2-deoxy-1-nitro-pyranose intermediate into a suitable donor can be achieved in a number of ways. Conversions to hemiacetals[83,84], halides[85,86], acetate[87], trichloroacetimidate[88,89], pentenyl glycoside[90], phosphate[91,92], thioglycosides[93,94] and xanthates[87] are among the most common approaches that have been used efficiently in the oligosaccharide synthesis. The azido moiety can be reduced under a variety of reaction conditions includes catalytic hydrogenation[95-97], treatment with 1,3-propanedithiol[98], staudinger ligation[99,100] or birch reduction[101,102]. Then the free amine can be converted into acetamido or other *N*HR or *N*R_2 derivatives by simple protecting group chemistry.

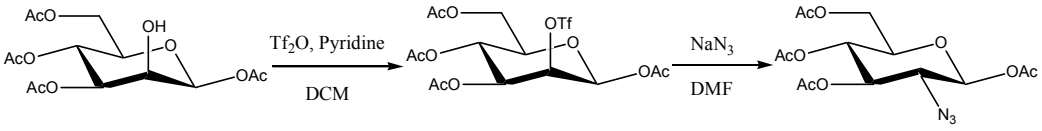
The unique reactivity of the three membered rings makes aziridine derivatives useful intermediates for the synthesis of 2-amino glycosides. Lafont and Descotes utilized the activated aziridines for the synthesis of 2-amino sugars by phosphoramidation method[103,104]. After this Griffith and Danishefsky explored the sulfonamide glycosylation method[105] with glycals (**Scheme 1.4**).



Scheme 1.4 Glycosylation based on phosphoramidation and sulfonamidation method

1.4.3 Synthesis of 2-deoxyglycosides by nucleophilic displacement method at C-2.

The nucleophilic displacement reaction is an important tool for the introduction of substituents into sugar framework. These reactions proceed via the bimolecular mechanism and result in the inversion of configuration. The displacement reactions with less reactive sulfonic esters like mesyl and tosyl are often limited to the more accessible primary carbon atoms[106-108]. If highly reactive triflates are employed as substrates, substitution reactions at either primary or secondary are often achieved under mild conditions. S_N2 displacement of a suitable triflate glycosyl derivative with the azide ion appears to be a viable alternative to the existing technologies for the preparation of 2-azido-2-deoxyglycosides [109-111] (**Scheme 1.5**).



Scheme 1.5 Nucleophilic displacement reaction using triflate

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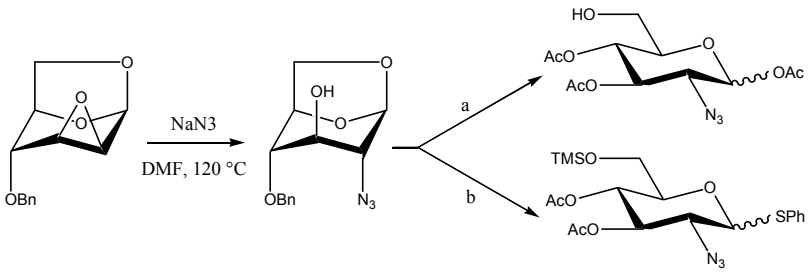
Series of epoxide derivatives developed by Cerny and Stanek occupy an important place in the nucleophilic displacement reactions[112] (**Scheme 1.6**). Recently introduced, modified one-pot procedure enhances the usefulness of this protocol[113]. To date, this anhydrosugar approach to 2-amino-glycosides has been applied to the synthesis of glycosaminoglycans[114], lipid A disaccharides[115,116], *N*-acetyllactosamines[117] and umbelliferone glycosides of *N*-acetyl glucosamine and chitobioses[118].



a) Ac_2O, AcOH, H_2SO_4, b) (i) Ac_2O/pyridine, (ii) TMSSPh, ZnI_2

Scheme 1.6 Nucleophilic displacement reaction using epoxides

1.4.4 Synthesis of glycosides using amino protecting groups

Naturally occurring 2-amino-2-deoxy glycopyranosides are often *N*-acetylated and are linked via 1,2-trans-glycosidic linkages. The use of *N*-acetylated donors leads to the formation of relatively unreactive oxazoline[78] intermediate that often remains as a major by-product. The high nucleophilicity of the lone pair of electrons on nitrogen atom of the acetamido group also presents a complication by attracting electrophilic species that often results in decreased reactivity or additional byproduct formation. A general way to decrease the reactivity of the amino group is to temporarily protect the amino functionality by electron withdrawing amide, carbamate or imine.

The choice of the amino protecting group is influenced by the fact that protecting group at C-2 can directly influence the stereochemical outcome of the reaction due to the property of neighboring group participation. This property helps to form the 1,2-trans glycosidic linkage whereas the non participating nature of the amino protecting group leads to the formation of 1,2-cis glycosidic bond. The use of non-participatory moiety alone does not guarantee 1,2-cis selectivity; for example the use of 2-azido-1-phosphate donors provided excellent 1,2-trans selectivity even in the presence of non-participating system[92]. In general, the ideal amine protecting group should be stable to a wide range of reaction conditions, impart sufficient reactivity, stereoselectivity, high yield in glycosylation reaction and readily removed under mild reaction conditions.

1.4.4.1 Haloacetamido derivatives

Halogen substituted 2-acetamido donors (2-chloroacetamido-2-deoxyglucopyranosyl) will form weaker, and therefore more reactive oxazoline intermediates by virtue of its electron withdrawing character[119]. As a result this type of glycosyl donors (*N*HCOCH$_2$Cl) would be better electrophiles and hence more reactive in glycosylation reactions. The effectiveness of this method has been shown in the glycosylation reaction of primary and secondary glycosyl acceptors[120,121]. 2-deoxy-2-dichloroacetamido (*N*HCOCHCl$_2$) derivatives were introduced as glycosyl donors to address the challenge associated with *N*-acetamido derivatives (*N*HCOCH$_3$). It is believed that the powerful electron withdrawing character of dichloroacetyl group has the appropriate structure to address this challenge[122].

Since the glycosyl trichloroacetimidates are excellent glycosyl donors[123], the bicyclic derivative also served well as a potentially reactive glycosyl donor for the synthesis of 1,2-trans-2-amino-2-deoxyglycosides[124,125]. Another advantage of the trichloroacetamide group

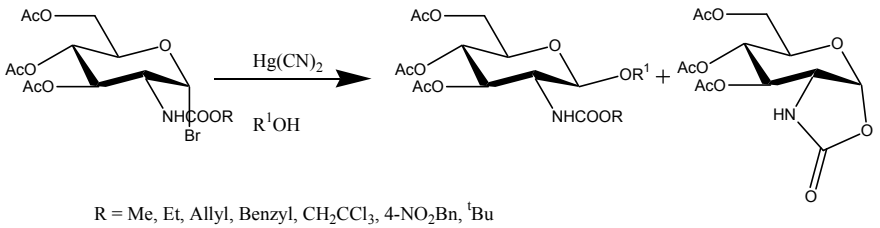
(*N*HCOCCl$_3$) was the ease of its deprotection under variety of reaction conditions amongst which are Bu$_3$SnH-AIBN[126], hydrogenolysis[127] or cleavage with NaOH followed by reaceylation[128]. The trifluoroacetamido derivatives also shown increased donor properties[124] during the glycosylation reactions. The reported glycosyl donors bearing *N*-trifluoroacetyl group are the acetates[129], bromides[130], phosphates[131] and recently S-benzoxazolyl[132]. These glycosyl donors have been well utilized in the synthesis of chiro-inositols[133], C-glycosyl phosphonates[134], diosgenin[135] derivatives and Lipid A analogues[136]. Schmidt et al. reported the *N*,*N*-diacetyl protected sugar derivatives for the β-linkage formation in order to address the reactivity of the *N*-acetamido protected glycosyl donors[137].

1.4.4.2 Alkoxycarbonyl derivatives

2-Alkoxycarbonyl-2-deoxy derivatives found widespread use as glycosyl donors due to their ease of formation as well as orthognality during the *N*-deprotection. The carbamates derivatives which were utilized as glycosyl donors include benzyl carbamates (*N*HCbz or *N*HZ)[138], allyl carbamates (*N*HAlloc)[139], trichloroethyl carbamates (*N*HTroc)[140], *N*-methoxycarbonyl[141], tert-butyloxycarbonyl (*N*HBoc)[142] and p-nitrobenzyloxycarbonyl (*N*HPNZ)[143,144].

The first reported glycosylation with Cbz protected sugars involved the activation of bromides[138,145]. But it led to the formation of oxazolidinone (**Scheme 1.7**) and migration of benzyl group to the aglycone[138] also observed. More potent glycosyl donors bearing the Cbz moiety have been developed including dimethylphosphinothioates[146], phosphorodiamidimidothioate[147], trichloroacetimidates[148], 4-pentenyl glycosides[149] and phosphates[149]. The Cbz protected glycosyl donors have been used in the successful preparation of heparin derivatives[150] and neoglycoconjugates[151] etc. The allyloxycarbamoyl protective group also posses participating properties in 1,2-trans glycosides formation. It have been used in the synthesis of glycopeptide assemblies[152] and *N*-acetylneuraminic acid derivatives[153].

The *N*HTroc (*N*-2,2,2-trichloroethoxycarbonyl) group have been utilized extensively in the solution and solid phase oligosaccharide synthesis due to the reactive donor properties compare to the other carbamate derivatives. More over its stability towards the alcoholysis under basic condition permits convenient transformations into other carbamates. Removal of



R = Me, Et, Allyl, Benzyl, CH_2CCl_3, 4-NO_2Bn, ^{t}Bu

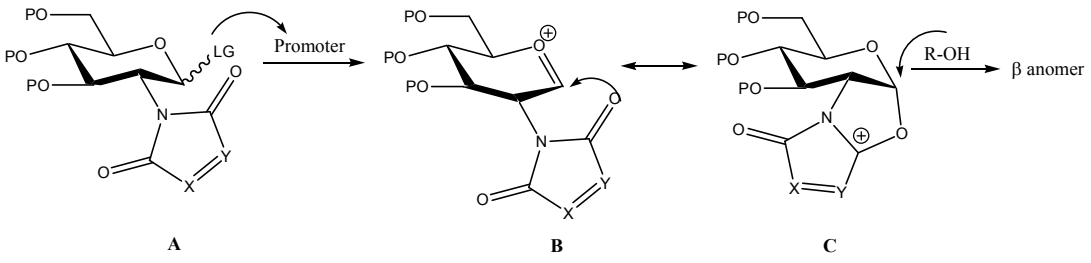
R^1 = ^{i}Pr, Cyclohexyl, ^{t}Bu, CH_2CCl_3

Scheme 1.7 Formation of oxazolidinone[142]

the *N*HTroc was achieved by reductive elimination using Zn in AcOH. It have been used as trichloroacetimidates for the synthesis of carbohydrate antigens[154] and hyaluronan trisaccharides[155], as fluorides for the synthesis of glycosylamines[156] and tumor-associated antigens[157], as thioglycosides for the synthesis of *N*-acetyllactosamine oligomers[158] and vancomycin[159] and as sulphoxides for the preparation of 2-aminoglycals[160]. Schmidt et al.[161] explored the *N*-acetyl-*N*-2,2,2-trichloroethoxycarbonyl (*N*AcTroc) protected thioglycosides as efficient donors for the glycosylation reactions. The *p*-nitrobenzyoxycarbonyl group[143] (*N*HPNZ) have been recently utilized in the 2-amino glycoside synthesis and this protecting group was not well explored towards the synthesis of complex oligosaccharides irrespective of its mild deprotection conditions.

1.4.4.3 Disubstituted 2-amino-2-deoxyglycosyl donors

N-phthalimido[78] have been used widely in the solution as well as solid phase oligosaccharide synthesis. The electrophilic activation of the bivalently protected glycosyl donor **A** yields an oxocarbenium ion **B**, which can form the oxazolinium intermediate **C**. The reactive intermediate **C** can only be attacked from the β-face by the nucleophile and cannot form the oxazoline (**Scheme 1.8**).



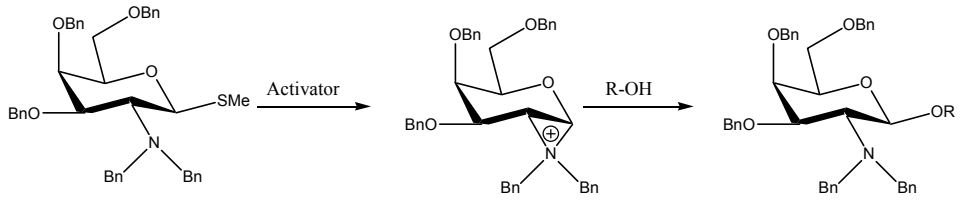
Scheme 1.8 Glycosylation of *N*-phthalimido derivatives

N-phthalimido protected donors and acceptors have been used extensively to prepare oligosaccharides. Gangliosides GD2 and GQ1b[162], glycoprotein residues[163] and blood group determinants[164] are to name a few. The strong basic condition and high temperature required for the cleavage (hydrazine hydrate in EtOH) was the main drawback associated with this protecting group. This led to the usage of tetrachlorophthalimido (TCP) derivatives as building blocks for the amino sugar synthesis[165]. Removal of the TCP was achieved by using either ethylene diamine[166] or by $NaBH_4$ in isopropanol[167].

Schmidt and coworkers[168] introduced the use of *N*-dimethylmaleoyl (DMM) derivatives for the synthesis of 2-aminoglycosides. DMM moiety provides anchimeric assistance for the formation of β-linkage and stable towards the acids and non-nucleophilic bases. This protecting group have also been used to synthesize *N*-glycan derivatives[169], human milk oligosaccharides[170] etc. The cleavage of DMM was performed in the presence of weakly basic and then under acidic conditions.

Boons et al.[171] developed the 2,5-dimethylpyrrole group for the synthesis of 2-aminoglycosides. The dimethylpyrrole moiety is not capable of anchimeric assistance, and the observed β-selectivity was attributed to the steric bulk. The dimethylpyrrole protecting group was cleaved in the presence of hydroxylamine and the phthalimido group was found to be stable under this conditions.

2-*N*,*N*-dibenzylamino moiety was also used as the amine protecting group for the synthesis of amino sugars[172]. The stereoselectivity was governed by the formation of dibenzyl aziridine intermediate and steric bulkiness as well (**Scheme 1.9**). The main advantage of this protecting group was the removal along with the *O*-benzyl substituents.



Scheme 1.9 Synthesis of glycosides using *N*,*N*-dibenzylamino group.

Recently one patent was published for the synthesis of chitin dimers utilizing benzyloxycarbonyl (*N*HCbz) and trichloroacetamide (*N*HCOCCl$_3$) as the participating amine protecting group for β-linkage formation[173]. Schmidt et al.[174] reported the synthesis of chitotetraose and chitohexaose based on the *N*-dimethylmaleoyl (*N*DMM) protecting group

DDAA, AADD, DAAD, ADDA, ADAD and DADA are the six chitotetraose sequences present in the chitinous organisms. The three sequences AADD, DADA and ADDA were synthesized by solution phase methodology using *N*-phthalimido (*N*Phth) and *N*-dimethylmaleoyl (*N*DMM) protection by our group[175].

1.5 Solid Phase synthesis of oligosaccharides.

Solid-phase synthesis is a methodology in which synthetic transformations takes place with one of the reactants attached to the insoluble matrix. The first solid-phase peptide synthesis[176] was reported by Merrifield in 1963 in which he performed the peptide synthesis using polystyrene resin, today referred to as Merrifield's resin. The advantages of solid-phase synthesis prompted researchers to explore oligosaccharide synthesis on solid supports. These include maximized yields by the use of excess reagents, ease of purification and synthesis speed.

The level of complexity associated with the synthesis of oligosaccharides on a polymer support is much greater than the other two classes of repeating biopolymers. Oligopeptides and oligonucleotides consist of only linear chains whereas oligosaccharides have the potential elongation, are often branched, requiring flexible protecting group strategies for the effective differentiation of hydroxyl and amine functionalities. The formation of new stereogenic center in every glycosylation step further complicates oligosaccharide synthesis. Thus, a series of issues have to be considered before planning the synthesis.

(1) Selection of an overall strategy for the attachment of sugar derivative to polymer support through the "reducing" end or the "nonreducing end",

(2) Choice of the solid support material,

(3) Selection of a linker, which has to be stable enough during the glycosylation and in the same time easily cleavable when desired,

(4) An extensive orthogonal protecting group strategy,

(5) Stereospecific and high-yielding coupling reactions.

1.5.1 Earlier studies on solid-phase oligosaccharide synthesis

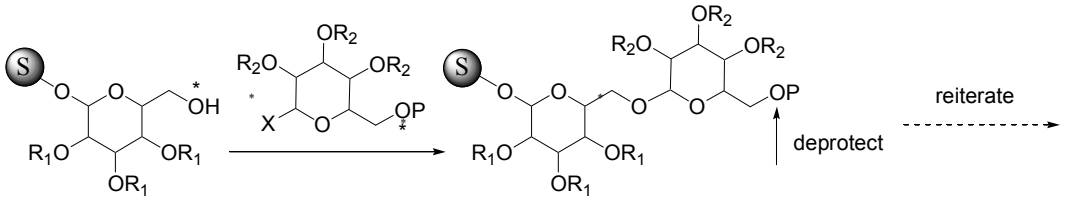
Frecet and schuerch were the first to report on the synthesis of di and trisaccharides on a solid support[177]. In addition to studies utilizing functionalized Merrifield's resin, controlled

pore glass (CPG) was applied to solid phase oligosaccharide synthesis as a non-swelling inorganic support by schuerch[178]. After 1990's the solid phase oligosaccharide synthesis (SPOS) has shown great promise[179].

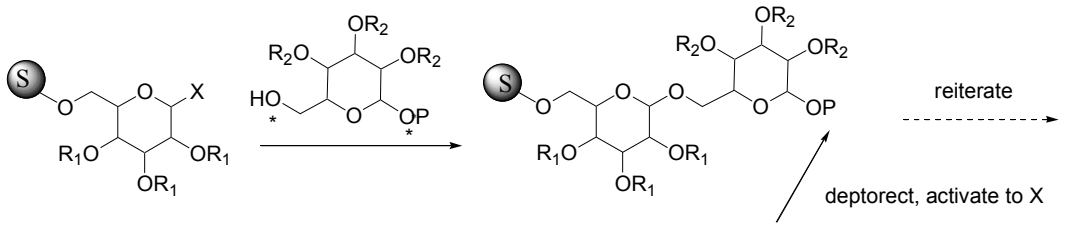
1.5.2 Synthetic strategies

Important aspect of oligosaccharide synthesis is a glycosylation reaction involving a reactive species (the glycosyl donor) and a nucleophile (the glycosyl acceptor). Attachment of glycosyl donor is achieved by connecting the non-reducing end of the first carbohydrate moiety to the solid support (donor-bound strategy). Otherwise, the anomeric position of the glycosyl acceptor is fixed to the support (acceptor-bound strategy). Any one of these possible strategies (**Scheme 1.10**) or the bidirectional synthesis has been utilized for solid phase oligosaccharide synthesis.

Acceptor-bound approach:



Donor-bound approach:

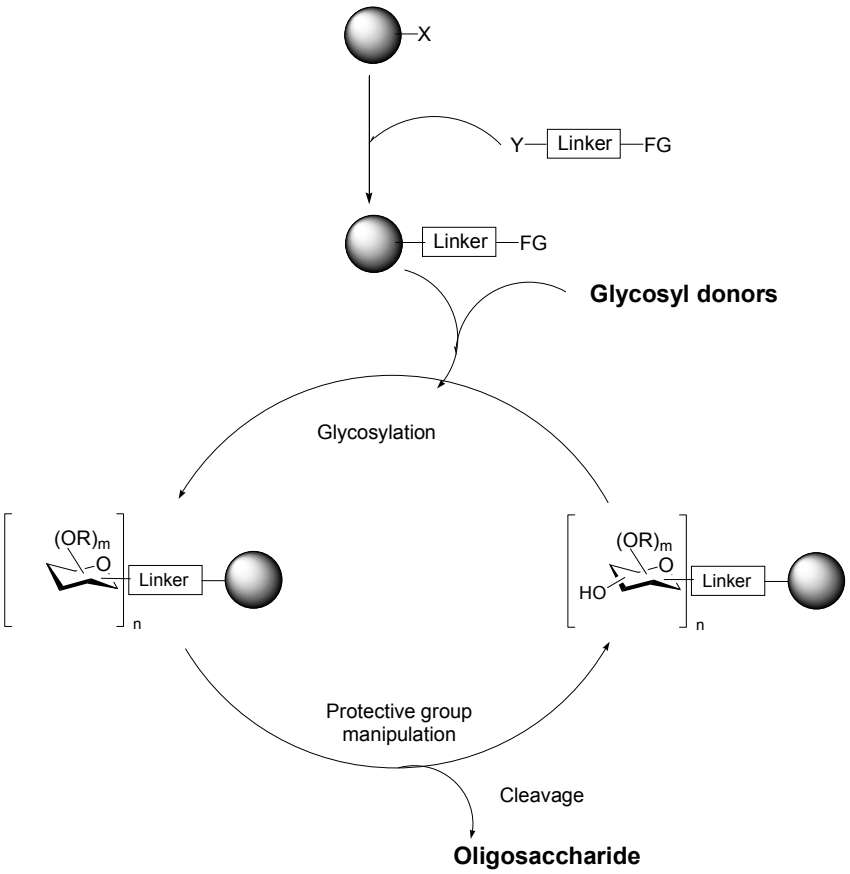


S solid support and linker, P-unique protecting group, X-activating group, * uniquely differentiated hydroxyl group.

Scheme 1.10 Acceptor and donor bound strategy.

Numerous synthetic strategies have been developed and considerable progress has been made in the field of solid-phase oligosaccharide synthesis. In general, attachment of the first fully protected carbohydrate unit through its anomeric position to a solid support, via

linker, is the most efficient and frequently used strategy for solid-phase oligosaccharide synthesis. The attachment of the first carbohydrate moiety is subjected to protective group manipulation and the resulting glycosyl acceptor is subsequently glycosylated with a suitable donor, which in turn is subjected to protective group manipulation to yield a glycosyl acceptor. Glycosylation and protective group manipulations are performed in an iterative cycle and final cleavage from the linker releases oligosaccharide from the solid support (**Scheme 1.11**).



Scheme 1.11 General synthetic procedure for the solid-phase oligosaccharide synthesis

1.5.3 Supports for the solid-phase oligosaccharide synthesis

Supports of different macroscopic shapes have been used for the solid-phase synthesis. Most common and widely used one is the spherical particles, which are readily weighed,

filtered, dried and well suited for most of the applications. The other forms of insoluble support include sheets[180], crown-shaped pins[181] or small discs[182]. The general requirements for a support are mechanical stability and chemical inertness under the reaction conditions to be used.

Chemically functionalized supports are necessary for attaching the synthetic intermediates covalently to the support via linkers. Therefore understanding the resin properties is crucial for an efficient solid-phase oligosaccharide synthesis. Before planning the synthetic route, one has to consider the properties of the resin i.e. swelling, cross-linking, particle size, stability, functional groups, loading capacity, etc. Mechanical stability was required to avoid the breaking down of the polymer into smaller particles and at the same time supports need to be chemically functionalized, so that the synthetic intermediates are located within the support. The most frequently used resins are gel type resins and typically cross-linked polystyrene or poly(styrene-oxyethylene) graft copolymers. In organic solvents such resins furnish a solvent-swollen gel, which facilitates the diffusion of reagents through the polymer network to the reactive sites.

The most commonly utilized resin today is the Merrifield resin (1 or 2 % divinylbenzene cross-linked polystyrene, **Figure 1.2**). The remarkable feature of this resin includes high capacity of loading and excellent stability towards a wide range of reaction conditions. Since the main part of the reaction takes place inside the bead it is anticipated that the solvent depending properties and steric hindrance may affect the reactivity and the stereochemical nature. With the aim of fine tuning the physiochemical properties of polystyrene-based supports and thereby improving their suitability for solid-phase synthesis, cross-linking agents other than divinylbenzene have been investigated.

Poly-(ethylene glycol) (PEG) grafted polystyrene[183] (PS) supports which swell in both polar and non polar solvents. PEG grafted into cross-linked polystyrene can be prepared either by linking PEG to suitably functionalized polystyrene[184] or by polymerization of oxirane on a hydroxylated support. Tentagel[185] and Argogel[186] (**Figure 1.2**) are PEG grafted PS copolymers that have been extensively used because of their mechanical stability and swelling properties. The high content of PEG plays an important role to enhance the hydrophobicity and the molecular mobility of the resin, which leads to solvent like environment. Loadings of commercially available Tentagel and Argogel are in the range of 0.15-0.30 mmol/g. The low loading capacity is being an advantage in solid phase organic synthesis since a too high loading capacity may lead to incomplete reactions because of steric hindrance.

1.5.4 Linkers for solid-phase oligosaccharide synthesis

Linkers are molecules which keep the intermediates in solid-phase synthesis bound to the support. Linkers should enable a simple way of attachment to the starting materials, stable under variety of conditions employed for the iteration of the sugar assemblies and easily detachable after the synthetic steps without affecting the product[187,188]. The choice of linker attached to the polymeric support is critical in the solid-phase synthesis. Linkers enable the attachment of a variety of functional groups to a solid support and upon cleavage either the originally attached or a new functional group may be generated. In most of the solid-phase synthesis, the linkers are attached to a support by means of a spacer. The spacers facilitate the diffusion of the reagents to the resin-bound substrate by increasing its distance from the support.

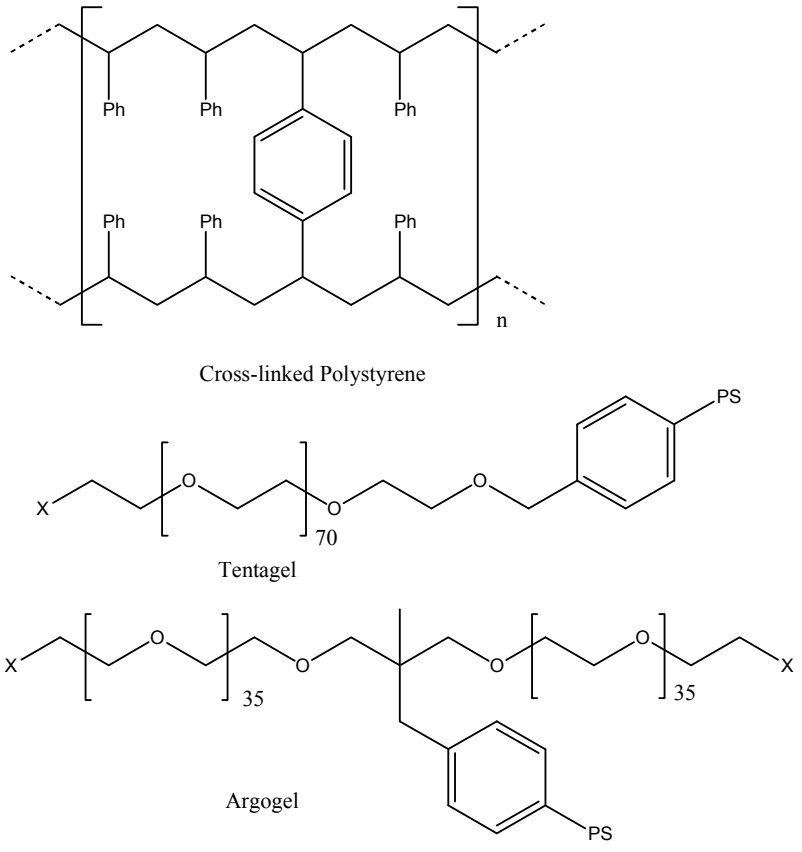


Figure 3 1.2: Structure of cross linked polystyrene, tentagel and argogel.

Silyl ethers are the convenient protective groups for hydroxyl groups due to the ease of removal under mild conditions. Diisopropyl arylsilane linkers have been used successfully in Danishefsky's donor-bound strategy employing glycal derived donors[189]. Also host of acid-

labile linkers known from the solid-phase peptide synthesis have been applied to solid-phase oligosaccharides assembly. Most acid-labile benzyl alcohol linkers suitable for the attachment of carboxylic acids to insoluble supports can also be used to attach aliphatic or aromatic alcohols as ethers.

Phenols can be etherified with resin-bound benzyl alcohols by the Mitsunobu reaction[190] or alternatively, by nucleophilic substitution of resin-bound benzyl halides. Aliphatic alcohols have been etherified with Wang resin by conversion of the latter into the trichloroacetimidate followed by nucleophilic substitution with the alcohol under slightly acidic conditions[191,192]. Ethers are generally inert towards the nucleophilic attack and are therefore suitable linkers for the solid-phase chemistry involving strong nucleophiles. Phenols etherified with hydroxymethyl polystyrene can be released by treatment with TFA. The TFA mediated cleavage of alcohols from supports occasionally leads to the formation of TFA esters of the released alcohols. This esterification can be avoided sometimes by using wet TFA (containing 5% water) instead of anhydrous TFA.

Both aliphatic alcohols and phenols have been immobilized as esters of support bound carboxylic acids. The esterification can be achieved by treatment of resin bound acids with alcohols and a carbodiimide, under Mitsunobu conditions or by acylation of alcohols with support bound acyl halides. Ester attachment of alcohols is particularly useful, when acidic reaction conditions are to be employed in a synthetic sequence. The alcohols can be quantitatively esterified by using excess of support bound acylating agent and it is easy to cap the excess agent by treating with methanol. Cleavage of support bound esters can be effected by a wide variety of reagents. These include saponification with alkali metal hydroxides[193], low-molecular weight amines[194] (Me-NH_2), mixtures of methanol and triethylamine[195] and hydrazine[196].

Thioglycosides are also used as linkers due to their ability of anomeric protection. This type of attachment to the carrier resin was utilized with trichloroacetimidate donors in the assembly of oligomers[197]. Several linkers related to *p*-methoxybenzyl (PMB) group that may be cleaved by oxidation have been introduced[198]. Several groups have used the idea of employing a photo cleavable linker[199] for chemical oligosaccharide synthesis. Photo labile *o*-nitrobenzylic linkers were used by K.C.Nikolaou and co-workers[200] and Fraser-Reid used the secondary *o*-nitrobenzyl ether linkage[201].

In addition to these, linker cleaved by olefin metathesis was utilized by Seeberger et al[202]. In this case carbohydrate moiety was connected through a glycosidic bond to octenediol-

functionalized Merrifield's resin. The octenediol linker was stable to a wide variety of conditions and was quantitatively cleaved by olefin cross metathesis in the presence of Grubb's catalyst to afford the fully protected oligosaccharide. Schmidt et al also utilized the ring closing metathesis reactions for the cleavage of linkers[203].

1.5.5 Use of different glycosylating agents

The key to the success of solid-phase glycosylation reaction is the glycosylating agents which can react efficiently. Since the purification is not possible during the glycosylation it is necessary that the glycosylation agents possess sufficient reactivity and selectivity.

1.5.5.1 The glycal assembly approach

The glycal assembly approach is one of the well explored methods in solution phase synthesis for the assembly of complex oligosaccharides, glycoconjugates, and glycosylated natural products[80]. Following their success, it was well adapted to the solid-phase synthesis[189]. Glycals minimize protecting group manipulations, serve as glycosyl acceptors, and may readily be converted to different glycosylating agents. It has been shown by the preparation of a linear hexasaccharide containing β-(1→3) glycosidic and β-(1→6)-galactosidic linkages[204]. Complete access to the Lewis^b antigen on solid support was achieved using the iodosulfonamidation and thioethyl glycosyl donor[205]. The glycal method was further extended to access *N*-linked glycopeptides by solid-phase synthesis[206].

1.5.5.2 Glycosyl sulphoxides

The use of glycosyl sulphoxides was initiated by Kahne and explored in the preparation of single compounds and of a combinatorial library of di- and tetrasaccharides[207]. In the absence of C-2 participating group, anomeric sulphoxides gave high α-selectivity. The selective formation of β-glycosidic linkages was achieved with the aid of a C-2 pivaloyl participating group.

1.5.5.3 Glycosyl trichloroacetimidates

The enormous success of glycosyl trichloroacetimidates as glycosyl donors in solution phase synthesis are due to its versatility, high yields and excellent selectivity in glycosylation

reactions. Krepinsky reported the first successful use of trichloroacetimidate glycosyl donors for the synthesis of a disaccharide on a soluble PEG support[208]. Schmidt et al utilized this strategy for the formation of the thiol ether linkage on polymer support (Merrifield's resin)[209]. Trichloroacetimidate donors also performed well with other solid supports materials. The performance of trichloroacetimidates in glycosylation reactions with acceptors bound to different polymeric supports have been well explored. Excellent coupling yields were reported with polystyrene or controlled pore glass (CPG), but PEG containing polymers found to perform poorly in these reactions. Reaction temperature, excess of donor and sometimes double glycosylation were found to be crucial if coupling efficiencies were to exceed 95 %. A capping step was introduced after each glycosylation to block the unreacted acceptor sites.

1.5.5.4 Other glycosylating agents

Other glycosylating agents which are well utilized in the solid phase synthesis are thioglycosides, thiofluorides, n-pentenyl glycosides and glycosyl phosphates. Thioglycosides are stable over longer time even in room temperature but the only disadvantage was its high toxicity of the activators. But these methods have a widespread use in the synthesis of oligosaccharides. This method was well utilized in both soluble[210] as well as in insoluble support[200]. The requirement of heavy metal salts activation makes the glycosyl fluoride method difficult on polymeric supports. For the α-fucosyl linkages this method was proved to be effective due to the high selectivity and good yields[211]. n-pentenyl glycosides were successfully utilized by Fraser-Reid at al.[212] in solid-phase synthesis using Polystyrene and Tentagel resins. Glycosyl phosphates are extremely reactive glycosyl donors that can be activated at low temperatures to form a variety of linkages in very high yields. This has been well demonstrated by Seeberger et al. in solution phase[213] as well as solid phase synthesis of oligosaccharides[214].

1.5.6 Cap technique

Compared to the coupling reactions in peptide and nucleotide synthesis, glycosylation reactions established for carbohydrate synthesis are much less effective. After few steps of glycosylation, significant amount of unreacted carbohydrate sequences (deletion sequences) will be present in the resin. After the final cleavage, the target oligosaccharides were mixed together with the deletion sequences. Purifying the desired oligosaccharide from these

mixtures would be challenging. For this purpose Cap and Tag[215], Capping reagent[216] and recently Cap and Capture technique[217] were developed and applied efficiently in SPOS.

1.5.7 Automation of solid-phase synthesis

Nowadays Oligonucleotides and Oligopeptides are routinely prepared in an efficient manner on automated synthesizers with solid-phase strategies. Seeberger et al.[213] first reported the synthesis of oligosaccharides using an automated synthesizer by utilizing solid-phase approach. The utility of the automated method has been demonstrated with glycosyl trichloroacetimidate and glycosyl building blocks. Temporary protecting groups such as levulinoyl esters, silyl ethers and acetate were shown to be compatible with automation procedure.

1.5.8 Glycosylation reactions in microreactor

Recently microfluidic based glycosidic reaction was reported by Seeberger et al.[218]. Glycosylation reaction was performed in continuous flow micro reactors and the optimization of yield, reaction time and temperatures were reported. Glycosyl trichloroacetimidates and glycosyl phosphates were well utilized to form the α(1→6) & α(1→2) linkages in mannose derivatives[219].

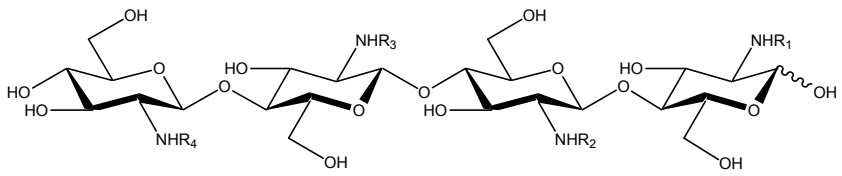
2. Aim of the Work

Recently partially acetylated chitooligosaccharides gained importance because of the fact that these oligosaccharides can be used to find out the enzyme (glycoside hydrolases family) specificity based on their hydrolytic ability. More over these oligosaccharides are useful for studying the embryogenesis of vertebrates. For in-depth studies of structure-activity relationships, structurally defined oligosaccharides are needed. The chemical synthesis of these partially acetylated chitooligosaccharides is a reliable approach to control the chain size and also the order of repeating units (GlcNAc and GlcN).

The aim of the present thesis was to develop general methods for the synthesis of three tetrasaccharides composed of two GlcNAc and two GlcN residues. To this end, methods are needed which allow a flexible sequential construction of any possible sequence of these monosaccharide building blocks. Thus the tasks consisted of

- developing a synthetic strategy, involving orthogonal *N*-protecting groups
- to explore proper *O*-protecting groups and their removal
- to study the activation of glycoside donors and stereoselective glycosylation
- to elaborate schemes for oligosaccharide synthesis suitable for both, in solution and solid phases
- to evaluate the strategies for their applicability toward the construction of higher oligosaccharides.

The three *N,N'*-diacetyl chitotetraoses are

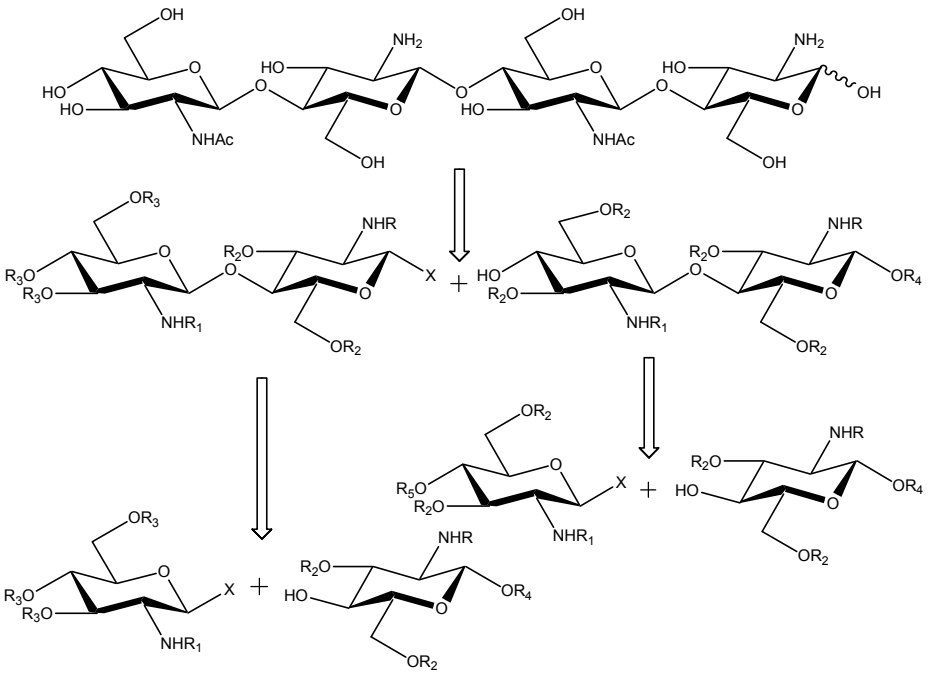


R_1 = Ac, R_2 = Ac, R_3 = H, R_4 = H **(GlcN-GlcN-GlcNAc-GlcNAc, 5)**
R_1 = H, R_2 = Ac, R_3 = Ac, R_4 = H **(GlcN-GlcNAc-GlcNAc-GlcN, 6)**
R_1 = H, R_2 = Ac, R_3 = H, R_4 = Ac **(GlcNAc-GlcN-GlcNAc-GlcN, 7)**

3.1. Solution phase synthesis

3.1.1 Retro synthetic analysis

The most important concept behind the oligosaccharide synthesis is the glycosylation reaction involving a reactive species (glycosyl donor) and a nucleophile (glycosyl acceptor). Chito oligosaccharides are composed of β-(1→4) linked GlcN (=D) and GlcNAc (=A) units. Because of strong nucleophilic character of amino functionality, protection of amino group is important towards the synthesis of the building blocks. The choice of amino protecting group provides the control over stereochemistry of the glycosidic bond between sugar units. The Retro synthetic analysis of the sequence ADAD (throughout this discussion, non-reducing end is depicted in the left hand side and reducing end depicted in the right hand side) is shown in the **Scheme 3.1.1**.



Scheme 3.1.1 Retro synthetic analysis of the sequence ADAD (7)

D-glucosamine (**8**) is a basic constituent of chitin and chitosan from which it can be easily obtained by hydrolysis. Its hydrochloride salt serves as the starting material for the building block synthesis. As discussed earlier in the introduction, many amino protecting groups have been developed for the 1,2-*trans* glycosylation which for D-gluco amino sugars requires glycosyl donors with participating protective groups in the C-2 position. In the formation of β-anomer, strong electron withdrawing character of the *N*-substituent as well as

the glycosidic bond formation which occurs generally via neighboring group participation plays a major role. Phthalimido[78], tetrachlorophthalimido[77,220], *N*,*N*-diacetylamino[137], trichloroacetylamino[126] groups are quite a few examples for this case. These glucosamine derivatives also exhibit increased glycosyl donor properties. However all these groups also have some disadvantages over others which have already been discussed in detail. The final deprotection in all the carbohydrate building block synthesis is the removal of stable *O*-benzyl ether groups by hydrogenolysis. In order to achieve the β-(1→4) glycosidic bond, the glycosyl acceptor must posses the free hydroxyl functionality in the C-4 position. In the retro synthetic analysis shown in **Scheme 3.1.1**, the C-6 and C-3 hydroxyl can be protected as their corresponding stable *O*-benzyl ethers.

The carbamate functionality has been used for protection of the amino group in peptide, protein and carbohydrate synthesis[221]. Benzyloxycarbonyl (Cbz or Z), p-nitrobenzyloxycarbonyl (PNZ), allyloxycarbonyl (All) are few examples used in the synthesis. Boullanger et al.[142] reported a detailed study on glycosylation of *N*-alkoxycarbonyl derivatives of glucosamine including Z and PNZ. When the β-acetates of this carbamate was used as a donor in the presence of Lewis acid, β-glycosides were obtained stereoselectively without the formation of the oxazolidinone (**Scheme 1.7**).

Among these carbamates Z and PNZ [143,144] are the two orthogonal protecting groups owing to their deprotection conditions. Z and PNZ can be removed by hydrogenolysis along with *O*-benzyl protecting groups at the end of a synthesis. At the same time, PNZ can be removed under mild conditions without affecting the commonly used *N*- and *O*- ether, acyl, and carbamoyl protecting groups. Selective reduction of nitro group to the electron-donating amine substituent followed by the 1,6 elimination yields the free amino substituent in C-2 position, which in turn can be easily protected as its corresponding NHAc derivative. The mild conditions employed for removal of PNZ avoid the vigorous conditions required for removal of the *N*-Phthalimido (N-Phth) protection [222] which is widely used in the synthesis of the amino sugars. PNZ can serve as the ideal protecting group to generate acetate derivative (*N*HPNZ → NH_2 → *N*HAc) and Z can serve as the free amino substituent (*N*HCbz → NH_2) in final deprotection of the oligosaccharide synthesis. It is anticipated that these protecting groups should be stable under the conditions of the glycosylation reactions and, because of neighboring group participation, control the stereoselectivity of the reaction. Moreover Z and PNZ have not been utilized in the complex, multi step synthesis and solid phase oligosaccharide synthesis which have gained more attention in recent years. Due to those

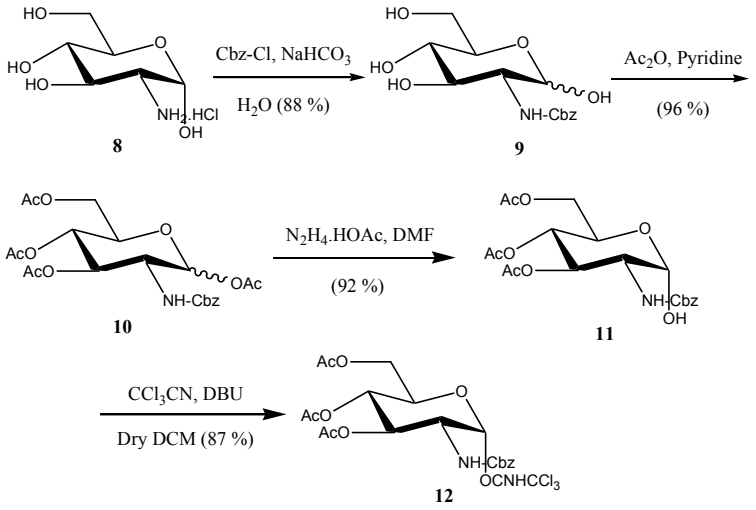
considerations, we decided to explore these protecting groups in the solution and solid phase synthesis of partially acetylated chitotetraoses.

Recently, *N*-PNZ protection was utilized along with thioglycoside donors[223]. However, the *N*-Z group was not stable under the conditions normally employed for the formation of thioglycosidic donors[224]. Therefore, in this work, the trichloroacetimidate activation of the donor was employed, due to its ease of formation and also exploitation of the same in the host of glycosidic linkages in solution as well as in the solid support[75].

The anomeric hydroxyl group of the acceptor is protected as the corresponding tert-butyldimethylsilyl ether which is stable under various conditions and can be removed easily. C-4 hydroxyl group is protected using the levulinoyl (Lev) protecting group[225] which serves as the temporary protecting group due to its unique removal by means of hydrazine acetate. The proposed formation of tetrasaccharide (**Scheme 3.1.1**) is planned by preparing two types of disaccharides which are converted to the appropriate donors and acceptors.

3.1.2 Preparation of *N*-Cbz protected glucosamine donor 12

α-D-Glucosamine hydrochloride salt was used as starting material for the synthesis of monosaccharide building blocks. A slightly modified procedure from the literature[226] was used to prepare the compound **9**. Treatment of **8** with benzyloxycarbonyl chloride in the presence of sodium bicarbonate in water medium yielded *N*-benzyloxycarbonyl protected glucosamine **9** in 88 % yield. Acetylation of **9** by means of Ac_2O in pyridine furnished tetraacetate **10** as a mixture of α/β isomers in 4:1. The anomeric acetyl group was selectively



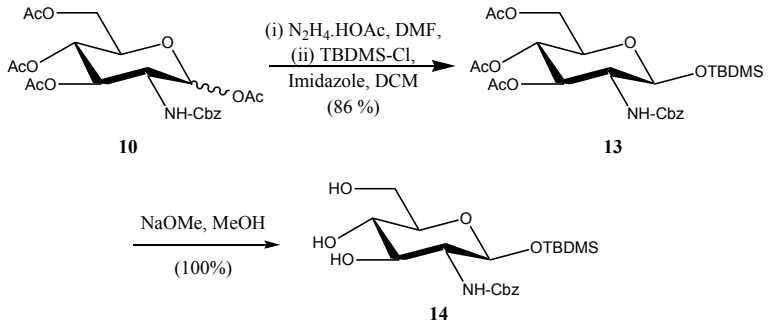
Scheme 3.1.2 Synthesis of *N*-Cbz protected donor 12

removed using hydrazine acetate in DMF to afford hemiacetal **11**. Reaction of **11** with trichloroacetonitrile in the presence of 1,8-diaza[5.4.0]bicycloundec-7-ene (DBU) exclusively afforded α-trichloroacetimidate (**12**) in 87 % yield (**Scheme 3.1.2**).

The exclusive formation of α-trichloroacetimidate is, in this case, mainly attributed by the favorable thermodynamic conformation[75]. Differences in the rate of formation of α- and β-*O*-glycosyl trichloroacetimidates are explained by the reversibility of the reaction and differing kinetic stabilities of the anomers. Generally β-trichloroacetimidates are favored when K_2CO_3 or Na_2CO_3 are used for the deprotection of the anomeric hydroxyl group. It catalyses the addition of trichloroacetonitrile rapidly and quantitatively, and also with only a small effect on the retro-reaction which leads to the exclusive formation of β-trichloroacetimidate. On the other hand, usage of DBU and NaH as a base resulted in the retro-reaction of the oxide ion which anomerized to the α-oxy anion to form ultimately the stable α-trichloroacetimidates.

3.1.3 Synthesis of 1-*O*-TBDMS-2-*N*-*Z*-glucosamine

Treatment of **10** with hydrazine acetate in the presence of DMF afforded hemiacetal **10**, which was used without further purification. Reaction with TBDMS-Cl and imidazole yielded exclusively the β-anomer of the corresponding TBDMS derivative **13**. Deacetylation of **13** under Zemplen[227] conditions afforded tert-butyldimethylsilyl 2-deoxy-*N*-benzyloxycarbonylamino-β-D-glucopyranoside (**14)** in quantitative yield **(Scheme 3.1.3)**.



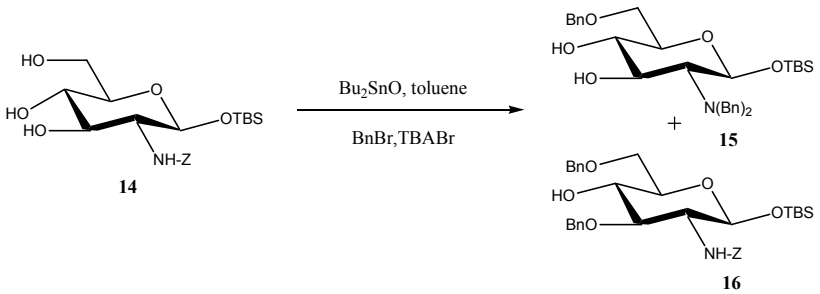
Scheme 3.1.3 Synthesis of compound 14

3.1.4 Synthesis of 3,6-di-*O*-benzylated acceptor 16

Synthetic applications of stannylenes have followed the elegant studies of Moffatt[228] and Ogawa[229,230] who showed that the inherent difference in the nucleophilicities of carbohydrate hydroxyls can be amplified by the formation of trialkyltin ethers. Further it is

noted that while acylation proceeds without any catalyst, alkylation is a sluggish reaction and needs assistance from tetrabutylammonium halides[231]. The remarkable features of these reactions are the regioselectivity (reaction of primary hydroxyl groups prevails over those on secondary or tertiary ones) and stereoselectivity (equatorial hydroxyl groups, and not axial ones, react preferentially). Both di-n-butyltin oxide (Bu_2SnO) and bis(tri-n-butyltin)oxide [$(n\text{-}Bu_3Sn)_2O$] are suitable for regioselective benzylation. The preparation of 3,6-di-*O*-benzyl derivatives of *N*-acetyl[232], *N*-phthalimido[233] and *N*-dimethylmaleimide[168] with dibutyltin oxide was reported in the literature. The 3,6-di-*O*-benzyl-2-*N*-Z derivatives were not yet described by this method.

Compound **14** was treated with dibutyltin oxide in refluxing toluene (Dean Stark apparatus) to afford the stannylene derivative which was then treated with benzyl bromide and tetrabutylammonium bromide and heating gently to ca. 120 °C (**Scheme 3.1.4**).

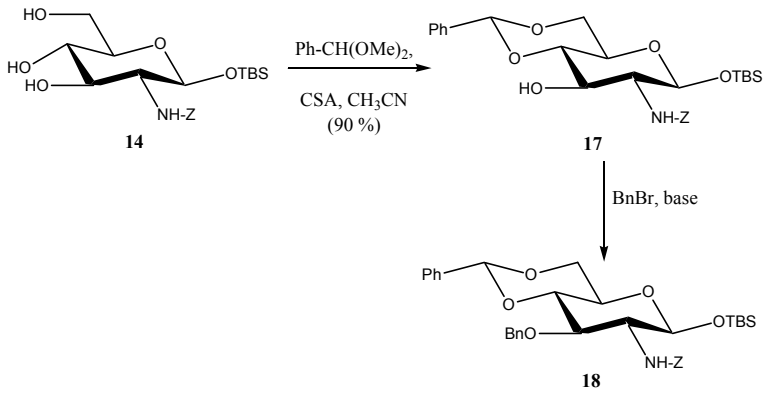


Scheme 3.1.4 Reaction with Stannylene derivatives

MALDI-TOF MS of crude reaction mixture showed the presence of a mixture of compounds **15** and **16**. The main product isolated after work-up was the undesired tribenzyl derivative **15**, as revealed by ^1H, ^{13}C, H-H COSY, HMBC and HMQC NMR. Obviously, the benzyloxycarbonyl group is not stable under refluxing conditions. Similar results observed after changing the solvent from toluene to benzene. Likewise, preparation of stannylene acetal with dibutyltin oxide in refluxing MeOH, followed by reaction with DMF, BnBr and heating the reaction mixture at ca. 100 °C for 2 hours[234], gave similar results.

Thus, a different approach was explored. Treatment of **14** with benzaldehyde dimethylacetal in the presence of catalytic amount of camphorsulphonic acid (CSA) afforded the 4,6-*O*-benzylidene derivative **17** (**Scheme 3.1.5**). Benzylation of C-3 hydroxyl group using 2.0 equiv. of NaH and 2.0 equiv. of benzyl bromide in dry THF yielded **18** in 44 % yield (**Table 3.1.1**), along with the starting material as revealed by ^1H and ^{13}C NMR. Increasing the amount of NaH to 3 equiv. did not improve the yield. Further increase of NaH

would result in the benzylation of secondary amine as reported in the literature[172]. Also, treatment of compound **14** with benzyl bromide in the presence of KOH in refluxing dioxane afforded **18** in only 49 % yield. A slightly improved yield (64 % with 66 % of conversion) was obtained when the reaction was performed with Ag_2O in dry DCM[235]. The conversion ratio did not improve after stirring the reaction mixture at room temperature for 10 days or increasing the amount of Ag_2O.



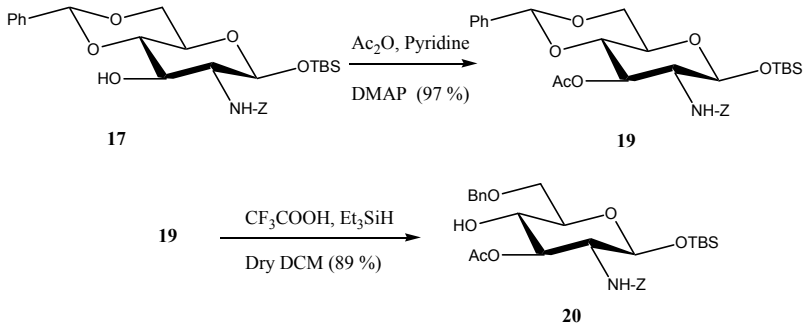
Scheme 3.1.5 Synthesis of Compound 11

Table 3.1.1 Conditions used for the benzylation at C-3 position

Entry	Reagents	Solvent	Temperature (°C)	Time	Yield % (Conversion)
1	BnBr, NaH	THF	RT	1 day	44 (64)
2	BnBr, KOH	dioxane	reflux	12 h	49
3	BnBr, Ag_2O	DCM	RT	5 days	64 (66)

3.1.5 Synthesis of *N*-Z-Acceptor 20

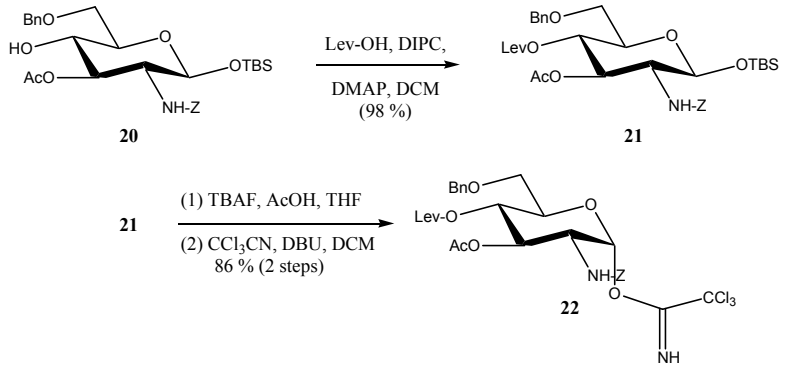
Insufficient yield for the C-3 benzylation reaction and the longer reaction time in case of Ag_2O prompted us to alter our approach towards the synthesis of oligosaccharides (**Scheme 3.1.1**). Our new approach involved the protection of C-3 hydroxyl group as an acetate ester which would provide a much easier synthesis than the one reported in literature[173]. Compound **18** was treated with Ac_2O and pyridine in dry DCM in the presence of DMAP afforded acetate **19** in excellent yield. Regioselective reductive cleavage of benzylidene acetal (**19**) with CF_3COOH/Et_3SiH[236] at 0 °C furnished 6-*O*-Bn acceptor **20** in good yield (**Scheme 3.1.6**).



Scheme 3.1.6 Synthesis of *N*-Z-Acceptor 20

3.1.6 Synthesis of N-Z-Donor 22

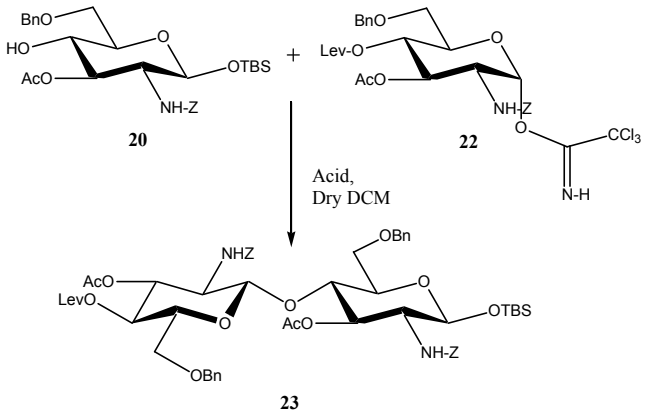
Compound **20** was treated with Levulinic acid and DMAP in the presence of DIPC yielded the orthogonally protected glucosamine **21** in excellent yield[149] (**Scheme 3.1.7**). The anomeric TBDMS group was removed using TBAF in the presence of acetic acid[237] followed by the reaction of crude product with trichloroacetonitrile in the presence of DBU in dry DCM afforded the α-trichloroacetimidate donor **22**.



Scheme 3.1.7 Synthesis of N-Z-Donor 22

3.1.7 Synthesis of *N,N'*-di-Z protected disaccharide 23

With the aim to optimize the reaction conditions for the stereoselective β-(1→4) glycosidic bond formation for the synthesis of protected disaccharide **23** (**Scheme 3.1.8**), the reaction conditions were varied with respect to the equivalents of Lewis and Brønsted acids, as well as the reaction temperature, the equivalents of donor and the reaction time, as summarized in (**Table 3.1.2**).



Scheme 3.1.8 Synthesis of protected disaccharide 23

Table 3.1.2 Synthesis of protected disaccharide 23

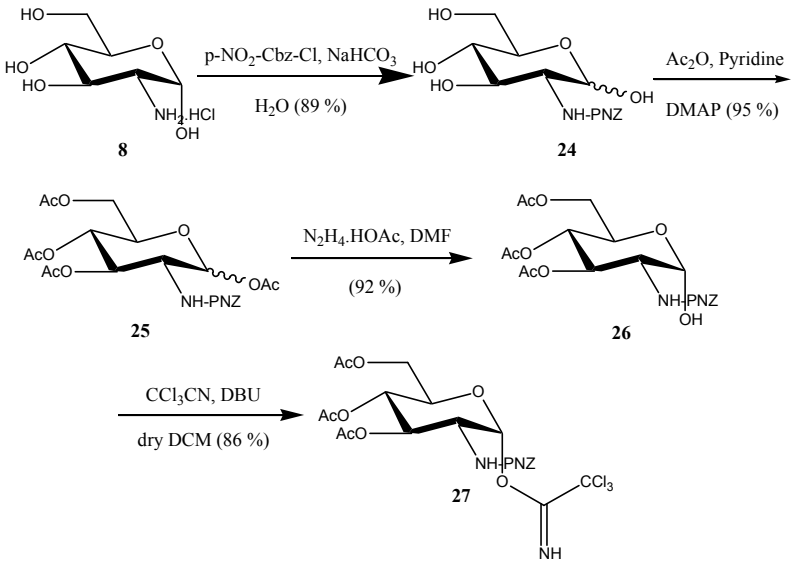
Entry	Equiv. of Donor	Acid	Equiv. of Acid	Temperature (°C)	Time (h)	Yield (%)
1	1.2	TMSOTf	0.12	-30	1	21
2	1.3	TMSOTf	0.5	-78	1	17
3	1.2	$EtAlCl_2$	0.5	-78	2	No Rxn.
4	1.2	$BF_3{\cdot}Et_2O$	0.5	-42	6	64
5	1.2	$BF_3{\cdot}Et_2O$	0.2	-50 to -30	1	66
6	1.2	$BF_3{\cdot}Et_2O$	0.5	-50 to -30	1	66
7	1.3	TfOH	0.2	-78	1	No Rxn.
8	1.3	TESOTf	0.24	-65	2	16
9	**1.3**	**$BF_3{\cdot}Et_2O$**	**0.3**	**-50 to -25**	**1**	**86**

TMSOTf[149], $BF_3{\cdot}Et_2O$[143,173,238] and TESOTf[144] have been utilized successfully in the glycosylation reactions between *N*-alkoxycarbonyl protected glycosyl acceptors and donors. In our case $BF_3{\cdot}Et_2O$ proved to be the most efficient Lewis acid catalyst to facilitate the glycosylation reactions. Formation of an oxazolidinone[142] has been described to occur during the glycosylation of several alcohols (2-propanol, cyclohexanol, tert-butyl alcohol & trichloroethanol) with *N*-alkoxycarbonyl (methyl, ethyl, allyl, benzyl (Z), trichloroethyl (Troc) and p-nitrobenzyl (PNZ)) protected glucosaminyl bromide. However, this side reaction was not observed in our case. Having established the convenience, and high yielding experimental conditions for the synthesis of *N*-benzyloxycarbonyl protected building blocks, we focused to prepare the *N*-p-nitrobenzyloxycarbonyl protected glycosyl acceptors and donors.

3.1.8 Synthesis of *N*-PNZ protected donor 27

As shown in **Scheme 3.1.9** glucosamine hydrochloride **8** was treated with sodium bicarbonate and *p*-nitrobenzyl chloroformate in water, yielding the alkoxy protected derivative **24**. The crude product **24** was treated with acetic anhydride in pyridine to afford the

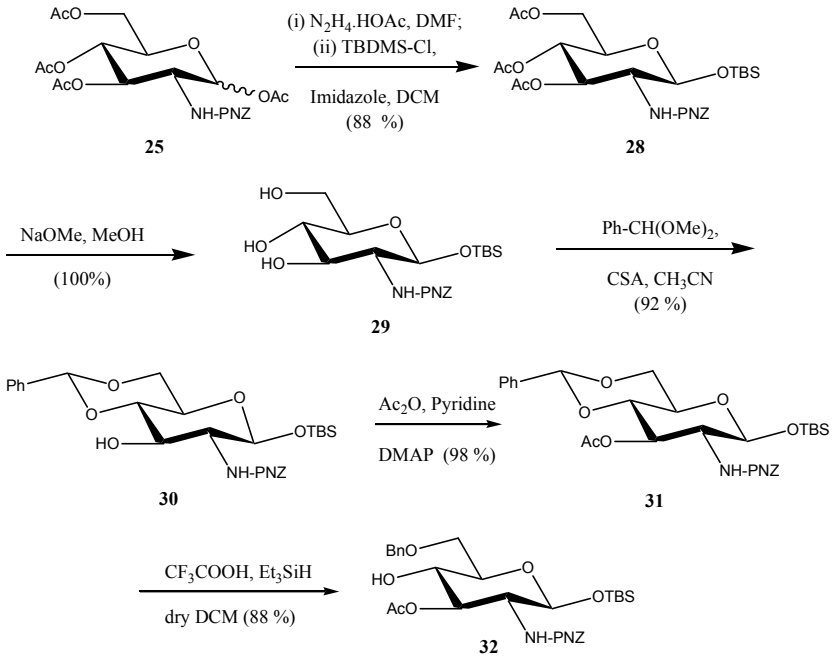
N-PNZ protected tetraacetate **25** in 95 % yield, anomeric ratio α : β = 4:1. Selective removal of anomeric *O*-acetyl group using hydrazine acetate in DMF afforded hemiacetal **26**, which upon treatment with trichloroacetonitrile in the presence of DBU yielded exclusively the α-anomeric donor **27** [143,144].



Scheme 3.1.9 Synthesis of *N*-PNZ donor 27

3.1.9 Synthesis of *N*-PNZ acceptor 32

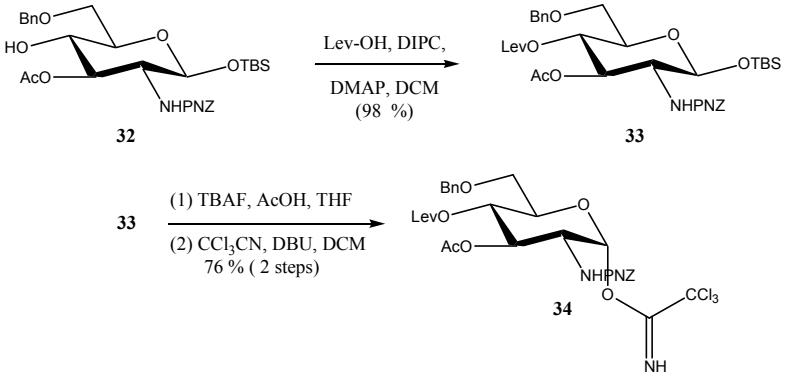
Tetraacetate **25** was treated with hydrazine acetate in DMF to afford the hemiacetal **26**, which was used without further purification. Reaction with TBDMS and imidazole afforded exclusively the β-anomer **28**. Deacetylation under Zemplen conditions furnished **29** in quantitative yield. Treatment of **29** with benzaldehyde dimethylacetal in the presence of CSA afforded the benzylidene acetal **30.** Reaction with acetic anhydride and pyridine in the presence of catalytic amount of DMAP provided the orthogonally protected glucosamine derivative **31**. Regioselective reductive cleavage of the benzylidene acetal **31** was performed with TFA and Triethylsilane which furnished acceptor **32** in good yield (**Scheme 3.1.10**).



Scheme 3.1.10 Synthesis of *N*-PNZ acceptor 32

3.1.10 Synthesis of *N*-PNZ donor 34

Compound **32** was treated with levulinic acid and DMAP in the presence of DIPC in dry DCM yielded *N*-PNZ derivative **33** (**Scheme 3.1.11**). The TBS ether was cleaved with TBAF in the presence of acetic acid in THF afford the hemiacetal which was used without further purification. Trichloroacetonitrile and DBU were added to the reaction mixture, which furnished the α-anomeric donor **34** in 76 % yield.



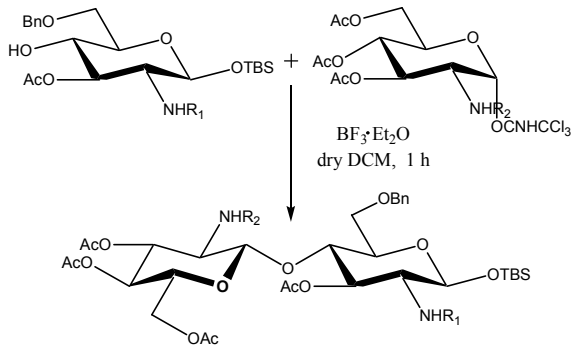
Scheme 3.1.11 Synthesis of *N*-PNZ donor 34

3.1.11 Synthesis of protected disaccharides

As discussed in the introduction, the synthesis of disaccharides reported in the

literature required many steps and results in rather low overall yields[173]. With our reaction scheme, the numbers of steps were considerably lower and the yields were generally quite high. This prompts us to synthesize the dimers in a simple and straight forward approach.

The acceptors **20** and **32** reacted with the donors **12** and **27** (1.3 equiv) respectively, in the presence of $BF_3 \cdot Et_2O$ (0.3 equiv) at -50 °C to -25 °C in dry DCM to yield different types of *N,O*-protected chitobioses (**Scheme 3.1.12**).



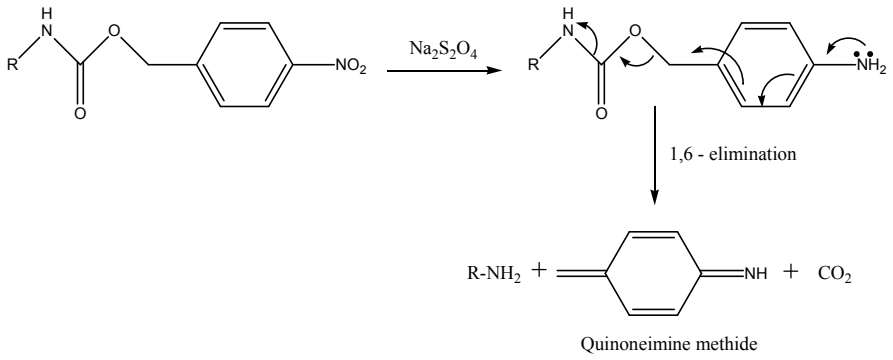
20 R_1 = Z, **12** R_2 = Z → **35** R_1 = Z, R_2 = Z (87 %)
32 R_1 = PNZ, **12** R_2 = Z → **36** R_1 = PNZ, R_2 = Z (91 %)
20 R_1 = Z, **27** R_2 = PNZ → **37** R_1 = Z, R_2 = PNZ (91%)
32 R_1 = PNZ, **27** R_2 = PNZ → **38** R_1 = PNZ, R_2 = PNZ (77 %)

Scheme 3.1.12 Synthesis of protected disaccharides

3.1.12 Conversion of *N*-PNZ into *N*-acetyl

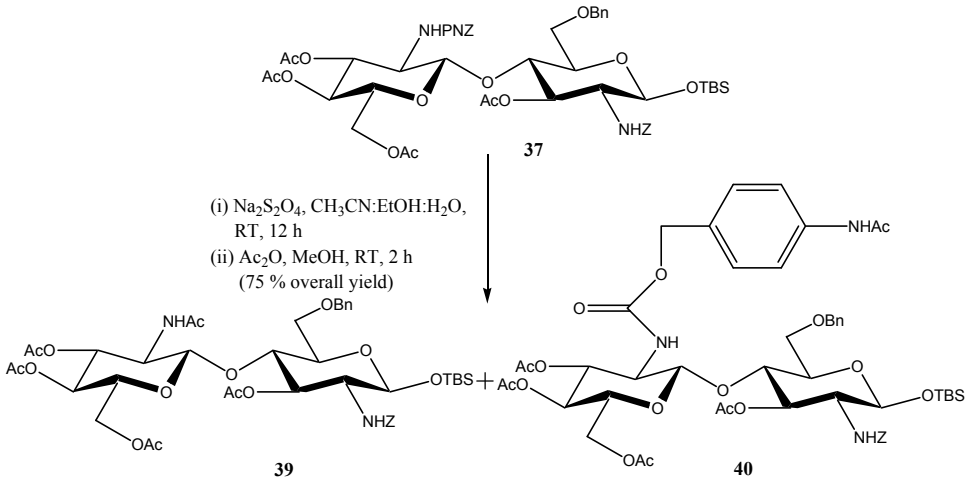
The PNZ group can be removed by a number of methods, such as catalytic hydrogenation, reduction with Zn/HCl[239], Zn/AcOH[240], Na_2S[241], $SnCl_2$[242] or sodium dithionite ($Na_2S_2O_4$)[243] in non-acidic or non-aqueous media. The first step is the reduction of the nitro group to give the p-aminobenzyloxycarbonyl derivative, which undergoes spontaneous 1,6 elimination to afford the free amine and the corresponding quinoneimine methide (**Scheme 3.1.13**). The free amine can be easily converted into acetamide by the standard acetylation procedure.

Although the effective nitro reducing agent sodium dithionite is typically used under basic conditions, there are some reports of its application in neutral media[143,144]. Disaccharide **37** was dissolved in MeCN:EtOH:H_2O (1:1:1) and then treated with sodium dithionite (**Scheme 3.1.14**). After the removal of the solvents, the residue was redissolved in MeOH and treated with Ac_2O to yield the *N'*-acetamido derivative **39**. The NMR spectrum of the crude



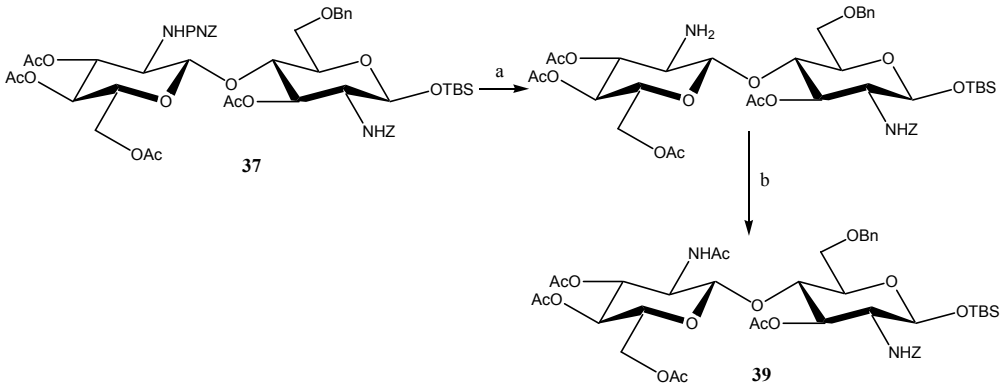
Scheme 3.1.13 Reductive cleavage of the PNZ group

product and the MALDI-TOF MS revealed the presence of *N*-acetylated p-aminobenzylated derivative (**40**) along with the product. This shows that the reduction of nitro group occurs fast, as revealed by TLC analysis, and that the 1,6-electron shift proceeds in a much slower rate.



Scheme 3.1.14 Reduction of PNZ derivative

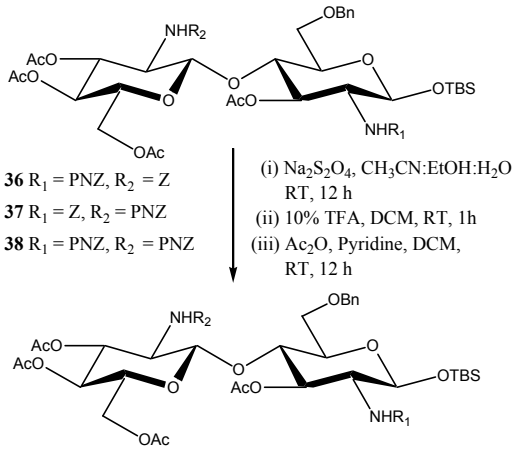
When the reaction was carried out in a two step process, i.e. reduction with sodium dithionite, followed by isolation of the free amine (yield: 60 %) and subsequent acetylation, the protected *N*'-acetamido disaccharide **39** was obtained in 96 % yield **(Scheme 3.1.15).** The yield is rather low, as compared to literature reports[143,144]. Alternative procedures of handling the sodium dithionite under basic conditions (NaOH and K_2CO_3) cannot be considered for these disaccharides, due to the presence of labile *O*-acetate protecting groups.



a) $Na_2S_2O_4$, CH_3CN:EtOH:H_2O, RT, 12 h, 60%; b) Ac_2O, Pyridine, DCM, RT,12 h, 96 %.

Scheme 3.1.15 Formation of *N'*-acetamido disaccharide 39

Seeberger et al.[244] have reported on halobenzyl ether as protecting groups which can be converted into labile arylamines by Pd-catalyzed amination and cleaved by brief exposure to Lewis acids, protic acids or oxidants. Selective removal was achieved in the presence of silyl ethers, alkyl and aryl esters, PMB ethers, acetals and glycal double bonds. We applied this type of exposure to protic acid for the improvement of 1,6-elimination from the electron rich p-aminobenzyloxycarbonyl derivatives.



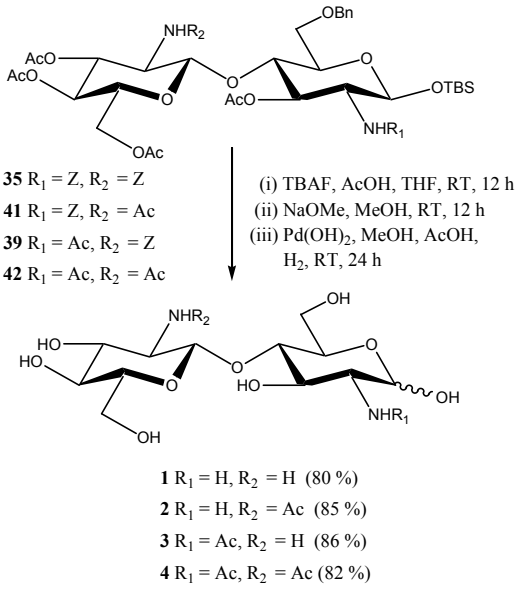
Scheme 3.1.16 Synthesis of protected *N*-acetamido disaccharides

The crude product from the reduction with sodium dithionite (solvents removed and then workup performed using H_2O and CH_2Cl_2) was redissolved in dry CH_2Cl_2 and then

treated with 1% AcOH/CH_2Cl_2, 10% AcOH/CH_2Cl_2. In both the cases there was no significant improvement of the 1,6-elimination. Finally, a complete reaction was observed when the product mixture (solvents removed and then workup performed using H_2O and CH_2Cl_2) was redissolved in dry CH_2Cl_2 and then treated with 10% TFA/CH_2Cl_2 for 1 h, followed by usual workup. Acetylation using Ac_2O/Pyridine gave the *N*-acetamido disaccharides in high overall yield (**Scheme 3.1.16**).

3.1.13 Synthesis of the free disaccharides

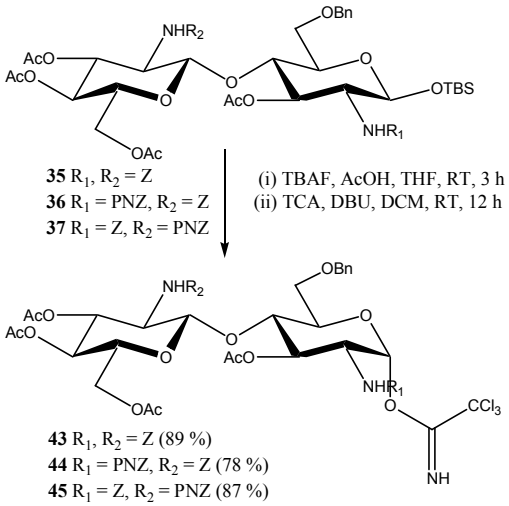
The anomeric silyl ether was cleaved using TBAF in the presence of acetic acid. Deacetylation under Zemplen conditions, followed by hydrogenolysis with $Pd(OH)_2$ (Pearlman's catalyst) in MeOH/AcOH (**Scheme 3.1.17**). The crude product was purified by GPC on a BioGel-P2 column to give the free disaccharides as anomeric mixture showing the correct mass by MALDI-TOF MS.



Scheme 3.1.17 Synthesis of free disaccharides

3.1.14 Synthesis of disaccharide donors

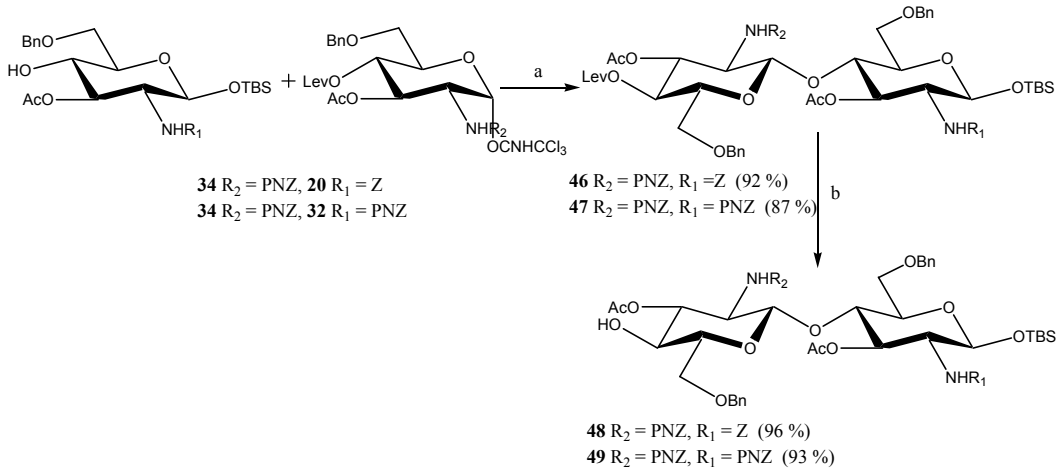
As discussed in the retro synthetic analysis (**Scheme 3.1.1**), the disaccharide donors were prepared from the disaccharides having the non reducing end protected as 1-*O*-TBS ethers. The TBS group was selectively cleaved in the presence of TBAF and acetic acid, and then it was treated with trichloroacetonitrile and DBU to afford the donor (**Scheme 3.1.18**).



Scheme 3.1.18 Synthesis of disaccharide donors

3.1.15 Synthesis of disaccharide acceptors

The acceptors **20** and **32** reacted with donor **34** (1.3 equiv) in the presence of $BF_3{\cdot}Et_2O$ as the Lewis acid catalyst at -50 °C to -30 °C in dry CH_2Cl_2 to yield the 1-*O*-TBS ether protected reducing end disaccharides (**Scheme 3.1.19**). The temporary protecting group levulinoyl ester was selectively cleaved using hydrazine acetate dissolved in MeOH to furnish the disaccharide acceptors.

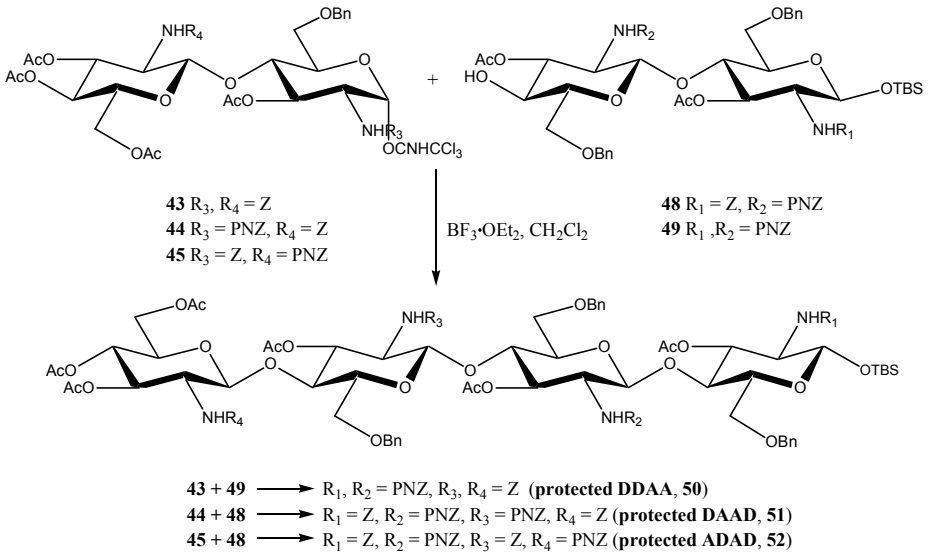


a) $BF_3{\cdot}Et_2O$, DCM, -50 to -30 °C, 1h; b) Hydrazine acetate, DCM, 1 h.

Scheme 3.1.19 Synthesis of 1-*O*-TBS protected disaccharide acceptors

3.1.16 Synthesis of 1-*O*-TBS protected tetrasaccharides

After successful preparation of dimeric donor derivatives (**43**, **44** and **45**) and acceptor derivatives (**48** and **49**), we shifted our focus on the preparation of protected tetrasaccharide sequences **DDAA**, **DAAD** and **ADAD**. $BF_3{\cdot}Et_2O$ was used as the Lewis acid for activation of donor molecules and dry CH_2Cl_2 was used as the solvent for (2+2) glycosylation with β-(1→4) bond formation (**Scheme 3.1.20**). In order to find out the effective glycosylation conditions, varying quantities of donors and acids were used. The results are summarized in **Table 3.1.3**.



Scheme 3.1.20 Synthesis of 1-*O*-TBS protected tetrasaccharides

Table 3.1.3 Synthesis of tetrasaccharides

Entry	Equiv. Of Donor	Equiv. Of Acid	Temperature (°C)	Time (h)	Yield (%)	Protected tetrasaccharide
1	1.5	0.5	-50 to -25	4	38	ADAD (**52**)
2	2.0	1.0	-50 to -25	6	45	ADAD (**52**)
3	**2.2**	**1.0**	**-50 to -25**	**2**	**47**	**ADAD** (**52**)
4	1.5	0.5	-50 to -25	2	40	DAAD (**51**)
5	1.5	3.0	-78 to -30	4	18	DAAD (**51**)
6	**2.0**	**1.0**	**-50 to -25**	**6**	**45**	**DAAD** (**51**)
7	1.5	0.5	-50 to -25	6	45	DDAA[a] (**50**)
8	1.5	0.5	-50 to 0	4	<10[b]	DDAA
9	1.5	0.5[c]	-50 to -25	4	-	-[d]

[a] MALDI-TOF MS shows the presence of two byproducts along with protected tetrasaccharide
[b] Major reaction observed was the deprotection of anomeric TBS group (TLC)
[c] TMSOTf used as Lewis acid catalyst
[d] Byproducts are the only products observed in MALDI-TOF MS

As shown in **Table 3.1.3**, the maximum yield of the glycosylation reaction was as low as 47 %. The anomeric position of the disaccharide acceptors (**48** and **49**) were protected as its silyl ether. The anomeric silyl ether is not stable at 0 °C and higher temperatures under the Lewis acid $BF_3{\cdot}Et_2O$[245,246] and therefore the glycosylation reactions must be performed at low temperatures.

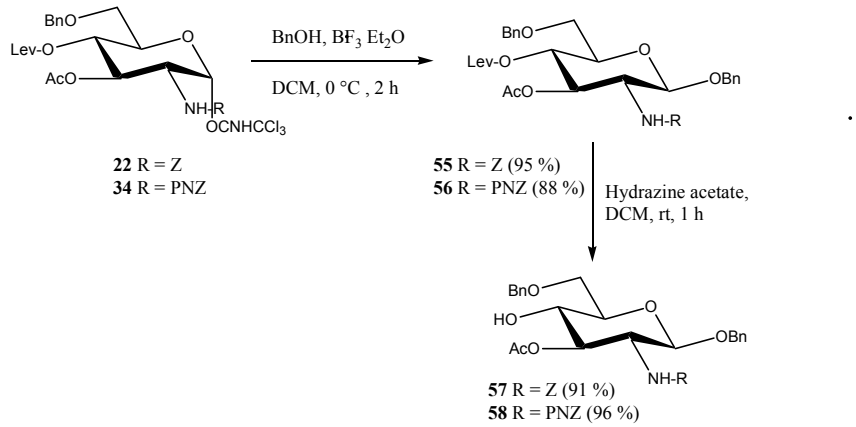
Attempted glycosylation reaction for the preparation of protected tetrasaccharide sequence DDAA using acceptor **49** and donor **43** under $BF_3{\cdot}Et_2O$ activation gave a mixture of products which could not be purified by chromatography. MALDI-TOF MS revealed the presence of a mixture of three products, as revealed by the pseudo molecular sodiated peaks at *m/z* = 1948.03 (product), 1719.92 and 1629.88 (byproducts).

Thus the main component is the expected tetrasaccharide **50** and the byproducts are (**53,** *m/z* = 1629.88 and **54,** *m/z* = 1719.92**)** most likely formed by the mechanism shown in **Scheme 3.1.33**. Another attempted glycosylation of the acceptor **49** and donor **43** under TMSOTf activation gave only the byproducts **53** and **54**. The byproduct formation under the glycosylation condition is quite surprising and one of the possible reasons could be the low nucleophilicity of acceptor alcohols.

The yield of the glycosylation was low and the observation of mixture of products prompted us to focus our attention for the alternative protective group in the anomeric position which will be quite stable under the glycosylation conditions, even at elevated temperatures. Since the final deprotection step (**Scheme 3.1.1**) is the removal of benzyl ethers, we decided to protect the anomeric hydroxyl group as benzyl ether.

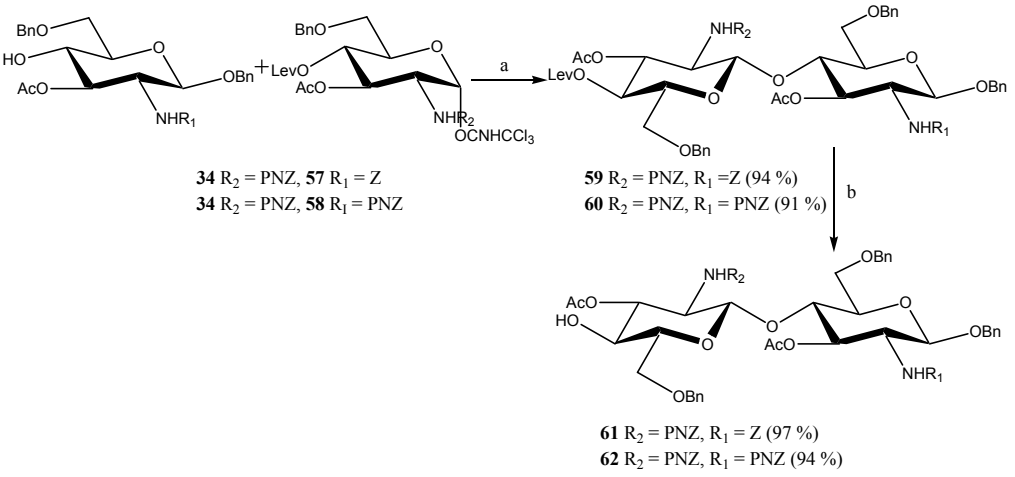
3.1.17 Preparation of acceptors possessing anomeric 1-*O*-Bn ethers

Donors **22** and **34** were glycosylated with benzyl alcohol under standard glycosylation conditions. Subsequent cleavage of the levulinoyl ester using the hydrazine acetate yielded the monomeric acceptors **57** and **58** in excellent yields (**Scheme 3.1.21**).



Scheme 3.1.21 Synthesis of monomeric 1-*O*-Bn protected acceptors

The *O*-Benzyl protected (**57** and **58**) acceptors were treated with donor **34** to yield the disaccharides. Cleavage of the levulinoyl ester with hydrazine acetate furnished the *O*-benzyl protected dimeric acceptors **61** and **62** (**Scheme 3.1.22**).



a) $BF_3 \cdot Et_2O$, DCM, 0 °C, 2 h, b) Hydrazine acetate, DCM, rt, 1 h.

Scheme 3.1.22 Synthesis of disaccharide acceptors

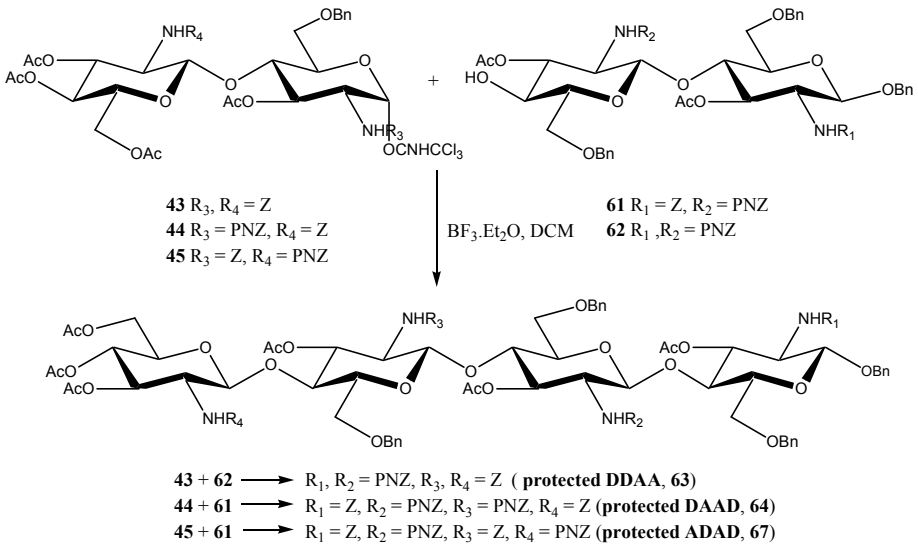
3.1.18 Synthesis of *O*-benzyl protected tetrasaccharides

The tetrasaccharide preparation was thoroughly examined using the same conditions which we employed for the anomeric TBS derivatives (**Scheme 3.1.23**). The glycosylation reaction was performed at 0 °C as well as at room temperature. The results are summarized in **Table 3.1.4, Table 3.1.5** and **Table 3.1.6** for the protected tetrasaccharides **DDAA**, **DAAD** and **ADAD** respectively.

Table 3.1.4 Glycosylation reaction for the protected DDAA (63)

Entry	Equiv. of Donor	Equiv. of Acid	Temperature (°C)	Time (h)	Yield (%)[a]
1	2.0	1.0	-50 to 0	3	57
2	**2.5**	**1.25**	**0 to rt**	**12**	**77**
3	1.5	0.5	0	7	34
4	1.5	0.5	0 to rt	14	30
5	2.0	1.0	0 to rt	15	67
6	1.5	0.5	-50 to -25	6	38
7	2.0	1.0	-50 to rt	12	40

[a] Mixture of three products (protected tetrasaccharide and two byproducts).



Scheme 3.1.23 Synthesis of Tetrasaccharides

In an attempt to synthesize the protected (**Table 3.1.4**) tetrasaccharide **DDAA**, coupling of acceptor **62** and donor **43** under $BF_3 \cdot Et_2O$ activation, gave a mixture of three products. Purification over silica gel column chromatography was proved to be not successful. MALDI-TOF MS (**Figure 3.1.1**) revealed the presence of three products, which have been identified from the pseudo molecular sodiated peaks at *m/z* = 1923.68 (product, **63**), *m/z* = 1629.57 (byproduct, **53**) and *m/z* = 1719.62 (byproduct, **54**). MALDI-TOF MS revealed that the peak values (*m/z*) observed for the byproducts, in the case of protected tetrasaccharide **50** as well as **63** are identical.

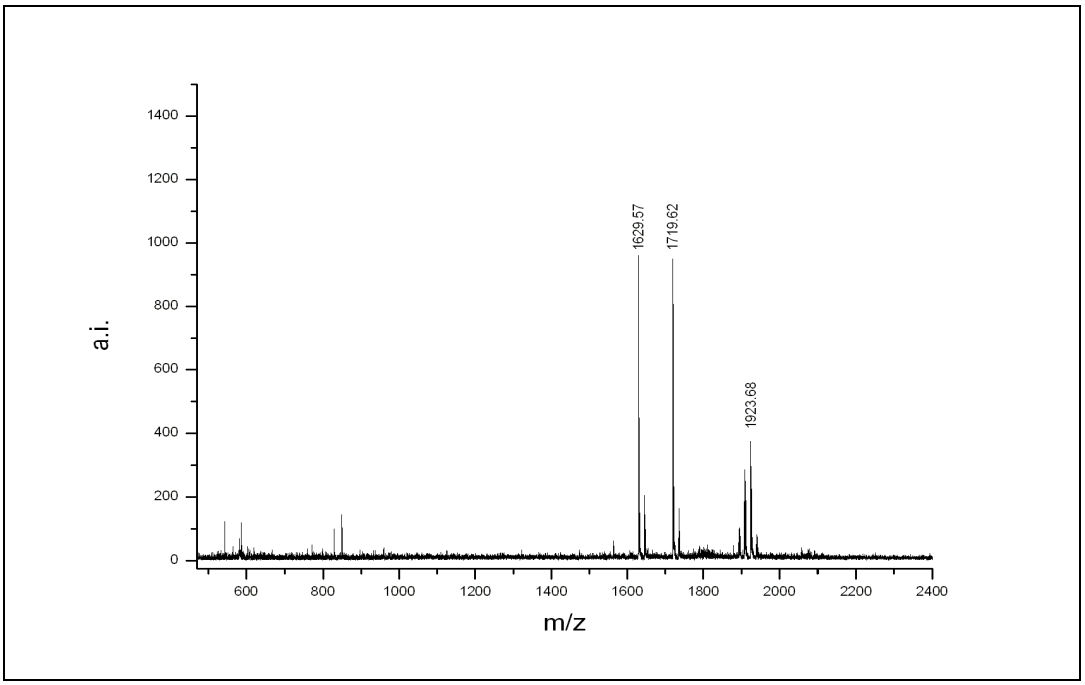


Figure 3.1.1 MALDI-TOF MS of the chromatographed protected tetrasaccharide DDAA (63)

Table 3.1.5 Glycosylation reaction for the protected DAAD (64)

Entry	Equiv. of Donor	Equiv. of Acid	Temperature (°C)	Time (h)	Yield (%)
1	**2.2**	**1.0**	**0 to rt**	**15**	**74**[a]
2	1.5	0.3	0 to rt	13	30[a]
3	2.2	1.0	-50 to -25	2	43
4	**2.0**	**1.0**	**-50 to -25**	**5**	**52**
5	2.0	1.0	-50 to rt	13	41[a]
6	2.0	1.0[b]	-30 to rt	7	-[c]

[a] Mixture of three products (protected tetrasaccharide and two byproducts)
[b] TESOTf used as Lewis acid catalyst
[c] Byproducts are the only products observed in MALDI-TOF MS

Coupling of acceptor **61** with donor **44** under $BF_3 \cdot Et_2O$ activation at 0°C to rt yielded a mixture of products (**Table 3.1.5**). MALDI-TOF MS of chromatographed product revealed the presence of expected tetrasaccharide (**64**) together with two more byproducts (**65** and **66**). This have been identified by pseudo molecular sodiated peaks at *m/z* = 1923.78 (**64**, protected **DAAD**), *m/z* = 1674.61 (**65**, byproduct) and *m/z* = 1809.64 (**66**, byproduct). The pure protected tetrasaccharide was obtained in moderate yield, when the coupling reaction was performed at -50 °C to -25 °C. An attempted coupling reaction with TESOTf yielded only the byproducts.

Coupling of acceptor **61** with donor **45** under $BF_3 \cdot Et_2O$ activation at 0 °C to rt

afforded a mixture of products (**Table 3.1.6**). MALDI-TOF MS of chromatographed product revealed the presence of three products, which have been identified from the pseudo molecular sodiated peaks at *m/z* = 1923.56 (protected **ADAD**, **67**), *m/z* = 1719.46 (byproduct, **68**) and *m/z* = 1809.50 (byproduct, **69**). The pure protected tetrasaccharide (**67**) was obtained in reasonable yield when the glycosylation was performed at -50 to -30 °C. An attempted synthesis of protected tetrasaccharide **69** using TESOTf yielded only the byproducts **68** and **69**.

Table 3.1.6 Glycosylation reaction for the protected ADAD (67)

Entry	Equiv. of Donor	Equiv. of Acid	Temperature (°C)	Time (h)	Yield (%)
1	1.5	0.5	-20 to 0	4	29 [a]
2	2.0	1.0	-50 to 0	3	58 [a]
3	1.5	0.5	0 to rt	13	27 [a]
4	**2.2**	**1.0**	**0 to rt**	**15**	**74** [a]
5	2.0	1.0	0 to rt	15	68 [a]
6	2.0	1.0	-50 to rt	14	60 [a]
7	**2.2**	**1.0**	**-50 to -30**	**3**	**48**
8	2.0	2.0	-50 to -25	6	31
9	1.3	0.24[b]	0 to rt	14	-[c]

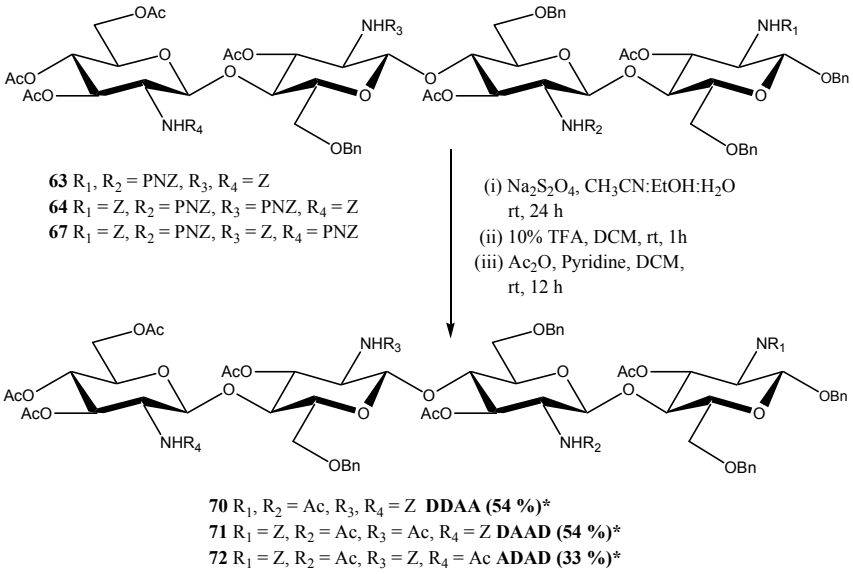
[a] Mixture of three products (protected tetrasaccharide and two byproducts)
[b] TESOTf used as Lewis acid catalyst
[c] Byproducts are the only products observed in MALDI-TOF MS

Without having any lead into the formation of byproducts, we decided to perform the PNZ reduction with the mixture itself, hoping that the purification might be easier after the acetylation step. The protected **DDAA** (**63**) was subjected to reduction with sodium dithionite (40 equiv.). However, even after 2 days of stirring, some amount of starting material remained in the reaction mixture. After workup, treatment with TFA, acetylation and chromatography, an acetylated product and the two byproducts **53** and **54** were isolated. From this observation, it is clear that the byproduct did not have any PNZ group. On the basis of these observations, we believe that the byproduct formed between the donors itself, as the donor was composed only with the Z as the amine protecting group. Eventually the byproduct should be the tetrasaccharide formed between the two donor molecules.

Further to prove this point, the deacetylation of the byproduct **53** (*m/z* = 1629.70) was performed in the presence of NH_3/MeOH at room temperature for 2 days. The mass spectrum revealed (*m/z* = 1293.61) that it contained 8 acetate groups. The donor (**43**), acceptor (**62**) and tetrasaccharide DDAA (**63**) had four, two and six acetate groups respectively. Thus,

observation of eight acetate groups suggests that the byproduct was a tetrasaccharide formed by the dimerization of the donor.

Irrespective of the byproducts formed during the glycosylation, pure acetylated products were isolated after the reductive step with sodium dithionite. In case of protected **DDAA** (**63)**, the pure diacetylated tetrasaccharide **70** was obtained in 54% overall yield after two steps together with 17% of byproducts **53** and **54** (**Scheme 3.1.24**). The NMR spectra of all tetrasaccharides containing the Z and PNZ moiety gave broad peaks which made the assignment difficult. The β-linkage was confirmed by ^{13}C, ^{1}H, HMQC experiments for the *N,N'*-diacetyl tetrasaccharides after the reductive cleavage step. [^{1}H NMR ($CDCl_3$): **70,** δ = 4.36 ($J_{1,2}$ 7.5 Hz, H-1_a), δ = 4.20 ($J_{1,2}$ 7.5 Hz, H-1_b), δ = 4.14 ($J_{1,2}$ 8.5 Hz, H-1_c), δ = 3.95 ($J_{1,2}$ 8.0 Hz, H-1_d)] .



***Overall yield after two steps (Glycosylation and reductive step)**

Scheme 3.1.24 Preparation of *N,N'*-diacetylated tetrasaccharides

In case of protected tetrasaccharide **DAAD**, the pure diacetylated tetrasaccharide **71** was obtained in 54% overall yield after two steps. [^{1}H NMR ($CDCl_3$): **71**, δ = 4.31 ($J_{1,2}$ 8.0 Hz, H-1_a), δ = 4.22 ($J_{1,2}$ 8.0 Hz, H-1_b), δ = 4.20 ($J_{1,2}$ 7.5 Hz, H-1_c), δ = 4.09 ($J_{1,2}$ 8.0 Hz, H-1_d)]. In case of protected tetrasaccharide **ADAD**, the diacetylated tetrasaccharide **72** was obtained as a mixture of products. MALDI-TOF MS revealed the reduction of byproducts **68** and **69** along with the tetrasaccharide **67**.

Silica gel column chromatography using the $EtOH/CHCl_3$ or $MeOH/CH_2Cl_2$ did not

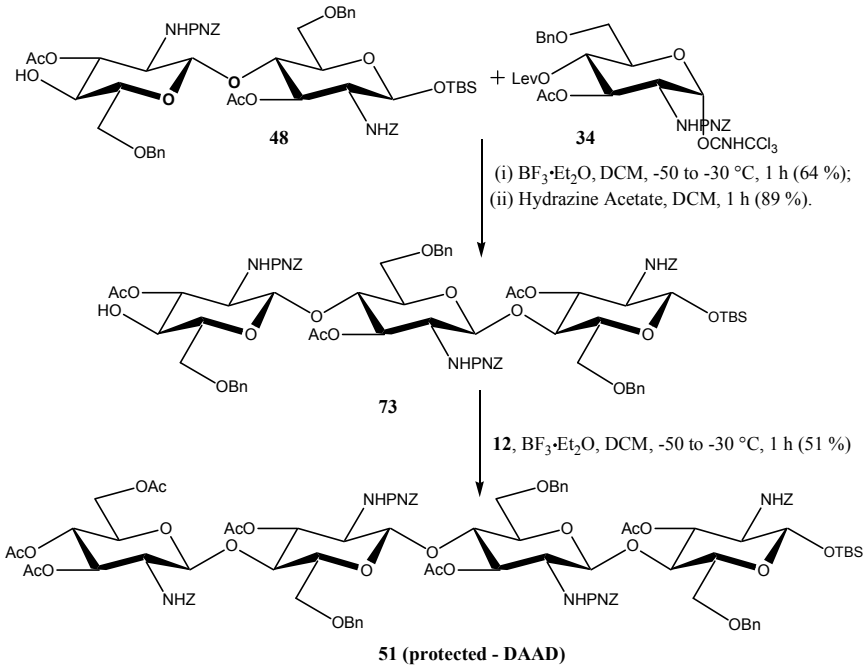
provide the acetylated product **72** as a pure compound. Due to the partial solubility of **72** in CH_3CN, purification by HPLC (RP-18, CH_3CN/H_2O) was not possible. Finally the separation of byproducts was achieved by preparative RP-18 TLC (H_2O/CH_3CN = 85/15), which afforded pure **72** in 33% overall yield for two steps [^{1}H NMR ($CDCl_3$): **72**, δ = 4.35 ($J_{1,2}$ 8.0 Hz, H-1_a, δ = 4.32 ($J_{1,2}$ 7.5 Hz, H-1_b), δ = 4.30 ($J_{1,2}$ 8.0 Hz, H-1_c), δ = 4.03 ($J_{1,2}$ 7.5 Hz, H-1_d)].

The glycosylation reactions between 1-*O*-TBS and 1-*O*-Bn protected monomeric acceptors with monomeric donors to afford the disaccharides were generally high yielding and in the same time byproduct formation was never observed. But, the observation of byproducts in the case of protected tetrasaccharides could be due to the low reactivity of *N*-alkoxy protected dimeric acceptor derivatives towards glycosylation reactions.

3.1.19 Tetrasaccharide formation using (3+1) glycosylation strategy

After observing the role of nucleophilicity in the glycosidic bond formation, we decided to study the same property in case of trisaccharide acceptors. Our strategy was to prepare a trisaccharide acceptor and further glycosylation with a monosaccharide donor to form the tetrasaccharide. We anticipated that this approach could minimize the byproduct formation.

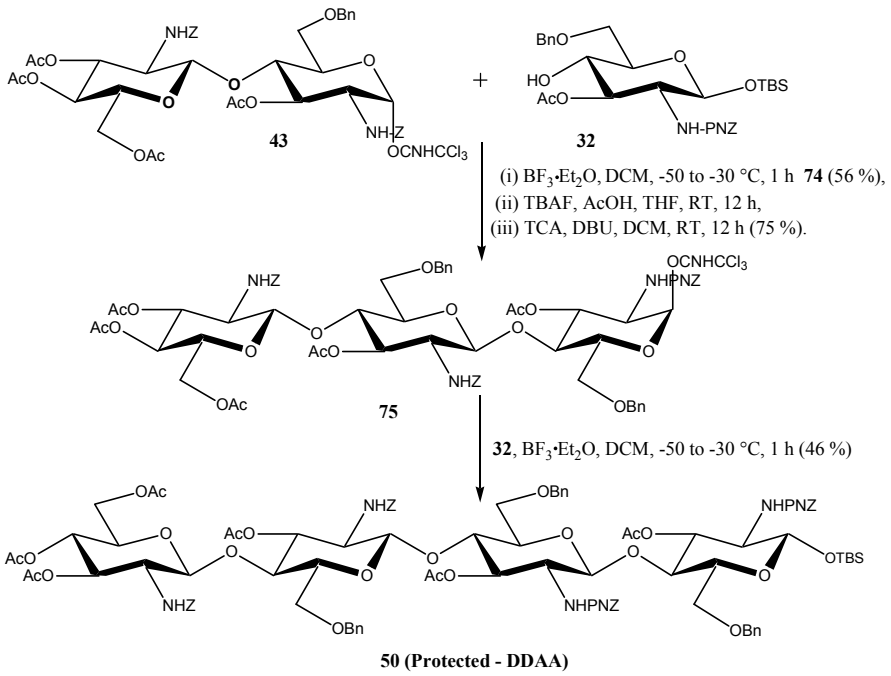
First we studied the reaction of the 1-*O*-TBS protected acceptor **48** with the monomeric donor **34** (1.5 equiv) in the presence of $BF_3{\cdot}Et_2O$ (0.3 equiv). The levulinoyl ester was removed by means of hydrazine acetate to afford the trisaccharide acceptor **73** (**Scheme 3.1.25**) which was glycosylated with donor **12** (2.5 equiv.) and $BF_3{\cdot}Et_2O$ (1.5 equiv) under standard conditions to yield the protected tetrasaccharide **DAAD** (**64**) in a yield of 51 %. Due to the presence of 1-*O*-TBS group the temperature was maintained in the range of -50 to -30 °C in both glycosylation reactions. In accordance with the lower reactivity of higher oligosaccharides, the yield of the trisaccharide was higher than that of the tetrasaccharide.



Scheme 3.1.25 Formation of tetrasaccharide using trimeric acceptor

Subsequently we decided to study the behavior of the monomeric acceptor alcohols by performing glycosylation reaction between dimeric and then trimeric donors to form the tetrasaccharides. The trisaccharide DDA-1-*O*-TBS (**74**) was prepared from the glycosylation of acceptor **32** with donor **43** (1.2 equiv) in the presence of $BF_3{\cdot}Et_2O$ (0.5 equiv). The trisaccharide derivative **74** was transformed into its corresponding trichloroacetimidate using the standard procedure to yield the trimeric donor DDA (**75**). The next glycosylation was performed using the acceptor **32** (1.5 equiv) and donor **75** (1.0 equiv) under $BF_3{\cdot}Et_2O$ (0.5 equiv) activation to afford the protected tetrasaccharide **DDAA** (**50**) (**Scheme 3.1.26**). Even though the yields for the glycosylation reactions were not high, we succeeded in the synthesis of pure tetrasaccharide **50** without the formation of byproduct. As discussed above, the protected tetrasaccharide **DDAA (50)** was never isolated in its pure form after the (2+2) glycosidic reaction irrespective of the anomeric protecting groups 1-*O*-Bn (**Table 3.1.4**) as well as 1-*O*-TBS (**Table 3.1.3)**.

The (3+1) glycosylation strategy was also applied to the 1-*O*-Bn ethers **61** and **62**. We expected that this strategy will provide some useful information regarding the reactivity of the monomeric donors at 0 – 25 °C. The glycosylation was performed between the dimeric acceptor **61** and donor **34** using the standard glycosylation conditions to form the protected trisaccharide acceptor AAD-1-*O*-Bn **77** (**Scheme 3.1.27**). Likewise, acceptor **61** reacted with

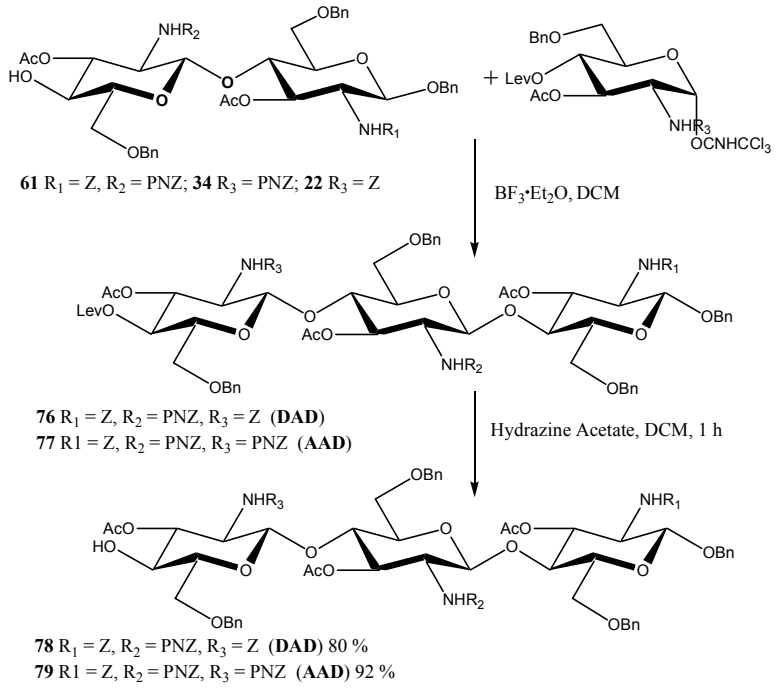


Scheme 3.1.26 Glycosylation reaction between trimeric donor and acceptor

donor **22** to afford the protected trisaccharide derivative DAD-1-*O*-Bn **67**. However, in this case, the product isolated after column chromatography was a mixture of trisaccharide **76** along with the two byproducts, as observed similarly in the case of disaccharide donors (MALDI-TOF MS: *m/z* = 1073.58 and 983.50).

It's obvious that the *N*-Cbz protected donor undergoes dissociation to form the byproducts more easily than in the case of the *N*-PNZ group. Higher yields were obtained when the glycosylation reaction was performed in the range of -45 °C to -25 °C (**Table 3.1.7**). Formation of byproducts was observed only during the glycosylation of protected trisaccharide DAD-1-*O*-Bn **76**, irrespective of the temperature control. So it was evident that the reactivity of the acceptor alcohol **61** is lower than that of responsible for the glycosylation to be more effective and also controls the formation of byproducts.

A different approach was explored by the synthesis of tetrasaccharides, using the trisaccharide acceptors **78** and **79** (**Scheme 3.1.28**). The glycosylation was performed under standard glycosylation conditions to yield the protected tetrasaccharides **DAAD** (**64**) and **ADAD** (**67**). In most of the cases reaction proceeded with byproduct formation (**Table 3.1.8**).

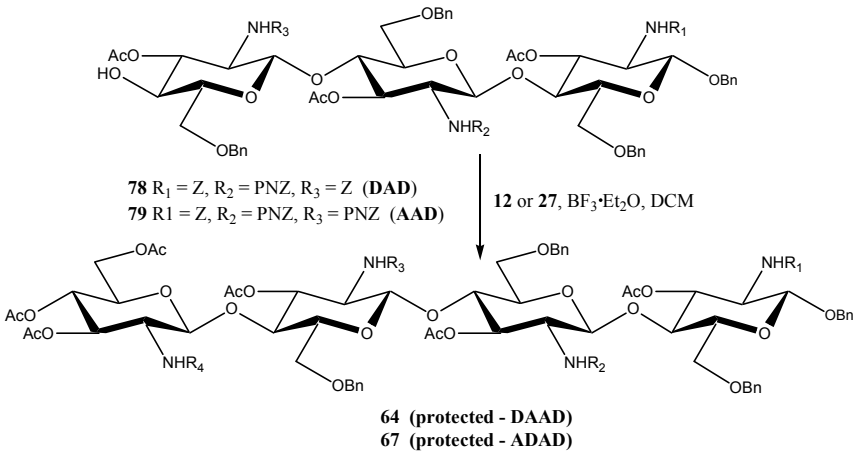


Scheme 3.1.27 Synthesis of 1-*O*-Bn trisaccharide acceptors

Table 3.1.7 Results of trisaccharide formation

Entry	Acceptor (equiv)	Donor (equiv)	$BF_3{\cdot}OEt_2$ (equiv)	Temperature (°C)	Time (h)	Product	Yield (%)
1	**61** (1.0)	**22** (2.0)	1.0	-50 to 0	3	**76**	49[a]
2	**61** (1.0)	**22** (2.0)	1.0	-45 to -25	2	**76**	50[a]
3	**61** (1.0)	**34** (1.3)	0.3	-30 to 0	1	**77**	38
4	**61** (1.0)	**34** (2.0)	1.0	-45 to -25	2	**77**	61

[a] Mixture of three products (protected trisaccharide and two byproducts).



Scheme 3.1.28 Glycosylation using 1-*O*-Bn trisaccharide acceptors

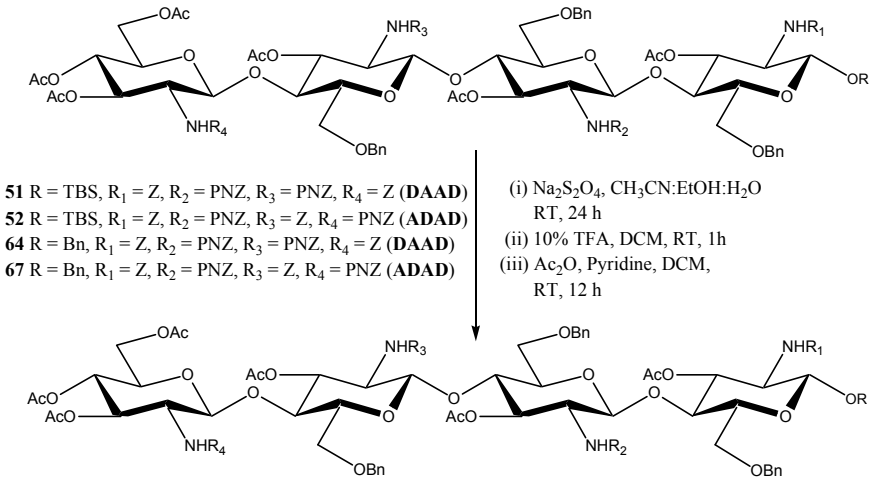
Table 3.1.8 Protected tetrasaccharide synthesis using trisaccharide acceptors

Entry	Acceptor (equiv)	Donor (equiv)	$BF_3{\cdot}Et_2O$ (equiv)	Temperature (°C)	Time (h)	Product	Yield (%)
1	**79** (1.0)	**12** (2.5)	1.50	-50 to 0	4	**64**	50[a]
2	**78** (1.0)	**27** (2.5)	1.25	-50 to 0	4	**67**	39
3	**79** (1.0)	**12** (2.5)	1.25	0 to rt	14	**64**	25[a]
4	**79** (1.0)	**12** (2.5)	1.25	-50 to -25	3	**64**	53

[a] Mixture of three products (protected tetrasaccharide and two byproducts *m/z* = 775.17, 883.24)

The C-4 hydroxyl group in *N*-acetylglucosamine derivative is a very poor nucleophile (glycosyl acceptors) in glycosylation reactions[86]. Crich et al.[247] reported a comparative study for the reactivity of C-4 hydroxyl groups towards the glycosylation reactions using *N*-Acetyl, *N*-azido and *N*-phthalimido protected acceptors. We believe that these carbamates (Z and PNZ) protected amino sugars also fall under the category of low reactivity glycosyl acceptors in the synthesis of higher oligosaccharides.

After exploring the various strategies for the synthesis of tetrasaccharides it is concluded that (2+2) glycosylation is superior to (3+1) glycosylation. As the protected tetrasaccharides **DDAA** (**63**) and **DAAD** (**64**) could be purified after the reductive cleavage of the *N*-PNZ group, it was better to perform the (2+2) glycosylation in order to obtain good yield. For the protected **ADAD (67)** sequence where the purification was rather tedious, the best option was to perform the glycosylation at low temperatures in order to obtain the pure product irrespective of the moderate yield.

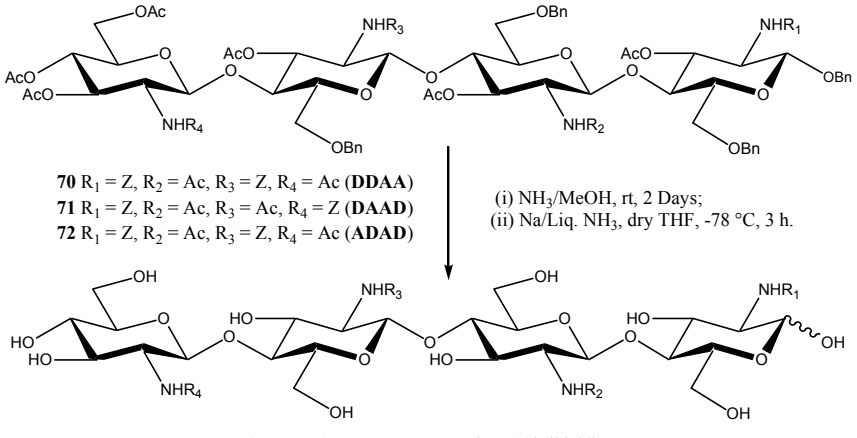


Scheme 3.1.29 Reductive cleavage using sodium dithionite

The reductive step using sodium dithionite was performed with pure products of protected tetrasaccharide **DAAD** and **ADAD** to afford acetylated derivatives in good yields (**Scheme 3.1.29**).

3.1.20 Final deprotection of protecting groups

Removal of *O*-acetyl groups of protected tetrasaccharides **70**, **71** and **72** by means of NaOMe in MeOH did not proceed to completeness, as judged by MALDI-TOF MS. Even strong basic conditions, i.e., NaOH solution, did not provide the expected results. Treatment of Na/Liq. NH_3, followed by quenching of the excess metal by addition of MeOH at -78 °C[248,249] removed some of the *O*-benzyl, but not all of the *O*-acetyl groups. Finally, the protected tetrasaccharides were treated with NH_3/MeOH for two days at rt. MALDI-TOF MS revealed mixtures of the fully *O*-deacetylated, *O*-benzylated tetrasaccharide and a mono-*O*-acetate. These mixtures were reacted with Na/ Liq. NH_3 in dry THF at -78 °C for 3 h, followed by addition of MeOH and stirring overnight. The reaction mixture was treated with Dowex (H^+) and then lyophilized. Further purification was done by exclusion chromatography (Biogel P2 extrafine, elution with water) to afford the pure tetrasaccharide in excellent yield (**Scheme 3.1.30**). By this way, a facile and high yielding deprotection protocol was achieved successfully.

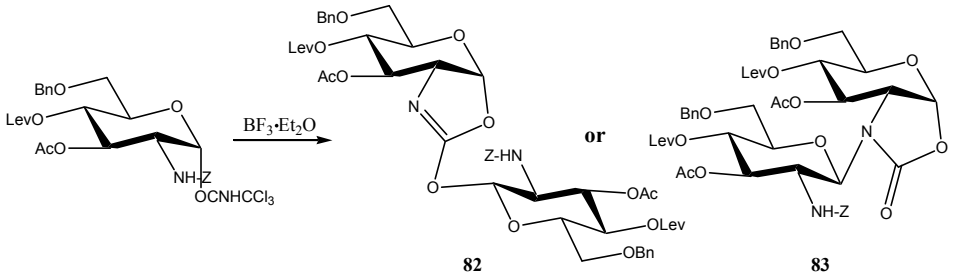


Scheme 3.1.30 Synthesis of free tetrasaccharides

3.1.21 Structure of byproducts formed during the glycosylation reaction

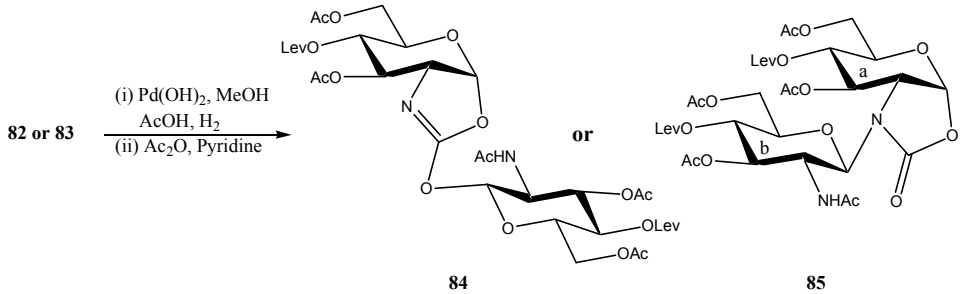
As discussed earlier in **Section 3.1.19**, coupling of donor **22** with acceptor **61** gave, besides the desired protected trisaccharide **76**, two byproducts which were detected by MALDI-TOF MS at *m/z* = 983.6 and 1073.7 $[M+Na]^+$ which account for molecular weights of 960.35 and 1050.40 respectively.

Assuming that the compound of M = 960.35 could have been formed by dimerization of donor, structures **82** and **83** were considered for this component (**Scheme 3.1.31**). Catalytic hydrogenation followed by *N*-acetylation gave a product of *m/z* = 795.30 $[M+Na]^+$ and the possible structures **84** and **85** were considered for this transformation (**Scheme 3.1.32**). The ^{13}C NMR spectrum, including HMQC and HMBC, showed signals of two anomeric carbon atoms at δ = 95.6 ppm ($^1J_{C1a,H1a}$ 188 Hz[250]) and δ = 84.0 ppm ($^1J_{C1b,H1b}$ 157.2 Hz). According to the literature, the coupling constant generally observed for $^1J_{C1-H1}$ is 170 Hz for an α- and 161-163 Hz for a β-glycosidic linkage[250].



$C_{49}H_{56}N_2O_{18}$ Calcd.: M - 960.35;
MALDI-TOF MS: calcd.: *m/z* = 983.34 $[M+Na]^+$, found: *m/z* = 983.6 $[M+Na]^+$

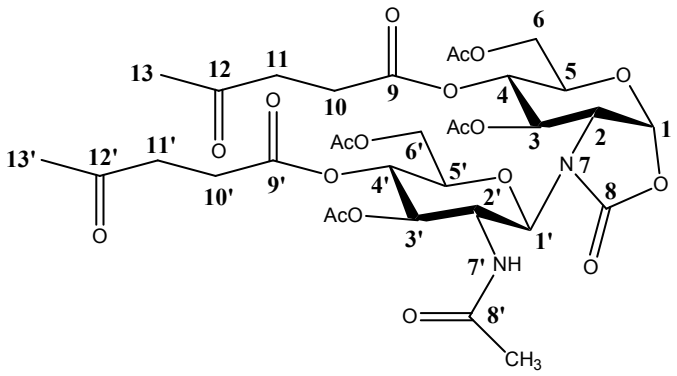
Scheme 3.1.31 Structures considered for the byproduct of M = 960.35



$C_{33}H_{44}N_2O_{19}$ Calcd.: M - 772.25;
MALDI-TOF MS: calcd.: *m/z* = 795.24 $[M+Na]^+$, found: *m/z* = 795.30 $[M+Na]^+$

Scheme 3.1.32 Structures considered for the product of hydrogenation and *N*-acetylation of 82 or 83 respectively.

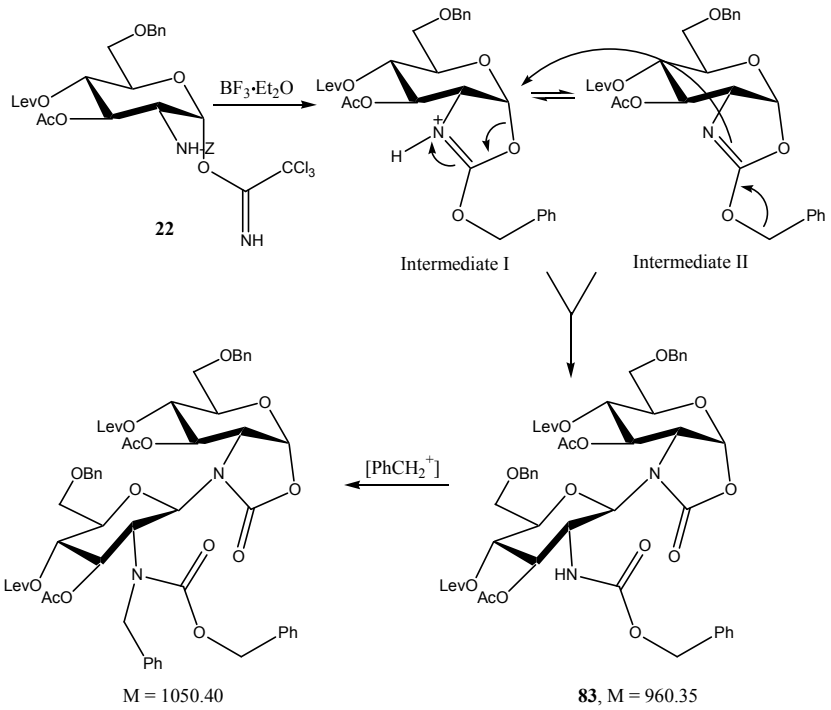
Based on ^{13}C NMR assignments, structure **84** (**Scheme 3.1.32**), and thus **82** (**Scheme 3.1.31**) is excluded. A possible mechanism for the formation of **83** is outline in **Scheme 3.1.33**.



85

Table 3.1.9 NMR spectroscopic values for Compound 85

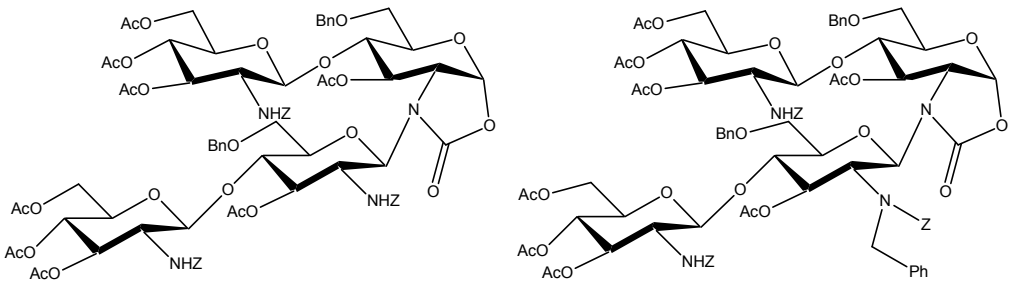
^{1}H (500 MHz, CD_2Cl_2)			^{13}C (300 MHz, CD_2Cl_2)		
δ ppm	**Coupling constants (Hz)**	**H**	**δ ppm**	**Coupling constants (Hz)**	**C**
5.85	d ($^{3}J_{1,2}$ = 7.5)	H-1	95.2	$^{1}J_{C1H1}$ = 188	C-1
4.57	ddd ($^{3}J_{2,1}$ = 7.5, $^{3}J_{2,3}$ =3.0, $^{4}J_{2,4}$ = 1.0)	H-2	53.2	$^{1}J_{C2H2}$ = 152.1	C-2
5.49	dd ($^{3}J_{3,2}$ = 4.5, $^{3}J_{3,4}$ = 2.5)	H-3	68.8	$^{1}J_{C3H3}$ = 156.1	C-3
4.93	ddd ($^{3}J_{4,3}$ = 7.0, $^{3}J_{4,5}$ = 4.0, $^{4}J_{4,2}$ = 1.0)	H-4	67.6	$^{1}J_{C4H4}$ = 152.2	C-4
4.05 – 4.12	m	H-5	69.3	$^{1}J_{C5H5}$ = 149.4	C-5
4.22 – 4.26	m	H-$6_{A,B}$	63.4	$^{1}J_{C6H6}$ = 151.9	C-6
5.17	d ($^{3}J_{1',2'}$ = 9.0)	H-1’	83.7	$^{1}J_{C1'H1'}$ = 157.2	C-1’
5.40	d ($^{3}J_{NH,2}$ = 9.5)	NH-7’	157		C-8[142,251]
4.35	ddd ($^{3}J_{2',1'}$ = 9.0, $^{3}J_{2',3'}$ = 10.0, $^{3}J_{2',NH}$ = 9.5)	H-2’	51.0	$^{1}J_{C2'H2'}$ = 144.6	C-2’
5.16	dd ($^{3}J_{3',2'}$ = $^{3}J_{3',4'}$ = 9.5	H-3’	72.8	$^{1}J_{C3'H3'}$ = 152.2	C-3’
5.09	dd ($^{3}J_{4',3'}$ = $^{3}J_{4',5'}$ = 9.5)	H-4’	67.9	$^{1}J_{C4'H4'}$ = 155.9	C-4’
3.79	ddd, ($^{3}J_{5',4'}$ = 9.5, $^{3}J_{5',6A'}$ = 4.0, $^{3}J_{5',6B'}$ = 2.5)	H-5’	74.6	$^{1}J_{C5'H5'}$ = 145.2	C-5’
4.05 – 4.12	m	H-$6'_{A,B}$	62.1	$^{1}J_{C6'H6'}$ = 150.4	C-6’



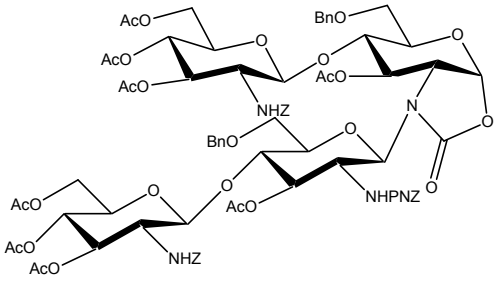
Scheme 3.1.33 Proposed mechanism for the formation of dimeric byproducts

In an analogous way, tetrasaccharide byproducts could be formed during the coupling of the disaccharide donors **43**, **44** and **45** respectively. The proposed structures are shown in **Scheme 3.1.34**.

Byproduct formed during the synthesis of protected DDAA



Byproduct formed during the synthesis of protected DAAD



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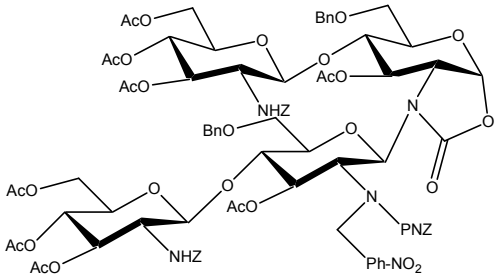

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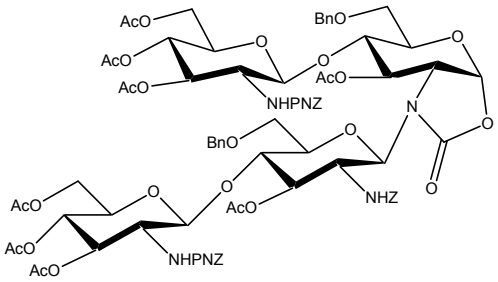
Byproduct formed during the synthesis of protected ADAD



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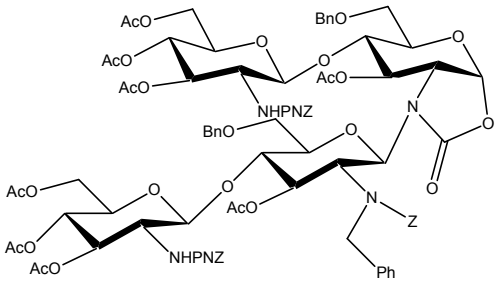


69

Scheme 3.1.34 Structures of tetrasaccharide byproducts

Table 3.1.10 Peaks (*m/z*) in MALDI-TOF MS of products formed during glycosylation reaction

Entry	Compound	Molecular Formula	Calculated Mass	MALDI-TOF MS $[M+Na]^+$	
				calculated	observed (*m/z*)
1	**53**	$C_{79}H_{90}N_4O_{32}$	1606.55	1629.54	1629.57
2	**54**	$C_{86}H_{96}N_4O_{32}$	1696.60	1719.59	1719.62
3	**65**	$C_{79}H_{89}N_5O_{34}$	1651.54	1674.53	1674.61
4	**66**	$C_{86}H_{94}N_6O_{36}$	1786.57	1809.56	1809.64
5	**68**	$C_{79}H_{88}N_6O_{36}$	1696.52	1719.51	1719.46
6	**69**	$C_{86}H_{94}N_6O_{36}$	1786.57	1809.56	1809.50

In conclusion, the amine protecting groups Z and PNZ has been utilized in the glycosidic bond formation with the corresponding acceptors and donors. Both exhibit sufficient reactivity towards the disaccharide formation. However, the reactivity is more crucial for the formation of tetrasaccharides. Irrespective of this effect the desired tetrasaccharides were prepared successfully and finally the deprotection yielded the tetrasaccharides in good yield.

3.2 Solid phase synthesis

Solid phase synthesis (SPS) is a rapid and efficient method to synthesize oligosaccharides[179]. As discussed in the introduction, it has gained much attention in recent years in the field of glycoconjugate chemistry. Exploring this method, a large number of oligosaccharides have been synthesized. Till now there was no report for the preparation of partially acetylated chitooligosaccharides or chitin derivatives by SPS. At the same time amino protected Z and PNZ sugar derivatives were not utilized as donors in SPS. After successfully developing a protocol for the synthesis of partially acetylated chito oligosaccharides by solution phase methodology, we also intended to prepare these oligosaccharides by SPS.

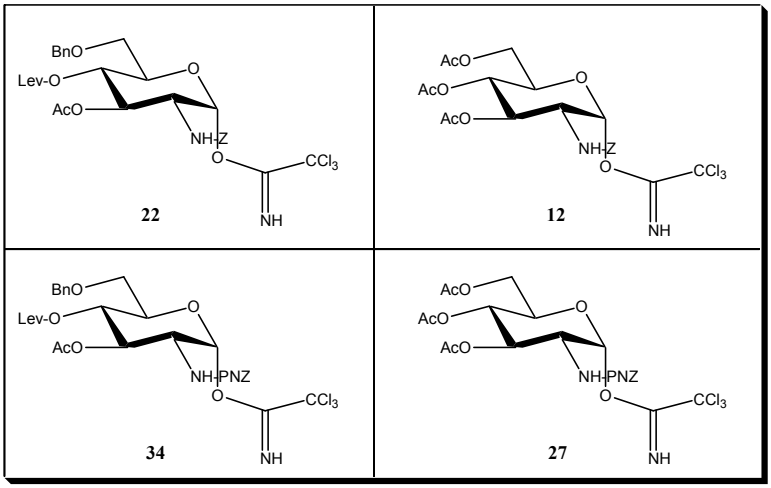


Figure 3.2.1 Donors for the SPS

We have shown the effectiveness of the glycosyl trichloroacetimidates as donors in the glycosidic bond formation and this method was utilized in SPS. The choice of polymer support and linkers were found to be crucial for high yielding glycosylation reactions. The conditions employed for glycosidic bond formation plays an important role in the choice of the support material. The monomeric trichloroacetimidate donors prepared for the solution phase synthesis were also utilized in SPS (**Figure 2.2.1**).

According to the literature[179], coupling of glycosyl donor or the glycosyl acceptor to the solid phase have been well explored in the SPS. Anchoring of the acceptor allowed for an excess of the reactive donor to be used to drive the reaction almost to completion. However, side reactions typically occurred by decomposition of the reactive species. The acceptor bound approach has more advantages and this method has been successfully utilized in the

automated oligosaccharide synthesis. It has further advantage that it provides the free reducing end sugar, thus allowing for further reactions, such as attachment of proteins.

A majority of linkers developed for the solid phase peptide synthesis will not be directly applicable to SPS of oligosaccharides, because their stability under glycosylation conditions are limited[188]. After extensive investigations on the compatibility of all the parameters involved, such as type of solid support, glycosylation method and protecting group strategy, we chose the following system:

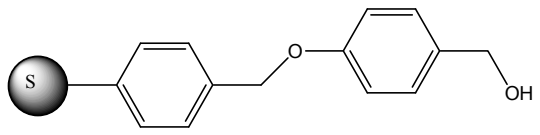
1) A polystyrene resin functionalized with Wang-OH[252] linker commercially available as Wang Resin was chosen. For comparative purpose, ethylene oxide grafted polystyrene functionalized with Wang-OH linker called as Tentagel Wang resin[253] was also studied.

2) Both Wang resins are stable towards low temperature glycosylation reactions with Lewis acid promoters[191]. They are highly resistant to basic conditions and can be readily cleaved with trifluoroacetic acid.

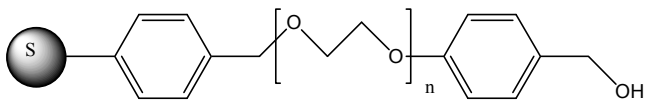
3) *O*-Glycosyl trichloroacetimidates as donors allow glycosylation under the above conditions.

4) Levulinoyl ester was used as an orthogonal protecting group, which can be cleaved efficiently by means of hydrazine acetate, thereby liberating the free hydroxyl site for further glycosylation.

The model synthetic sequence for the assembly of target tetrasaccharide based on glycosyl acceptor approach has been shown in **Scheme 3.2.1** and the structure of the resins are shown in **Figure 3.2.2**.

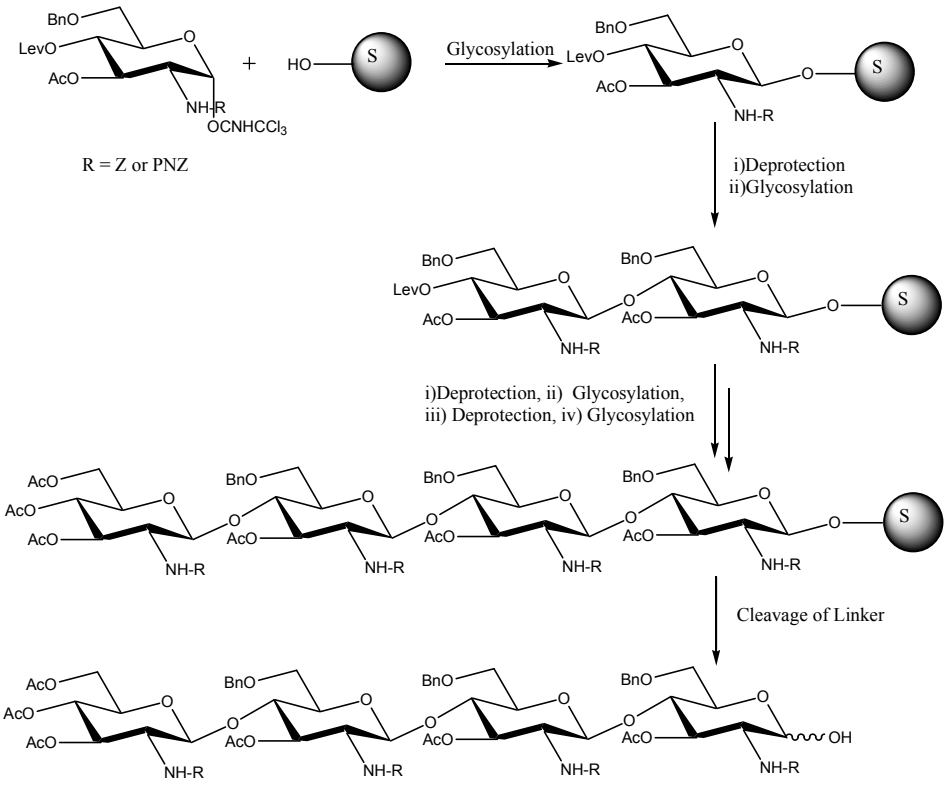


Wang Resin



Tentagel Wang Resin

Figure 3.2.2 Structures of the resin used in the SPOS



Scheme 3.2.1 Tetrasaccharide assembly based on glycosyl acceptor approach

In order to maintain anhydrous condition for the glycosylation, solid phase reactions were performed with a custom-made glass tube fitted with a frit at the bottom and a septum at the top. Glycosylation reactions were performed using 3.0 equiv of donor and 1.0 equiv of $BF_3{\cdot}Et_2O$ as the Lewis acid promoter for the activation of trichloroacetimidate donor. After the glycosylation reaction, resin was washed, filtered, dried under the vacuum overnight and from the weight gain, extent of glycosylation was calculated. The supernatant of an analytical cleavage (10% TFA/DCM) reaction from 2 to 4 mg of saccharide bound resin can be analyzed by MALDI-TOF MS, thus confirms the presence of any unreacted acceptor site after glycosylation reaction and also the presence of any levulinoyl group intact saccharide units.

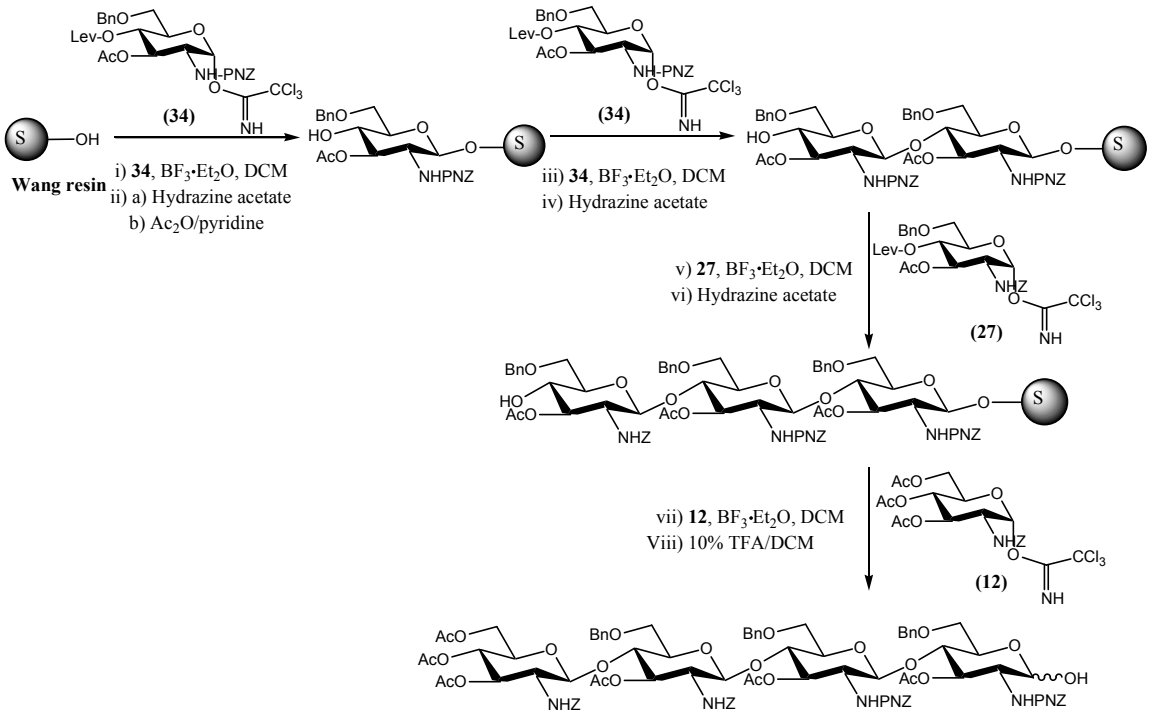
3.2.1 Synthesis of protected tetrasaccharide DDAA

The glycosylation was performed with Wang resin (loading 0.6 mmol/g) using donor **34** and $BF_3{\cdot}Et_2O$ at rt for 1 h. The yield calculated from weight of the resin was ca. 60%. Repetition of the glycosylation reaction resulted increased the yield to ca. 95%. The remaining active sites present in the resin were effectively capped by acetylation using Ac_2O and pyridine (**Scheme 3.2.2**).

3. Results and Discussion

Cleavage of the levulinoyl ester was performed using hydrazine acetate (20 equiv) dissolved in MeOH[254]. Generally the deprotection was performed using a mixture of hydrazine with AcOH and pyridine[213]. Complete deprotection was achieved within 2 h as identified by MALDI-TOF MS.

The iteration of sugar assembly was performed using the donors **34**, **22** and **12** respectively followed by the cleavage of the levulinoyl ester prior to glycosylation reactions. Both glycosylation and the deprotection were performed twice for one hour each. The cleavage of the sugar derivative from the resin was achieved by treating the resin with 10% TFA in DCM for 2 h and the same procedure repeated once again. After the cleavage, the solutions containing the sugar derivatives were washed with Sat. $NaHCO_3$ solution. MALDI-TOF MS of the crude product showed a mixture of tetra, tri and disaccharide derivatives. Column chromatography (40-70% EtOAc/Hexane) afforded a mixture of the tetra and trisaccharides in 4% overall yield after 8 steps, i.e. in the average 67% per step. MALDI-TOF MS of the residue from washings after glycosylation reaction revealed the byproduct formation between the donors as discussed in the solution phase synthesis (**Scheme 3.1.21**).



Scheme 3.2.2 SPS for the protected DDAA

The advantage of Tentagel over the polystyrene resin was its low loading capacity. The low loading capacity had been an advantage in SPS since a too high loading capacity may lead to incomplete reactions because of steric hindrance. Tentagel Wang resin was utilized for the preparation of protected tetrasaccharide **DDAA** as shown in **Scheme 3.2.2**, and the results were not encouraging.

3.2.2 Glycosylation reactions at low temperatures

For this purpose the experimental flask was slightly modified by changing the frit into the side arm. After glycosylation reaction resin was washed using appropriate solvent by pressurizing the tube with nitrogen gas. In order to find out the feasibility of glycosylation at low temperatures following steps were performed as shown in **Scheme 3.2.2** for the synthesis of protected tetrasaccharide **ADAD**. For all the protected tetrasaccharides, glycosylation reaction (-50 to -30 °C), acetylation (RT) and deprotection (RT) of levulinoyl ester were performed for 1 h.

1) Glycosylation of Wang resin with donor **22,** followed by acetylation and cleavage of the levulinoyl ester.

2) Glycosylation with donor **34,** followed by cleavage of levulinoyl ester.

3) Glycosylation with donor **22,** followed by cleavage of levulinoyl ester.

4) Glycosylation with donor **27**.

5) Cleavage of the product from the resin using 10% TFA/DCM.

MALFI-TOF MS of the crude product showed the presence of the protected tetrasaccharide together with lower oligomers which could not be separated by column chromatography on silica gel, even after acetylation. The yield of the tetrasaccharide product, as calculated from peak areas after separation by HPLC on a RP-18 column (65 % CH_3CN/H_2O) was 11% after 8 steps (76% per step). This shows that the glycosylation reaction gives better yield at low temperatures than performing at room temperature. Convinced by this observation we intended to explore the double glycosylation method, i.e. repetition of each coupling and deprotection reaction.

For the preparation of protected tetrasaccharide **ADAD** (**86**) the following steps were performed as shown in **Scheme 3.2.2.**

1) Double glycosylation of Wang resin with donor **22,** followed by acetylation and double cleavage of levulinoyl ester.

2) Double glycosylation with donor **34,** followed by double cleavage of levulinoyl

ester.

3) Double glycosylation with donor **22,** followed by double cleavage of levulinoyl ester.

4) Double glycosylation with donor **27**.

5) Cleavage of the product from resin using 10% TFA/DCM.

The crude mixture was purified by silica gel column chromatography and the fraction containing tetrasaccharides were analyzed by HPLC. Yield calculated from the peak area was 52% for 8 steps (92% per step).

Encouraged by this result we concentrated on the effectiveness of the Tentagel resin by the same experimental protocol. After the complete iteration and cleavage for the protected tetrasaccharide **ADAD**, crude product was analyzed by HPLC. The extent of product formation calculated from the peak area shows only 12% as compared to 45 % in the case of Wang resin. The reason for the low yield could be the presence of ethylene glycol (spacer) repeated units, which increases the number of nucleophilic sites. During the glycosylation reaction this property can direct the incoming Lewis acid to coordinate with the oxygen atom rather than activating the donor molecules.

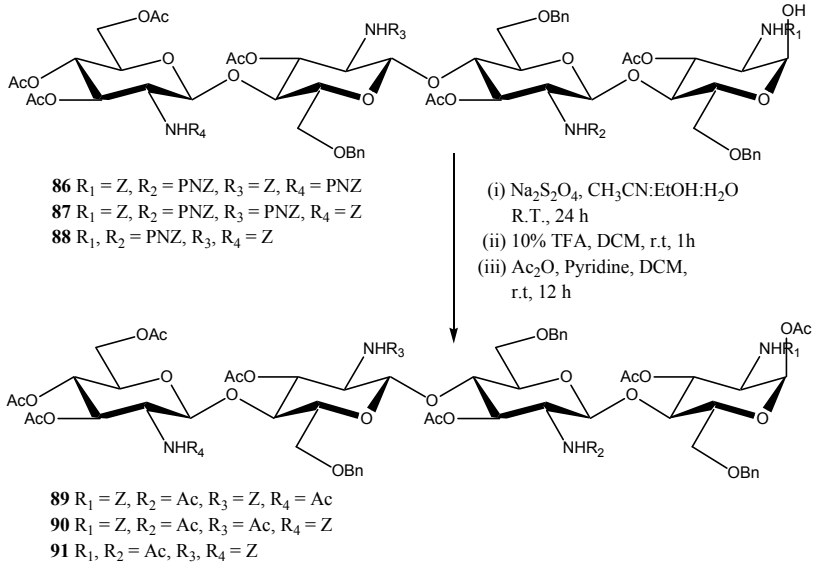
Having established a high yielding protocol using the Wang resin, we focused to prepare the other two protected tetrasaccharides **DAAD** and **DDAA**. The same sets of experiments were performed once again using wang resin with appropriate donors followed by the cleavage using TFA. The results are summarized in **Table 3.2.1**.

Though the yield for the tetrasaccharide formation is better by the application of the double glycosylation/deprotection protocol with wang resin, the purification remains a challenging task. The tetrasaccharide products were always isolated as a mixture along with trisaccharide derivatives. This was the main reason for reporting yield (**Table 2.2.1.)** by calculating peak areas of HPLC chromatogram. The NMR spectrum of tetrasaccharides containing Z and PNZ as amine protecting groups were not well resolved to calculate the coupling constant for the anomeric protons. The reductive cleavage step for the conversion of PNZ protecting group into the corresponding *N*HAc derivatives using sodium dithionite was performed. After reduction, pure *N*-acetamido tetrasaccharides were isolated using column chromatography. This resulted in the calculation of yield per step by incorporating the reductive reaction and thus increasing the total no of steps for the tetrasaccharide preparation into nine. (**Scheme 3.2.3** and **Table 3.2.2**)

Table 3.2.1 Preparation of protected tetrasaccharides by SPS.

Entry	Protected tetrasaccharide	Resin	Experimental Conditions[a]	Product Formation[b]	Yield[c] (% per step)
1	ADAD (**86**)	Wang	Single glycosylation and deprotection	36%	11% (76)
2	ADAD (**86**)	Wang	Double glycosylation and double deprotection	45%	52% (92)
3	ADAD (**86**)	Tentagel	Double glycosylation and double deprotection	12%	7% (71)
4	DAAD (**87**)	Wang	Double glycosylation and double deprotection	38%	39% (89)
5	DDAA (**88**)	Wang	Double glycosylation and double deprotection	38%	45% (91)

[a]Glycosylation performed at -50 to -30 °C and deprotection performed at rt twice for 1 h.
[b]Extent of product formation calculated from peak area of HPLC of the crude product.
[c]yield calculated from peak area of HPLC for the purified fraction containing tetrasaccharides.



Scheme 3.2.3 Reductive cleavage using sodium dithionite

Table 3.2.2 Yield for the tetrasaccharides by SPOS method

Entry	Protected tetrasaccharide (*N,N'*-diacetylated)	Overall yield after 9 steps (%)	Yield per step (%)
1	ADAD (**89**)	43	91
2	DAAD (**90**)	37	90
3	DDAA (**91**)	32	88

In summary, solid-phase oligosaccharide synthesis with *N*HCbz and *N*HPNZ protected glucosamine donors has been studied. With Wang resin, excellent yields were obtained throughout the iterative assemblies. At low temperatures the intermediate alcohols possessed excellent reactivity towards the donor molecules, which resulted in high yield of the glycosylation reactions. The maximum yield observed in case of protected tetrasaccharide **ADAD** (**86**) proves the efficiency of this method as compared with solution phase synthesis. This simple and straight forward methodology will be useful for future automation techniques. Further extension of this method is possible and we are convinced that the preparation of higher chitooligosaccharides will be quite efficient.

For the first time, Z and PNZ groups have been explored as glycosyl donors in the SPS. The high yield per step clearly indicates that the reactivity of the acceptor alcohols having Z and PNZ as amine protecting groups in the SPS. We believe that this methodology will be very effective during the iterative cycle and the cleavage under mild conditions makes this procedure a viable option for further exploration.

4. Summary

Convenient and high yielding protocols were developed for the preparation of *N*-benzyloxycarbonyl (*N*HCbz) and *N*-p-nitrobenzyloxycarbonyl (*N*HPNZ) glucosamine protected glycosyl acceptors and donors. Among the Lewis and Brønsted acids examined for the disaccharide coupling reaction $BF_3{\cdot}Et_2O$ was found to be the most efficient catalyst for the glycosylation reaction. The deprotection of the *N*HPNZ moiety was achieved by reduction with sodium dithionite followed by treatment with TFA before reacetylation of free amine to ensure the complete removal of 1,6 elimination product. Following the general deprotection procedure, chitobioses and partially acetylated chitobioses were obtained in high yield. Comparing with the literature report, our synthetic strategy was simple, high yielding during the building block synthesis and the number of steps to achieve the target compound were also lesser.

The disaccharide donors and acceptors were readily prepared by synthesizing the appropriate disaccharide derivatives. In order to study the reactivity of glycosyl acceptors, reducing end were protected as its corresponding *O*-*tert*-butyldimethylsilyl ether (*O*-TBDMS) and *O*-benzyl ether (*O*-Bn). The disaccharide acceptors and disaccharide donors were coupled in a (2+2) glycosylation manner to afford the tetrasaccharides. The reaction proceeded with formation of byproducts which were identified as dimers of the donors, as proved by NMR (1D and 2D techniques). A plausible mechanism is proposed for the formation of the byproducts. We believe that this is the first time this type of byproduct isolated during the glycosylation reaction. The observed glycosylation reaction occurred between the C-1 of the donor molecule and C-2 nitrogen atom of the acceptor molecule

In order to address the byproduct formation, series of glycosylations were performed. The temperature control, reactivity of glycosyl acceptors and the protecting group compatibility under glycosylation conditions proved to be the major factor in the byproduct formation. Further to prove the reactivity of the glycosyl acceptors and the glycosyl donors, (3+1) glycosylation reaction was performed utilizing glycosylation of a monomeric acceptor with a trimeric donor, as well as trimeric acceptor with a monomeric donor.

For this purpose trisaccharide donors and trisaccharide acceptors were developed. This shows the effectiveness of the protecting group pattern which have been chosen for the oligosaccharide synthesis and their compatibility over wide variety of transformations. Formation of byproduct was found to be rapid with *N*HCbz donors than with *N*HPNZ donors. Finally a high yielding deprotection procedure was developed for the complete removal of protecting groups and successfully applied in the preparation of three partially acetylated

chitotetraoses (**DDAA, DAAD and ADAD**).

To demonstrate the utility of the *N*HCbz and *N*HPNZ donors, solid-phase synthesis were performed using the Wang and Tentagel Wang resins. The glycosylation reactions were performed at various temperatures and glycosylation reactions at low temperature proved to be highly successful. Among the resins utilized for the synthesis, Wang resin was found to be efficient than the Tentagel Wang resin. The double glycosylation and double deprotection methods were found to be high yielding. Cleavage of the linker followed by reduction with sodium dithionite, pure protected *N,N'*-diacetylated tetrasaccharides were obtained in excellent yields after nine steps (90 % per step). For the first time *N*HCbz and *N*HPNZ donors were utilized and in the same time partially acetylated chito tetrasaccharides have also been synthesized by solid-phase methodology.

We have shown that the *N*HCbz and *N*HPNZ glycosyl donors and glycosyl acceptors can be utilized for the synthesis of tetrasaccharides. More over the presence of acetate ester in C-3 position further opens up the possibility for the synthesis of branched oligosaccharides. The high yield per step in the solid-phase oligosaccharide synthesis proves the versatility of this approach and we believe that this method will be suitable for the synthesis of higher chitooligosaccharides and also useful for the automation process.

5. Experimental Section

5.1 General Remarks

Solvents

Solvents used in the reactions were dried according to the standard methods by distillation over drying agents. DMF was heated under reflux for 3 hours in the presence of CaH_2, distilled and stored over molecular sieves (4 Å). Methanol was treated with magnesium turnings, heated under reflux for 5 hours, distilled and stored over molecular sieves (4 Å). THF was freshly distilled from sodiumbenzophenone ketyl under nitrogen. DCM was freshly distilled from P_2O_5 under nitrogen. All other chemical reagents were purchased from Acros and used without further purification.

Chromatography

- **Thin layer chromatography (TLC)** was performed using aluminum plates covered with SiO_2 (Merck 60, F_{254}). The chromatograms were developed under UV light and/or by treatment of the TLC plate with the following reagent by gentle heating with a heat gun (ammonium molybdate 20g and cerium (IV) sulfate 0.4 g in 10% aq. sulphuric acid 400 ml).
- **Flash column chromatography** was performed using SiO_2 60 (Merck, 230-400 mesh).
- **Size-exclusion chromatography** was carried out on BioGel P2 (extra fine) procured from Biorad laboratories.

Analytical data

- **Melting points** were measured with an Electrothermal IA9100 and were uncorrected.

- **Optical rotations** were measured with a Jasco Dip-1000 polarimeter.

- **Infrared spectra** were recorded with a Perkin Elmer FT-IR 16 PC. The absorption bands were reported in wave number (cm^{-1}).

- **NMR** spectra were recorded using Bruker ARX 300 and ARX500 MHz spectrometers. The chemical shifts are reported in parts per million (δ) relative to the deuterated solvent peak: $CDCl_3$ (δ_H: 7.27, δ_C: 77.0), CD_2Cl_2 (δ_H: 5.32, δ_C: 53.8). Spectra obtained in D_2O are reported reference to HOD signal at 4.79 ppm for ^1H spectra. For ^{13}C spectra sodium 3-(trimethylsilyl)propionate-d_4 was used as an external standard for calibration of the chemical shift. Coupling constants (*J*) were reported in Hertz (Hz). For the characterization of the observed signal multiplicities, following abbreviations were used: s (singlet), bs (broad singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet).

- **MALDI-MS** were measured with a Bruker Reflex II mass spectrometer using 2,4,6-trihydroxyacetophenone (THAP) or 2,5.dihydroxybenzoic acid (DHB) as a matrix. A 0.5 µl of aliquot of matrix solution was spotted on the sample holder and allowed to dry. Addition of 0.5 µl aliquot of oligosaccharide sample solution was co-spotted on the matrix, dried, and analyzed in the positive ion mode.

- **Exact Masses** were recorded by ESI-MS on Micromass Q-TOF mass spectrometer from waters Inc.

- **Elemental analyses** were performed using an Autoanalysator CHNS-932 or Elementaranalysator CHNOS VarioEL III.

- **HPLC** analysis was performed with Waters Model 590 using preparative Lichrosorb RP-18 column (5 µm, 250 x 4 mm). Product separated using the following conditions: λ = 207 nm, 65 % CH_3CN/H_2O, flow rate = 1.2 ml/min.

- Shaking was performed using the IKA-VIBRAX-VXR instrument for the solid-phase synthesis.

5.2. General experimental procedures

Procedure A (Glycosylation reaction)

A mixture of glycosyl trichloroacetimidate and glycosyl acceptor (1.0 mmol) were coevaporated with toluene, dried under vacuum for 1 h, and dissolved in 10 ml of dry DCM. The solution was cooled to appropriate temperature and then appropriate Lewis acid was added. After the stipulated time Et_3N was added to quench the reaction, the solution was concentrated under reduced pressure, and the crude product was purified by flash silica gel column chromatography to afford the desired saccharides.

Procedure B (*N*HPNZ → *N*HAc)

Compound (0.1 mmol) was dissolved in 6 ml CH_3CN, EtOH, H_2O (v/v/v 1:1:1). sodium dithionite (140 mg) was added and the stirring was continued at room temperature for overnight. Then solvent was concentrated and redissolved in DCM/H_2O (30 ml). Organic layer separated and the aqueous layer back extracted with DCM (2 x 15 ml). Combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Residue was redissolved in 5 ml of dry DCM, and then treated with 1ml of 10% TFA/DCM. After 1 h reaction mixture was quenched by the addition of sat. $NaHCO_3$ and then extracted with DCM (2 x 10 ml). Combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Again the resulting residue was redissolved in dry

DCM, treated with Ac_2O/pyridine (1:2, 1.5 ml) and stirred at room temperature for overnight. Then solvents removed and azeotropically distilled over toluene and the resulting residue was purified by silica gel column chromatography.

Procedure C (Removal of levulinoyl ester)

A solution of hydrazine acetate (1.15 mmol) in MeOH (1 ml) was added to a solution of levulinoyl derivative (1 mmol) in 10 ml dry DCM and the resulting solution was stirred for 2 h at room temperature. Then the reaction mixture concentrated and product purified by silica gel column chromatography.

Procedure D (Preparation of trichloroacetimidate donor)

To a stirred solution of *O*-TBDMS derivative (1 mmol) in dry THF (10 ml) were added dropwise and simultaneously TBAF (1.0 M solution in THF, 1.5 mmol) and AcOH (1.5 mmol). After the stipulated time, reaction mixture was diluted with H_2O (50 ml) and extracted with EtOAc (2 x 100 ml). Combined organic extracts were washed with sat aq. $NaHCO_3$ (100 ml), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Residue was redissolved in a solution of DCM (10 ml), CCl_3CN (10 mmol) and DBU (0.3 mmol) added and stirred at room temperature for overnight. The reaction mixture was concentrated in vacuo, and the resulting residue purified by silica gel column chromatography to yield the product.

Procedure E (Preparation of free disaccharides)

To a stirred solution of *O*-TBDMS derivative (0.1 mmol) in THF (4 ml) were added dropwise and simultaneously TBAF (1.0 M solution in THF, 0.2 mmol) and AcOH (0.2 mmol) and stirred continuously at room temperature for overnight. Then solvents removed under reduced pressure and product purified using silica gel column chromatography. Then the product redissolved in 8 ml of MeOH/DCM (1:1), treated with NaOMe (0.4 mmol) and stirred at room temperature for overnight. Then the reaction mixture was neutralized with Amberlite IR120 (H^+) resin, filtered, solvent removed under reduced pressure and dried under vacuum to yield the product. Then the product was treated with $Pd(OH)_2$ (0.2 mmol) in 3 ml of MeOH/AcOH (9:1) and then flushed with hydrogen atmosphere. Then it was stirred for overnight under a hydrogen atmosphere. The solution was filtered using celite and washed with MeOH/H_2O (3:1) and purified on a Biogel P-2 column, using H_2O as eluent. All fractions were analyzed by MALDI-TOF MS and then the product containing fractions were collected and lyophilized to yield the free disaccharides as mixture of anomers.

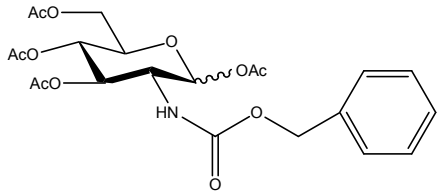
Procedure F (Preparation of free tetrasaccharides)

Protected tetrasaccharide (0.02 mmol) was treated with 20 ml of NH_3/MeOH and the resultant mixture was stirred under room temperature for 2 days. Then solvent was removed under the stream of nitrogen gas, residue azeotropically dried with toluene (3 x 5ml) and dried

under vacuum for 2 hours. Then it was treated with 3 ml of THF and NH_3 condensed (15 ml) into the reaction flask at -78 °C. After 10 minutes small pieces of Na metal (~ 50 mg) were added to the flask, affording a solution with a deep blue color. After 3 h reaction was quenched by adding 5 ml of MeOH (If the blue color disappears before the mention time, again some pieces of Na metal added to maintain the deep blue color). The reaction mixture was stirred continuously for overnight while the temperature slowly increases to room temperature in order to evaporate the ammonia. Dowex 50-X8 acidic resin was added carefully until pH 7 was reached. The mixture was filtered, rinsed with MeOH/H_2O and then concentrated under reduced pressure and then redissolved in H_2O followed by lyophilization to yield the crude compound. Then it was purified on a Biogel P-2 column, using H_2O as eluent. All fractions were analyzed by MALDI-TOF MS and then product containing fractions were collected and lyophilized to yield the free *N,N*-diacetylated tetrasaccharides as mixture of anomers.

5.3 Experimental details

1,3,4,6-Tetra-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-α/β-D-glucopyranoside (10)



To a solution of D-glucosamine hydrochloride **8** (10.78 g, 50 mmol) in H_2O (100 ml) were added $NaHCO_3$ (10.5 g, 125 mmol) and benzyloxycarbonyl chloride (11.87 g, 75 mmol). After being stirred at room temperature for overnight, the product (**9**) was filtered and washed with ice cold water and dried under vacuum (13.76 g, 88 %). Then the solid was redispersed in pyridine (68.8 g, 869mmol) with vigorous stirring and then cooled to 0 °C. Ac_2O (66.48 g, 651 mmol) was added in drops using the dropping funnel and stirred continuously at RT. for overnight. Then cold EtOH (30 ml) was slowly added to quench the excess Ac_2O, which was then reduced by rotary evaporation. The residue was redissolved in toluene (3 x 50 ml) and concentrated for the azeotropic removal of pyridine. The remaining slurry was redissolved in EtOAc (250 ml) and washed with distilled H_2O (2 x 250 ml) and brine (250 ml), then dried over anhydrous Na_2SO_4, filtered and solvent was removed under reduced pressure. The residue was purified over silica gel column chromatography using 30 – 45 % EtOAc/Hexane solvent gradient which afforded compound **10** as a white solid (α/β - 4/1 , 20.27 g, 97 %).

R_f 0.46 (50 % EtOAc/Hexane), m.p. 54.0 – 55.5.

IR (NaCl Plates) 3368, 2966, 1753 cm^{-1}.

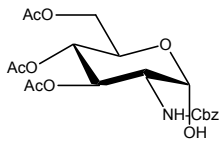
^1H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.35 (m, 6H), 6.21 (d, *J* = 3.6 Hz, 0.8 H, $H_1\alpha$), 5.68 (d, *J* = 9.0 Hz, 0.2 H, $H_1\beta$), 5.24 (d, *J* = 9.6 Hz, 1H), 5.18 (d, *J* = 8.7 Hz, 2H), 5.15 (d, *J* = 4.2 Hz, 1H), 5.01 – 5.12 (m, 3H), 4.93 (d, *J* = 9.3 Hz, 1H), 4.7 – 4.30 (m, 2H), 4.07 (d, *J* = 2.7 Hz, 1H), 3.97 – 4.04 (m, 2H), 2.16 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.93 (s, 3H).

^{13}C NMR is in accordance with literature data[255].

ESI-MS *m/z* $(M + H)^+$ calcd 482.1662, obsd 482.1644.

$C_{22}H_{27}NO_{11}$ (481.1584) calcd C 54.88, H 5.65, N 2.91; found C 54.68, H 5.61, N 2.81.

3,4,6-Tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (11)



A solution of **10** (7.1 g, 14.76 mmol) and hydrazine acetate (1.56 g, 16.98 mmol) in dry DMF (40 ml) was stirred at room temperature. After 1 h reaction mixture was diluted with DCM (150 ml) and washed with ice cold saturated $NaHCO_3$ (4 x 150 ml). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography using 35 – 45 % EtOAc/Hexane solvent gradient to yield the product **11** as a white solid (5.96 g, 92 %)

R_f 0.38 (50 % EtOAc/Hexane), m.p. 53.5 – 54.5, $[\alpha]^{24}_D$ +56.25 (*c* = 0.50, $CHCl_3$).

IR (NaCl Plates) 3365, 2959, 1748 cm^{-1}.

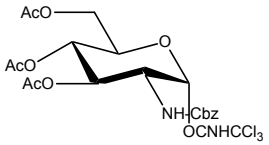
^1H NMR (300 MHz, $CDCl_3$) δ 7.23 – 7.30 (s, 5H), 5.21 – 5.31 (m,3H), 5.07 – 5.15 (m, 3H), 5.02 (d, *J* = 12.3 Hz, 1H), 4.19 – 4.22 (m, 2H), 4.11 – 4.13 (m, 1H), 4.03 (ddd, *J* = 3.3, 6.9, 9.0 Hz, 1H), 2.08 (s, 3H), 2.02 (s, 3H), 1.90 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 170.8, 169.4, 155.8, 136.1, 128.5, 128.1, 128.0, 91.7, 70.9, 68.3, 67.5, 66.8, 62.0, 53.9, 20.6, 20.5, 20.5.

ESI-MS *m/z* $(M + H)^+$ calcd 440.1557, obsd 440.1573.

$C_{20}H_{25}NO_{10}$ (439.1478) calcd C 54.67, H 5.73, N 3.19; found C 54.43, H 5.80, N 3.08.

3,4,6-Tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl trichloroacetamidate (12)



A mixture of **11** (5.62 g, 12.80 mmol), CCl_3CN (12.90 g, 89.60 mmol) and DBU (0.39 g, 2.56 mmol) in dry DCM (100 ml) was stirred at r.t for overnight. Then reaction mixture was concentrated under reduced pressure and then the residual syrup was purified by silica gel chromatography using 30 % EtOAc/Hexane to yield **12** as a yellow solid (6.49 g, 87 %).

R_f 0.57 (40 % EtOAc/Hexane), m.p. 55.0 – 56.5, $[\alpha]^{24}_D$ +58.90 (*c* = 0.52, $CHCl_3$).

IR (NaCl Plates) 3336, 2957, 1748, 1679 cm^{-1}

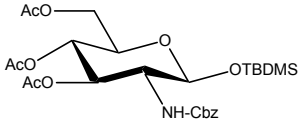
^1H NMR (300 MHz, $CDCl_3$) δ 8.77 (s, 1H), 7.3 (s, 5H), 6.40 (d, *J* = 3.6 Hz, 1H), 5.19 – 5.33 (m, 2H), 5.09 (AB quartet, *J* = 12.3 Hz, 2H), 4.95 (d, *J* = 9.3 Hz, 1H), 4.23 – 4.33 (m, 2H), 4.09 – 4.15 (m, 2H), 2.07 (s, 3H), 2.04 (s, 3H), 1.94 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 170.5, 169.2, 160.3, 155.6, 136.0, 128.5, 128.3, 128.1, 94.9, 90.7, 71.6, 70.2, 67.5, 67.2, 61.5, 53.5, 20.6, 20.5, 20.4.

ESI-MS *m/z* $(M + Na)^+$ calcd 605.0472, obsd 605.0478

$C_{22}H_{25}Cl_3N_2O_{10}$ (582.0575) calcd C 45.26, H 4.32, N 4.80; found C 45.46, H 4.42, N 4.89.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (13)



A solution of **10** (21.8 g, 45 mmol) and hydrazine acetate (4.75 g, 51.7 mmol) in dry DMF (100 ml) was stirred at room temperature. After 1 h reaction mixture was diluted with DCM (250 ml) and washed with ice cold sat. aq. $NaHCO_3$ (4 x 250 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to yield compound **11**. Compound **11** was redissolved in dry DCM without purification and then imidazole (6.12 g, 90 mmol) and *tert*-butylchlorodimethylsilane (7.79 g, 51.6 mmol) was added. After stirring at rt for overnight the reaction mixture was diluted with water (150 ml), then aqueous layer was extracted with DCM (2 x 100 ml). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure to yield the crude product. The crude product was purified by silica gel chromatography using 25 % EtOAc/Hexane to yield **13** as a white solid (21.54 g, 86 %).

R_f 0.54 (30 % EtOAc/Hexane), m.p. 132.0 – 134.0, $[\alpha]^{24}_D$ +7.44 (*c* = 0.52, $CHCl_3$).

IR (NaCl Plates) 3292, 2957, 1748, 1692 cm^{-1};

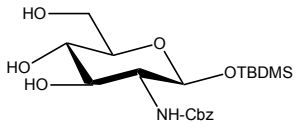
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (s, 5H), 5.21-5.23 (m, 1H), 5.07 (s, 2H), 5.02 (dd, *J* = 9.6, 9.6 Hz, 1H), 4.86 (d, *J* = 8.1 Hz, 1H), 4.79 (d, *J* = 9.0 Hz, 1H), 4.16 (dd, *J* = 6.0 Hz, 12.0 Hz), 4.12 (dd, *J* = 2.4 Hz, 12.0 Hz, 1H), 3.58 – 3.72 (m, 2H), 2.09 (s,3H), 2.02 (s,3H), 1.96 (s, 3H), 0.87 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 170.4, 169.4, 155.5, 136.2, 128.4, 128.0, 96.2, 72.0, 69.4, 66.8, 62.4, 58.0, 25.4, 20.6, 20.5, 20.4, 18.2, -3.9, -4.9.

ESI-MS *m/z* $(M + H)^+$ calcd 554.2422, obsd 554.2434.

$C_{26}H_{39}NO_{10}Si$ (553.2343) calcd C 56.40, H 7.10, N 2.53; found C 56.14, H 7.25, N 2.47.

tert-Butyldimethylsilyl 2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (14)



Compound **6** (16.4 g, 29.5 mmol) was dissolved in dry MeOH (200 ml) and NaOMe (0.16 g, 2.95 mmol) was added and stirred at RT. After 3 h the reaction mixture was neutralized with Amberlite IR120 (H^+) resin. The resin was filtered and solvent was removed under reduced pressure and then dried under vacuum to yield the compound **14** as a white solid (12.65 g, Quantitative).

R_f 0.22 (50 % EtOAc/Hexane), m.p. 51.0 – 52.0, $[\alpha]^{24}_D$ -40.38 (*c* = 0.52, $CHCl_3$).

IR (NaCl Plates) 3344, 2955, 1700 cm^{-1}.

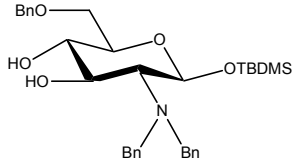
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 (s, 5 H), 5.93 (d, *J* = 9.0 Hz, 1H), 5.13 (d, *J* = 12.3 Hz, 1H), 4.89 (d, *J* = 12.0 Hz, 1H), 4.62 (d, *J* = 7.5 Hz, 1H), 3.74 – 3.86 (m, 2H), 3.63 – 3.68 (m, 2H), 3.41 (d, *J* = 8.4 Hz, 1H), 3.29 (d, *J* = 8.4 Hz, 1H), 0.83 (s, 9H), 0.07 (s, 3H), 0.04 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 156.8, 136.8, 128.8, 128.5, 97.0, 75.8, 74.4, 71.1, 67.3, 62.4, 60.2, 25.1, 18.3, -3.7, -4.9.

ESI-MS *m/z* $(M + H)^+$ calcd 428.2105, obsd 428.2100.

$C_{20}H_{33}NO_7Si$ (427.2026) calcd C 56.18, H 7.78, N 3.28; found C 55.93, H 7.94, N 3.23.

tert-Butyldimethylsilyl 6-*O*-benzyl-*N,N*-dibenzyl-2-deoxy-β-D-glucopyranoside (15)



A suspension of Compound **14** (1 g, 2.34 mmol) and dibutyltin oxide (1.46 g, 5.85 mmol) in toluene 20 ml was heated under reflux (Dean-Stark Apparatus). After 12 h tetrabutylammonium bromide (1.51 g, 4.68 mmol) and benzyl bromide (1g, 5.85 mmol) were added and the mixture was gently refluxed. After 3 h the reaction mixture was cooled, filtered and concentrated under reduced pressure. The residue was dissolved in EtOAc, filtered and again concentrated under reduced pressure. Then the residue was purified by silica gel column chromatography (5-25 % EtOAc/Hexane) to yield the product **8** as yellow liquid (0.59 g, 42 %).

R_f 0.33 (30 % EtOAc/Hexane), $[\alpha]^{24}_D$ +20.16 (*c* = 1.0, $CHCl_3$).

IR (Neat) 3418, 3062, 2927, 2855 cm^{-1}.

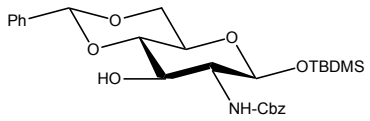
^1H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.22 (m, 15H), 4.96 (d, *J* = 9.1 Hz, 1H, H-1), 4.55 (AB Quartet, *J* = 12.3 Hz, 2H, O-C*H*$_2$-Ph), 3.87 (AB Quartet, *J* = 12.6 Hz, 2H, N-C*H*$_2$-Ph), 3.71 (dd, *J* = 10.5, 3.6 Hz, 1H, H-6$_A$), 3.63 (dd, *J* = 10.2, 5.4 Hz, 1H, H-6$_B$), 3.52 (dd, *J* = 10.5, 9.3 Hz, 1H, H-3), 3.46 -3.33 (m, 2H, H-5, H-4), 3.26 (bs, 1H, C-3 *OH*), 2.78 (bs, 1H, C-4 *OH*), 2.53 (dd, *J* = 10.2, 8.3 Hz, 1H, H-2), 1.05 (s, 9H, (Si-C-(C*H*$_3$)$_3$)), 0.24 (s, 3H, Si-*CH*$_3$), 0.23 (s, 3H, Si-*CH*$_3$).

^{13}C NMR (75 MHz, $CDCl_3$) δ 140.9, 139.2, 138.0, 129.2, 128.5, 128.3, 127.6, 127.0, 97.0 (C-1), 74.6 (C-5), 73.5 (O-*C*H$_2$-Ph), 72.6 (C-4), 71.6 (C-3), 70.3 (C-6), 63.9 (C-2), 54.2 (N-*C*H$_2$-Ph), 26.0 (Si-C-(*C*H$_3$)$_3$), 17.9 (Si-*C*-(CH$_3$)$_3$), -3.5 (Si-*C*H3), -5.1 (Si-*C*H$_3$).

ESI-MS *m/z* $(M + H)^+$ calcd 564.3145, obsd 564.3140.

$C_{33}H_{45}NO_5Si$ (563.3067) calcd C 70.30, H 8.04, N 2.48; found C 70.01, H 7.90, N 2.38.

tert-Butyldimethylsilyl 4,6,-*O*-benzylidene-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (17)



Compound **14** (10 g, 23.4 mmol) was dissolved in CH_3CN (150 ml) and Benzaldehyde dimethylacetal (4.27 g, 28 mmol) was added followed by the addition of camphorsulphonic acid (0.54 g, 2.34 mmol). After stirring overnight at RT, Et_3N was added and then solvents were removed under reduced pressure. Then crude compound was purified by silica gel chromatography using 25 % EtOAc/Hexane to yield the compound **17** as a white solid (10.83 g, 90 %).

R_f 0.51 (30 % EtOAc/Hexane), m.p. 183.0 – 184.0, $[\alpha]^{24}_D$ -35.58 (*c* = 0.5, $CHCl_3$).

IR (NaCl Plates) 3313, 2955, 1686 cm^{-1}.

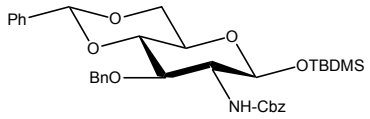
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 10H), 5.5 (s, H), 5.04 - 5.08 (m, 3H), 4.82 (d, *J* = 4.2 Hz, 1H), 4.27 (dd, *J* = 4.8 Hz, 10.2 Hz, 1H), 3.75 (dd, *J* = 10.2, 10.2 Hz, 1H), 3.54 (dd, *J* = 9.3, 9.3 Hz, 1H), 3.26 – 3.45 (m, 3H), 0.70 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 156.8, 137.6, 136.6, 129.6, 128.9, 128.7, 128.6, 102.3, 96.7, 81.9, 71.3, 69.1, 67.5, 66.7, 61.3, 25.9, 18.3, -3.8, -4.9.

ESI-MS *m/z* $(M + H)^+$ calcd 516.2418, obsd 516.2452.

$C_{27}H_{37}NO_7Si$ (515.2339) calcd C 62.89, H 7.23, N 2.72; found C 62.77, H 7.10, N 2.66.

tert-Butyldimethylsilyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (18)



To a stirred solution of compound **17** (5 g, 9.7 mmol) in DCM 100 ml were added activated 4 Å molecular sieves (5g), benzyl bromide (6.74 g, 29.1), and Ag_2O (4.14 g, 24.2 mmol). The flask was shielded from light, and the solution was stirred for 5 days. The solution was filtered through a pad of silica gel, and the filtrate concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (10 % EtOAc/Hexane) to yield compound **18** as a white viscous solid (3.74 g, 64 %).

R_f 0.62 (20 % EtOAc/Hexane).

IR (NaCl Plates) 2856, 1697 cm^{-1}.

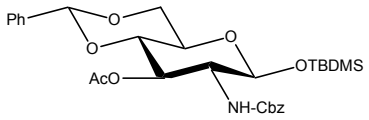
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.27 (m, 15H), 5.58 (s, 1H), 5.12 – 5.02 (m, 3H), 4.88 (d, *J* = 11.7 Hz, 2H), 4.67 (d, *J* = 11.7 Hz, 1H), 4.31 (dd, *J* = 10.8, 4.8 Hz, 1H), 4.10 (bs, 1H), 3.81 (dd, *J* = 10.2, 10.2 Hz, 1H), 3.73 (dd, *J* = 9.3 Hz, 1H), 3.53 – 3.45 (m, 1H), 3.23 (bs, 1H), 0.89 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 156.6, 138.3, 137.4, 136.4, 128.5, 128.3, 128.2, 128.1, 126.1, 101.2, 96.0, 82.6, 77.2, 74.3, 68.8, 66.7, 66.0, 60.2, 25.5, 17.9, -4.4, -5.4.

ESI-MS *m/z* $(M + H)^+$ calcd 516.2418, obsd 516.2452.

$C_{34}H_{43}NO_7Si$ (605.2809) calcd C 67.19, H 7.46, N 2.30; found C 67.39, H 7.55, N 2.33.

tert-Butyldimethylsilyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-benzyloxy-carbonylamino-2-deoxy-β-D-glucopyranoside (19)



Compound **17** (9 g, 17.5 mmol) was dissolved in dry DCM (120 ml) and pyridine (6.9 g, 87 mmol), DMAP (0.21 g, 1.73 mmol) and Ac_2O (8.9 g, 87 mmol) were added. The reaction mixture was stirred overnight and then water (100 ml) was added and stirred for 1 h. Then the organic layer was extracted with water (2 x 100 ml), 1N HCl (2 x 100 ml), and saturated $NaHCO_3$ (2 x 100 ml). The organic phase was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude compound was purified by silica gel column chromatography using 20 % EtOAc/Hexane to yield the compound **19** as a white solid (9.44 g, 97 %).

R_f 0.42 (20 % EtOAc/Hexane), m.p. 62.0 – 63.0, $[\alpha]^{24}_D$ -42.45 (*c* = 0.52, $CHCl_3$).

IR (NaCl Plates) 3334, 2957, 1730 $cm^{-1.}$

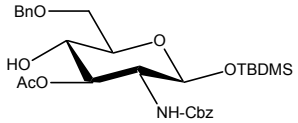
^1H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.45 (m, 2H), 7.32 – 7.35 (m, 8H), 5.48 (s, 1H), 5.24 (d, *J* = 9.9 Hz, 1H), 5.07 (AB Quartet, *J* = 12.0 Hz, 2H), 4.96 (d, *J* = 9.0 Hz, 1H), 4.73 (d, *J* = 7.8 Hz, 1H), 4.25 (dd, *J*= 5.2 Hz, 10.2 Hz, 1H), 3.78 (dd, *J* = 10.2, 10.2 Hz, 1H), 3.65 – 3.71 (m, 2H), 3.43 – 3.51 (m, 1H), 1.98 (s, 3H), 0.86 (s, 9H), 0.09 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 156.2, 137.5, 136.8, 129.5, 128.9, 128.6, 126.5, 101.8, 97.8, 79.3, 72.0, 69.1, 67.2, 66.9, 59.1, 25.9, 21.6, 18.3, -3.9, -4.9.

ESI-MS *m/z* $(M + Na)^+$ calcd 580.2680, obsd 580.2332.

$C_{29}H_{39}NO_8Si$ (557.2445) calcd C 62.45, H 7.05, N 2.51; found C 62.43, H 7.39, N 2.52.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (20)



Compound **19** (6.56 g, 11.77 mmol) and Et_3SiH (6.84 g, 58.86 mmol) were dissolved in dry DCM (100 ml) under N_2 atmosphere. Then TFA (6.71 g, 58.86 mmol) was added in drops at 0 °C and stirred continuously at the same temperature. After 6 h saturated $NaHCO_3$ (100 ml) was added to quench the reaction. Then organic layer was separated and aqueous phase was extracted with DCM (2 x 50 ml), combined organic extracts were dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure to yield the crude product. The crude compound was purified by silica gel column chromatography using 30 % EtOAc/Hexane to yield the compound **20** as a white solid (3.08 g, 89 %).

R_f 0.57 (50 % EtOAc/Hexane), m.p. 107.0 – 108.5, $[\alpha]^{24}_D$ -24.39 (*c* = 1.03, $CHCl_3$).

IR (NaCl Plates) 3285, 2956, 1747, 1694 cm^{-1}.

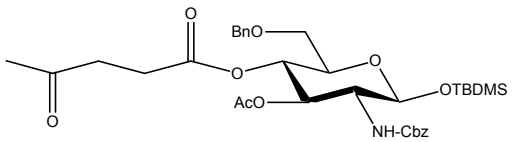
^1H NMR (300 MHz, $CDCl_3$) δ 7.28 – 7.36 (m, 10H), 5.06 (s, 3H), 4.97 (dd, *J* = 9.8, 10.8 Hz, 2H), 4.67 (d, *J* = 7.8 Hz, 1H), 4.57 (AB Quartet, *J* = 12.3 Hz, 2H), 3.75 (d, *J* = 4.8 Hz, 2H), 3.70 (dd, *J* = 9.0, 9.6 Hz, 1H), 3.58 – 3.65 (m, 1H), 3.45 – 3.55 (m, 2H), 2.01 (s, 3H), 0.89 (s, 3H), 0.1 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 155.9, 137.6, 136.4, 128.4, 128.0, 127.8, 127.6, 96.6, 75.2, 74.0, 73.7, 71.1, 70.5, 66.7, 57.7, 25.5, 20.8, 17.9, -4.2, -5.4.

ESI-MS *m/z* $(M + H)^+$ calcd 560.2680, obsd 560.2690.

$C_{29}H_{41}NO_8Si$ (559.2601) calcd C 62.23, H 7.38, N 2.50; found C 62.14, H 7.61, N 2.50.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-4-*O*-levulinyl-β-D-glucopyranoside (21)



To a stirred solution of **20** (3.54 g, 6.08 mmol) in dry DCM (60 ml) at 0 °C was added Levulinic acid (0.74 g, 6.38 mmol) and DMAP (0.82 g, 6.69 mmol). After 10 min, DIPC (0.77 g, 6.08 mmol) was added, and the solution was left to slowly warm to RT. After 6 h, the solvent mixture was concentrated under reduced pressure, and then the residue was purified by silica gel chromatography using 30 % EtOAc/Hexane to yield the compound **21** as a white solid (3.92 g, 98 %)

R_f 0.35 (30 % EtOAc/Hexane), m.p. 94.0 – 95.0, $[\alpha]^{24}_D$ +8.15 (*c* = 0.53, $CHCl_3$).

IR (NaCl Plates) 3283, 2930, 1744, 1718, 1693 cm^{-1}.

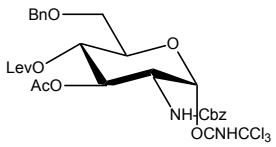
^1H NMR (300 MHz, $CDCl_3$) δ 7.26 – 7.32 (m, 10H), 5.19 (d, *J* = 9.3 Hz, 1H), 5.07 (s, 2H), 5.02 (d, *J* = 9.3 Hz, 1H), 4.70 – 4.85 (m, 2H), 4.53 (s, 2H), 3.58 – 3.68 (m, 3H), 3.56 (d, *J* = 1.8 Hz, 1H), 2.53 – 2.71 (m, 2H), 2.32 – 2.49 (m, 2H), 2.13 (s, 3H), 1.99 (s, 3H), 0.86 (s, 9H), 0.12 (s, 3H), 0.07 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 206.3, 171.8, 171.5, 156.1, 138.5, 136.8, 128.9, 128.7, 128.5, 128.0, 96.8, 73.9, 73.8, 72.5, 70.2, 69.8, 67.2, 58.5, 38.3, 30.1, 28.2, 25.9, 21.1, 18.3, -3.8, -4.9.

ESI-MS *m/z* $(M + H)^+$ calcd 658.3048, obsd 658.3013.

$C_{34}H_{47}NO_{10}Si$ (657.2969) calcd C 62.08, H 7.20, N 2.13; found C 61.97, H 7.32, N 2.08.

3-*O*-Acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-4-*O*-levulinyl-α-D-glucopyranosyl trichloroacetimidate (22)



Following the general procedure **D** compound **21** (3.92 g, 5.96 mmol) was desilylated at 0 °C for 3 h and then transformed into trichloroacetimidate donor. Purification by silica gel column chromatography (30 % EtOAc/Hexane) afforded the compound **22** as a yellow liquid (3.445 g, 84 %).

R_f 0.32 (30 % EtOAc/Hexane), $[\alpha]^{24}_D$ +64.70 (*c* = 0.62, $CHCl_3$).

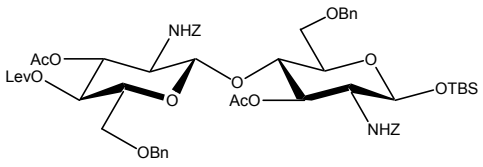
IR (Neat) 3340, 2928, 1751 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.26 – 7.31 (m, 10H), 6.41 (d, *J* = 3.3 Hz, 1H), 5.27 – 5.36 (m, 2H), 5.08 (AB Quartet, *J* = 12.3 Hz, 2H), 4.95 (d, *J* = 9.3 Hz, 1H), 4.49 (AB Quartet, *J* = 12.0 Hz, 2H), 4.25 (ddd, *J* = 3.0, 6.0, 9.3 Hz, 3.6 Hz, 1H), 4.07 – 4.11 (m, 1H), 3.61 (dd, *J* = 3.0 Hz, 11.4 Hz, 1H), 3.55 (dd, *J* = 3.6 Hz, 11.4 Hz, 1H), 2.56 – 2.75 (m, 2H), 2.28 – 2.49 (m, 2H), 2.13 (s, 3H), 1.98 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 171.1, 160.4, 155.6, 137.7, 136.1, 128.5, 128.3, 128.2, 128.1, 127.9, 127.6, 95.1, 90.8, 73.4, 71.6, 70.5, 68.2, 68.1, 67.1, 53.7, 37.6, 29.6, 27.7, 20.6.

ESI-MS *m/z* $(M + Na)^+$ calcd 709.1098, obsd 709.1069.
$C_{30}H_{33}Cl_3N_2O_{10}$ (686.1201) calcd C 52.38, H 4.83, N 4.07; found C 52.26, H 4.80, N 3.97.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-4-*O*-levulinyl-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonyl-amino-2-deoxy-β-D-glucopyranoside (23)



Coupling of *N*-Cbz acceptor **20** (0.543 g, 0.97 mmol) and *N*-Cbz protected trichloroacetimidate donor **22** (0.870 g, 1.26 mmol) following general procedure **A** for 1 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.041 g, 0.29 mmol) afforded the disaccharide **23** as a white solid (0.904 g, 86 %) after purification over silica gel chromatography using 30 – 50 % EtOAc/Hexane.

R_f 0.41 (40 % EtOAc/Hexane), m.p. 69.0 – 70.0, $[\alpha]^{24}_D$ +2.78 (*c* = 0.53, $CHCl_3$).

IR (NaCl Plates) 3332, 2955, 1748 cm^{-1}.

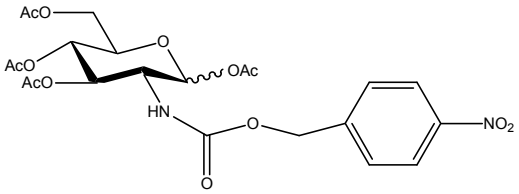
^1H NMR (300 MHz, $CDCl_3$) δ 7.26 – 7.38 (m, 20H), 5.02 – 5.08 (m, 3H), 5.00 (d, *J* = 9.5 Hz, 2H), 4.93 – 4.96 (m, 2H), 4.79 (d, *J* = 9.5 Hz, 1H), 4.70 (d, *J* = 12.0 Hz, 1H), 4.53 (bs, 1H), 4.42 (AB Quartet, *J* = 12.0 Hz, 2H), 4.35 (d, *J* = 12.0 Hz, 2H), 4.23 (bs, 1H), 3.86 (dd, *J* = 5.1, 6.6 Hz, 1H), 3.63 (ddd, *J* = 9.0, 9.0, 10.0 Hz, 1H), 3.56 (dd, *J* = 10.5, 3.0 Hz, 1H), 3.49 (dd, *J* = 10.5, 4.0 Hz, 1H), 3.56 – 3.45 (m, 3H), 3.26 (d, *J* = 11.0 Hz, 1H), 3.18 (d, *J* = 8.5 Hz, 1H), 2.58 – 2.69 (m, 2H), 2.29 – 2.42 (m, 2H), 2.13 (s, 3H), 1.93 (s, 3H), 1.87 (s, 3H), 0.86 (s, 9H), 0.10 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 206.5, 171.6, 171.4, 171.0, 156.2, 155.8, 138.8, 137.2, 129.0, 128.8, 128.7, 128.5, 128.4, 128.0, 101.2, 97.1, 75.6, 75.5, 74.8, 73.9, 73.7, 73.6, 73.2, 72.7, 69.5, 69.1, 67.9, 67.0, 58.3, 56.7, 38.0, 29.8, 28.2, 25.7, 20.9, 20.8, 18.2, -4.1, -5.2.

ESI-MS *m/z* $(M + H)^+$ calcd 1085.4679, obsd 1085.4626.

$C_{57}H_{72}N_2O_{17}Si$ (1084.4600) calcd C 63.08, H 6.69, N 2.58; found C 62.72, H 6.60, N 2.64.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-α/β-D-glucopyranoside (25)



To a solution of D-glucosamine hydrochloride **8** (5.5g, 25.5 mmol) in H_2O (50 ml) were added $NaHCO_3$ (4.29 g, 51.0 mmol) and *p*-nitrobenzyloxycarbonyl chloride (5.5 g, 25.5 mmol). After being stirred at RT for 48 h, the product (**24**) was filtered and washed with ice cold water and dried under vacuum. Then the solid was redispersed in pyridine (40.42 g, 510 mmol) with vigorous stirring and then cooled to 0 °C. Ac_2O (39.04 g, 383 mmol) was added in drops using the dropping funnel and stirred continuously at RT for overnight. Then cold EtOH (15 ml) was slowly added to quench the excess Ac_2O, which was then reduced by rotary evaporation. The residue was redissolved in toluene (2 x 20 ml) and concentrated for

the azeotropic removal of pyridine. The remaining slurry was redissolved in EtOAc (200 ml) and washed with distilled H_2O (2 x 200 ml) and brine (200 ml), then dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to yield the crude compound. The product was purified by silica gel chromatography using 30 – 40 % EtOAc/Hexane solvent gradient to yield the compound **25** as a white solid (α/β - 4/1 , 11.47 g, 85 %).

R_f 0.32 (50 % EtOAc/Hexane), m.p. 69.0 – 70.0.

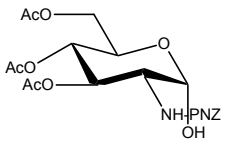
IR (NaCl Plates) 3336, 2958, 1752, 1525 cm^{-1}.

^1H and ^{13}C NMR is in accordance with the literature data[238].

ESI-MS *m/z* $(M + Na)^+$ calcd 549.1333, obsd 549.1305.

$C_{22}H_{26}N_2O_{13}$ (526.1435) calcd C 50.19, H 4.98, N 5.32; found C 50.14, H 4.83, N 5.23.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranoside (26)



A solution of **25** (7.75 g, 14.73 mmol) and hydrazine acetate (1.56 g, 16.94 mmol) in dry DMF (40 ml) was stirred at room temperature. After 1 h reaction mixture was diluted with DCM (150 ml) and washed with ice cold saturated $NaHCO_3$ (4 x 150 ml). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated in rotary evaporator to yield the crude compound. The crude was purified by silica gel column chromatography using 40 – 50 % EtOAc/Hexane solvent gradient to yield the product **26** as a white solid (6.56 g, 92 %).

R_f 0.27 (50 % EtOAc/Hexane); m.p. 62.0 – 63.0; $[\alpha]^{24}_D$ +39.69 (*c* = 0.5, $CHCl_3$).

IR (NaCl Plates) 3346, 1738, 1519 cm^{-1}.

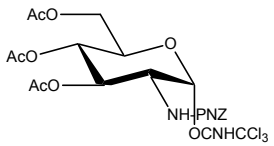
^1H NMR is in accordance with literature data[238].

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 171.3, 169.8, 155.7, 148.0, 144.0, 128.0, 124.0, 92.2, 71.3, 68.7, 68.0, 65.8, 62.5, 54.5, 21.2, 21.1, 21.0

ESI-MS *m/z* $(M + Na)^+$ calcd 507.1227, obsd 507.1199.

$C_{20}H_{24}N_2O_{12}$ (484.1329) calcd C 49.59, H 4.99, N 5.78; found C 49.19, H 4.97, N 5.60.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-α-D-glucopyranosyl trichloroacetamidate (27)



A mixture of **26** (4.5 g, 9.29 mmol), CCl_3CN (13.4 g, 92.9 mmol) and DBU (0.28 g, 1.86 mmol) in dry DCM (100 ml) was stirred at RT. After 14 hours the reaction mixture was concentrated in vacuum, and the residual syrup was purified by silica gel column chromatography using 40 % EtOAc/Hexane to yield **27** as a yellow solid (5.02 g, 86 %).

R_f 0.49 (50 % EtOAc/Hexane), m.p. 61.5 – 62.5, $[\alpha]^{24}_D$ +62.17 (*c* = 0.52, $CHCl_3$).

IR (NaCl Plates) 3313, 1738, 1676, 1519 cm^{-1}.

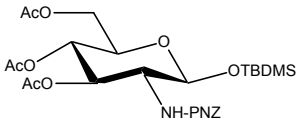
^1H NMR is in accordance with literature data[144].

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 171.0, 169.8, 160.7, 155.5, 148.1, 143.7, 128.5, 128.1, 95.1, 91.1, 71.0, 70.6, 67.8, 66.0, 61.8, 54.1, 21.0, 20.9.

ESI-MS *m/z* $(M + Na)^+$ calcd 650.0323, obsd 650.0286.

$C_{22}H_{24}Cl_3N_3O_{12}$ (627.0426) calcd C 42.02, H 3.85, N 6.68; found C 41.86, H 3.90, N 6.67.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranoside (28)



A solution of **25** (17 g, 32.29 mmol) and hydrazine acetate (3.416 g, 37.14 mmol) in dry DMF (80 ml) was stirred at room temperature. After 1 h reaction mixture was diluted with DCM (200 ml) and washed with ice cold sat. aq. $NaHCO_3$ (4 x 200 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to yield compound **26**. Compound **26** was redissolved in dry DCM (200 ml) without purification and then imidazole (4.397 g, 64.58 mmol) and *tert*-butylchlorodimethylsilane (5.84 g, 38.75 mmol) was added. After stirring at r.t for overnight the reaction mixture was diluted with water (150 ml), then aqueous layer was extracted with DCM (2 x 100 ml). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, concentrated under reduced pressure to yield the

crude product. The crude product was purified by silica gel column chromatography using 25 % EtOAc/Hexane to yield **28** as a white solid (17.087 g, 88 %).

R_f = 0.60 (50 % EtOAc/Hexane), m.p. 127.5 – 128.5, $[\alpha]^{24}_D$ +4.35 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 3360, 2956, 1751, 1527 cm^{-1}.

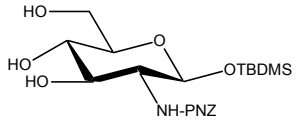
^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.7 Hz, 2H), 7.48 (d, *J* = 8.4 Hz, 2H), 5.11 – 5.28 (m, 3H), 4.96 – 5.05 (m, 2H), 4.82 (d, *J* = 6.6 Hz, 1H), 4.20 (dd, *J* = 6.0 Hz, 12.0 Hz, 1H), 4.12 (dd, *J* = 2.7 Hz, 12.0 Hz, 1H), 3.70 (ddd, *J* = 2.4, 6.0, 9.9 Hz, 1H), 3.57 (d, *J* = 8.4 Hz, 1H), 2.07 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 0.85 (s, 9H), 0.10 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 170.9, 169.8, 155.6, 148.1, 144.1, 128.5, 124.1, 96.5, 72.4, 72.2, 69.5, 65.7, 62.9, 58.7, 25.8, 21.1, 21.0, 18.3, -3.8, -4.9.

ESI-MS *m/z* $(M + Na)^+$ calcd 621.2092, obsd 621.2106.

$C_{26}H_{38}N_2O_{12}Si$ (598.2194) calcd C 52.16, H 6.40, N 4.68; found C 52.10, H 6.54, N 4.68.

tert-Butyldimethylsilyl 2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranoside (29)



Compound **28** (17.0 g, 28.39 mmol) was dissolved in dry MeOH (100 ml) and NaOMe (0.08 g, 1.42 mmol) was added and stirred at RT. After 3 h reaction mixture was neutralized with Amberlite IR120 (H^+) resin. The resin was filtered and solvent was removed under rotary evaporator and compound **29** (14.01 g, Quantitative) was dried in vacuum.

R_f 0.54 (100 % EtOAc), m.p. 60.0 – 61.5, $[\alpha]^{24}_D$ -24.21 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 3349, 2931, 1708, 1524 cm^{-1}.

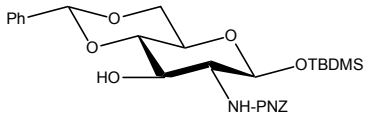
^1H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 6.06 (d, *J* = 8.7 Hz, 1H), 5.15 (d, *J* = 13.2 Hz, 1H), 5.03 (d, *J* = 13.5 Hz, 1H), 4.65 (d, *J* = 7.2 Hz, 1H), 4.30 (bs, 3H), 3.81 (dd, *J* = 10.8 Hz, 2H), 3.67 (s, 2H), 3.39 (d, *J* = 6.9 Hz, 1H), 3.3 (bs, 1H), 0.80 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 157.3, 147.9, 143.9, 128.5, 124.0, 96.9, 75.8, 74.4, 70.8, 65.9, 62.0, 60.2, 25.9, 18.3, -3.8, -4.9.

ESI-MS *m/z* $(M + Na)^+$ calcd 495.1775, obsd 495.1760.

$C_{20}H_{32}N_2O_9Si$ (472.1877) calcd C 50.83, H 6.38, N 5.93; found C 50.52, H 6.42, N 5.85.

tert-Butyldimethylsilyl 4,6,-*O*-benzylidene-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranoside (30)



Compound **29 (**7.8 g, 16.51 mmol) was dissolved in CH_3CN (150 ml) and benzaldehyde dimethylacetal (2.89 g, 18.98 mmol) was added followed by the addition of Camphorsulphonic acid (0.46 g, 1.98 mmol). After stirring overnight at r.t, Et_3N was added and the solvents evaporated. Then the crude compound was purified by silica gel column chromatography using 20 - 30 % EtOAc/Hexane to yield the compound **30** as a white solid (8.49 g, 92 %).

R_f 0.46 (40 % EtOAc/Hexane), m.p. 148.5 – 149.5, $[\alpha]^{24}_D$ -30.73 (*c* = 0.52, $CHCl_3$).

IR (NaCl Plates) 3321, 2954, 1710, 1524 cm^{-1}.

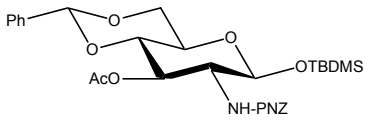
^1H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.7 Hz, 2H), 7.41 – 7.49 (m, 4H), 7.35 (m, 3H), 5.51 (s, 3H), 5.10 – 5.23 (m, 3H), 4.80 (bs, 1H), 4.28 (dd, *J* = 4.8 Hz, 10.5 Hz, 1H), 4.10 (bs, 1H), 3.76 (dd, *J* = 10.2, 10.2 Hz, 1H), 3.54 (dd, *J* = 9.0, 9.0 Hz, 1H), 3.39 – 3.47 (m, 2H), 3.23 – 3.28 (m, 1H), 0.86 (s, 9H), 0.10 (s, 3H), 0.07 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 156.3, 147.6, 143.6, 137.0, 129.7, 129.3, 129.0, 128.3, 128.2, 126.3, 123.7, 101.9, 96.3, 81.5, 70.9, 68.6, 66.3, 65.5, 60.0, 26.9, 25.5, 17.8, -3.7, -4.9.

ESI-MS *m/z* $(M + Na)^+$ calcd 583.2088, obsd 583.2122.

$C_{27}H_{36}N_2O_9Si$ (560.2190) calcd C 57.84, H 6.47, N 5.00; found C 57.92, H 6.28, N 4.92.

tert-Butyldimethylsilyl 3-*O*-acetyl-4,6,-*O*-benzylidene-2-deoxy-2-*p*-nitrobenzyloxy-carbonylamino-β-D-glucopyranoside (31)



Compound **30** (8.0 g, 14.27 mmol) was dissolved in dry DCM (100 ml) and pyridine (4.52 g, 57.08 mmol), DMAP (0.17 g, 1.43 mmol) and Ac_2O (4.37 g, 42.80 mmol) were added. The reaction mixture was stirred overnight and then water (100 ml) was added and stirred for 1 h. The organic layer was extracted with water (2 x 100 ml), 1N HCl (2 x 100 ml), and saturated $NaHCO_3$ (2 x 100 ml). The organic phase was dried over Na_2SO_4, filtered and the solvent was removed using rotary evaporator. The crude compound was purified by silica gel column

chromatography using 20 % EtOAc/Hexane to yield the compound **31** as a white solid (8.47 g, 98 %).

R_f 0.30 (20 % EtOAc/Hexane), m.p. 170.0 – 171.5, $[\alpha]^{24}_D$ -37.59 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 3300, 2935, 1730, 1700, 1526 cm^{-1}.

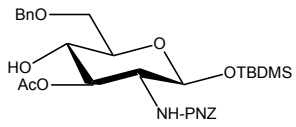
^1H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.4 Hz, 2H), 7.42 – 7.49 (m, 4H), 7.33 – 7.36 (m, 3H), 5.50 (s, 1H), 5.26 (dd, *J* = 9.9, 9.9 Hz, 1H), 5.20 (d, *J* = 9.6 Hz, 1H) 5.17 (s, 1H), 4.76 (d, *J* = 7.8 HZ, 1H), 4.24 (dd, *J* = 4.5 Hz, 8.7 Hz, 1H), 3.78 (dd, *J* = 10.2, 10.2 Hz, 1H), 3.70 (dd, *J* = 9.6, 9.6 Hz, 1H), 3.53 (d, *J* = 9.9 Hz, 1H), 3.47 (ddd, *J* = 5.1, 5.1, 9.9 Hz, 1H), 2.02 (s, 3H), 0.85 (s, 9H), 0.09 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 155.8, 148.0, 144.2, 137.3, 129.5, 128.6, 128.5, 126.6, 124.1, 101.8, 97.6, 79.2, 71.8, 69.0, 66.9, 65.6, 59.2, 25.8, 21.3, 18.2, -3.7, -5.0.

ESI-MS *m/z* $(M + Na)^+$ calcd 625.2193, obsd 625.2192.

$C_{29}H_{38}N_2O_{10}Si$ (602.2296) calcd C 57.79, H 6.35, N 4.65; found C 57.67, H 6.30, N 4.58.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranoside (32)



Compound **31** (8.82 g, 14.60 mmol) and Et_3SiH (10.21 g, 87.8 mmol) were dissolved in dry DCM (120 ml) under N_2 atmosphere. Then TFA (10.01 g, 87.8 mmol) was added in drops at 0 °C and stirred continuously at the same temperature. After 6 h saturated $NaHCO_3$ (120 ml) was added to quench the reaction. Then organic layer was separated and aqueous phase was extracted with DCM (2 x 100 ml), combined organic extracts were dried over anhydrous Na_2SO_4, filtered, concentrated to yield the crude product. The crude compound was purified by silica gel column chromatography using 30 - 40 % EtOAc/Hexane to yield the compound **32** as a white viscous solid (7.81 g, 88 %).

R_f 0.41 (50 % EtOAc/Hexane), m.p. 45.5 – 46.5, $[\alpha]^{24}_D$ -26.17 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 3334, 2932, 1723, 1524 cm^{-1}.

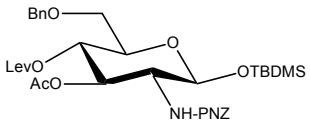
^1H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.7 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 7.28 – 7.35 (m, 5H), 5.16 (m, 3H), 5.03 (dd, *J* = 9.9, 9.9 Hz, 1H), 4.69 (d, *J* = 6.6 Hz, 1H), 4.57 (AB Quartet, *J* = 12.3 Hz, 2H), 3.76 (dd, *J* = 1.5, 5.7 Hz, 2H), 3.71 (d, *J* = 9.3 Hz, 1H), 3.49 – 3.64 (m, 2H), 3.11 (bs, 1H), 2.05 (s, 3H), 0.84 (s, 9H), 0.10 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 155.4, 147.5, 143.9, 137.5, 128.4, 128.0, 127.8, 127.6, 123.6, 96.5, 75.1, 73.9, 73.7, 70.9, 70.5, 65.1, 57.8, 25.4, 20.9, 17.8, -4.2, -5.4.

ESI-MS *m/z* $(M + Na)^+$ calcd 627.2350, obsd 627.2319.

$C_{29}H_{40}N_2O_{10}Si$ (604.2452) calcd C 57.60, H 6.67, N 4.63; found C 57.42, H 6.77, N 4.70.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-levulinyl-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranoside (33)



To a stirred solution of **32** (5.32 g, 8.80 mmol) in dry DCM (80 ml) at 0 °C was added levulinic acid (1.07 g, 9.24 mmol) and DMAP (1.18 g, 9.68 mmol). After 10 min, DIPC (1.11 g, 8.80 mmol) was added, and the solution was left slowly to attain the RT. After 6 h, the reaction mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography using 30 – 40 % EtOAc/Hexane to yield the compound **33** as a white solid (6.05 g, 98 %).

R_f 0.32 (40 % EtOAc/Hexane), m.p. 102.0 – 103.0, $[\alpha]^{24}_D$ +5.85 (*c* = 0.5, $CHCl_3$).

IR (NaCl Plates) 3281, 2932, 1746, 1523 cm^{-1}.

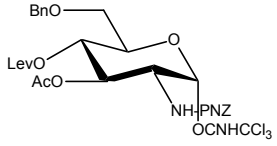
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.7 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 7.27 – 7.33 (m, 5H), 5.12 – 5.23 (m, 3H), 5.06 (dd, *J* = 9.3, 9.3 Hz, 2H), 4.79 (d, *J* = 6.9 Hz, 1H), 4.52 (s, 2H), 3.70 (m, 2H), 3.64 – 3.70 (m, 2H), 3.56 – 3.61 (m, 2H), 2.53 – 2.74 (m, 2H), 2.29 – 2.49 (m, 2H), 2.13 (s, 3H), 2.04 (s, 3H), 0.85 (s, 3H), 0.12 (s, 3H), 0.07 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 206.4, 171.8, 155.7, 148.0, 144.3, 138.4, 128.7, 128.5, 128.0, 127.9, 124.1, 96.7, 73.9, 73.8, 72.5, 69.8, 65.6, 58.7, 38.0, 30.0, 28.2, 25.9, 21.1, 18.2, -3.7, -5.0.

ESI-MS *m/z* $(M + Na)^+$ calcd 725.2718, obsd 725.2720.

$C_{34}H_{46}N_2O_{12}Si$ (702.2820) calcd C 58.10, H 6.60, N 3.99; found C 57.78, H 6.55, N 4.03.

3-*O*-Acetyl-6-*O*-benzyl-2-deoxy-4-*O*-levulinyl-2-*p*-nitrobenzyloxycarbonylamino-α-D-glucopyranosyl trichloroacetimidate (34)



Following the general procedure **D** compound **33** (5.49 g, 7.81 mmol) was desilylated at 0 °C for 3 h and then transformed into trichloroacetimidate donor. Purification by silica gel column chromatography (40 % EtOAc/Hexane) afforded the compound **34** as a yellow viscous solid (4.37 g, 76 %).

R_f 0.35 (50 % EtOAc/Hexane), m.p. 42.0 – 43.0, $[\alpha]^{24}_D$ +73.29 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 3314, 2949, 1751, 1676, 1522 cm^{-1}.

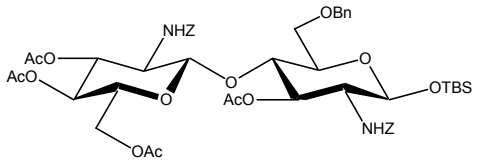
^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.7 Hz, 2H), 7.60 (d, *J* = 8.7 Hz, 2H), 7.27 – 7.33 (m, 5H), 6.43 (d, *J* = 3.6 Hz, 1H), 5.29 – 5.39 (m, 2H), 5.18 (s, 2H), 5.18 (d, *J* = 9.0 Hz, 1H), 4.51 (AB Quartet, *J* = 12.0 Hz, 2H), 4.20 (ddd, *J* = 3.6, 9.6, 9.6 Hz, 1H), 4.07 – 4.16 (m, 2H), 3.65 (d, *J* = 3.9 Hz, 11.1 Hz, 1H), 3.62 (dd, *J* = 3.0 Hz, 11.1 Hz, 1H), 2.58 – 2.79 (m, 2H), 2.28 – 2.52 (m, 2H), 2.15 (s, 3H), 2.05 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 171.1, 160.4, 155.1, 147.7, 143.4, 137.7, 128.3, 128.1, 127.9, 127.6, 123.7, 94.9, 90.8, 73.5, 71.5, 70.6, 68.0, 67.9, 65.5, 53.8, 37.5, 29.6, 27.7, 20.7.

ESI-MS *m/z* $(M + Na)^+$ calcd 754.0949, obsd 754.0978.

$C_{30}H_{32}Cl_3N_3O_{12}$ (731.1052) calcd 49.16, H 4.40, N 5.73; found C 48.85, H 4.57, N 5.68.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (35)



Coupling of *N*-Cbz acceptor **20** (0.934 g, 1.67 mmol) and *N*-Cbz protected trichloroacetimidate donor **12** (1.264 g, 2.17 mmol) following general procedure **A** for 1 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.057 g, 0.40 mmol) afforded the disaccharide **35** as a white solid (1.422g, 87 %) after purification over silica gel column chromatography using 30 – 40 % EtOAc/Hexane.

R_f 0.30 (40 % EtOAc/Hexane), m.p. 96.0 – 98.0, $[\alpha]^{24}_D$ -22.55 (*c* = 0.50, $CHCl_3$).

IR (NaCl Plates) 3332, 2955, 1748 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.29 (m, 15 H), 5.05 – 5.14 (m, 3H), 4.90 (dd, *J* = 9.5 Hz, 1H), 4.84 (s, 1H), 4.72 (d, *J* = 11.5 Hz, 1H), 4.55 4.58 (m, 1H), 4.20 – 4.36 (m, 2H), 3.96 d, *J*= 11.0 Hz, 1H), 3.85 (bs, 1H), 3.61 (ddd, *J* = 9.5, 9.0, 9.0 Hz, 1H), 3.45 (m, 3H), 3.29 (d, *J*

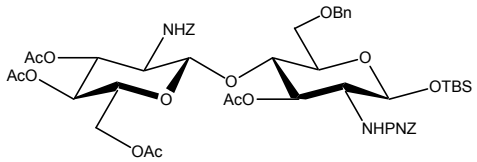
= 7.0 Hz, 1H), 3.19 - 3.25 (m, 1H), 2.05 (s, 3H), 2.01 (s, 3H), 1.94 (s, 3H), 1.91 (s, 3H), 0.87 (s, 9H), 0.12 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.4, 170.3, 169.5, 155.8, 155.3, 136.4, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0 100., 96.7, 75.1, 74.2, 74.0, 73.4, 72.1, 71.3, 68.4, 67.0, 66.7, 65.9, 61.8, 57.8, 55.9, 25.5, 20.7, 20.6, 20.5, 17.9, -4.2, -5.4.

ESI-MS *m/z* $(M + H)^+$ calcd 981.4053, obsd 981.4008.

$C_{49}H_{64}N_2O_{17}Si$ (980.3974) calcd C 59.98, H 6.57, N 2.86; found C 59.78, H 6.57, N 2.85.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranoside (36)



Coupling of *N*-PNZ acceptor **32** (0.576 g, 0.95 mmol) and *N*-Cbz protected trichloroacetimidate donor **12** (0.721 g, 1.24 mmol) following general procedure **A** for 1 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.041 g, 0.29 mmol) afforded the disaccharide **36** as a white solid (0.91g, 91 %) after purification over silica gel column chromatography using 30 – 50 % EtOAc/Hexane.

R_f 0.22 (40 % EtOAc/Hexane), m.p. 73.5 – 74.5, $[\alpha]^{24}_D$ -19.14 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 3335, 2930, 1722 cm^{-1}.

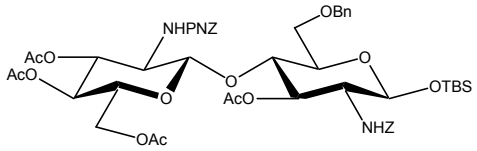
^{1}H NMR (300 MHz, CD_2Cl_2) δ 8.18 (d, *J* = 8.7 Hz, 2H), 7.49 (d, *J* = 9.0 Hz, 2H), 7.30 – 7.44 (m, 10H), 5.16 (s, 2H), 4.88 – 5.09 (m, 6H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 10.2 Hz, 1H), 4.30 – 4.39 (m, 3H), 3.97 (dd, *J* = 12.3Hz, 2.1 Hz, 1H), 3.88 (dd, *J* = 9.9, 9.3 Hz, 1H), 3.75 (d, *J* = 4.2 Hz, 1H), 3.46 – 3.54 (m, 4H), 3.35 (d, *J* = 10.5 Hz, 1H), 3.22 (d, *J* = 8.1 Hz, 1H), 2.04 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.91 (s, 3H), 0.85 (s, 9H), 0.11 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.3, 170.8, 170.7, 169.8, 155.9, 155.8, 148.0, 144.6, 137.1, 129.1, 128.8, 128.7, 128.5, 128.0, 124.0, 101.1, 96.9, 75.4, 74.7, 73.8, 72.6, 72.5, 71.9, 68.7, 67.1, 67.0, 65.5, 62.2, 58.5, 56.5, 25.7, 20.9, 20.8, 20.7, 18.1, -4.1, -5.3.

ESI-MS *m/z* $(M + H)^+$ calcd 1026.3903, obsd 1026.3864.

$C_{49}H_{63}N_3O_{19}Si$ (1025.3825) calcd C 57.35, H 6.19, N 4.10; found C 56.90, H 6.09, N 4.10.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (37)



Coupling of *N*-Cbz acceptor **20** (1.0 g, 1.79 mmol) and *N*-PNZ protected trichloroacetimidate donor **27** (1.39 g, 2.21 mmol) following general procedure **A** for 1 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.058 g, 0.41 mmol) afforded the disaccharide **37** as a white solid (1.64g, 91 %) after purification over silica gel column chromatography using 30 – 40 % EtOAc/Hexane.

R_f 0.22 (40 % EtOAc/Hexane), m.p. 85.5 – 86.5, $[\alpha]^{24}_D$ -23.30 (*c* = 0.50, $CHCl_3$).

IR (NaCl Plates) 3332, 2856, 1724, 1522 cm^{-1}.

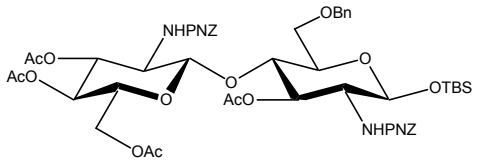
^1H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.7 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H),7.33 – 7.39 (m, 10H), 5.10 – 5.21 (m, 2H), 4.99 (m, 4H), 4.91 (dd, *J* = 9.3, 9.3 Hz, 2H), 4.73 (d, *J* = 12.3 Hz, 1H), 4.60 (m, 1H), 4.37 (d, *J* = 4.2 Hz, 1H), 4.34 (d, *J* = 3.3 Hz, 1H), 4.31 (d, *J* = 4.8 Hz, 1H), 3.97 (dd, *J* = 2.1 Hz, 10.2 Hz, 1H), 3.86 (dd, *J* =9.0, 9.0 Hz, 1H), 3.54 – 3.63 (m, 1H), 3.45 – 3.47 (m, 3H), 3.32 (d, *J* = 9.6 Hz, 1H), 3.21 (m, 1H), 2.05 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.91 (s, 3H), 0.85 (s, 9H), 0.11 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 170.5, 170.4, 169.5, 155.4, 155.3, 147.6, 143.9, 137.7, 136.5, 128.8, 128.5, 128.1, 123.7, 100.5, 96.6, 75.0, 74.3, 73.5, 72.3, 72.2, 71.5, 68.5, 67.2, 66.8, 65.2, 61.9, 58.1, 56.1, 25.5, 20.7, 20.6, 20.5, 17.9, -4.1, -5.3.

ESI-MS *m/z* $(M + H)^+$ calcd 1026.3903, obsd 1026.3894.

$C_{49}H_{63}N_3O_{19}Si$ (1025.3825) calcd C 57.35, H 6.19, N 4.10; found C 56.89, H 6.24, N 4.11.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-p-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranoside (38)



Coupling of *N*-PNZ acceptor **32** (0.551 g, 0.91 mmol) and *N*-PNZ protected trichloroacetimidate donor **27** (0.71 g, 1.13 mmol) following general procedure **A** for 1 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.041 g, 0.29 mmol) afforded the disaccharide **38** as a white solid (0.76g, 77 %) after purification over silica gel column chromatography using 30 – 40 % EtOAc/Hexane.

R_f 0.14 (40 % EtOAc/Hexane), m.p. 92.5 – 93.5, $[\alpha]^{24}_D$ -27.49 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 2857, 1742, 1520 cm^{-1}.

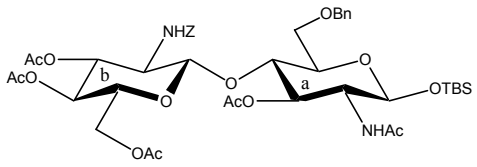
^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.7 Hz, 4H), 7.33 – 7.49 (m, 9H), 5.17 (s, 2H), 5.00 – 5.18 (m, 3H), 4.98 (d, *J* = 9.3 Hz, 1H), 4.92 (d, *J* = 9.6 Hz, 2H), 4.75 (d, *J* = 11.1 Hz, 1H), 4.40 – 4.72 (m, 3H), 4.35 (dd, *J* = 4.5 Hz, 12.3 Hz, 1H), 3.98 (dd, *J* = 1.8 Hz, 12.3 Hz, 1H), 3.89 (dd, *J* = 9.3, 9.3 Hz, 1H), 3.59 (d, *J* = 9.9 Hz, 2H), 3.48 (d, *J* = 9.3 Hz, 3H), 3.30 – 3.48 (m, 2H), 2.06 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.94 (s, 3H), 0.84 (s, 9H), 0.09 (s, 3H), 0.03 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 170.4, 170.3, 169.4, 155.3, 155.0, 147.7, 147.6, 143.8, 143.6, 137.6, 137.5, 128.8, 128.6, 128.5, 128.4, 128.1, 123.7, 100.1, 96.5, 77.2, 75.1, 74.4, 73.6, 72.2, 71.5, 68.3, 67.5, 65.3, 65.2, 61.8, 58.2, 56.3, 25.5, 20.6, 20.5, 17.8, -4.3, -5.5.

ESI-MS *m/z* $(M + Na)^+$ calcd 1093.3574, obsd 1093.3557.

$C_{49}H_{62}N_4O_{21}Si$ (1070.3676) calcd C 54.94, H 5.83, N 5.23; found C 54.89, H 5.96, N 5.23.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (41)



Compound **36** (0.103 g, 0.1 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.209 g, 1.2 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (60 – 80 % EtOAc/Hexane) to afford the compound **41** as a white solid (0.068 g, 76 % yield).

R_f 0.19 (60 % EtOAc/Hexane), m.p. 96.5 – 97.5, $[\alpha]^{24}_D$ -32.77 (*c* = 1.01, $CHCl_3$).

IR (NaCl Plates) 2856, 1746 cm^{-1}.

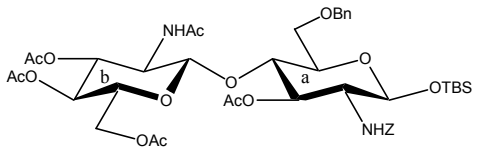
^1H NMR (300 MHz, CD_2Cl_2) δ 7.33 – 7.40 (m, 10H, 2Ph), 5.59 (d, $J_{NHa,2a}$ = 9.0 Hz, 1H, NH_a,), 4.89 – 5.09 (m, 5H, H-3a, H-3b, H-4a, Z-CH_2-Ph), 4.69 (d, J_{gem} = 12.0 Hz, 1H, C*H*H-Ph), 4.61 (d, $J_{1a,2a}$ = 9.0 Hz, 1H, H-1a), 4.50 (d, $J_{NHb,2b}$ = 7.8 Hz, 1H, NH-b), 4.45 (d, J_{gem} = 12.0 Hz, 2H, CH*H*Ph, H-1_b (HMQC)), 4.33 (dd, $J_{6bA,5b}$ = 4.5, $J_{6bA,6bB}$ = 12.6 Hz, 1H, H-6_bA), 3.97 (dd, $J_{6bB,5b}$ = 2.1, $J_{6bB,6bA}$ = 12.6 Hz, 1H, H-6_bB), 3.87 (dd, $J_{4b,3b}$ = 9.3, $J_{4b,5b}$ = 10.3 Hz, 1H, H-4_b), 3.81 (ddd, $J_{2a,1a}$ = 9.0, $J_{2a,3a}$ = 9.3, $J_{2a,NHa}$ = 9.3 Hz, 1H), 3.37 – 3.50 (m, 4H, H-2_a, H-5_a, H-6_aA, H-6_aA), 3.26 (d, $J_{5b,4b}$ = 7.5 Hz, 1H, H-5_b), 2.02 (s, 3H, CH_3-CO-O), 2.01 (s, 3H, CH_3-CO-O), 1.98 (s, 3H, CH_3-CO-O), 1.90 (s, 3H, CH_3-CO-O), 1.88 (s, 3H, CH_3-CO-NH), 0.89 (s, 9H, SiC(CH_3)$_3$), 0.13 (s, 3H, SiCH_3), 0.09 (s, 3H, SiCH_3).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2 (CH_3-*C*O-NH), 170.5 (*C*H_3-*C*O-O), 170.4 (*C*H_3-*C*O-O), 169.7 (*C*H_3-*C*O-O), 169.4 (*C*H_3-*C*O-O), 155.5 (Z-*C*O-NH), 137.8, 136.4 (*C*-Ph), 128.7, 128.4, 128.3,128.2 (*C*H-Ph), 100.7 (C-1_b), 96.4 (C-1_a), 75.1 (C-4_b), 74.4 (C-5_b), 73.4 (*C*H_2-Ph), 72.6 (C-4_a), 72.2 (C-5_a), 71.5 (C-3_b), 68.3 (Z-*C*H_2-Ph), 67.5 (C-6_a), 66.7 (C-3a), 61.8 (C-6_b), 56.1 (C-2_a), 55.7 (C-2_b), 25.5 (SiC(*C*H_3)$_3$-C), 23.2 (NH-CO-*C*H_3), 20.6, 20.5, 20.4 (*C*H_3-CO-O), 17.9 (Si*C*(CH_3)$_3$), -3.7 (Si-*C*H_3), -4.8 (Si-*C*H_3).

ESI-MS *m/z* $(M + H)^+$ calcd 889.3790, obsd 889.3760.

$C_{43}H_{60}N_2O_{16}Si$ (888.3712) calcd C 58.09, H 6.80, N 3.15; found C 57.78, H 6.66, N 3.09.

tert-Butyldimethylsilyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl -(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (39)



Compound **37** (0.103 g, 0.1 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.209 g, 1.2 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (60 – 70 % EtOAc/Hexane) to afford the compound **39** as a white solid (0.071 g, 80 % yield).

R_f 0.49 (8 % EtOH/$CHCl_3$), m.p. 88.5 – 89.5, $[\alpha]^{24}_D$ -20.18 (*c* = 1.03, $CHCl_3$).

IR (NaCl Plates) 2856, 1744 cm^{-1}.

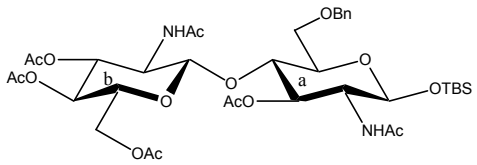
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 – 7.48 (m, 10H, 2Ph), 5.19 (d, $J_{NHa,1a}$ = 8.4 Hz, 1H, NH_a), 5.00 – 5.13 (m, 5H, NH_b, H-3_b, H-4_a, Z-CH_2-Ph), 4.94 (dd, $J_{3a,2a}$ = 9.6, $J_{3a,4a}$ = 10.5 Hz, 1H, H-3_a), 4.81 (d, J_{gem} = 12.0 Hz, 1H, *O*-CH*H*-Ph), 4.62 (d, $J_{1a,2a}$ = 7.8 Hz, 1H$_a$), 4.56 (d, $J_{1b,2b}$ = 8.1 Hz, 1H, H-1_b), 4.46 (d, J_{gem} = 12.3 Hz, 1H, *O*-C*H*H-Ph), 4.34 (dd, $J_{6Aa,5a}$ = 4.5 Hz, $J_{6Aa,6Ba}$ = 12.0 Hz, 1H, H-6A$_a$), 3.98 (dd, $J_{6Ba,5a}$ = 2.1, $J_{6Ba,6Aa}$ =12.0 Hz, 1H, H-6B$_a$), 3.87 (dd, $J_{4b,3b}$ = 9.0, $J_{4b,5b}$ = 11.1 Hz, 1H, H-4_b), 3.54 – 3.70 (m, 5H, H-6A$_b$, H-6B$_b$, H-2_a, H-2_b, H-5_a), 3.47 (ddd, $J_{5b,4b}$ = 9.6 , $J_{5b,6Ab}$ = 7.2, $J_{5b,6Bb}$ = 2.4 Hz, 1H, H-5_b), 2.05 (s, 3H, C*H*$_3$CO-O), 2.01 (s, 3H, C*H*$_3$CO-O), 1.99 (s, 3H, C*H*$_3$CO-O), 1.91 (s, 3H, C*H*$_3$CO-O), 1.73 (s, 3H, C*H*$_3$CO-NH), 0.87 (s, 9H, SiC(C*H*$_3$)$_3$), 0.11 (s, 3H, SiC*H*$_3$), 0.06 (s, 3H, SiC*H*$_3$).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2 (CH_3-*C*O-NH), 170.5 (CH_3-*C*O-O), 170.3 (CH_3-*C*O-O), 169.7 (CH_3-*C*O-O), 169.4 (CH_3-*C*O-O), 155.5 (Z-NH-*C*O-O), 137.7, 136.4 (Ph-*C*), 128.6, 128.4, 128.3, 128.2, 128.1 (Ph-*C*H), 100.6 (C-1_b), 96.4 (C-1_a), 75.2 (C-4_b), 74.3 (C-5_b), 73.4 (Ph-*C*H_2), 72.6 (C-5_a), 72.1 (C-3_a), 71.4 (C-3_b), 68.4 (C-4_a), 67.6 (C-6_a), 66.7 (Z-*C*H_2), 61.8 (C-6_b), 56.1 (C-2_a), 55.7 (C-2_b), 25.4 (SiC(*C*H_3)$_3$-C), 23.2 (NH-CO-*C*H_3), 20.6 (*C*H_3-CO-O), 20.5 (*C*H_3-CO-O), 20.4 (*C*H_3-CO-O), 17.8 (Si*C*(CH_3)$_3$), -4.2 (Si-*C*H_3), -5.3 (Si-*C*H_3).

ESI-MS *m/z* $(M + H)^+$ calcd 889.3790, obsd 889.3795.

$C_{43}H_{60}N_2O_{16}Si$ (888.3712) calcd C 58.09, H 6.80, N 3.15; found C 57.95, H 6.56, N 3.10.

tert-Butyldimethylsilyl 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-glucopyranoside (42)



Compound **38** (0.107 g, 0.1 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.418 g, 2.4 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (2 – 5 % EtOH/$CHCl_3$) to afford the compound **42** as a white solid (0.066 g, 83 % yield).

R_f 0.49 (8 % EtOH/$CHCl_3$), m.p. 256.0 – 258.0, $[\alpha]^{24}_D$ -42.67 (*c* = 0.50, $CHCl_3$).

IR (NaCl Plates) 2860, 1746 cm^{-1}.

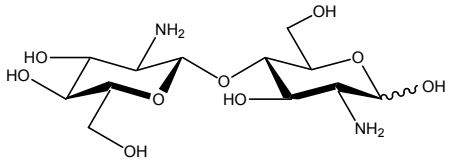
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.48 (m, 5H, 1Ph), 5.82 (d, $J_{NHa,1a}$ = 9.3 Hz, 1H, NH_a), 5.12 (d, $J_{NHb,1b}$ = 9.0 Hz, 1H, NH_b), 4.93 – 5.09 (m, 3H, H-3_a, H-3_b, H-4_a), 4.79 (d, J_{gem} = 12.0 Hz, 1H, C*H*HPh), 4.63 (d, $J_{1a,2a}$ = 7.5 Hz, 1H, H-1_a), 4.53 (d, $J_{1b,2b}$ = 8.7 Hz, 1H, H-1_b), 4.47 (d, J_{gem} = 12.0 Hz, 1H, CH*H*Ph), 4.34 (dd, $J_{6Ab,5b}$ = 4.5, $J_{6Ab,6Bb}$ = 12.3 Hz, 1H, H6A_b), 4.00 – 4.04 (m, 1H, H6B_b), 3.95 – 3.98 (m, 1H, H-2_a), 3.89 (dd, $J_{4b,3b}$ = 9.0, $J_{4b,5b}$ = 9.9 Hz, 1H, H-4_b), 3.73 (ddd, $J_{2b,1b}$ = 8.7, $J_{2b,3b}$ = 9.0, $J_{2b,NHb}$ = 9.9 Hz, 1H, H-2_b), 3.62 – 3.71 (m, 2H, H-6AB_a), 3.56 (ddd, $J_{5a,4a}$ = 2.4, $J_{5a,6Aa}$ = 5.1, $J_{5a,6Ba}$ = 9.6 Hz, 1H, H-5_a), 3.48 (ddd, $J_{5b,4b}$ = 2.7, $J_{5b,6Ab}$ = 6.9, $J_{5b,6Bb}$ = 9.0 Hz, 1H, H-5_b), 2.06 (s, 3H, C*H*$_3$CO-O), 2.02 (s, 3H, C*H*$_3$CO-O), 2.01 (s, 3H, C*H*$_3$CO-O), 1.98 (s, 3H, C*H*$_3$CO-O), 1.92 (s, 3H, C*H*$_3$CO-NH), 1.74 (s, 3H, C*H*$_3$CO-NH), 0.88 (s, 9H, SiC(C*H*$_3$)$_3$), 0.13 (s, 3H, SiC*H*$_3$), 0.09 (s, 3H, SiC*H*$_3$).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1 (CH_3-*C*O-NH), 170.7 (CH_3-*C*O-NH), 170.5 (CH_3-*C*O-O), 169.9 (CH_3-*C*O-O), 169.8 (CH_3-*C*O-O), 169.4 (CH_3-*C*O-O), 137.8 (*C*-Ph), 128.7, 128.5 (*C*H-Ph), 100.4 (C-1_b), 96.4 (C-1_a), 75.2 (C-4_b), 74.4 (C-5_b), 73.8 (*C*H_2-Ph), 72.7 (C-3_a), 72.5 (C-3_b), 71.6 (C-4_a), 68.3 (C-6_a), 68.1 (Z-*C*H_2-Ph), 61.9 (C-6_b), 55.6 (C-2_a), 54.7 (C-2_b), 25.5 (SiC(*C*H_3)$_3$-C), 23.2 (NH-CO-*C*H_3), 23.1 (NH-CO-*C*H_3), 20.7 (*C*H_3-CO-O), 20.6 (*C*H_3-CO-O), 20.5 (*C*H_3-CO-O), 17.9 (Si*C*(CH_3)$_3$), -4.2 (Si-*C*H_3), -5.3 (Si-*C*H_3).

ESI-MS *m/z* $(M + H)^+$ 797.3528, obsd 797.3505.

$C_{37}H_{56}N_2O_{15}Si$ (796.3450) calcd C 55.76, H 7.08, N 3.52; found C 55.63, H 6.97, N 3.43.

2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranose (1, DD)



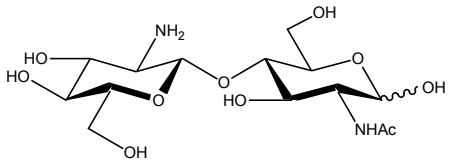
Following the general procedure **E**,1-*O*-TBSMS derivative **35** (0.060 g, 0.069 mmol) was deprotected to yield the free disaccharide **1** (0.019 g, 80 %).

^{1}H NMR (300 MHz, D_2O) δ 5.43 (d, *J* = 3.6 Hz, 0.6H, H-1$_a$α), 4.98 (d, *J* = 8.4 Hz, 0.4 H, H-1$_a$β), 4.94 (d, *J* = 8.4 Hz, 1H, H-1b), 3.65 – 4.06 (m, 12H), 3.45 – 3.51 (m, 3H), 3.33 (dd, *J* = 10.5, 3.6 Hz, 1H), 3.07 – 3.14 (m, 2H), 2.80 – 2.86 (m, 1H).

^{13}C NMR (75 MHz, D_2O) δ 98.2, 93.1, 89.3, 77.2, 76.9, 76.8, 75.0, 72.3, 70.8, 70.4, 70.0, 68.4, 60.8, 60.7, 60.5, 57.1, 56.4, 54.6.

ESI-MS *m/z* $(M + H)^+$ 341.1560, obsd 341.1563.

2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranose (2, DA)



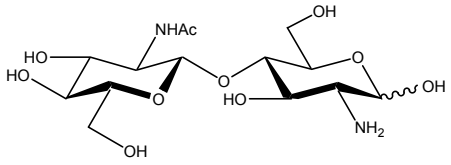
Following the general procedure **E,** 1-*O*-TBSMS derivative **41** (0.054 g, 0.061 mmol) was deprotected to yield the free disaccharide **2** (0.020 g, 85 %).

^{1}H NMR is in accordance with the literature data[64,173].

^{13}C NMR (75 MHz, D_2O) δ 175.2, 174.9, 98.7, 95.2, 90.8, 78.1, 77.9, 76.7, 74.9, 73.6, 71.2, 70.4, 70.0, 69.3, 61.0, 60.8, 60.7, 56.6, 54.5, 54.3, 22.6, 22.3.

ESI-MS *m/z* $(M + H)^+$ 383.1666, obsd 383.1665.

2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranose (3, AD)

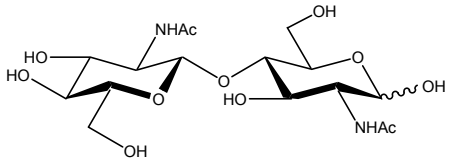


Following the general procedure **E** 1-*O*-TBSMS derivative **39** (0.050 g, 0.056 mmol) was deprotected to yield the free disaccharide **3** (0.018 g, 86 %).

ESI-MS *m/z* $(M + H)^+$ 383.1666, obsd 383.1671.

^{1}H and ^{13}C NMR is in accordance with the literature data[65,256].

2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranose (4, AA)



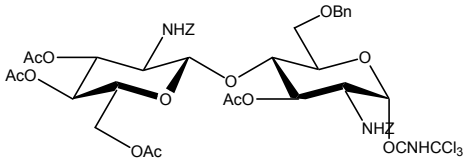
Following the general procedure **E** 1-*O*-TBSMS derivative **42** (0.046 g, 0.058 mmol) was deprotected to yield the free disaccharide **4** (0.018 g, 82 %).

^{1}H NMR is in accordance with the literature data[64,256].

^{13}C NMR (75 MHz, D_2O) δ 175.2, 175.0, 174.9, 101.9, 95.2, 90.9, 80.3, 79.8, 76.3, 74.9, 73.9, 73.0, 70.4, 70.1, 69.7, 60.9, 60.6, 60.4, 56.5, 56.0, 54.0, 22.5, 22.4, 22.3.

ESI-MS *m/z* $(M + H)^+$ 425.1771, obsd 425.1759.

3,4,6-Tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl trichloroacetimidate (43)



Following the general procedure **D** compound **35** (1.30 g, 1.33 mmol) was desilylated at 0 °C for 3 h and then transformed into trichloroacetimidate donor. Purification by silica gel column chromatography (40 – 50 % EtOAc/Hexane) afforded the compound **43** as a yellow solid (1.133 g, 89 %).

R_f 0.30 (40 % EtOAc/Hexane), m.p. 72.0 – 73.0, $[\alpha]^{24}_D$ +24.43 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 3322, 2951, 1739, 1674 cm^{-1}.

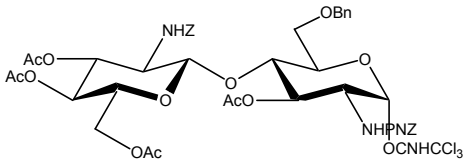
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.25 – 7.40 (m, 15 H), 6.42 (d, *J* = 3.6 Hz, 1H), 5.18 (dd, *J* = 9.9, 11.7 Hz, 1H), 5.06 (AB Quartet, *J* = 12.0 Hz, 2H), 5.01 (s, 3H), 4. 93 (d, *J* = 9.3 Hz, 1H), 4.87 (d, *J* = 9.3 Hz, 1H), 4.69 (d, *J* = 9.0 Hz, 1H), 4.35 (d, *J* = 4.5 Hz, 1H), 4.27 – 4.32 (m, 2H), 4.17 (ddd, *J* = 3.3, 7.5, 9.9 Hz, 10.2 Hz, 1H), 4.03 (dd, *J* = 9.6, 9.9 Hz, 2H), 3.95 (dd, *J* = 1.5 Hz, 12.0 Hz, 1H), 3.77 (d, *J* = 9.9 Hz, 1H), 3.33 – 3.47 (m, 4H), 2.04 (s, 3H), 2.00 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 170.5, 170.3, 169.4, 160.6, 155.7, 155.3, 137.4, 136.1, 128.8, 128.7, 128.6, 128.5, 128.2, 128.1, 127.9, 99.9, 95.3, 90.8, 74.0, 7.9, 73.4, 72.6, 72.1, 71.3, 70.7, 70.4, 68.4, 66.9, 66.7, 66.4,61.8, 56.0, 53.7, 20.6, 20.5, 20.4,20.3.

ESI-MS *m/z* $(M - CCl_3CONH)^+$ calcd 849.3082, obsd 849.3105.

$C_{45}H_{50}Cl_3N_3O_{17}$ (1009.2206) calcd C 53.45, H 4,98, N 4.16; found C 53.05, H 4.96, N 4.21.

3,4,6-Tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-α-D-glucopyranosyl trichloroacetimidate (44)



Following the general procedure **D** compound **36** (1.32 g, 1.38 mmol) was desilylated at 0 °C and then slowly temperature increased to room temperature and stirred overnight. Then it was transformed into trichloroacetimidate donor. Purification by silica gel column chromatography (40 – 50 % EtOAc/Hexane) afforded the compound **44** as a yellow solid (0.99 g, 78 %).

R_f 0.41 (50 % EtOAc/Hexane), m.p. 84.0 – 85.0, $[\alpha]^{24}_D$ +22.64 (*c* = 0.53, $CHCl_3$).

IR (NaCl Plates) 3324, 1740,1520 cm^{-1}.

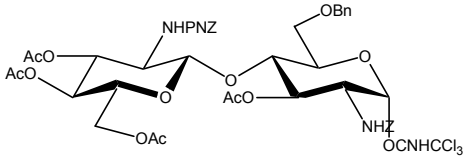
^1H NMR (300 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.20 (d, *J* = 8.7 Hz, 2H), 7.26 – 7.46 (m, 12H), 6.36 (d, *J* = 3.6 Hz, 1H), 5.22 (d, *J* = 11.1 Hz, 1H), 5.17 (s, 2H), 5.08 (d, *J* = 9.3 Hz, 1H), 5.02 (s, 2H), 4.90 (dd, *J* = 9.6, 10.2 Hz, 2H), 4.70 (d, *J* = 9.9 Hz, 1H), 4.37 (dd, *J* = 4.2 Hz, 12.0 Hz, 2H), 4.30 (d, *J* = 12.0 Hz, 1H), 4.16 (ddd, *J* = 3.3, 7.5, 9.9 Hz, 10.5 Hz, 1H), 4.05 (dd, *J* = 9.6, 10.2 Hz, 1H), 3.96 (d, *J* = 11.4 Hz, 1H), 3.78 (d, *J* = 9.9 H, 1H), 3.30 – 3.56 (m, 4H), 2.05 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.91 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 17.0, 170.4, 170.2, 169.4, 160.7, 155.2, 155.1, 147.6, 143.4, 137.4, 136.4, 128.9, 128.8, 128.4, 128.2, 127.9, 123.7, 100.3, 95.1, 90.8, 73.7, 73.3, 72.5, 72.1, 71.4, 70.4, 68.3, 66.7, 66.2, 65.4, 61.7, 56.0, 53.8, 20.7, 20.6, 20.5, 20.4.

ESI-MS *m/z* $(M - CCl_3CONH)^+$ calcd 894.2933, obsd 849.2881.

$C_{45}H_{49}Cl_3N_3O_{19}$ (1054.2057) calcd C 51.17, H 4.68, N 5.30; found C 50.90, H 4.78, N 5.29.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl trichloroacetimidate (45)



Following the general procedure **D** compound **37** (1.24 g, 1.20 mmol) was desilylated at 0 °C and then slowly temperature increased to room temperature and stirred overnight. Then it was transformed into trichloroacetimidate donor. Purification by silica gel column chromatography (40 – 50 % EtOAc/Hexane) afforded the compound **45** as a yellow solid (1.09 g, 87 %).

R_f 0.32 (50 % EtOAc/Hexane), m.p. 78.0 – 79.0, $[\alpha]^{24}_D$ +12.64 (*c* = 0.52, $CHCl_3$).

IR (NaCl Plates) 3321, 1739, 1519 cm^{-1}.

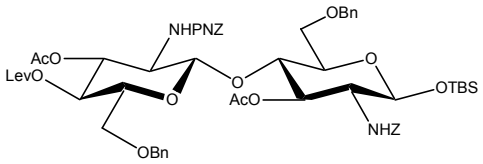
^1H NMR (500 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.19 - 8.23 (m, 2H), 7.31 – 7.48 (m, 12H), 6.34 (d, *J* = 3.5 Hz, 1H), 5.18 – 5.21 (m, 2H), 5.21 (d, *J* = 11.5 Hz, 3H), 5.04 (d, *J* = 12.0 Hz, 2H), 4.91 (d, *J* = 8.0 Hz, 2H), 4.73 (d, *J* = 7.5 Hz, 1H), 4.47 (s, 1H), 4.30 – 4.37 (m, 3H), 4.20 (s, 1H), 4.06 (dd, *J* = 10.0, 10.5 Hz, 1H), 3.97 (d, *J* = 12.0 Hz, 1H), 3.58 (m, 1H), 3.46 (ddd, *J* = 2.5, 7.5, 10.5 Hz, 2H), 3.38 (bs, 1H), 2.06 (s, 3H), 2.02 (s,3H), 1.94 (s, 3H), 1.93 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.4, 170.7, 170.6, 169.8, 160.9, 156.1, 155.4, 148.1, 144.3, 138.1, 136.9, 129.2, 128.8, 128.5, 128.3, 123.7, 100.6, 95.6, 91.2, 74.6, 73.8, 73.1, 72.5, 72.1, 70.7, 68.7, 67.3, 65.8, 65.7, 62.1, 56.8, 54.0, 20.8, 20.7.

ESI-MS *m/z* $(M - CCl_3CONH)^+$ calcd 894.2933, obsd 894.2916.

$C_{45}H_{49}Cl_3N_3O_{19}$ (1054.2057) calcd C 51.17, H 4.68, N 5.30; found C 51.14, H 4.65, N 5.30.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-4-*O*-levulinyl-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxy-carbonylamino-2-deoxy-β-D-glucopyranoside (46)



Coupling of *N*-Cbz acceptor **20** (0.56 g, 1.0 mmol) and *N*-PNZ protected trichloroacetimidate donor **34** (0.950 g, 1.3 mmol) following general procedure **A** for 1 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.034 g, 0.24 mmol) afforded the disaccharide **46** as a white solid (1.04g, 92 %) after purification over silica gel column chromatography using 35 – 50 % EtOAc/Hexane.

R_f 0.16 (40 % EtOAc/Hexane), m.p. 72.0 – 73.0, $[\alpha]^{24}_D$ -3.88 (*c* = 0.50, $CHCl_3$).

IR (NaCl Plates) 3340, 2857, 1716 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.7 Hz, 2H), 7.44 (d, *J* = 8.7 Hz, 2H), 7.28 – 7.30 (m, 15H), 5.07 – 5.19 (m, 5H), 4.97 – 5.03 (m, 2H), 4.82 (d, *J* = 9.3 Hz, 1H), 4.71 (d, *J* = 9.3 Hz, 1H), 4.57 (d, *J* = 8.7 Hz, 1H), 4.50 (d, *J* = 8.1 Hz, 1H), 4.40 (AB Quartet, *J* = 12.0 Hz, 3H), 3.89 (dd, *J* = 9.6, 10.5 Hz, 1H), 3.65 (dd, *J* = 3.0 Hz, 11.4 Hz, 1H), 3.61 (d, *J* = 3.0 Hz, 1H), 3.57 (d, *J* = 2.4 Hz, 2H), 3.52 (d, *J* = 5.4 Hz, *2*H), 3.47 (d, *J* = 2.4 Hz, 2H), 3.35 (bs, 1H),

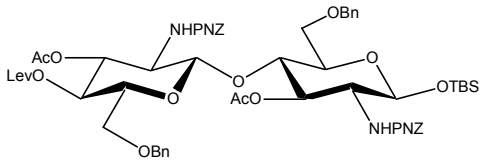
2.56 – 2.74 (m, 2H), 2.28 – 2.49 (m, 2H), 2.13 (s, 3H), 1.96 (s, 3H), 1.89 (s, 3H), 0.86 (s, 9H), 0.09 (s, 3H), 0.04 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 180.3, 171.2, 171.1, 155.8, 155.0, 147.7, 137.6, 136.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 123.7, 99.9, 96.6, 77,2, 75.2, 74.5, 73.6, 73.4, 72.7, 72.2, 69.3, 68.7, 67.7, 66.7, 65.2, 57.9, 56.5, 37.6, 29.6, 27.8, 25.5, 20.7, 20.6, 17.9, -4.3, -5.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1130.4529, obsd 1130.4495.

$C_{57}H_{71}N_3O_{19}Si$ (1129.4451) calcd C 60.57, H 6.33, N 3.72; found C 60.30, H 6.13, N 3.85.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-4-*O*-levulinyl-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranoside (47)



Coupling of *N*-PNZ acceptor **32** (0.61 g, 1.0 mmol) and *N*-PNZ protected trichloroacetimidate donor **34** (0.95 g, 1.3 mmol) following general procedure **A** for 1 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.034 g, 0.24 mmol) afforded the disaccharide **47** as a white solid (1.025g, 87 %) after purification over silica gel column chromatography using 30 – 50 % EtOAc/Hexane.

R_f 0.14 (40 % EtOAc/Hexane), m.p. 75.5 – 76.5, $[\alpha]^{24}_D$ -8.88 (*c* = 0.50, $CHCl_3$).

IR (NaCl Plates) 3332, 2931, 1748, 1523 cm^{-1}.

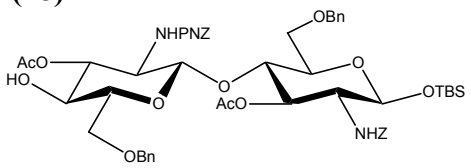
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, *J* = 9.0 Hz, 4H), 7.47 (d, *J* = 9.0 Hz, 4H), 7.27 – 7.38 (m, 10 H), 5.16 (s, 3H), 5.10 (bs, 1H), 5.03 (dd, *J* = 9.6, 10.2 Hz, 3H), 4.96 (d, *J* = 8.7 Hz, 1H), 4.72 (d, *J* = 11.4 Hz, 2H), 4.59 (d, *J* = 7.2 Hz, 1H), 4.44 (AB Quartet, *J* = 12.0 Hz, 3H), 3.89 (dd, *J* = 9.0, 9.6 Hz, 1H), 3.57 – 3.66 (m, 3H), 3.48 – 3.54 (m, 4H), 3.38 (m, 2H), 2.57 – 2.74 (m, 2H), 2.29 – 2.47 (m, 2H), 2.14 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 0.84 (s, 9H), 0.09 (s, 3H), 0.03 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 171.2, 171.1, 170.7, 155.3, 155.1, 147.7, 147.6, 143.9, 143.7, 137.6, 128.7, 128.3, 128.1, 127.9, 127.7, 123.8, 123.7, 100.2, 96.5, 77.2, 75.1, 74.5, 73.6, 73.4, 72.7, 72.1, 69.2, 68.7, 67.9, 65.2, 65.1, 58.1, 56.4, 37.6, 29.6, 27.8, 25.5, 20.7, 20.6, 17.9, -4.2, -5.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1175.4380, obsd 1175.4331.

$C_{57}H_{70}N_4O_{21}Si$ (1174.4302) calcd C 58.25, H 6.00, N 4.77; found C 58.02, H 5.83, N 4.77.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (48)



Following the general procedure **C** disaccharide **46** (0.94 g, 0.83 mmol) was delevulinated and then crude product purified using silica gel chromatography (40 – 60 % EtOAc/Hexane) to afford the disaccharide acceptor **48** as a white solid (0.82 g, 96%).

R_f 0.19 (40 % EtOAc/Hexane), m.p. 78.5 – 79.5. $[\alpha]^{24}_D$ -26.60 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 3335, 2856, 1716 cm^{-1}.

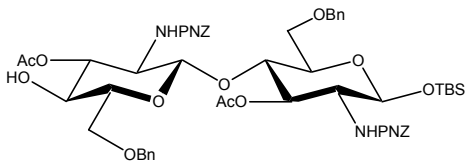
^1H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J* = 9.0 Hz, 2H), 7.28 – 7. 36 (m, 17H), 4.97 – 5.19 (m, 5H), 4.83 (d, *J* = 9.3 Hz, 2H), 4.72 (d, *J* = 11.7 Hz, 2H), 4.56 (m, 1H), 4.51 (AB Quartet, *J* = 12.0 Hz, 2H), 4.30 – 4.44 (m, 2H), 3.85 (dd, *J* = 9.3, 10.2 Hz, 1H), 3.74 (dd, *J* = 4.5 Hz, 10.2 Hz, 1H), 3.67 (d, *J* = 4.8 Hz, 1H), 3.60 – 3.65 (m, 3H), 3.51 (d, *J* = 10.8 Hz, 1H), 3.60 (d, *J* = 9.0 Hz, 1H), 3.35 (bs, 2H), 2.98 (s, 1H), 1.99 (s, 3H), 1.89 (s, 3H), 0.86 (s, 9H), 0.09 (s, 3H), 0.04 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 170.9, 155.9, 155.3, 147.6, 143.8, 137.6, 137.3, 136.4, 12.7, 128.5, 128.4, 127.9, 127.7, 123.7, 100.6, 96.7, 77.2, 75.3, 75.2, 74.5, 73.6, 73.4, 72.3, 70.7, 70.2, 67.9, 66.7, 65.2, 57.9, 56.1, 25.5, 20.8, 20.7, 17.9, -4.3, -5.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1032.4162, obsd 1032.4125.

$C_{52}H_{65}N_3O_{17}Si$ (1031.4083) calcd C 60.51, H 6.35, N 4.07; found C 60.21, H 6.23, N 4.09.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxy-carbonylamino-β-D-glucopyranoside (49)



Following the general procedure **C** disaccharide **47** (0.958 g, 0.82 mmol) was delevulinated and then crude product purified using silica gel column chromatography (40 – 60 % EtOAc/Hexane) to afford the disaccharide acceptor **49** as a white solid (0.82 g, 93%).

R_f 0.16 (50 % EtOAc/Hexane), m.p. 78.0 – 79.0, $[\alpha]^{24}_D$ -28.70 (*c* = 0.50, $CHCl_3$).

IR (NaCl Plates) 3345, 2929, 1722, 1519 cm^{-1}.

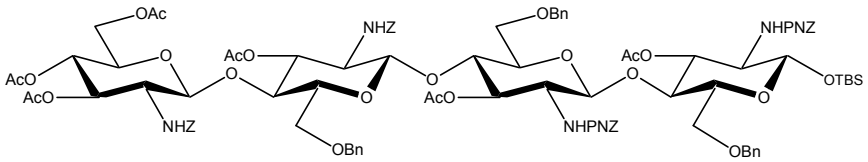
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J* = 8.7 Hz, 4H), 7.26 – 7.49 (m, 14 H), 5.09 – 5.16 (m, 3H), 5.03 (d, *J* = 9.6 Hz, 1H), 4.94 (d, *J* = 9.6 Hz, 1H), 4.80 (m, 1H), 4.72 (d, *J* = 12.9 Hz, 1H), 4.59 (m, 1H), 4.52 (AB Quartet, *J* = 12.0 Hz, 3H), 4.35 – 4.44 (m, 2H), 3.86 (dd, *J* = 9.0, 9.6 Hz, 1H), 3.74 (dd, *J* = 4.5 Hz, 9.6 Hz, 1H), 3.68 (d, *J* = 4.8 Hz, 1H), 3.62 – 3.65 (m, 2H), 3.59 (d, *J* = 2.7 Hz, 1H), 3.49 (d, *J* = 10.8 Hz, 2H), 3.43 (d, *J* = 9.9 Hz, 1H), 3.34 (m, 2H), 2.95 (bs, 1H), 2.00 (s, 3H), 1.92 (s, 3H), 0.84 (s, 9H), 0.09 (s, 3H), 0.03 (s, 3H)

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 170.9, 155.3, 155.2, 147.6, 147.5, 143.9, 143.8, 137.5, 137.2, 128.8, 128.5, 128.1, 128.0, 127.7, 123.7, 100.7, 96.4, 77.2, 75.1, 74.5, 73.6, 73.4, 72.3, 70.8, 70.1, 67.7, 67.6, 65.2, 65.1, 58.0, 56.0, 25.4, 20.8, 20.7, 17.8, -4.2, -5.4;

ESI-MS *m/z* $(M + H)^+$ calcd 1077.4012, obsd 1077.3983.

$C_{52}H_{64}N_4O_{19}Si$ (1076.3934) calcd C 57.98, H 5.99, N 5.20; found C 57.71, H 5.84, N 5.25.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxy-carbonylamino -β-D-glucopyranoside (50)



Coupling of disaccharide acceptor **49** (0.102 g, 0.095 mmol) and disaccharide trichloroacetimidate donor **43** (0.144 g, 1.4 mmol) following general procedure **A** for 6 hour at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.007 g, 0.048 mmol) afforded the tetrasaccharide **50** as a mixture along with by products **53** and **54** in 45% yield (0.081g) after purification over silica gel column chromatography using 30 – 50 % EtOAc/Hexane.

R_f 0.24 (50 % EtOAc/Hexane)

IR (NaCl Plates) 2858, 1743, 1521 cm^{-1}.

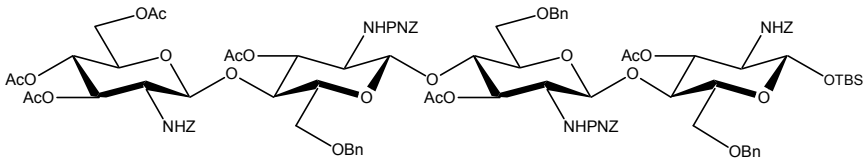
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.7 Hz, 4H), 7.26 – 7.50 (m, 29H), 5.19 (bs, 2H), 4.99 – 5.12 (m, 6H), 4.92 (d, *J* = 10.1 Hz, 2H), 4.85 (d, *J* = 8.4 Hz, 1H), 4.74 (d, *J* = 12.3 Hz, 2H), 4.56 – 4.63 (m, 5H), 4.44 (d, *J* = 12.0 Hz, 2H), 4.31 (dd, *J* = 12.3, 4.2 Hz, 1H), 4.18 (dd, *J* = 12.0, 2.7 Hz, 3H), 3.90 – 3.94 (m, 3H), 3.85 (dd, *J* = 9.3, 9.3 Hz, 2H), 3.71 – 3.82 (m, 3H), 3.60 (dd, *J* = 10.8, 3.0 Hz, 2H), 3.50 (d, *J* = 10.2 Hz, 2H), 3.34 – 3.45 (m, 8H), 2.98 (bs, 1H), 2.92 (d, *J* = 9.1 Hz, 1H), 2.05 (s, 6H), 2.00 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 1.76 (s, 3H), 0.85 (s, 9H), 0.10 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 170.8, 170.5, 170.4, 170.3, 169.4, 155.5, 155.4, 155.3, 155.2, 147.6, 143.9, 137.4, 137.1, 136.8, 136.6, 136.4, 129.1, 129.0, 128.9, 128.8, 128.6,

128.5, 128.3, 128.2, 128.1, 128.0, 123.7, 100.8, 100.5, 100.1, 96.6, 77.2, 75.1, 75.0, 74.6, 74.0, 73.9, 73.7, 73.6, 73.3, 73.1, 72.7, 72.4, 72.1, 71.3, 68.4, 67.1, 67.0, 66.9, 66.8, 66.6, 65.2, 65.0, 61.7, 58.1, 56.3, 56.1, 56.0, 25.5, 20.7, 20.6, 20.5, 20.4, 17.9, -4.2, -5.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1925.7016, obsd 1925.6993.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-p-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxy-carbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonyl-amino -2-deoxy-β-D-glucopyranoside (51)



Coupling of disaccharide acceptor **48** (0.085 g, 0.082 mmol) and disaccharide trichloroacetimidate donor **44** (0.174 g, 0.165 mmol) following general procedure **A** for 6 h at -50 to -25 °C using $BF_3 \cdot Et_2O$ (0.034 g, 0.082 mmol) afforded the tetrasaccharide **51** as a white solid (0.072 g, 45 %) after purification over silica gel column chromatography using 40 – 50 % EtOAc/Hexane.

R_f 0.19 (50 % EtOAc/Hexane), m.p. 103.0 – 105.0, $[\alpha]^{24}_D$ -29.06 (*c* = 1.70, $CHCl_3$).

IR (NaCl Plates) 3345, 2929, 1722, 1519 cm^{-1}.

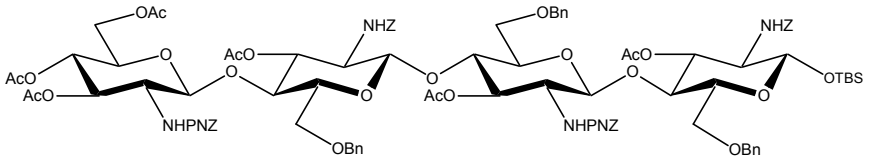
^1H NMR (300 MHz, CD_2Cl_2) δ 8.22 (d, *J* = 8.7 Hz, 2H), 8.16 – 8.19 (m, 2H), 7.33 – 7.48 (m, 29 H), 4.97 – 5.20 (m, 9H), 4.79 – 4.92 (m, 4H), 4.66 (d, *J* = 11.7 Hz, 3H), 4.59 (d, *J* = 11.3 Hz, 2H), 4.53 (d, *J* = 6.3 Hz, 1H), 4.44 (d, *J* = 12.3 Hz, 1H), 4.28 – 4.34 (m, 3H), 4.24 (d, *J* = 12.0 Hz, 2H), 4.14 (bs, 2H), 4.05 (d, *J* = 6.6 Hz, 1H), 3.95 (d, *J* = 2.1 Hz, 1H), 3.89 (d, *J* = 9.6 Hz, 1H), 3.82 (ddd, *J* = 2.7, 6.9, 9.6 Hz, 2H), 3.35 – 3.59 (m, 12H), 3.22 (bs, 1H), 3.02 (d, *J* = 9.3 Hz, 1H), 2.00 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H), 1.90 (s, 3H), 1.71 (s, 3H), 0.87 (s, 9H), 0.11 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.4, 171.2, 170.9, 170.7, 170.6, 169.8, 156.2, 155.8, 155.7, 155.5, 148.1, 148.0, 144.5, 144.4, 138.3, 137.9, 137.6, 137.2, 137.1, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.4, 128.3, 128.2, 124.1, 124.0, 101.3, 101.0, 100.6, 97.1, 75.9, 75.7, 75.0, 74.8, 74.4, 74.2, 73.9, 73.8, 73.7, 73.4, 72.9, 72.8, 72.5, 71.8, 68.7, 68.3, 68.2, 67.6, 67.5, 67.1, 67.0, 65.6, 62.1, 58.5, 58.3, 56.6, 56.5, 25.7, 21.0, 20.9, 20.8, 20.7, 18.2, -4.1, -5.3.

ESI-MS *m/z* $(M + H)^+$ calcd 1925.7016, obsd 1925.6892;

$C_{95}H_{112}N_6O_{35}Si$ (1924.6938) calcd C 59.24, H 5.86, N 4.36; found C 58.96, H 5.72, N 4.37.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-p-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (52)



Coupling of disaccharide acceptor **48** (0.103 g, 0.10 mmol) and disaccharide trichloroacetimidate donor **45** (0.232 g, 0.22 mmol) following general procedure **A** for 2 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.014 g, 0.10 mmol) afforded the tetrasaccharide **52** as a white solid (0.09 g, 47 %) after purification over silica gel column chromatography using 45 – 65 % EtOAc/Hexane.

R_f 0.49 (60 % EtOAc/Hexane), m.p. 107.0 – 109.0, $[\alpha]^{24}_D$ -13.98 (*c* = 1.30, $CHCl_3$).

IR (NaCl Plates) 2857, 1725, 1521 cm^{-1}.

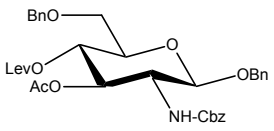
^1H NMR (300 MHz, CD_2Cl_2) δ 8.23 (d, *J* = 8.7 Hz, 2H), 8.16 – 8.19 (m, 2H), 7.33 – 7.50 (m, 29H), 4.97 – 5.22 (m, 9H), 4.79 – 4.93 (m, 4H), 4.70 (d, *J* = 12.0 Hz, 1H), 4.62 (d, *J* = 12.0 Hz, 2H), 4.59 (d, *J* = 9.9 Hz, 3H), 4.46 (d, *J* = 12.0 Hz, 2H), 4.34 (d, *J* = 4.2 Hz, 1H), 4.22 – 4.30 (m, 4H), 4.11 (d, *J* = 6.0 Hz, 1H), 3.77 – 3.96 (m, 5H), 3.56 (dd, *J* = 9.3, 9.3 Hz, 3H), 3.33 – 3.53 (m, 9H), 3.09 (d, *J* = 9.3 Hz, 2H), 2.02 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.91 (s, 3H), 1.78 (s, 3H), 0.87 (s, 9H), 0.11 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.4, 171.2, 170.8, 170.7, 170.6, 169.7, 156.2, 155.9, 155.6, 155.4, 148.1, 148.0, 145.6, 145.3, 138.2, 137.8, 137.2, 137.1, 129.3, 129.2, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 124.1, 124.0, 101.4, 101.1, 100.3, 97.1, 75.8, 75.0, 74.7, 74.6, 74.4, 74.3, 74.1, 73.9, 73.8, 73.5, 72.9, 72.6, 72.5, 71.9, 68.6, 68.1, 67.8, 67.7, 67.5, 67.0, 65.7, 65.6, 62.0, 58.3, 56.7, 56.5, 56.4, 25.8, 20.9, 20.8, 20.6, 18.2, -4.1, -5.3.

ESI-MS *m/z* $(M + H)^+$ calcd 1925.7016, obsd 1925.7090;

$C_{95}H_{112}N_6O_{35}Si$ (1924.6938) Calcd C 59.24, H 5.86, N 4.36; found C 58.95, H 5.72, N 4.33.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-4-*O*-levulinyl-β-D-glucopyranoside (55)



N-Cbz protected trichloroacetimidate **22** (0.7 g, 1.02 mmol) was coevaporated with dry toluene (2 x 5 ml) and then dried under vacuum for 1 h. 10 ml of dry DCM was added under

N_2 atmosphere and then BnOH (0.17 g, 1.53 mmol) added and the resulting solution was cooled to 0 °C. After 10 minutes $BF_3{\cdot}Et_2O$ (0.043 g, 0.30 mmol) was added in drops and stirred for 2 h. The reaction mixture was neutralized with Et_3N and then concentrated under reduced pressure to yield the crude compound. The crude product was purified by silica gel column chromatography using 40 – 50 % EtOAc/Hexane to yield the compound **55** as a white solid (0.61 g, 95 % yield).

R_f 0.24 (40 % EtOAc/Hexane); m.p. 93.0 – 95.0, $[\alpha]^{24}_D$ -12.27 (*c*= 0.53, $CHCl_3$).

IR (NaCl Plates) 3336, 2882, 1745, 1703 cm^{-1}.

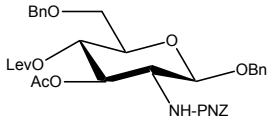
^1H NMR (300 MHz, $CDCl_3$) δ 7.24 – 7.34 (m, 15H), 5.17 (d, *J* =9.9 Hz, 1H), 5.03 – 5.12 (m, 3H), 4.87 (d, *J* = 12.3 Hz, 1H), 4.70 – 4.84 (m, 1H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.55 (s, 2H), 3.73 (dd, *J* = 9.0 Hz, 1H), 3.55 – 3.64 (m, 3H), 2.71 – 2.53 (m, 2H), 2.27 – 2.46 (m, 2H), 2.11 (s, 3H), 1.98 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 171.4, 171.0, 155.8, 137.9, 136.4, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 99.7, 73.6, 73.4, 72.1, 70.6, 69.7, 69.2, 66.9, 56.2, 37.6, 29.6, 27.8, 20.6.

ESI-MS *m/z* $(M + Na)^+$ calcd 656.2472, obsd 656.2443.

$C_{35}H_{39}NO_{10}$ (633.2574) calcd C 66.34, H 6.20, N 2.21; found C 66.14, H 6.33, N 2.27.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-levulinyl-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranoside (56)



N-PNZ protected trichloroacetimidate **34** (1.64 g, 2.24 mmol) was co evaporated with dry toluene (2 x 8 ml) and then dried under vacuum for 1 h. 20 ml of dry DCM was added under N_2 atmosphere and then BnOH (0.36 g, 3.36 mmol) added and the resulting solution was cooled to 0 °C. After 10 minutes $BF_3{\cdot}Et_2O$ (0.08 g, 0.56 mmol) was added in drops and stirred for 3h. The reaction mixture was neutralized with Et_3N and then concentrated under reduced pressure to yield the crude compound. The crude product was purified by silica gel column chromatography using 30 – 50 % EtOAc/Hexane to yield the compound **56** as a white solid (1.34 g, 88 % yield).

R_f 0.27 (50 % EtOAc/Hexane), m.p. 147.0 – 148.0, $[\alpha]^{24}_D$ -24.77 ($_C$ = 0.54, $CHCl_3$).

IR (NaCl Plates) 3363, 2892, 1750, 1713, 1521 cm^{-1}.

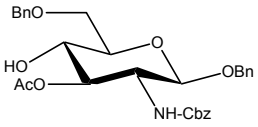
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, J = 8.7 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.26 – 7.34 (m, 10H), 5.18 (bs, 3H), 5.09 (d, *J* = 9.0 Hz, 1H), 4.91 (d, *J* = 12.0 Hz, 2H), 4.60 (d, *J* = 12.3 Hz, 2H), 4.55 (s, 2H), 3.58 – 3.72 (m, 4H), 2.54 – 2.74 (m, 2H), 2.28 – 2.48 (m, 2H), 2.13 (s, 3H), 2.02 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 171.3, 170.9, 155.3, 147.5, 143.8, 137.9, 136.8, 128.4, 128.3, 127.9, 127.8, 127.7, 123.6, 99.6, 73.6, 73.4, 72.1, 70.7, 69.5, 69.0, 65.2, 56.3, 37.6, 29.6, 27.8, 20.7.

ESI-MS *m/z* $(M + Na)^+$ calcd 701.2322, obsd 701.2314.

$C_{35}H_{38}N_2O_{12}$ (678.2425) calcd C 61.94, H 5.64, N 4.13; found C 61.57, H 5.70, N 4.13.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (57)



Following the general procedure **C** disaccharide **55** (1.1 g, 1.73 mmol) was delevulinated and then crude product purified using silica gel column chromatography (40 – 50 % EtOAc/Hexane) to afford the disaccharide acceptor **57** as a white solid (0.85 g, 91%).

R_f 0.32 (50 % EtOAc/Hexane), m.p. 105.0 – 106.0, $[\alpha]^{24}_D$ -59.87 (*c* = 0.50, $CHCl_3$).

IR (NaCl Plates) 3334, 2870, 1691 cm^{-1}.

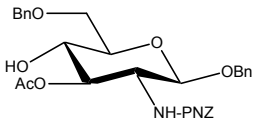
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 - 7.31 (m, 15H), 5.05 (AB Quartet, *J* = 12.3 Hz, 2H), 4.92 (d, *J* = 9.3 Hz, 2H), 4.82 (d, *J* = 9.3 Hz, 1H), 4.58 (AB Quartet, *J* = 12.0 Hz, 2H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.44 (d, *J* = 6.9 Hz, 1H), 3.74 (d, *J* = 4.8 Hz, 2H), 3.64 – 3.71 (m, 2H), 3.34 – 3.45 (m, 1H), 2.97 (bs, 1H), 1.94 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 156.0, 137.7, 136.5, 128.4, 128.3, 127.9, 127.8, 27.7, 100.0, 75.2, 74.3, 73.7, 70.8, 70.5, 70.2, 66.8, 55.7, 20.8.

ESI-MS *m/z* $(M + Na)^+$ calcd 558.2104, obsd 558.2116.

$C_{30}H_{33}NO_8$ (535.2206) calcd C 67.28, H 6.21, N 2.62; found C 66.90, H 6.20, N 2.53.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranoside (58)



Following the general procedure **C** disaccharide **56** (1.25 g, 1.84 mmol) was delevulinated and then product purified using silica gel chromatography (40 – 50 % EtOAc/Hexane) to afford the disaccharide acceptor **58** as a white viscous solid (1.03 g, 96%).

R_f 0.35 (60 % EtOAc/Hexane), m.p. 43.0 – 44.0, $[\alpha]^{24}_D$ -43.87 (*c* = 0.52, $CHCl_3$).

IR (NaCl Plates) 3320, 2867, 1705, 1521 cm^{-1}.

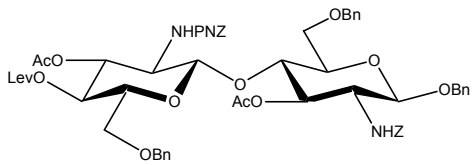
^1H NMR (300 MHz, $CDCl_3$) δ 8.11 (m, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.23 – 7.35 (m, 10H), 5.17 (m, 2H), 5.06 (d, *J* = 9.6 Hz, 1H), 4.98 (d, *J* = 8.4 HZ, 1H) 4.88 (d, *J* = 9.3 Hz, 1H), 4.59 (AB Quartet, *J* = 12.0 Hz, 3H), 4.56 (d, *J* = 12.0 Hz, 1H), 4.49 (d, *J* = 7.2 Hz, 1H), 3.68 – 3.84 (m, 4H), 3.47 – 3.53 (m, 1H), 3.05 (bs, 1H), 2.04 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 155.6, 147.5, 143.9, 137.5, 136.9, 128.5, 128.4, 127.9, 127.8, 127.7, 123.6, 100.0, 75.1, 74.1, 73.8, 70.8, 70.5, 70.2, 65.2, 55.9, 20.9.

ESI-MS *m/z* $(M + Na)^+$ calcd 603.1955, obsd 603.1976.

$C_{30}H_{33}N_2O_{10}$ (580.2057) calcd C 62.06, H 5.56, N 4.83; found C 62.20, H 5.62, N 4.71.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-4-*O*-levulinyl-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (59)



Coupling of *N*-Cbz acceptor **57** (0.80 g, 1.5 mmol) and *N*-PNZ protected trichloroacetimidate donor **34** (1.32 g, 1.80 mmol) following general procedure **A** for 2 h at 0 °C using $BF_3{\cdot}Et_2O$ (0.053 g, 0.37 mmol) afforded the disaccharide **59** as a white solid (1.56 g, 94 %) after purification over silica gel column chromatography using 40 – 50 % EtOAc/Hexane.

R_f 0.22 (50 % EtOAc/Hexane), m.p. 154.0 – 155.0, $[\alpha]^{24}_D$ -35.13 (*c* = 0.47, $CHCl_3$).

IR (NaCl Plates) 3329, 2871, 1747, 1523 cm^{-1}.

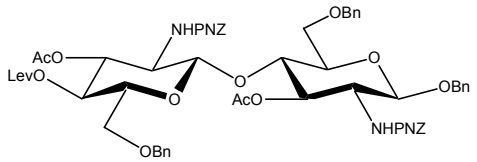
^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 7.8 Hz, 2H), 7.13 - 7.44 (m, 22H), 5.09 – 5.23 (m, 4H), 4.92 – 5.05 (m, 3H), 4.87 (d, *J* = 12.3 Hz, 1H), 4.75 (dd, *J* = 9.3, 10.2 Hz, 2H), 4.56 (d, *J* = 12.3 Hz, 1H), 4.30 – 4.47 (m, 5H), 3.90 (dd, *J* = 9.6, 9.6 Hz, 1H), 3.70 (bs, 2H), 3.60 (d, *J* = 4.5 Hz, 1H), 3.56 (d, *J* = 3.3 Hz, 1H), 3.53 (d, *J* = 4.2 Hz, 1H), 3.47 – 3.51 (m, 1H), 3.30 – 3.45 (m, 3H), 2.56 – 2.73 (m, 2H), 2.27 – 2.47 (m, 2H), 2.13 (s, 3H), 1.96 (s, 3H), 1.88 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 171.2, 170.9, 155.9, 154.9, 147.6, 137.6, 137.0, 136.5, 128.4, 1283, 128.1, 128.0, 127.9, 127.8, 127.7, 123.7, 100.2, 99.9, 74.9, 74.5, 73.6, 73.4, 72.6, 72.1, 72.0, 70.7, 70.6, 69.3, 68.7, 66.8, 65.2, 56.4, 55.9, 37.6, 29.6, 27.7, 20.6, 20.5.

ESI-MS *m/z* $(M + H)^+$ calcd 1106.4134, obsd 1106.4105.

$C_{58}H_{63}N_3O_{19}$ (1105.4056) calcd C 62.98, H 5.74, N 3.80; found C 62.67, H 5.66, N 3.77.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-4-*O*-levulinyl-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranoside (60):



Coupling of *N*-PNZ acceptor **58** (0.36 g, 0.62 mmol) and *N*-PNZ protected trichloroacetimidate donor **34** (0.56 g, 0.77 mmol) following general procedure **A** for 2 h at 0 °C using $BF_3{\cdot}Et_2O$ (0.021 g, 0.15 mmol) afforded the disaccharide **60** as a white solid (0.65 g, 91 %) after purification over silica gel column chromatography using 40 – 50 % EtOAc/Hexane.

R_f 0.14 (40 % EtOAc/Hexane), m.p. 181.0 – 182.0, $[\alpha]^{24}_D$ -34.35 (*c* = 0.51, $CHCl_3$).

IR (NaCl Plates) 3337, 2939, 1746, 1522 cm^{-1}.

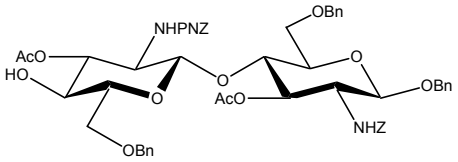
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, *J* = 7.5 Hz, 4H), 8.13 (m, 2H), 7.26 – 7.44 (m, 19H), 5.18 (s, 2H), 5.11 (bs, 1H), 5.02 (dd, *J* = 9.9, 9.0 Hz, 3H), 4.89 (d, *J* = 12.3 Hz, 2H), 4.70 – 4.81 (m, 1H), 4.50 - 4.57 (m, 2H), 4.37 – 4.43 (m, 5H), 3.92 (dd, *J* = 9.0, 10.5 Hz, 1H), 3.74 (d, *J* = 9.3 Hz, 1H), 3.61 (d, *J* = 3.3 Hz, 1H), 3.58 (d, *J* = 2.7 Hz, 1H), 3.53 (d, *J* = 4.5 Hz, 2H), 3.49 (d, *J* = 4.8 Hz, 1H), 3.43 (bs, 2H), 3.31 (bs, 1H), 2.58 – 2.75 (m, 2H), 2.31 – 2.47 (m, 2H), 2.14 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 171.2, 171.0, 167.5, 155.4, 155.0, 147.7, 147.5, 144.0, 141.0, 137.6, 128.8, 128.4, 128.3, 128.1, 127.9. 127.8, 127.7, 123.7, 123.6, 100.3, 100.2, 77.2, 74.7, 74.5, 73.7, 73.4, 72.7, 72.1, 70.6, 69.2, 68.7, 65.3, 65.2, 63.9, 57.6, 56.3, 37.6, 29.6, 27.8, 20.7, 20.6.

ESI-MS *m/z* $(M + Na)^+$ calcd 1173.3804, obsd 1173.3845.

$C_{58}H_{62}N_4O_{21}$ (1150.3907) calcd C 60.52, H 5.43, N 4.87; found C 60.21, H 5.40, N 4.96.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (61)



Following the general procedure **C** disaccharide **59** (1.56 g, 1.54 mmol) was delevulinated and then crude product purified using silica gel column chromatography (45 – 60 % EtOAc/Hexane) to afford the disaccharide acceptor **61** as a white solid (1.37 g, 97%).

R_f 0.29 (60 % EtOAc/Hexane), m.p. 69.0 – 70.0, $[\alpha]^{24}_D$ -61.01 (*c* = 0.45, $CHCl_3$).

IR (NaCl Plates) 3334, 2868, 1698, 1522 cm^{-1}.

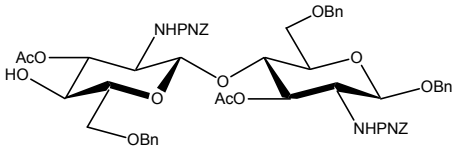
^1H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J* = 7.5 Hz, 2H), 7.25 – 7.44 (m, 22H), 5.09 (s, 4H), 4.96 - 5.03 (m, 1H), 4.86 (d, *J* = 12.3 Hz, 1H), 4.76 (dd, *J* = 8.4, 10.2 Hz, 3H), 4.55 (d, *J* = 12.3 Hz, 1H), 4.51 (d, *J* = 11.7 Hz, 2H), 4.39 (d, *J* = 12.6 Hz, 2H), 4.28 (bs, 1H), 3.86 (dd, *J* = 9.3, 10.5 Hz, 1H), 3.74 (dd, *J* = 4.5 Hz, 9.9 Hz, 2H), 3.66 (d, *J* = 5.7 Hz, 1H), 3.61 (d, *J* = 8.1 Hz, 2H), 3.52 (d, *J* = 10.5 Hz, 1H), 3.41 (d, *J* = 9.3 Hz, 2H), 3.20 (bs, 2H), 1.99 (s, 3H), 1.88 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 170.8, 155.9, 155.2, 147.7, 143.5, 137.4, 137.0, 136.4, 128.8, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 27.8, 127.7, 123.6, 100.5, 100.1, 77.2, 75.1, 75.0, 74.5, 73.7, 73.3, 72.4, 72.2, 70.7, 70.6, 70.2, 66.8, 65.2, 56.0, 55.9, 20.7, 20.6.

ESI-MS *m/z* $(M + H)^+$ calcd 1008.3766, obsd 1008.3741.

$C_{53}H_{57}N_3O_{17}$ (1007.3688) calcd C 63.15, H 5.70, N 4.17; found C 62.94, H 5.54, N 4.11.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranoside (62)



Following the general procedure **C** disaccharide **60** (0.58 g, 0.51 mmol) was delevulinated and then crude product purified using silica gel column chromatography (50 – 60 % EtOAc/Hexane) to afford the disaccharide acceptor **62** as a white solid (0.50 g, 94%).

R_f 0.14 (50 % EtOAc/Hexane), m.p. 84.5 – 85, $[\alpha]^{24}_D$ -55.97 (*c* = 0.52, $CHCl_3$).

IR (NaCl Plates) 3333, 2937, 1732, 1522 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 8.14 – 8.22 (m, 4H), 7.26 – 7.42 (m, 19H), 5.09 – 5.19 (m, 4H), 4.97 (d, *J* = 8.5 Hz, 2H), 4.88 (d, *J* = 12.0 Hz, 1H), 4.76 (bs, 2H), 4.51 (AB Quartet, *J* =

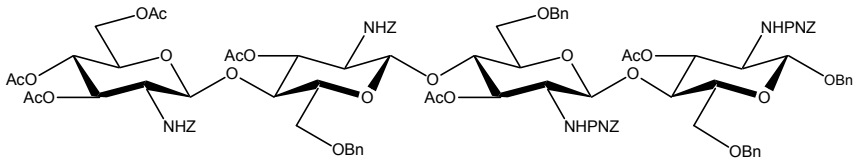
12.0 Hz, 4H), 4.40 (d, *J* = 11.0 Hz, 2H), 4.27 (bs, 1H), 3.80 (dd, *J* = 9.0, 10.0 Hz, 1H), 3.74 (dd, *J* = 4.0 Hz, 10.0 Hz, 2H), 3.66 (dd, *J* = 4.5 Hz, 9.5 Hz, 2H), 3.58 – 3.63 (m, 2H), 3.53 (d, *J* = 10.0 Hz, 1H), 3.42 (d, *J* = 9.5 Hz, 1H), 3.32 (bs, 2H), 1.99 (s, 3H), 1.90 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 170.8, 155.4, 155.2, 147.6, 147.5, 144.0, 143.8, 137.4, 137.1, 136.9, 128.8, 128.5, 128.3, 128.1, 128.0, 127.8, 127.6, 123.7, 123.6, 100.6, 100.1, 75.1, 74.9, 74.5, 73.6, 73.5, 73.4, 73.3, 72.3, 70.5, 70.1, 67.4, 65.5, 65.1, 55.9, 55.8, 20.7, 20.6.

ESI-MS *m/z* $(M + H)^+$ calcd 1053.3617, obsd 1053.3568.

$C_{53}H_{56}N_4O_{19}$ (1052.3539) calcd C 60.45, H 5.36, N 5.32; found C 59.96, H 5.35, N 5.32.

Benzyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitro-benzyloxycarbonyl-amino-β-D-glucopyranoside (63)



Coupling of disaccharide acceptor **62** (0.107 g, 0.10 mmol) and disaccharide trichloroacetimidate donor **43** (0.253 g, 0.25 mmol) following general procedure **A** for 12 h at -0 °C to RT using $BF_3{\cdot}Et_2O$ (0.017 g, 0.125mmol) afforded the tetrasaccharide **63** as a white solid (0.149 g, 77 %) along with byproducts **53** and **54** after purification over silica gel column chromatography using 50 – 70 % EtOAc/Hexane.

R_f 0.49 (60 % EtOAc/Hexane).

IR (NaCl Plates) 3335, 2862, 1730, 1519 cm^{-1}.

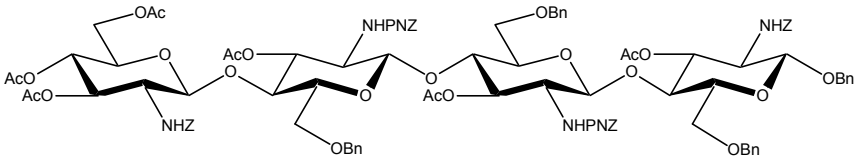
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 – 8.19 (m, 4H), 7.26 – 7.39 (m, 34H), 5.20 (d, *J* = 7.5 Hz, 2H), 5.00 – 5.18 (m, 7H), 4.90 (d, *J* = 12.0 Hz, 2H), 4.86 (d, *J* = 9.0 Hz, 1H), 4.76 (d, *J* = 10.0 Hz, 1H), 4.65 – 4.72 (m, 1H), 4.60 (d, *J* = 11.5 Hz, 2H), 4.57 (d, *J* = 12.0 Hz, 2H), 4.40 (bs, 3H), 4.30 (dd, *J* = 12.0, 4.5 Hz, 2H), 4.24 (bs, 1H), 4.18 (d, *J* = 12.0 Hz, 2H), 3.96 – 4.03 (m, 2H), 3.92 (d, *J* = 11.0 Hz, 2H), 3.86 (dd, *J* = 9.5, 9.5 Hz, 2H), 3.76 (dd, *J* = 9.0, 9.5 Hz, 1H), 3.73 (dd, *J* = 8.5, 9.0 Hz, 1H), 3.61 (d, *J* = 8.0 Hz, 1H), 3.54 (d, *J* = 10.5 Hz, 1H), 3.25 – 3.48 (m, 10H), 3.00 (bs, 1H), 2.92 (d, *J* = 6.5 Hz, 1H), 2.05 (s, 3H), 2.00 (s, 6H), 1.94 (s, 3H), 1.90 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.9, 170.5, 170.3, 170.1, 169.4, 155.5, 155.4, 155.3, 155.2, 147.7, 147.5, 144.0, 143.9, 137.1, 136.6, 136.4, 129.0, 128.6, 128.5, 128.3, 128.1, 127.9, 123.7, 100.8, 100.9, 100.6, 100.1, 74.8, 74.7, 74.6, 74.5, 74.0, 73.9, 73.8, 73.7, 73.5,

73.3, 73.0, 72.5, 72.4, 72.2, 72.1, 72.0, 71.3, 68.4, 66.9, 66.8, 66.6, 65.2, 65.1, 61.7, 56.1, 56.0, 55.9, 55.8, 20.7, 20.6, 20.5, 20.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1901.6621, obsd 1901.6587.

Benzyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-p-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (64)



Coupling of disaccharide acceptor **61** (0.101 g, 0.1 mmol) and disaccharide trichloroacetimidate donor **44** (0.232 g, 0.22 mmol) following general procedure **A** for 15 h at 0 °C to RT using $BF_3{\cdot}Et_2O$ (0.014 g, 0.10 mmol) afforded the tetrasaccharide **64** as a white solid (0.141 g, 74 %) after purification over silica gel column chromatography using 50 – 70 % EtOAc/Hexane.

R_f 0.46 (60 % EtOAc/Hexane), m.p. 223.0 – 225.0, $[\alpha]^{24}_D$ -29.37 (*c* = 1.40, $CHCl_3$).

IR (NaCl Plates) 3335, 2865, 1731, 1519 cm^{-1}.

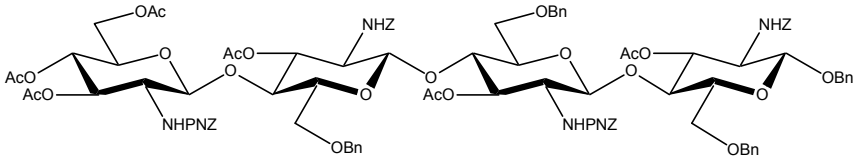
^1H NMR (500 MHz, CD_2Cl_2) δ 8.22 (d, *J* = 8.5 Hz, 2H), 8.19 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.5 Hz, 4H), 7.29 – 7.41 (m, 30H), 5.15 – 5.18 (m, 1H), 5.11 (d, *J* = 8.0 Hz, 2H), 5.07 (d, *J* = 9.0 Hz, 5H), 4.96 (dd, *J* = 10.5, 10.5 Hz, 1H), 4.88 (d, *J* = 4.5 Hz, 2H), 4.86 (d, *J* = 12.0 Hz, 2H), 4.71 (d, *J* = 12.5 Hz, 2H), 4.64 (d, *J* = 11.5 Hz, 2H), 4.59 (d, *J* = 8.0 Hz, 1H), 4.57 (d, *J* = 8.5 Hz, 1H), 4.38 – 4.50 (m, 3H), 4.32 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.28 (d, *J* = 11.5 Hz, 2H), 4.24 (d, *J* = 12.0 Hz, 2H), 4.12 (bs, 2H), 4.03 (bs, 1H), 3.92 (dd, *J* = 12.0, 2.5 Hz, 1H), 3.86 (d, *J* = 8.5 Hz, 1H), 3.81 (ddd, *J* = 2.0, 7.0, 10.0 Hz, 2H), 3.69 (dd, *J* = 8.5, 8.5 Hz, 1H), 3.47 – 3.59 (m, 5H), 3.41 (d, *J* = 9.0 Hz, 2H), 3.35 (bs, 4H), 3.18 (bs, 1H), 3.03 (d, *J* = 8.0 Hz, 1H), 2.01 (s, 3H), 1.98 (s, 6H), 1.97 (s, 3H), 1.93 (s, 3H), 1.90 (s, 3H).

^{13}C NMR (125 MHz, CD_2Cl_2) δ 171.3, 171.2, 170.9, 170.7, 170.6, 169.9, 156.3, 155.7, 155.6, 155.5, 148.1, 148.0, 144.6, 144.5, 138.2, 137.7, 137.6, 137.5, 137.2, 137.1, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 124.1, 124.0, 101.2, 100.9, 100.7, 100.6, 75.6, 74.9, 74.7, 74.4, 74.3, 74.1, 73.9, 73.8, 73.7, 73.4, 72.8, 72.7, 72.6, 72.4, 71.8, 71.7, 71.2, 68.6, 67.8, 67.5, 67.4, 67.1, 65.9, 65.6, 62.1, 56.5, 56.4, 56.3, 56.2, 20.9, 20.8, 20.7.

ESI-MS *m/z* $(M + H)^+$ calcd 1901.6621, obsd 1901.6674.

$C_{96}H_{104}N_6O_{35}$ (1900.6543) calcd C 60.63, H 5.51, N 4.42; found C 60.29, H 5.50, N 4.32.

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-p-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (67)



Coupling of disaccharide acceptor **61** (0.096 g, 0.095 mmol) and disaccharide trichloroacetimidate donor **45** (0.220 g, 0.208 mmol) following general procedure **A** for 15 h at -0 °C to RT using $BF_3{\cdot}Et_2O$ (0.013 g, 0.095 mmol) afforded the tetrasaccharide **67** as a white solid (0.134 g, 74 %) after purification over silica gel column chromatography using 2 % EtOH/$CHCl_3$.

R_f 0.62 (8 % EtOH/$CHCl_3$), m.p. 193.0 – 195.0, $[\alpha]^{24}_D$ -37.24 (*c* = 0.83, $CHCl_3$).

IR (NaCl Plates) 2910, 1724, 1519 cm^{-1}.

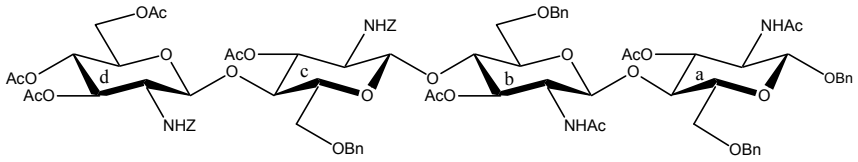
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, *J* = 8.0 Hz, 2H), 8.19 (d, *J* = 8.0 Hz, 2H), 7.26 – 7.47 (m, 34 H), 5.18 (d, *J* = 11.5 Hz, 1H), 5.02 – 5.14 (m, 7H), 4.94 – 4.98 (m, 2H), 4.88 (d, *J* = 11.5 Hz, 2H), 4.74 (bs, 3H), 4.58 (d, *J* = 12.0 Hz, 3H), 4.24 – 4.46 (m, 6H), 4.18 (d, *J* = 11,5 Hz, 2H), 4.00 – 4.14 (m, 2H), 3.93 (d, *J* = 11.5 Hz, 2H), 3.84 (dd, *J* = 10.0, 9.5 Hz, 1H), 3.79 (dd, *J* = 9.5, 10.0 Hz, 2H), 3.72 (bs, 1H), 3.58 (bs, 2H), 3.53 (d, *J* = 10.5 Hz, 2H), 3.20 – 3.48 (m, 8H), 3.00 (bs, 2H), 2.05 (s, 3H), 2.02 (s, 3H), 1.93 (s, 12H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.3, 171.2, 170.9, 170.7, 170.6, 169.8, 156.3, 155.9, 155.6, 155.4, 148.1, 148.0, 144.6, 144.4, 138.2, 137.8, 137.7, 137.2, 129.4, 129.3, 129.1, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 124.1, 124.0, 101.4, 101.3, 100.9, 100.4, 77.3, 75.6, 74.8, 74.7, 74.4, 74.2, 74.0, 73.8, 73.5, 72.9, 72.7, 72.6, 71.9, 71.8, 71,2, 68.7, 67.9, 67.8, 67.5, 67.1, 67.0, 65.7, 65.6, 62.1, 56.8, 56.7, 56.5, 56.4, 20.9, 20.8, 20.7.

ESI-MS *m/z* $(M + H)^+$ calcd 1901.6621, obsd 1901.6572.

$C_{96}H_{104}N_6O_{35}$ (1900.6543) calcd C 60.63, H 5.51, N 4.42; found C 60.25, H 5.48, N 4.38.

Benzyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (70)



Compound **63** (0.132 g, 0.07 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.483 g, 2.78 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (2 - 6 % EtOH/$CHCl_3$) to afford the compound **70** as a white solid (0.072 g, 64 % yield) and mixture of byproducts **53** and **54** (0.023 g, 17 %).

R_f 0.35 (100 % EtOAc), m.p. 211.5 – 213.0, $[\alpha]^{24}_D$ -59.23 (*c* = 0.25, $CHCl_3$).

IR (NaCl Plates) 3330, 2864, 1741, 1696 cm^{-1}.

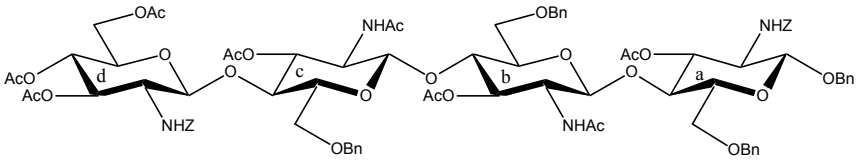
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.41 (m, 30H), 5.75 (d, *J* = 8.5 Hz, 1H), 5.07 (AB Quartet, *J* = 12.0 Hz, 4H), 5.04 (d, *J* = 13.0 Hz, 1H), 4.92 (d, *J* = 8.0 Hz, 1H), 4.85 – 4.89 (m, 3H), 4.77 (d, *J* = 12.0 Hz, 1H), 4.56 – 4.68 (m, 5H), 4.44 (d, *J* = 12.0 Hz, 1H), 4.36 (d, *J* = 7.5 Hz, 1, H-1_a), 4.31 (dd, *J* = 4.5 Hz, 12.0 Hz, 1H), 4.24 (d, *J* = 9.5 Hz, 1H), 4.20 (d, *J* = 7.5 Hz, 1H, H-1_b), 4.17 (d, *J* = 11.5 Hz, 1H), 4.14 (d, *J* = 8.5 Hz, 1H, H-1_c), 3.95 (d, *J* = 7.5 Hz, 1H, H-1_d) 3.92 (d, *J* = 7.5 Hz, 2H), 3.89 (d, *J* = 8.0 Hz, 1H), 3.84 (ddd, *J* = 9.5, 9.5, 10.0 Hz, 1H), 3.77 (dd, *J* = 9.0, 9.5 Hz, 2H), 3.65 (d, *J* = 2.5 Hz, 3H), 3.44 – 3.52 (m, 3H), 3.38 (ddd, *J* = 9.0, 10.0, 10.0 Hz, 3H), 3.28 – 3.34 (m, 2H), 3.03 (bs, 1H), 2.91 (d, *J* = 8.0 Hz, 1H), 2.04 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 1.89 (s, 3H), 1.71 (s, 3H).

^{13}C NMR (125 MHz, CD_2Cl_2) δ 171.4, 171.3, 170.8, 170.7, 170.5, 170.1, 170.0, 169.7, 155.8, 155.7, 138.2, 137.8, 137.7, 137.6, 137.1, 137.0, 129.1, 128.8, 128.7, 128.3, 128.1, 101.2, 100.8, 100.6, 100.4, 75.0, 74.8, 74.7, 74.3, 74.0, 73.6, 73.3, 73.1, 72.9, 72.8, 72.4, 71.8, 71.7, 71.5, 70.8, 68.7, 68.1, 67.4, 67.3, 67.0, 66.9, 62.0, 56.5, 56.4, 56.3, 53.4, 23.3, 23.2, 20.9, 20.8, 20.7, 20.6.

ESI-MS *m/z* $(M + H)^+$ calcd 1627.6395, obsd 1627.6503;

$C_{84}H_{98}N_4O_{29}$ (1626.6317) calcd C 61.98, H 6.07, N 3.44; found C 62.09, H 5.89, N 3.45.

Benzyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (71)



Compound **64** (0.096 g, 0.051 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.355 g, 2.04 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (4 - 6 % EtOH/$CHCl_3$) to afford the compound **71** as a white solid (0.064 g, 78 % yield).

R_f 0.32 (100 % EtOAc). m.p. 213.0 – 215.0 , $[\alpha]^{24}_D$ -50.48 (*c* = 0.25, $CHCl_3$).

IR (NaCl Plates) 2925, 1738, 1521 cm^{-1}.

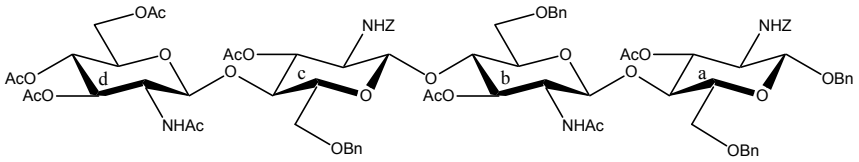
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 – 7.38 (m, 30H), 4.99 (d, *J* = 8.5 Hz, 4H), 4.91 (d, *J* = 9.0 Hz, 1H), 4.87 (d, *J* = 9.0 Hz, 1H), 4.76 – 4.84 (m, 4H), 4.69 (d, *J* = 12.0 Hz, 2H), 4.65 (d, *J* = 7.5 Hz, 1H), 4.61 (d, *J* = 11.0 Hz, 1H), 4.52 (d, *J* = 5.0 Hz, 1H), 4.49 (d, *J* = 12.0 Hz, 2H), 4.36 (d, *J* = 12.0 Hz, 1H), 4.31 (d, *J* = 8.0 Hz, 1H, H-1_a), 4.23 (d, *J* = 11.0 Hz, 1H), 4.22 (d, *J* = 8.0 Hz, 1H, H-1_b), 4.21 (d, *J* = 7.5 Hz, 1H, H-1_c), 4.17 (d, *J* = 12.0 Hz, 2H), 4.09 (d, *J* = 8.0 Hz, 1H, H-1_d), 4.00 (bs, 1H), 3.85 (dd, *J* = 2.0 Hz, 12.5Hz, 1H), 3.80 (dd, *J* = 9.5, 10.0 Hz, 1H), 3.73 (ddd, *J* = 9.0, 9.5, 9.5 Hz, 2H), 3.61 – 3.68 (m, 2H), 3.53 – 3.58 (m, 3H), 3.45 – 3.51 (m, 2H), 3.41 (d, *J* = 7.5 Hz, 1H), 3.32 – 3.37 (m, 2H), 3.27 – 3.29 (m, 2H), 3.18 (ddd, *J* = 2.5, 4.5, 10.0 Hz, 1H), 2.99 (ddd, *J* = 2.0, 4.0, 10.0 Hz, 1H), 1.93 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.90 (s, 3H), 1.89 (s, 3H), 1.81 (s, 3H), 1.61 (s, 3H), 1.58 (s, 3H).

^{13}C NMR (125 MHz, CD_2Cl_2) δ 171.3, 171.2, 171.1, 170.7, 170.6, 169.9, 169.8, 169.7, 156.3, 155.8, 138.3, 137.9, 137.8, 137.6, 137.2, 137.1, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 101.3, 100.9, 100.8, 100.6, 75.3, 74.9, 74.6, 74.5, 74.4, 74.2, 74.0, 73.8, 73.4, 73.1, 73.0, 72.8, 72.6, 71.8, 71.1, 68.7, 68.0, 67.8, 67.6, 67.1, 67.0, 62.1, 56.5, 56.2, 54.4, 54.3, 23.3, 23.2, 20.9, 20.8, 20.7.

ESI-MS *m/z* $(M + H)^+$ calcd 1627.6395, obsd 1627.6331.

$C_{84}H_{98}N_4O_{29}$ (1626.6317) calcd C 61.98, H 6.07, N 3.44; found C 61.73, H 6.17, N 3.45.

Benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl -(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (72)



Compound **67** (0.106 g, 0.056 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.388 g, 2.23 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (2 – 6 % EtOH/$CHCl_3$) to afford the compound **72** as a white solid (0.064 g, 70 % yield).

R_f 0.27 (100 % EtOAc), m.p. 233.0 – 235.0, $[\alpha]^{24}_D$ -40.63 (*c* = 0.90).

IR (NaCl Plates) 2920, 1742, 1520 cm^{-1}.

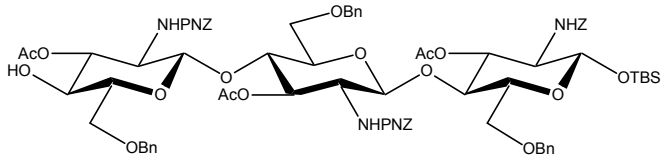
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 – 7.53 (m, 30H), 5.11 (AB Quartet, *J* = 12.5 Hz, 2H), 5.05 (dd, *J* = 11.5, 12.0 Hz, 2H), 4.93 (d, *J* = 2.0 Hz, 1H), 4.92 (d, *J* = 5.0 Hz, 2H), 4.89 (d, *J* = 2.5 Hz, 1H), 4.79 – 4.88 (m, 3H), 4.70 (d, *J* = 12.0 Hz, 1H), 4.65 (d, *J* = 10.5 Hz, 1H), 4.62 (d, *J* = 6.5 Hz, 1H), 4.58 (d, *J* = 9.0 Hz, 1H), 4.49 (d, *J* = 9.0 Hz, 1H), 4.41 (d, *J* = 12.5 Hz, 1H), 4.35 (d, *J* = 8.0 Hz, 1H, H-1_a), 4.32 (d, *J* = 7.5 Hz, 1H, H-1_b), 4.30 (d, *J* = 8.0 Hz, 1H, H-1_c), 4.26 (d, *J* = 12.0 Hz, 1H), 4.17 (d, *J* = 12.0 Hz, 1H), 4.13 (d, *J* = 8.0 Hz, 1H), 4.03 (d, *J* = 7.5 Hz, 1H, H-1_d), 3.93 (d, *J* = 11.0 Hz, 2H), 3.85 (dd, *J* = 9.5, 10.5 Hz, 2H), 3.74 – 3.82 (m, 3H), 3.57 – 3.70 (m, 4H), 3.51 – 3.54 (m, 2H), 3.44 (d, *J* = 7.0 Hz, 1H), 3.32 – 3.42 (m, 4H), 3.03 (d, *J* = 8.5 Hz, 2H), 2.06 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.91 (s, 3H), 1.72 (s, 3H), 1.68 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 170.5, 170.4, 170.2, 169.7, 169.5, 169.4, 169.3, 156.5, 156.1, 137.5, 137.3, 137.0, 136.9, 136.5, 129.2, 129.0, 128.9, 128.8, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 100.8, 100.6, 100.2, 99.7, 74.9, 74.5, 74.4, 74.0, 73.9, 73.8, 73.6, 73.5, 73.3, 73.1, 72.5, 72.4, 71.3, 71.2, 70.4, 68.3, 67.2, 67.1, 66.9, 66.7, 66.6, 61.7, 55.9, 55.7, 54.4, 53.8, 23.1, 23.0, 20.7, 20.6 (2), 20.5 (2), 20.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1627.6395, obsd 1627.6429.

$C_{84}H_{98}N_4O_{29}$ (1626.6317) calcd C 61.98, H 6.07, N 3.44; found C 62.17, H 5.62, N 3.40.

tert-Butyldimethylsilyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-p-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*p*-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl -(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (73)



Coupling of disaccharide acceptor **48** (0.40 g, 0.39 mmol) and *N*-PNZ trichloroacetimidate donor **34** (0.426 g, 0.58 mmol) following general procedure **A** for 1 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.022 g, 0.16 mmol) afforded the trisaccharide as a white solid (0.398 g, 64 %) after purification over silica gel chromatography using 40 – 50 % EtOAc/Hexane. Following the general procedure **C** trisaccharide (0.370 g, 0.231 mmol) was delevulinated and then product purified using silica gel chromatography (2 % EtOH/$CHCl_3$) to afford the trisaccharide acceptor **73 (AAD-*O*-TBS)** as a white solid (0.310 g, 89%).

R_f 0.32 (60 % EtOAc/Hexane), m.p. 99.5 – 101.0, $[\alpha]^{24}_D$ -23.74 (*c* = 1.05).

IR (NaCl Plates) 3342, 2857, 1721, 1521 cm^{-1}.

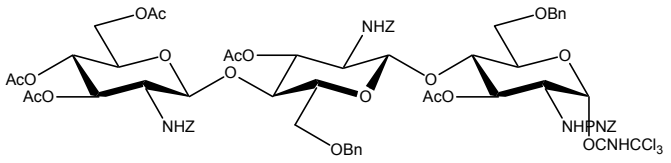
^{1}H NMR (300 MHz, CD_2Cl_2) δ 8.22 (d, *J* = 9.9 Hz, 2H), 2H), 8.16 – 8.19 (m, 2H), 7.27 – 7.49 (m, 24H), 5.20 (d, *J* = 12.5 Hz, 1H), 5.05 – 5.15 (m, 4H), 4.98 (dd, *J* = 9.0, 10.5 Hz, 2H), 4.85 (d, *J* = 9.9 Hz, 2H), 4.69 (d, *J* = 9.0 Hz, 2H), 4.62 (d, *J* = 11.1 Hz, 2H), 4.55 (d, *J* = 5.1 Hz, 1H), 4.51 (d, *J* = 4.8 Hz, 2H), 4.46 (d, *J* = 3.9 Hz, 1H), 4.41 (d, *J* = 6.9 Hz, 1H), 4.29 (d, *J* = 12.0 Hz, 1H), 4.20 (bs, 2H), 3.85 (ddd, *J* = 1.8, 7.8, 9.6 Hz, 2H), 3.65 – 3.75 (m, 3H), 3.62 (d, *J* = 3.3 Hz, 1H), 3.49 – 3.60 (m, 5H), 3.18 – 3.41 (m, 4H), 2.78 (d, *J* = 3.6 Hz, 1H), 1.99 (s, 3H), 1.98 (s, 3H), 1.88 (s, 3H), 0.87 (s, 9H), 0.11 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.6, 171.4, 171.1, 156.23, 155.6, 155.5, 148.1, 148.0, 144.5, 144.4, 138.1, 137.7, 137.1, 129.3, 129.2, 129.1, 129.0, 128.9, 128.6, 128.5, 128.4, 128.3, 128.1, 124.1, 124.0, 101.2, 100.8, 97.1, 76.8, 75.6, 75.5, 75.0, 74.9, 74.4, 74.0, 73.9, 73.8, 72.8, 72.6, 70.7, 70.3, 68.2, 68.1, 67.7, 67.1, 65.7, 58.3, 56.8, 56.3, 25.7, 21.0, 20.9, 18.2, -4.1, -5.3.

ESI-MS *m/z* $(M + Na)^+$ calcd 1526.5463, obsd 1526.5432.

$C_{75}H_{89}N_5O_{26}Si$ (1503.5565) calcd C 59.87, H 5.96, N 4.65; found C 59.62, H 5.91, N 4.80.

3,4,6-Tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-p-nitrobenzyloxycarbonylamino-α-D-glucopyranosyl trichloroacetimidate (75)



Coupling of *N*-PNZ acceptor **32** (0.121 g, 0.2 mmol) and disaccharide donor **43** (0.243 g, 0.24 mmol) following general procedure **A** for 1 h at -50 to -25 °C using $BF_3{\cdot}Et_2O$ (0.014 g, 0.1 mmol) afforded the trisaccharide **74** as a white solid (0.164 g, 56 %) after purification over silica gel chromatography using 30 – 40 % EtOAc/Hexane. Following the general procedure

D compound **74** (0.150 g, 0.103 mmol) was desilylated at room temperature and stirred for overnight. Then it was transformed into trichloroacetimidate donor. Purification by silica gel column chromatography (40 – 50 % EtOAc/Hexane) afforded the compound **75** as a white solid (0.114 g, 75 %).

R_f 0.54 (60 % EtOAc/Hexane), m.p. 103.0 – 105.0.

IR (NaCl Plates) 2860, 1723, 1519 cm^{-1}.

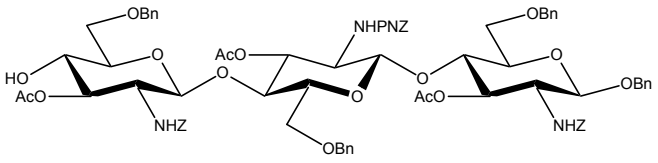
^1H NMR (300 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.25 (d, *J* = 8.4 Hz, 2H), 7.34 – 7.39 (m, 22H), 6.31 (d, *J* = 3.6 Hz, 1H), 4.88 – 5.16 (m, 8H), 4.80 (dd, *J* = 9.3, 10.5 Hz, 3H), 4.65 (d, *J* = 10.5 Hz, 2H), 4.51 (d, *J* = 11.7 Hz, 1H), 4.27 (d, *J* = 4.5 Hz, 1H), 4.21 (d, *J* = 6.3 Hz, 1H), 4.13 (d, *J* = 12.0 Hz, 2H), 3.92 (dd, *J* = 9.3, 10.2 Hz, 2H), 3.85 (d, *J* = 12.0 Hz, 2H), 3.72 (dd, *J* = 9.3, 11.7 Hz, 2H), 3.23 – 3.46 (m, 8H), 2.92 (d, *J* = 8.7 Hz, 1H), 1.97 (s, 3H), 1.93 (s, 6H), 1.84 (s, 3H), 1.82 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 170.4, 170.3, 169.4, 169.3, 160.3, 155.4, 155.3, 155.2, 147.6, 143.5, 137.2, 137.1, 136.5, 128.9, 128.5, 128.4, 128.3, 128.1, 127.9, 123.7, 101.0, 99.9, 95.2, 90.9, 77.1, 73.9, 73.7, 73.6, 73.1, 72.7, 72.4, 72.1, 71.2, 70.5, 68.4, 67.0, 66.8, 66.6, 66.3, 65.4, 61.7, 56.1, 56.0, 53.8, 20.6, 20.5, 20.4, 20.3.

ESI-MS *m/z* $(M - CCl_3CONH)^+$ calcd 1321.4564, obsd 1321.4539.

$C_{68}H_{74}Cl_3N_5O_{26}$ (1481.3688) calcd C 55.05, H 5.03, N 4.72; found C 54.71, H 4.86, N 4.70.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-p-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (78)



Coupling of disaccharide acceptor **61** (0.268 g, 0.266 mmol) and *N*-PNZ trichloroacetimidate donor **22** (0.366 g, 0.536 mmol) following general procedure **A** for 2 h at -45 to -25 °C using $BF_3{\cdot}Et_2O$ (0.038 g, 0.266 mmol) afforded the trisaccharide **76** as a white solid (0.204g, 50 %) after purification over silica gel chromatography using 30 – 50 % EtOAc/Hexane. Following the general procedure **C** trisaccharide (0.113 g, 0.074 mmol) was delevulinated and then product purified using silica gel chromatography (2 – 3 % EtOH/$CHCl_3$) to afford the trisaccharide acceptor **78 (DAD-*O*-Bn)** as a white solid (0.085 g, 80%).

R_f 0.46 (8 % EtOH/$CHCl_3$), m.p. 186.0 – 188.0, $[\alpha]^{24}_D$ -49.40 (*c* = 0.63).

IR (NaCl Plates) 3349, 1734, 1697, 1521 cm^{-1}.

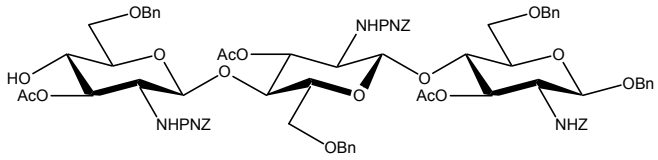
^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 7.2 Hz, 2H), 7.27 – 7.41 (m, 32 H), 4.96 – 5.21 (m, 7H), 4.90 (d, *J* = 12.3 Hz, 1H), 4.77 (d, *J* = 10.2 Hz, 2H), 4.62 (d, *J* = 8.1 Hz, 2H), 4.58 (d, *J* = 6.6 Hz, 2H), 4.51 (d, *J* = 3.0 Hz, 2H), 4.45 (d, *J* = 7.2 Hz, 1H), 4.37 (d, *J* = 12.0 Hz, 2H), 4.18 (d, *J* = 11.1 Hz, 1H), 3.85 (dd, *J* = 9.3, 9.9 Hz, 2H), 3.77 (dd, *J* = 8.7, 10.2 Hz, 2H), 3.70 (d, *J* = 3.6 Hz, 1H), 3.57 – 3.66 (m, 5H), 3.32 – 3.47 (m, 5H), 3.13 – 3.25 (m, 2H), 2.95 – 3.05 (m, 1H), 2.89 (bs, 1H), 1.99 (s, 6H), 1.85 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.7, 171.3, 171.1, 156.3, 155.9, 155.6, 148.0, 144.6, 138.2, 137.8, 137.7, 129.3, 128.8, 128.5, 128.4, 128.2, 124.0, 101.3, 101.1, 100.9, 75.6, 75.5, 75.0, 74.7, 74.4, 74.0, 73.5, 73.0, 72.7, 71.2, 70.8, 70.4, 67.9, 67.8, 67.5, 67.1, 66.9, 65.6, 65.5, 56.7, 56.4, 56.1, 21.0, 20.9.

ESI-MS *m/z* $(M + H)^+$ calcd 1435.5397, obsd 1435.5496.

$C_{76}H_{82}N_4O_{24}$ (1434.5319) calcd C 63.59, H 5.76, N 3.90; found C 63.54, H 5.74, N 3.83.

Benzyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-p-nitrobenzyloxycarbonylamino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-p-nitrobenzyloxycarbonyl-amino-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (79)



Coupling of disaccharide acceptor **61** (0.260 g, 0.258 mmol) and *N*-PNZ trichloroacetimidate donor **34** (0.378 g, 0.516 mmol) following general procedure **A** for 2 h at -45 to -25 °C using $BF_3{\cdot}Et_2O$ (0.037 g, 0.258 mmol) afforded the trisaccharide **77** as a white solid (0.249g, 61 %) after purification over silica gel chromatography using 40 – 50 % EtOAc/Hexane. Following the general procedure **C** trisaccharide (0.222 g, 0.141 mmol) was delevulinated and then product purified using silica gel chromatography (2 – 3 % EtOH/$CHCl_3$) to afford the trisaccharide acceptor **79 (AAD-*O*-Bn)** as a white solid (0.191 g, 92%).

R_f 0.43 (8 % EtOH/$CHCl_3$), m.p. 88.0 – 90.0, $[\alpha]^{24}_D$ -43.59 (*c* = 0.66).

IR (NaCl Plates) 3341, 1720, 1519 cm^{-1}.

^1H NMR (300 MHz, CD_2Cl_2) δ 8.26 (d, *J* = 9.0 Hz, 2H), 8.22 (d, *J* = 9.0 Hz, 2H), 7.33 – 7.52 (m, 29H), 5.09 – 5.27 (m, 6H), 5.03 (d, *J* = 10.8 Hz, 1H), 4.97 (d, *J* = 8.7 Hz, 1H), 4.90 (d, *J* = 11.3 Hz, 2H), 4.73 (d, *J* = 9.0 Hz, 1H), 4.67 (d, *J* = 11.4 Hz, 2H), 4.62 (d, *J* = 11.7 Hz, 2H), 4.55 (d, *J* = 3.3 Hz, 2H), 4.50 (d, *J* = 4.8 Hz, 1H), 4.44 (d, *J* = 6.3 Hz, 2H), 4.34 (d, *J* = 12.0

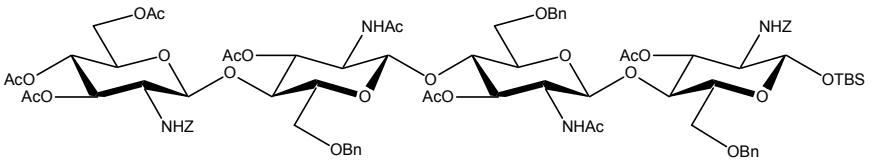
Hz, 2H), 4.28 (d, *J* = 7.5 Hz, 1H), 3.79 – 3.96 (m, 2H), 3.60 – 3.79 (m, 8H), 3.21 – 3.47 (m, 5H), 2.94 (d, *J* = 3.0 Hz, 1H), 2.01 (s, 6H), 1.93 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.7, 171.3, 171.1, 156.3, 155.6, 155.5, 148.0, 147.9, 144.5, 144.4, 138.3, 138.1, 137.8, 137.7, 137.2, 129.3, 129.1, 129.0, 128.8, 128.7, 128.5, 128.3, 128.2, 128.1, 128.0, 124.1, 124.0, 101.0, 100.8, 100.7, 75.7, 75.5, 75.4, 74.9, 74.8, 74.4, 74.2, 73.8, 73.6, 72.9, 72.7, 71.1, 70.5, 70.2, 67.9, 67.7, 67.0, 65.6, 56.7, 56.3, 56.2, 21.0, 20.9, 20.8.

ESI-MS *m/z* $(M + H)^+$ calcd 1480.5248, obsd 1480.5172.

$C_{76}H_{81}N_5O_{26}$ (1479.5170) calcd C 61.66, H 5.51, N 4.73; found C 61.43, H 5.39, N 4.69.

tert-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (80)



Compound **51** (0.237 g, 0.123 mmol) was dissolved in 9 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.857 g, 4.92 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (2 - 4 % EtOH/$CHCl_3$) to afford the compound **80** as a white solid (0.14 g, 72 % yield).

R_f 0.57 (100 % EtOAc), m.p. 121.0 – 123.0, $[\alpha]^{24}_D$ -23.72 (*c* = 0.85, $CHCl_3$).

IR (NaCl Plates) 2860, 1743, 1522 cm^{-1}.

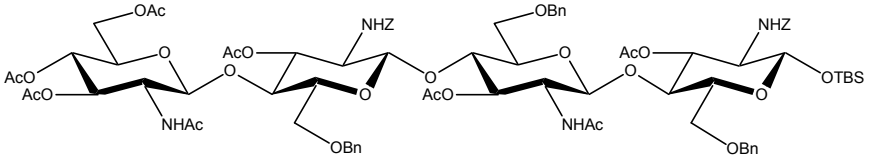
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 – 7.44 (m, 25 H), 5.15 (bs, 1H), 5.07 (AB Quartet, *J* = 12.0 Hz, 4H), 4.98 (dd, *J* = 10.0, 10.0 Hz, 1H), 4.84 – 4.90 (m, 3H), 4.79 (d, *J* = 12.0 Hz, 1H), 4.73 (d, *J* = 11.5 Hz, 2H), 4.63 (dd, *J* = 9.5, 10.0 Hz, 2H), 4.57 (d, *J* = 7.5 Hz, 2H), 4.42 (d, *J* = 12.0 Hz, 1H), 4.31 (dd, *J* = 12.0, 4.5 Hz, 1H), 4.27 (d, *J* = 11.5 Hz, 2H), 4.19 (d, *J* = 12.0 Hz, 2H), 4.09 (d, *J* = 8.0 Hz, 1H), 3.92 (dd, *J* = 10.5, 2.0 Hz, 2H), 3.85 (d, *J* = 9.5 Hz, 1H), 3. 80 (ddd, *J* = 4.0, 9.5, 9.5 Hz, 2H), 3.74 (dd, *J* = 18.5, 9.5 Hz, 1H), 3.68 (d, *J* = 8.5 Hz, 1H), 3.54 – 3.66 (m, 5H), 3.46 (d, *J* = 9.5 Hz, 1H), 3.42 (d, *J* = 9.0 Hz, 2H), 3.30 – 3.37 (m, 2H), 3.24 (d, *J* = 8.5 Hz, 1H), 2.99 (d, *J* = 9.5 Hz, 1H), 2.05 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.91 (s, 3H), 1.72 (s, 3H), 1.68 (s, 3H), 0.87 (s, 9H), 0.11 (s. 3H), 0.06 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.8, 170.4, 170.3, 169.8, 169.7, 169.4, 156.0, 155.3, 137.7, 137.2, 136.5, 136.4, 129.1, 129.0, 128.8, 128.6, 128.4, 128.1, 127.9, 101.0, 100.5, 100.2, 96.7, 75.2, 74.4, 74.1, 74.0, 73.9, 73.8, 73.7, 73.5, 73.1, 73.0, 72.6, 72.3, 72.2, 71.3, 68.3, 67.8, 67.4, 67.0, 66.8, 66.6, 61.7, 57.8, 56.0, 54.0, 53.8, 25.5, 23.1, 23.0, 20.6, 20.5, 20.4, 17.9, -4.2, -5.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1651.6790, obsd 1651.6705.

$C_{83}H_{106}N_4O_{29}Si$ (1650.6712) calcd C 60.35, H 6.47, N 3.39; found C 60.01, H 6.56, N 3.44.

tert-Butyldimethylsilyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (81)



Compound **52** (0.115 g, 0.06 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.415 g, 2.4 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (3 – 5 % EtOH/$CHCl_3$) to afford the compound **81** as a white solid (0.08 g, 81 % yield).

R_f 0.41 (100 % EtOAc), m.p. 127.0 – 1280, $[\alpha]^{24}_D$ -28.07 (*c* = 0.75 $CHCl_3$).

IR (NaCl Plates) 2860, 1742 1521cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (m, 25 H), 5.04 – 5.09 (m, 4H), 4.86 – 4.98 (m, 4H), 4.82 (d, *J* = 12.5 Hz, 1H), 4.76 (d, *J* = 7.0 Hz, 1H), 4.71 (d, *J* = 10.0 Hz, 1H), 4.68 (d, *J* = 7.0 Hz, 1H), 4.60 – 4.64 (m, 2H), 4.56 (d, *J* = 8.5 Hz, 1H), 4.48 (d, *J* = 9.0 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 1H), 4.36 (d, *J* = 8.5 Hz, 1H), 4.32 (dd, *J* = 12.0, 4.5 Hz, 1H), 4.28 (d, *J* = 12.0 Hz, 1H), 4.18 (d, *J* = 12.0 Hz, 2H), 4.05 (d, *J* = 7.5 Hz, 1H), 3.94 (dd, *J* = 12.0, 2.0 Hz, 2H), 3.84 (dd, *J* = 9.0, 9.5 Hz, 1H), 3.87 (ddd, *J* = 9.5, 9.5, 10.0 Hz, 3H), 3.69 – 3.73 (m, 1H), 3.67 (d, *J* = 9.5 Hz, 1H), 3.61 – 3.64 (m, 1H), 3.52 – 3.57 (m, 3H), 3.30 – 3.45 (m, 5H), 3.04 (dd, *J* = 8.5, 10.5 Hz, 2H), 2.06 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.91 (s, 3H), 1.72 (s, 3H), 1.70 (s, 3H), 0.87 (s, 9H), 0.12 (s, 3H), 0.06 (s, 3H).

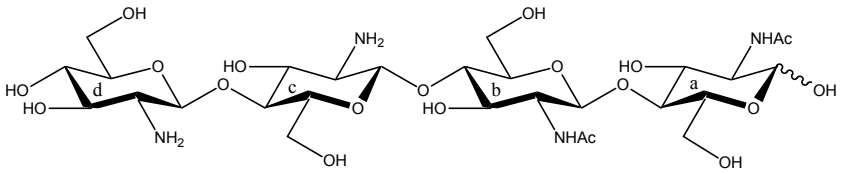
^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.6, 170.5, 170.4, 169.7, 169.4, 169.3, 156.0, 155.6, 137.6, 137.4, 137.0, 136.5, 136.4, 129.3, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 101.0, 100.7, 99.8, 97.0, 75.3, 75.2, 74.6, 74.1, 73.9, 73.8, 73.5, 73.4,

73.3, 73.2, 72.5, 72.4, 72.3, 71.4, 68.3, 67.7, 67.2, 67.1, 66.7, 66.2, 61.8, 57.8, 56.0, 54.5, 54.0, 25.6, 23.2, 23.1, 20.7, 20.6, 20.5, 20.4, 17.9, -4.2, -5.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1651.6790, obsd 1651.6770

$C_{83}H_{106}N_4O_{29}Si$ (1650.6712) calcd C 60.35, H 6.47, N 3.39; found C 59.98, H 6.56, N 3.41.

2-Amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl–(1→4)-2-acetamido-2-deoxy-α/β-D-glucopyranose (5, DDAA)



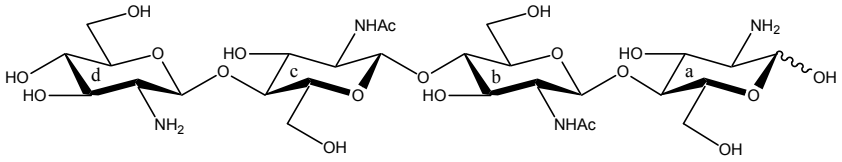
Following the general experimental procedure **F** protected tetrasaccharide **70** (0.027 g, 0.017 mmol) was deprotected to yield the free tetrasaccharide **5** as a colorless weightless solid (11.4 mg, 92 %).

^{1}H NMR (500 MHz, D_2O) δ 5.19 (d, J = 2.5 Hz, 0.6 H, H-1_aα), 4.70 (d, J = 8.0 Hz, 0.4H, H-1_aβ), 4.64 (d, J = 7.5 Hz, 1H, H-1_bβ), 4.61 (d, J = 7.0 Hz, 1H, H-1_cβ), 4.60 (d, J = 8.0 Hz, 1H, H-1_dβ), 3.88 – 3.94 (m, 6H), 3.73 – 3.83 (m, 9H), 3.61 – 3.70 (m, 6H), 3.42 – 3.53 (m, 4H), 2.80 (ddd, J = 7.5, 8.0, 9.0 Hz, 2H, H-2_c, H-2_d), 2.07 (s, 3H), 2.04 (s, 3H).

^{13}C NMR (75 MHz, D_2O) δ 175.2, 175.0, 174.9, 101.7 (J_{C1bH1b} = 163.0), 101.0 (J_{C1cH1c} = 163.7), 100.9 (J_{C1dH1d} = 163.6), 95.2 (J_{C1aH1a} = 163.0 (β)), 90.9 (J_{C1aH1a} = 172.7 (α)), 80.1, 79.7, 77.8, 76.7, 75.3, 75.0, 74.3, 73.1, 72.9, 72.2, 70.4, 70.3, 69.7, 61.9, 60.9, 60.5, 60.4, 56.7, 56.6, 55.8, 54.1, 53.2, 22.6, 22.5, 22.3.

ESI-MS *m/z* $(M + H)^+$ calcd 747.3148, obsd 747.3173.

2-Amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy- α/β -D-glucopyranose (6, DAAD)



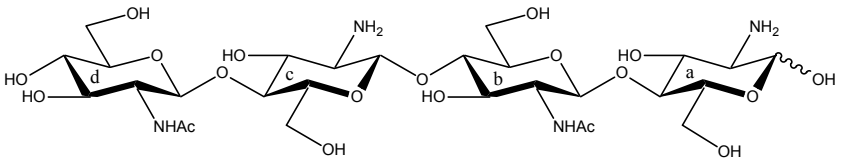
Following the general experimental procedure **F** protected tetrasaccharide **71** (0.021 g, 0.013 mmol) was deprotected to yield the free tetrasaccharide **6** as a colorless weightless solid (8.62 mg, 86 %).

^{1}H NMR (500 MHz, D_2O) δ 5.36 (d, *J* = 3.0 Hz, 0.5 H, H-1$_a$α), 4.60 (d, *J* = 7.5 Hz, 1H, H-1$_b$β), 4.57 (d, *J* = 7.5 Hz, 1H, H-1$_c$β), 4.56 (d, *J* = 8.0 Hz, 1H, H-1$_d$β), 3.88 – 3.95 (m, 5H), 3.71 – 3.86 (m, 12H), 3.61 – 3.67 (m, 7H), 3.49 – 3.57 (m, 4H), 3.40 – 3.47 (m, 3H), 3.15 (d, J = 10.0 Hz, 0.6H), 2.76 – 2.82 (m, 2H), 2.07 (s, 3H), 2.06 (s, 3H).

^{13}C NMR (75 MHz, D_2O) δ 175.0, 101.8 (J_{C1bH1b} = 163.9), 101.7 (J_{C1cH1c} = 164.3), 101.6 (J_{C1dH1d} = 163.0), 94.9 (J_{C1aH1a} = 164.4 (β)), 90.2 (J_{C1aH1a} = 173.4 (α)), 79.7, 79.6, 79.4, 78.2, 76.7, 75.2, 75.0, 74.9, 72.5, 72.3, 70.7, 70.0, 61.0, 60.6, 60.4, 60.3, 56.1, 55.8, 55.6, 55.5, 22.6, 22.5.

ESI-MS *m/z* $(M + H)^+$ calcd 747.3148, obsd 747.3136.

2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl–(1→4)-2-amino-2-deoxy- α/β -D-glucopyranose (7, ADAD)



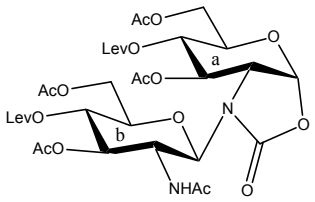
Following the general experimental procedure **F** protected tetrasaccharide **72** (0.021 g, 0.013 mmol) was deprotected to yield the free tetrasaccharide **7** as a colorless weightless solid (8.52 mg, 85 %).

^{1}H NMR (500 MHz, D_2O) δ 5.37 (d, *J* = 3.5 Hz, 0.6H, H-1$_a$α), 4.59 (d, *J* = 8.5 Hz, 1H, H-1$_b$), 4.58 (d, *J* = 7.0 Hz, 1H, H-1$_c$), 4.57 (d, *J* = 8.0 Hz, 1H, H-1$_d$), 3.90 – 97 (m, 4H), 3.73 – 3.86 (m, 11H), 3.56 – 3.68 (m, 13H), 3.44 – 3.51 (m, 3H), 3.19 (dd, *J* = 10.0, 3.5 Hz, 0.7H, H-2$_a$α), 2.85 (d, *J* = 8.0 Hz, 1H), 2.81 (dd, *J* = 9.0, 10.0 Hz, 1H), 2.07 (s, 6H).

^{13}C NMR (75 MHz, D_2O) δ 175.0, 102.0 (J_{C1bH1b} = 163.5), 101.8 (J_{C1cH1c} = 162.5), 101.7 (J_{C1dH1d} = 162.6), 94.9 (J_{C1aH1a} = 162.6 (β)), 90.2 (J_{C1aH1a} = 173.4 (α)), 79.5, 79.3, 79.0, 78.0, 76.3, 75.2, 75.0, 73.9, 72.2, 70.5, 70.2, 69.7, 61.9, 61.0, 60.6, 60.4, 60.3, 57.1, 56.5, 56.0, 55.8, 22.5.

ESI-MS *m/z* $(M + H)^+$ calcd 747.3148, obsd 747.3152.

2-(2-Acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-levulinyl-β-D-carboxylamino)-2-deoxy-α-D-glucopyranose Intramolecular 2,1-Ester-3,6-di-*O*-acetyl-4-*O*-levulinate (85)



Compound **83** (0.044 g, 0.046 mmol) was treated with $Pd(OH)_2$ (0.02 g, 0.14 mmol) in 3 ml of MeOH/AcOH (9:1) and then flushed with hydrogen atmosphere. Then it was stirred for overnight under a hydrogen atmosphere. The solution was filtered using celite and washed with MeOH and concentrated under reduced pressure to yield the crude compound. The crude compound was redissolved in 5 ml of DCM and then treated with Ac_2O/pyridine 1.5 ml (1:2) and stirred for overnight. Then solvents were removed under reduced pressure and the excess reagent was removed by coevaporation with toluene. The resulted residue was purified using silica gel column chromatography (3 – 5 % EtOH/$CHCl_3$) to yield the compound **85** as a white solid (0.020 g, 57 % yield).

R_f 0.27 (100 % EtOAc), m.p. 101.0 – 102.0, $[\alpha]^{24}_D$ -6.82 (*c* = 0.85 $CHCl_3$).

IR (NaCl Plates) 2928, 1739, 1539, 1365, 1221 cm^{-1}.

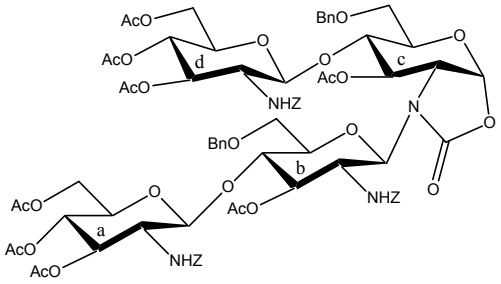
^{1}H NMR (500 MHz, CD_2Cl_2) δ 5.85 (d, $J_{1a,2a}$ = 7.5 Hz, 1H, H-1_a), 5.40 (d, $J_{NH,2b}$ = 9.3 Hz, 1H, NH_b), 5.49 (dd, $J_{3a,2a}$ = 4.5, $J_{3a,4a}$ = 2.5 Hz, 1H, H-3_a), 5.17 (d, $J_{1b,2b}$ = 9.5 Hz, 1H, H-1_b), 5.16 (dd, $J_{3b,2b}$= $J_{3b,4b}$ = 9.5 Hz, 1H, H-3_b), 5.09 (dd, J_{4b3b} = J_{4b5b} = 9.5 Hz, 1H, H-4_b), 4.93 (ddd, J_{4a3a} = 7.0, J_{4a5a} = 4.0, J_{4a2a} = 1.0 Hz, 1H, H-4_a), 4.57 (ddd, J_{2a1a} = 7.5, J_{2a3a} = 3.0, J_{2a4a} = 1.0 Hz, 1H, H-2_a), 4.35 (ddd, J_{2b1b} = 9.0, J_{2b3b} = 10.0, $J_{2b,NH}$ = 10.0 Hz, 1H, H-2_b), 4.22 – 4.26 (m, 2H, H-6_a [A,B]), 4.05 – 4.12 (m, 3H, H-6_b [A,B], H-5_a), 3.79 (ddd, J_{5b4b} = 9.5, $J_{5b,6Ab}$, = 4.0, $J_{5b,6Bb}$ = 2.5 Hz, 1H, H-5_b), 2.62 – 2.84 (m, 4H, CH_2-CO (Lev)), 2.39 - 2.58 (m, 4H, CH_2-CO-O (Lev)), 2.14 (6H, CH_3-CO (Lev)), 2.13 (3H, CH_3-CO), 2.08 (3H, CH_3-CO), 2.06 (3H, CH_3-CO), 2.02 (3H, CH_3-CO), 1.88 (3H, CH_3-CONH).

^{13}C NMR (300 MHz, CD_2Cl_2) δ 206.0, 205.9 (*CO*-CH_2 (Lev)), 171.7, 171.4, 171.1, 170.9, 170.8, 170.6, 169.0 (2-*CO*-O-CH_2 (Lev), 4-*CO*-CH_3, 1-NH-*CO*-CH_3), 155.3 (N-*C*O-O), 95.2 (C-1_a), 83.7 (C-1_b), 74.6 (C-5_b), 72.8 (C-3_b), 69.3 (C-5_a), 68.8 (C-3_a), 67.9 (C-4_b), 67.6 (C-4_a), 63.4 (C-6_a), 62.1 (C-6_b), 53.2 (C-2_a), 51.0 (C-2_b), 38.0, 37.9 (*CH*$_2$-C0-Lev), 29.8, 29.7 (*C*H$_3$-CO (Lev)), 28.1, 28.0 (*C*H$_2$-CO-O (Lev)), 23.2 (NH-CO-*CH*$_3$), 21.1, 20.9, 20.8, 20.7 (*CH*$_3$-CO).

ESI-MS *m/z* $(M + H)^+$ calcd 773.2617, obsd 773.2569

$C_{33}H_{44}N_2O_{19}$ (772.2538) calcd C 51.29, H 5.74, N 3.63; found C 51.61, H 5.86, N 3.63.

2-(3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-carboxylamino)-3,4,6-tri-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-deoxy-α-D-glucopyranose Intramolecular 2,1-Ester-3-*O*-acetyl-6-*O*-benzylate (53)



Coupling of disaccharide acceptor **48** (0.108 g, 0.10 mmol) and disaccharide trichloroacetimidate donor **43** (0.152 g, 0.15 mmol) following general procedure **A** for 4 h at -40 °C to -25 °C using TMSOTf (0.1 M in DCM) (0.0033 g, 0.015 mmol) afforded the tetrasaccharide byproduct (**53**) as a white solid (0.072 g, 45 %) after purification over silica gel column chromatography using 40 – 60 % EtOAc/Hexane.

R_f 0.14 (50 % EtOAc/Hexane), m.p. 99.0 – 101.0, $[\alpha]^{24}_D$ -19.09 (*c* = 1.25, $CHCl_3$).

IR (NaCl Plates) 1744, 1520, 1455, 1366, 1223 cm^{-1}.

^1H NMR (500 MHz, CD_2Cl_2) δ 7.28 (m, 25 H), 5.65 (d, *J* = 5.0 Hz, 1H, H-1_c), 5.36 (bs, 1H), 5.28 (bs, 1H), 4.99 – 5.04 (m, 6H), 4.95 (dd, *J* = 10.0, 10.0 Hz, 2H), 4.90 (dd, *J* = 9.0, 9.5 Hz, 2H), 4.77 (d, *J* = 10.0 Hz, 2H, H-1_b, H-1_d), 4.71 (d, *J* = 11.5 Hz, 1H), 4.51 (d, *J* = 11.5 Hz, 2H), 4.43 (d, *J* = 8.0 Hz, 1H, H-1_a), 4.41 (s, 1H), 4.37 (d, *J* = 9.5 Hz, 1H), 4.33 (d, *J* = 4.5 Hz, 2H), 4.30 (d, *J* = 4.5 Hz, 2H), 4.25 (bs, 1H), 3.89 – 4.00 (m, 5H), 3.74 (bs, 1H), 3.55 (bs, 2H), 3.41 – 3.50 (m, 5H), 3.33 (d, *J* = 9.0 Hz, 1H), 3.24 (d, *J* = 7.5 Hz, 1H), 2.02 (s, 6H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.89 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 170.5, 170.4, 170.2, 169.5, 169.4, 169.0, 168.9, 156.1, 155.4, 137.8, 137.5, 136.6, 136.3, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 127.6, 126.1, 100.1, 99.0, 95.7, 83.2, 74.2, 73.7, 73.3, 73.2, 72.0, 71.8, 71.4, 71.3, 70.7, 68.8, 68.4, 67.5, 66.9, 66.8, 66.7, 62.0, 61.7, 56.3, 56.1, 54.2, 52.2, 20.9, 20.7, 20.6, 20.5, 20.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1607.5616, obsd 1607.5563

$C_{79}H_{90}N_4O_{32}$ (1606.5538) calcd C 59.02, H 5.64, N 3.49; found C 58.74, H 5.75, N 3.63.

Solid Phase Synthesis

General procedure G (Glycosylation reaction)

Resin (200 mg) or acceptor bound resin was swollen in a solution of 3.0 equiv. donor (azeotropically dried by co-evaporation with toluene) in 1:1 mixture of DCM/Hexane (2ml/100 mg resin) and shaken for 15 min at appropriate temperature. Then $BF_3{\cdot}Et_2O$ (1.0 equiv) was added and the reaction mixture was shaken for 1 h. The resin was then washed with 3 x 10 ml each: DCM, THF. The resin was dried under vacuum for one hour prior to second glycosylation. After the second glycosylation using the above conditions the resin was washed with 3 x 10 ml each: DCM, 10% MeOH/DCM, THF and then dried under vacuum for one hour prior to deprotection.

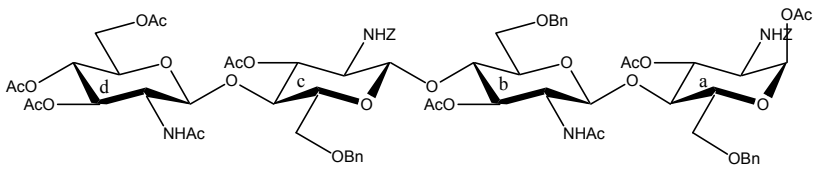
General Procedure H (Levulinoyl ester deprotection)

Levulinoyl ester protected sugar bound resin was swollen in DCM 2 ml and then hydrazine acetate (20 equiv.) dissolved in 2ml MeOH was added and shaken for 1 h. The resin was then washed with 3 x 5 ml each: 20% MeOH/DCM, MeOH, DCM, THF. The resin was dried under vacuum for one hour prior to second deprotection. After the second deprotection using the same condition and washing the resin dried under vacuum for overnight prior to the next glycosylation.

General Procedure I (Cleavage of resin)

Carbohydrate bound resin (300 – 400 mg) was swollen in DCM 5 ml and then 0.5 ml of TFA (~10 %) was added and shaken for 1 h. The resin was washed with 3 x 10 ml each: DCM, THF. The combined washings were treated with sat. aq. $NaHCO_3$ and then organic layer separated. The aqueous layer back extracted with DCM (2 x 15 ml) and then the combined organic extracts were dried over $Na_2S_2O_4$, filtered, concentrated under reduced pressure to yield the crude product.

Acetyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (89)



Step -1:

According to general procedure **G** Wang resin (0.2 g, 0.12mmol, 0.6mmol/g loading) was glycosylated with donor **22** (0.248 g, 0.36 mmol) using $BF_3{\cdot}Et_2O$ (0.017 g, 0.13 mmol). The resin was then washed and then glycosylated second time. After washing, the resin was swollen in 4ml of DCM and then treated with Ac_2O (0.245g, 2.4 mmol) and pyridine (0.380 g, 4.8 mmol) for 1 hour. The resin was then washed with 3 x 5ml each: DCM, 20% MeOH/DCM, 5%AcOH/THF, THF. According to general procedure **H** levulinoyl ester was deprotected twice with hydrazine acetate (0.221 mg, 2.4 mmol).

Step – 2:

The carbohydrate bound resin was glycosylated twice with donor **34** (0.264 g, 0.36 mmol) using general procedure **G** and then deprotection of levulinoyl ester performed using general procedure **H**.

Step – 3:

The carbohydrate bound resin was glycosylated twice with donor **22** (0.248 g, 0.36 mmol) using general procedure **G** and then deprotection of levulinoyl ester performed using general procedure **H**.

Step – 4:

The carbohydrate bound resin was glycosylated twice with donor **27** (0.226 g, 0.36 mmol) using general procedure **G.**

Step – 5:

Cleavage of the carbohydrate bound resin performed according to the general procedure **I**. Then the crude product was purified using silica gel column chromatography (2 % EtOH/$CHCl_3$) to afford the tetrasaccharide (**ADAD**) derivative **86** as a white solid (0.112 g, 52 % for 8 steps, 92 % per step). The MALDI-TOF MS showed that the product is a mixture composed of tetra and trisaccharide derivative. The yield presented above for the tetrasaccharide derivative was calculated using the peak area from the HPLC chromatogram.

Step – 6: (*N*HPNZ → *N*HAc)

Compound **86** (0.058 g, 0.032 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.223 g, 1.28 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (3 – 5 % EtOH/$CHCl_3$) to afford the compound **89** as a white solid (0.037 g, 74 % yield).

R_f 0.19 (100 % EtOAc), m.p. 132.0 – 134.0, $[\alpha]^{24}_D$ -7.78 (*c* = 1.2, $CHCl_3$).

IR (NaCl Plates) 3308, 2864, 1741 cm^{-1}.

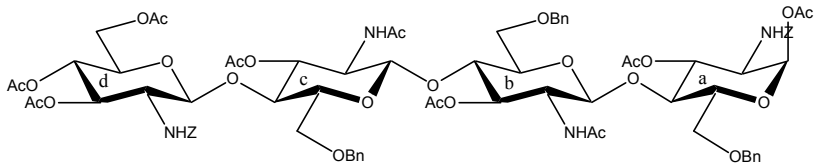
^1H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.51 (m, 25H), 6.19 (d, *J* = 3.5 Hz, 1H, H-1aα), 5.13 (d, *J* = 12.0 Hz, 2H), 5.08 (d, *J* = 11.0 Hz, 2H), 5.04 (d, *J* = 12.0 Hz, 2H), 4.95 (d, *J* = 9.0 Hz, 1H), 4.91 – 4.94 (m, 2H), 4.88 (d, *J* = 13.0 Hz, 1H), 4.70 (d, *J* = 12.0 Hz, 1H), 4.58 (d, *J* = 10.0 Hz, 2H), 4.47 (dd, *J* = 9.0,9.5 Hz, 2H), 4.35 (d, *J* = 8.0 Hz, 1H, H-1b), 4.30 – 4.34 (m, 2H), 4.27 (d, *J* = 11.5 Hz, 1H), 4.18 (d, *J* = 12.0 Hz, 1H), 4.12 (ddd, *J* = 3.5, 10.0, 10.0 Hz, 1H), 4.05 (d, *J* = 8.0 Hz, 1H, H-1c), 3.93 – 3.96 (m, 2H), 3.91 (d, *J* = 9.0 Hz, 1H, H-1d), 3.81 (dd, *J* = 8.5, 3.0 Hz, 1H), 3.75 – 3.80 (m, 2H), 3.71 (d, *J* = 10.0 Hz, 1H), 3.66 (d, *J* = 9.5 Hz, 1H), 3.58 (d, *J* = 10.0 Hz, 1H), 3.51 (bs, 3H), 3.46 (d, *J* = 10.5 Hz, 2H), 3.40 (bs, 3H), 3.03 (bs, 2H), 2.15 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.91 (s, 3H), 1.72 (s, 3H), 1.66 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 171.2, 170.9, 170.7, 170.6, 169.9, 169.8, 169.7, 169.5, 156.1, 155.9, 138.1, 138.0, 137.7, 137.2, 136.9, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.5, 128.3, 101.5, 101.2, 100.2, 91.3, 75.8, 74.9, 74.6, 74.5, 74.4, 74.2, 74.1, 73.6, 73.5, 72.8, 72.7, 72.5, 71.9, 70.9, 68.8, 67.7, 67.6, 67.3, 67.2, 62.1, 56.4, 54.9, 54.4, 54.0, 23.4, 23.3, 21.2, 20.9, 20.8, 20.7.

ESI-MS *m/z* $(M + H)^+$ calcd 1579.6031, obsd 1579.5996.

$C_{79}H_{94}N_4O_{30}$ (1578.5953) calcd C 60.07, H 6.00, N 3.55; found C 60.03, H 5.87, N 3.53.

Acetyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (90)



Step -1:

According to general procedure **G** Wang resin (0.15 g, 0.09mmol, 0.6mmol/g loading) was glycosylated with donor **22** (0.186 g, 0.27 mmol) using $BF_3{\cdot}Et_2O$ (0.013 g, 0.09 mmol). The resin was then washed and then glycosylated second time. After washing, the resin was swollen in 4ml of DCM and then treated with Ac_2O (0.245g, 1.8 mmol) and pyridine (0.380 g, 3.6 mmol) for 1 hour. The resin was then washed with 3 x 5ml each: DCM, 20% MeOH/DCM, 5%AcOH/THF, THF. According to general procedure **H** levulinoyl ester was deprotected twice with hydrazine acetate (0.221 mg, 1.8 mmol).

Step – 2:

The carbohydrate bound resin was glycosylated twice with donor **34** (0.198 g, 0.27 mmol) using general procedure **G** and then deprotection of levulinoyl ester performed using general procedure **H**.

Step – 3:

The carbohydrate bound resin was glycosylated twice with donor **34** (0.198 g, 0.27 mmol) using general procedure **G** and then deprotection of levulinoyl ester performed using general procedure **H**.

Step – 4:

The carbohydrate bound resin was glycosylated twice with donor **12** (0.157 g, 0.27 mmol) using general procedure **G.**

Step – 5:

Cleavage of the carbohydrate bound resin performed according to the general procedure **I**. Then the crude product was purified using silica gel column chromatography (2 % EtOH/$CHCl_3$) to afford the tetrasaccharide derivative (**DAAD**, **87**) (0.064 g, 39 % for 8 steps, 89 % per step). The MALDI-TOF MS showed that the product is a mixture composed of tetra and trisaccharide derivative. The yield presented above for the tetrasaccharide derivative was calculated using the peak area from the HPLC chromatogram.

Step – 6: (*N*HPNZ → *N*HAc)

Compound **87** (0.067 g, 0.037 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v 1:1:1) and then sodium dithionite (0.257 g, 1.476 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (3 – 5 % EtOH/$CHCl_3$) to afford the compound **90** as a white solid (0.038 g, 66 % yield).

R_f 0.32 (100 % EtOAc), m.p. 125.0 – 127.0, $[\alpha]^{24}_D$ -8.12 (*c* = 1.40, $CHCl_3$).

IR (NaCl Plates) 3302, 1741, 1522 cm^{-1}.

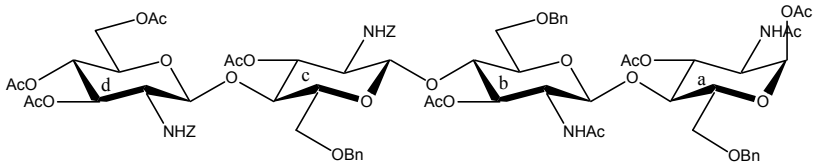
^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.31 – 7.48 (m, 25 H), 6.01 (d, *J* = 4.0 Hz, 1H, H-1aα), 5.13 (dd, *J* = 10.5, 11.5 Hz, 2H), 5.07 – 5.10 (m, 3H), 5.15 (d, *J* = 12.5 Hz, 1H), 4.98 (d, *J* = 10.0 Hz, 1H), 4.90 (d, *J* = 9.0 Hz, 1H), 4.87 (d, *J* = 4.0 Hz, 1H), 4.80 (d, *J* = 10.0 Hz, 1H), 4.76 (d, *J* = 12.0 Hz, 1H), 4.67 – 4.73 (m, 3H), 4.61 (d, *J* = 11.5 Hz, 1H), 4.55 (d, *J* = 9.0 Hz, 1H), 4.37 (d, *J* = 12.0 Hz, 1H), 4.31 (dd, *J* = 12.5, 4.5 Hz, 2H), 4.25 – 4.27 (m, 3H), 4.17 (d, *J* = 8.5 Hz, 1H), 4.08 (dd, *J* = 4.0, 10.0, 11.0 Hz, 2H), 3.92 – 3.98 (m, 2H), 3.73 – 3.85 (m, 4H), 3.54 – 3.66 (m, 4H), 3.36 – 3.49 (m, 4H), 3.27 (d, *J* = 9.5 Hz, 1H), 3.08 (d, *J* = 10.0 Hz, 1H), 2.11 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (s, 6H), 1.90 (s, 3H), 1.67 (s, 3H), 1.66 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.2, 170.9, 170.8, 170.5, 170.3, 169.7, 169.5, 169.4, 169.1, 155.7, 155.3, 137.3, 137.2, 130.0, 136.4, 136.1, 129.4, 129.2, 129.1, 129.0, 128.8, 128-6, 128.5, 128.4, 128.2, 128.1, 128.0, 101.2 (J_{C1bH1b} = 160.0 (β)), 100.5 (J_{C1cH1c} = 163.3 (β)), 99.9 (J_{C1dH1d} = 161.0 (β)), 91.0 (J_{C1aH1a} = 176.1 (α)), 77.2, 74.2, 74.1, 74.0, 73.9, 73.8, 73.7, 73.5, 73.1, 73.0, 72.2, 72.1, 71.3, 70.6, 68.3, 67.2, 67.1, 67.0, 66.8, 66.5, 61.7, 56.0, 54.0, 53.7, 53.1, 23.1, 23.0, 21.0, 20.7, 20.6, 20.5, 20.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1579.6031, obsd 1579.5961.

$C_{79}H_{94}N_4O_{30}$ (1578.5953) calcd C 60.07, H 6.00, N 3.55; found C 59.75, H 5.87, N 3.50.

Acetyl 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (91)



Step - 1:

According to general procedure **G** Wang resin (0.15 g, 0.09mmol, 0.6mmol/g loading) was glycosylated with donor **34** (0.198 g, 0.27 mmol) using $BF_3{\cdot}Et_2O$ (0.013 g, 0.09 mmol). The resin was then washed and then glycosylated second time. After washing, the resin was swollen in 4ml of DCM and then treated with Ac_2O (0.245g, 1.8 mmol) and pyridine (0.380 g, 3.6 mmol) for 1 hour. The resin was then washed with 3 x 5ml each: DCM, 20% MeOH/DCM, 5%AcOH/THF, THF. According to general procedure **H** levulinoyl ester was deprotected twice with hydrazine acetate (0.221 mg, 1.8 mmol).

Step – 2:

The carbohydrate bound resin was glycosylated twice with donor **34** (0.198 g, 0.27 mmol) using general procedure **G** and then deprotection of levulinoyl ester performed using general procedure **H**.

Step – 3:

The carbohydrate bound resin was glycosylated twice with donor **22** (0.186 g, 0.27 mmol) using general procedure **G** and then deprotection of levulinoyl ester performed using general procedure **H**.

Step – 4:

The carbohydrate bound resin was glycosylated twice with donor **12** (0.157 g, 0.27 mmol) using general procedure **G.**

Step – 5:

Cleavage of the carbohydrate bound resin performed according to the general procedure **I**. Then the crude product was purified using silica gel column chromatography (2 % EtOH/$CHCl_3$) to afford the tetrasaccharide derivative (**DDAA**, **88**) (0.073 g, 45 % for 8 steps, 91 % per step). The MALDI-TOF MS showed that the product is a mixture composed of tetra and trisaccharide derivative. The yield presented above for the tetrasaccharide derivative was calculated using the peak area from the HPLC chromatogram.

Step – 6: (*N*HPNZ → *N*HAc)

Compound **88** (0.053 g, 0.029 mmol) was dissolved in 6 ml of CH_3CN:EtOH:H_2O (v:v:v: 1:1:1) and then sodium dithionite (0.203 g, 1.168 mmol) was added and the reaction performed according to the general procedure **B**. Finally the residue was purified by silica gel column chromatography (4 – 6 % EtOH/$CHCl_3$) to afford the compound **91** as a white solid (0.030 g, 65 % yield).

R_f 0.22 (100 % EtOAc), m.p. 123.0 – 125.0, $[\alpha]^{24}_D$ -10.20 (*c* = 1.2, $CHCl_3$).

IR (NaCl Plates) 3306, 1743, 1519 cm^{-1}.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.30 – 7.45 (m, 25H), 6.07 (d, *J* = 3.5 Hz, 1H, H-1aα), 5.62 (d, *J* = 9.0 Hz, 1H), 5.14 (dd, *J* = 11.0, 9.5 Hz, 1H), 5.06 (bs, 2H), 5.04 (d, *J* = 4.0 Hz, 2H), 4.86 – 4.89 (m, 2H), 4.79 (d, *J* = 12.0 Hz, 1H), 4.69 (dd, *J* = 9.0, 10.0 Hz, 2H), 4.61 (dd, *J* = 11.5, 12.5 Hz, 2H), 4.33 – 4.39 (m, 2H), 4.29 (dd, *J* = 12.0, 4.0 Hz, 2H), 4.24 (dd, *J* = 11.5, 4.5 Hz, 3H), 4.19 (d, *J* = 8.0 Hz, 1H), 4.05 (bs, 2H), 3.97 (d, *J* = 9.5 Hz, 1H), 3.92 (d, *J* = 12.0 Hz, 1H), 3.81 (dd, *J* = 9.5, 10.0 Hz, 2H), 3.72 – 3.78 (m, 2H), 3.61 (dd, *J* = 11.5, 2.5 Hz, 1H), 3.43 – 3.55 (m, 6H), 3.36 (bs, 3H), 3.13 (d, *J* = 11.0 Hz, 1H), 3.00 (d, *J* = 10.0 Hz, 1H), 2.12 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H), 1.90 (s, 6H), 1. 66 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.9, 171.0, 170.5, 170.4, 170.3, 170.0, 169.7, 169.4, 169.1, 155.5, 155.3, 137.3, 137.1, 136.9, 136.6, 136.5, 129.3, 129.1, 129.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 101.4 (J_{C1bH1b} = 162.1 (β)), 100.9 (J_{C1cH1c} = 161.4 (β)), 100.0 (J_{C1dH1d} = 162.4 (β)), 90.9 (J_{C1aH1a} = 177.4 (α)), 77.2, 74.3, 74.0, 73.9, 73.6, 73.3, 73.2, 73.0, 72.5, 72.4, 72.2, 72.1, 71.3, 70.6, 68.4, 67.0, 66.8, 66.6, 66.5, 66.4, 61.8, 56.0, 55.9, 53.7, 51.3, 23.1, 23.0, 21.7, 20.7, 20.6, 20.5, 20.4.

ESI-MS *m/z* $(M + H)^+$ calcd 1579.6031, obsd 1579.6100.

$C_{79}H_{94}N_4O_{30}$ (1578.5953) calcd C 60.07, H 6.00, N 3.55; found C 59.80, H 6.09, N 3.56.

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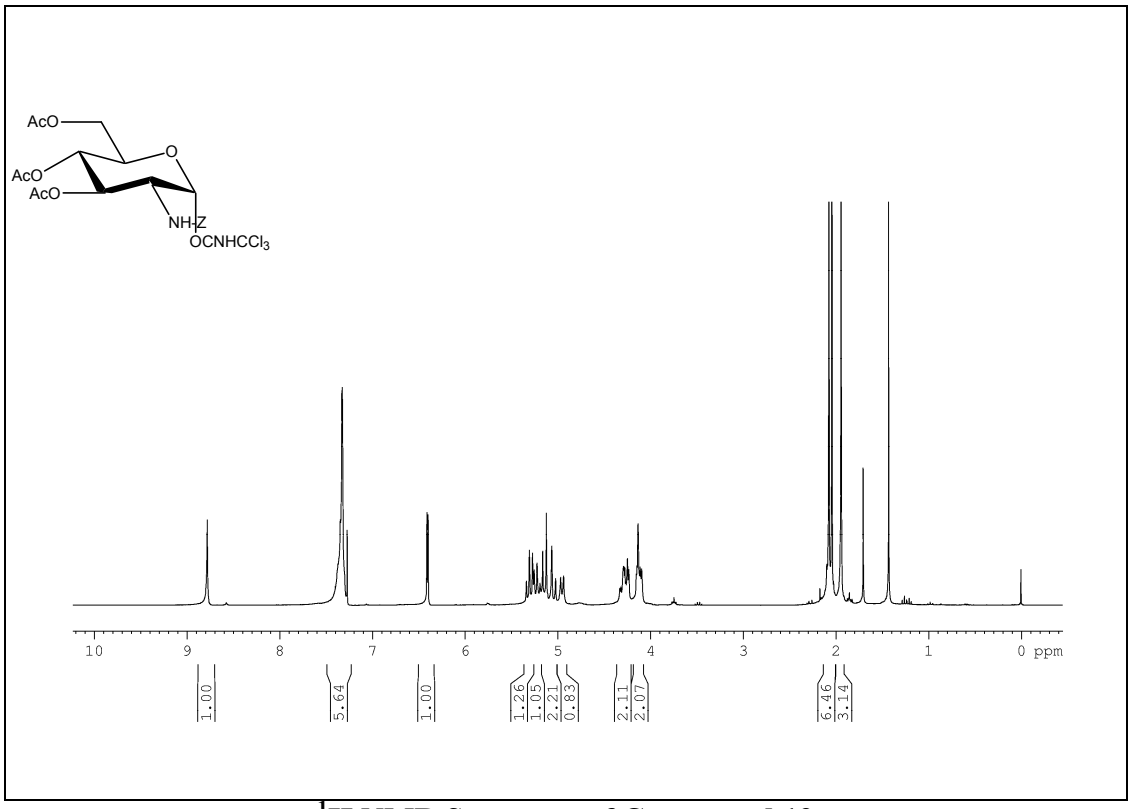
7.1 Abbreviations

Ac_2O	acetic anhydride
AcOH	acetic acid
Ac	acetyl
AIBN	2,2'-azo-bis(isobutyronitrile)
Alloc	allyloxycarbonyl
APT	attached proton test
aq	aqueous
$BF_3 \cdot OEt_2$	boron trifluoride diethylether complex
Boc	*tert*-butoxycarbonyl
bs	broad singlet
°C	degree celcius
CAN	ceric ammonium nitrate
Cbz (or) Z	benzyloxycarbonyl
COs	chitooligosaccharides
COSY	correlation spectroscopy
CPG	controlled pore glass
CSA	camphorsulphonic acid
d	doublet
DBU	1,8-diaza[5.4.0]bicycloundec-7-ene
DCM	dichloromethane
dd	double doublet
DEPT	distortionless enhancement by polarization transfer
DHB	2,5-dihydroxybenzoic acid
DIPC	*N,N'*-diisopropylcarbodiimide
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMM	dimethylmaleimide
DP	degree of polymerization
Dts	dithiasuccinoyl
equiv	equivalent
ESI	electronspray ionization
EtOAc	ethyl acetate

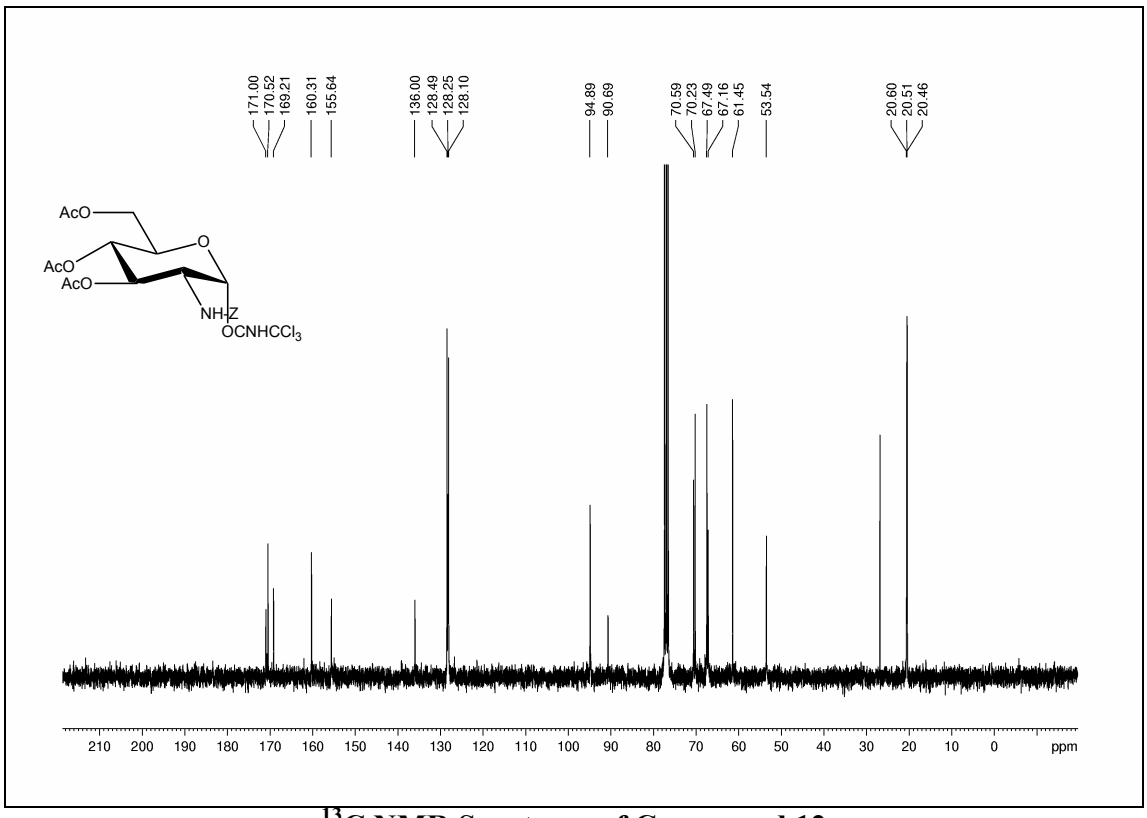
EtOH	ethanol
F_A	degree of acetylation
Glc	glucose
GlcN (**D**)	2-amino-2-deoxy-β-D-glucopyranose
GlcNAc (**A**)	2-acetamido-2-deoxy-β-D-glucopyranose
h	hour
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum correlation
HPLC	high-performance liquid chromatography
Hz	hertz
IR	infrared
J	coupling constant
LCOs	lipochitooligosaccharides
Lev	levulinoyl
LMWC	low molecular weight chitosan
M	molar
m	multiplet
MeOH	methanol
m.p.	melting point
MALDI-TOF	matrix assisted laser desorption ionization - time of flight
Me	methyl
min	minute
mmol	millimol
MS	mass spectroscopy
NaH	sodium hydride
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
PEG	poly-ethylene glycol
pent	pentenyl
Phth	phthaloyl
PMB	*p*-methoxybenzyl
PNZ	*p*-nitrobenzyloxycarbonyl
PS	polystyrene
ppm	parts per million

py	pyridine
rt	room temperature
s	singlet
SPS	solid-phase synthesis
SPOS	solid-phase oligosaccharide synthesis
t	triplet
TBABr	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBDMS (or) TBS	*tert*-butyldimethylsilylchloride
TCP	tetrachlorophthaloyl
TES	triethylsilane
TFA	trifluoroacetic acid
TESOTf	triethylsilyl trifluromethanesulfonate
TfOH	triflic acid
THAP	2,4,6-trihydroxyacetophenone
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethyl silane
TMSOTf	trimethylsilyl trifluromethanesulfonate
Troc	trichloroethoxycarbonyl
δ	chemical shift
[α]	specific rotation

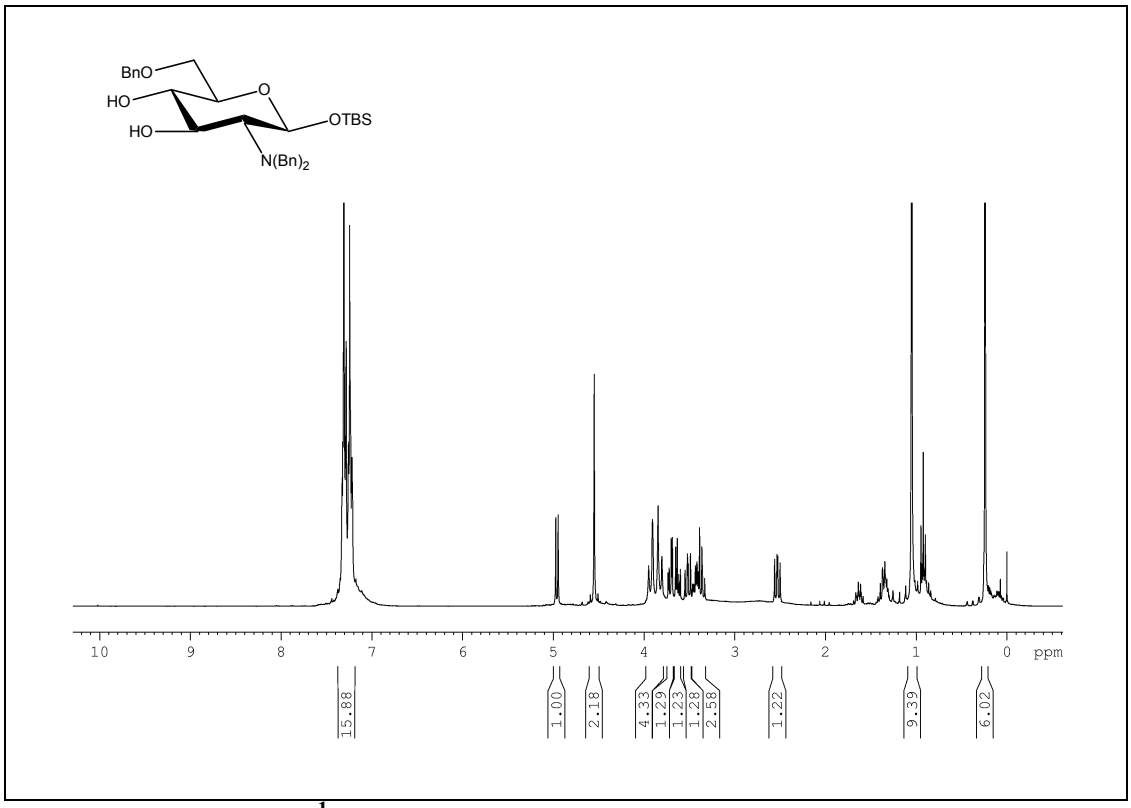
7.2 ^{1}H, ^{13}C, 2D, MALDI Spectra



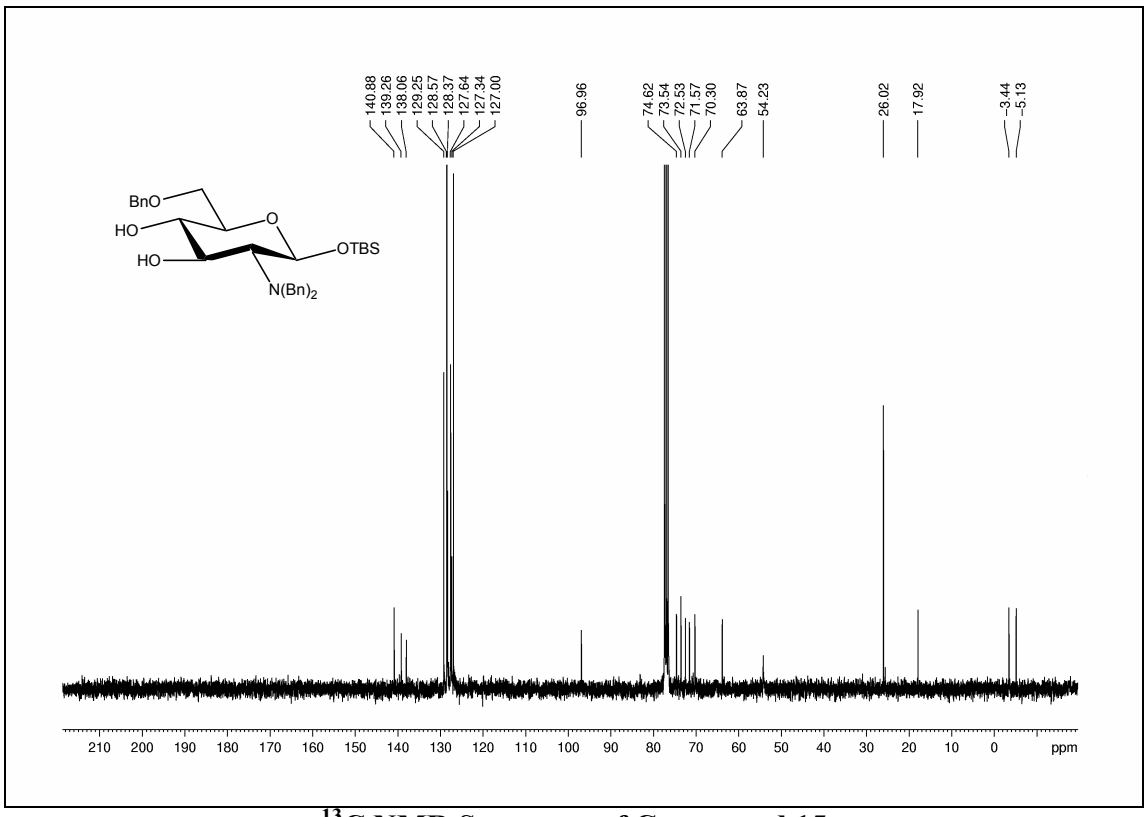
^{1}H NMR Spectrum of Compound 12



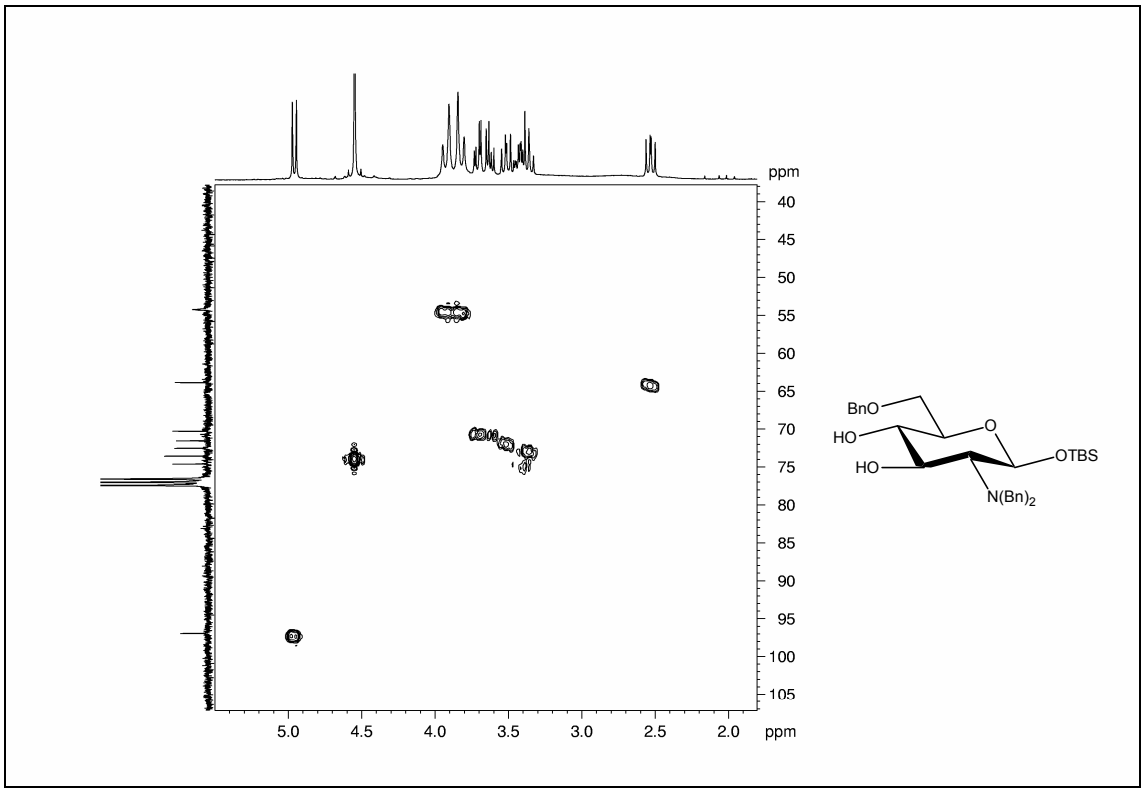
^{13}C NMR Spectrum of Compound 12



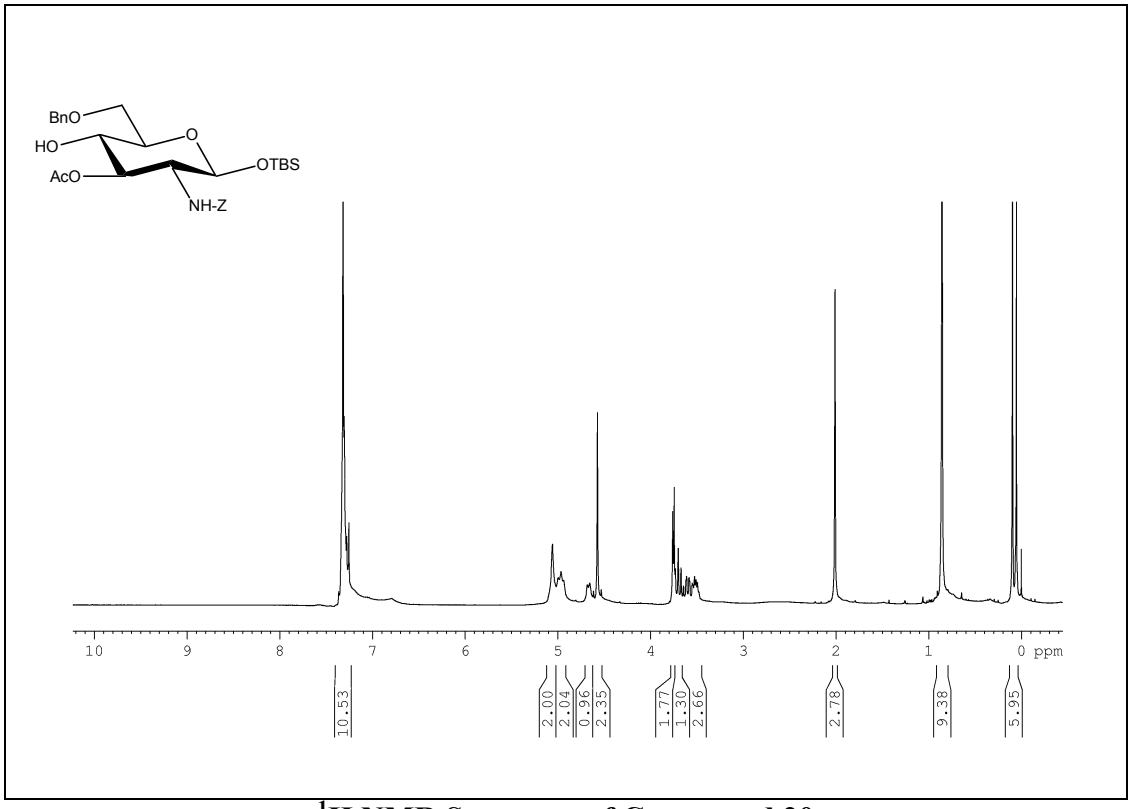
^{1}H NMR Spectrum of Compound 15



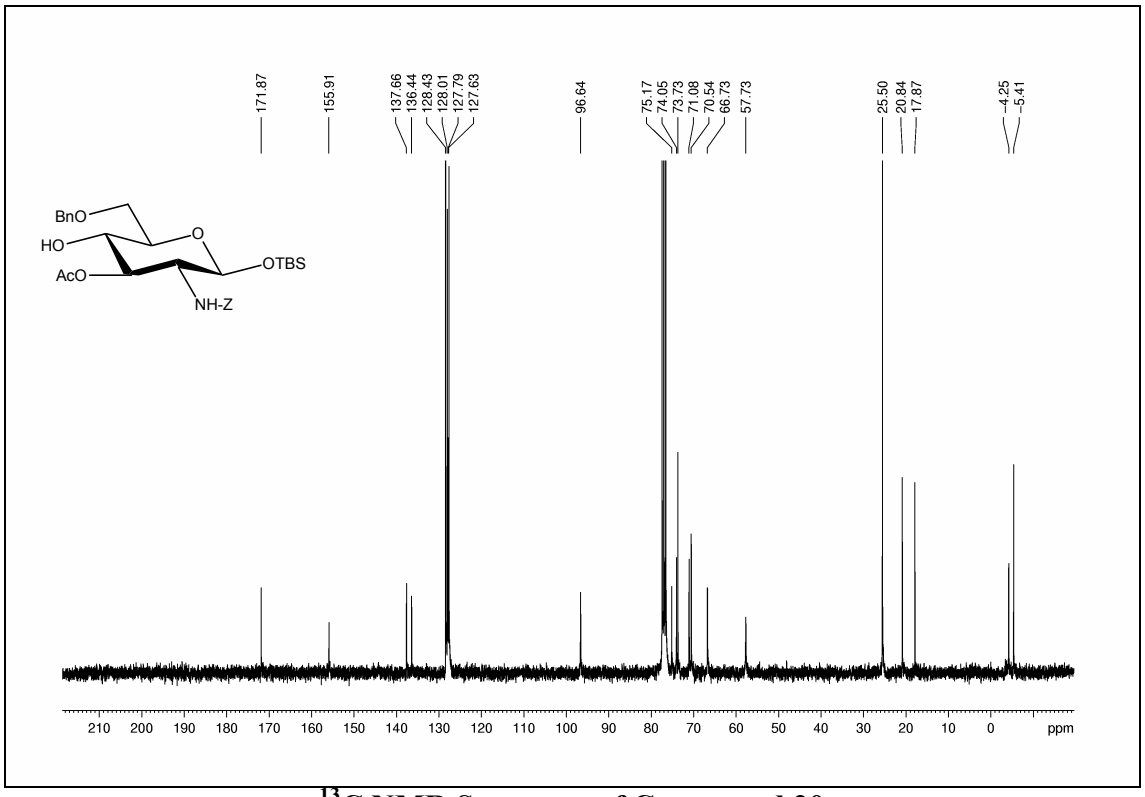
^{13}C NMR Spectrum of Compound 15



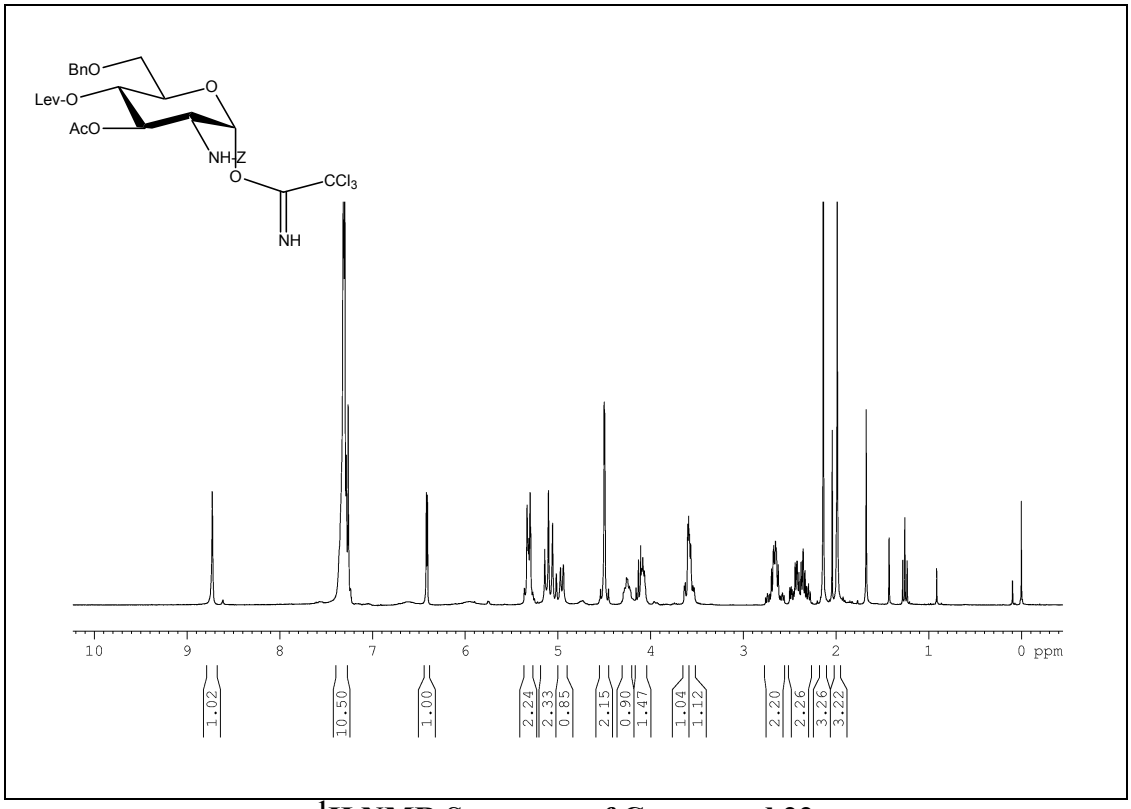
HMQC Spectrum of Compound 15



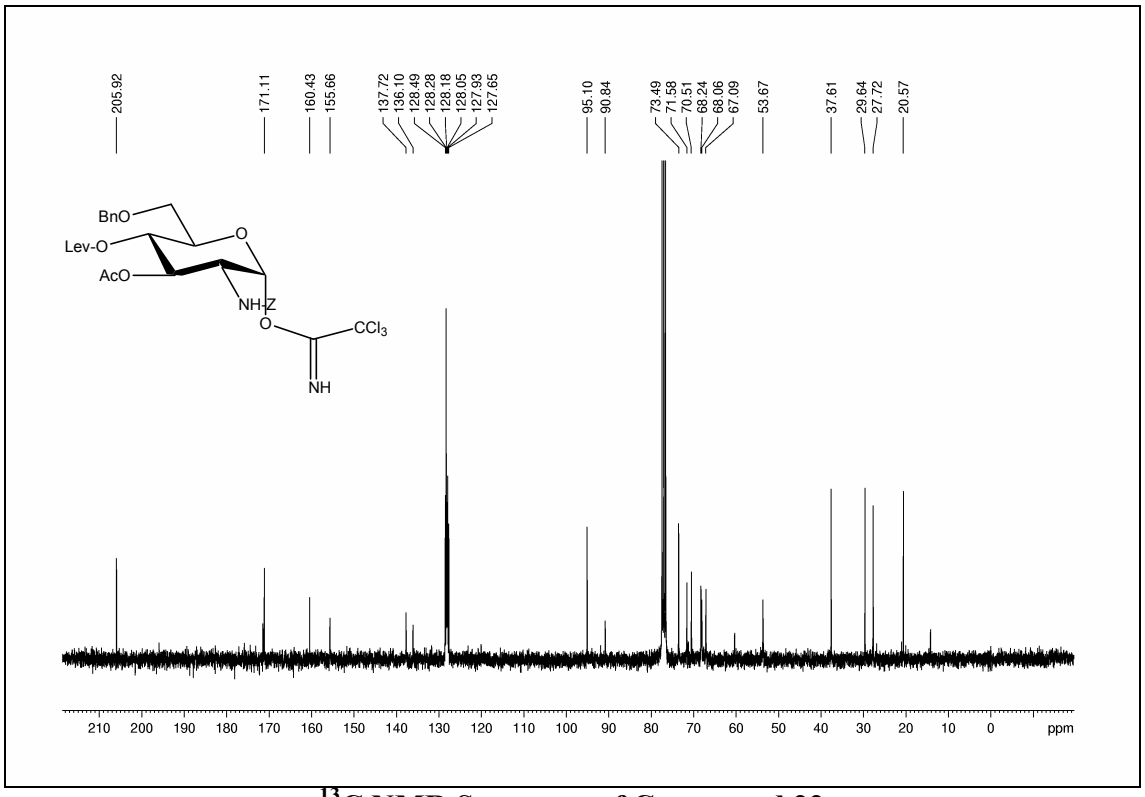
^{1}H NMR Spectrum of Compound 20



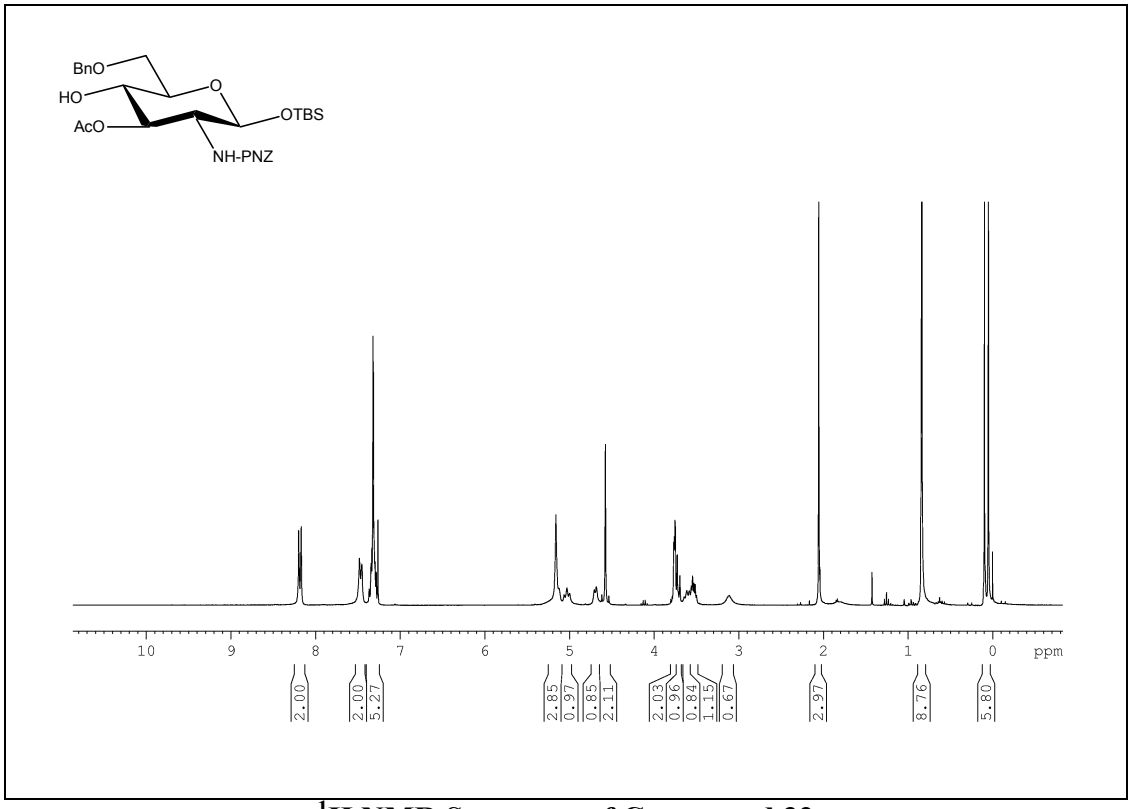
^{13}C NMR Spectrum of Compound 20



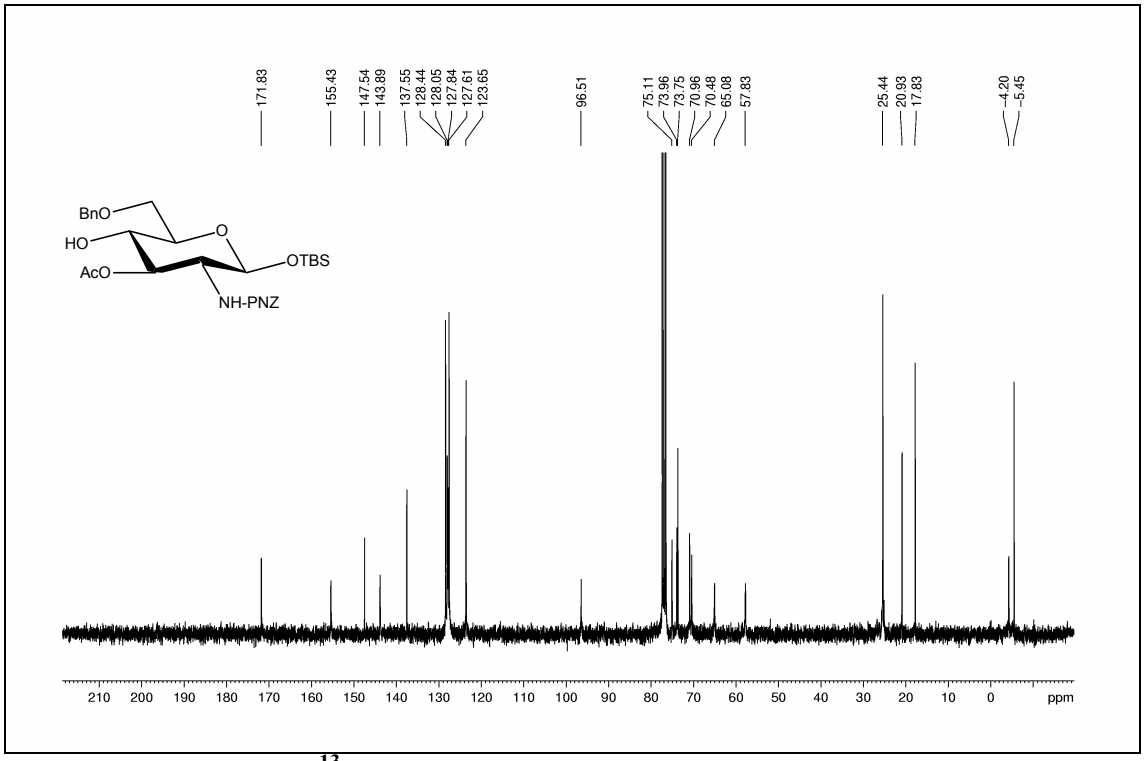
^{1}H NMR Spectrum of Compound 22



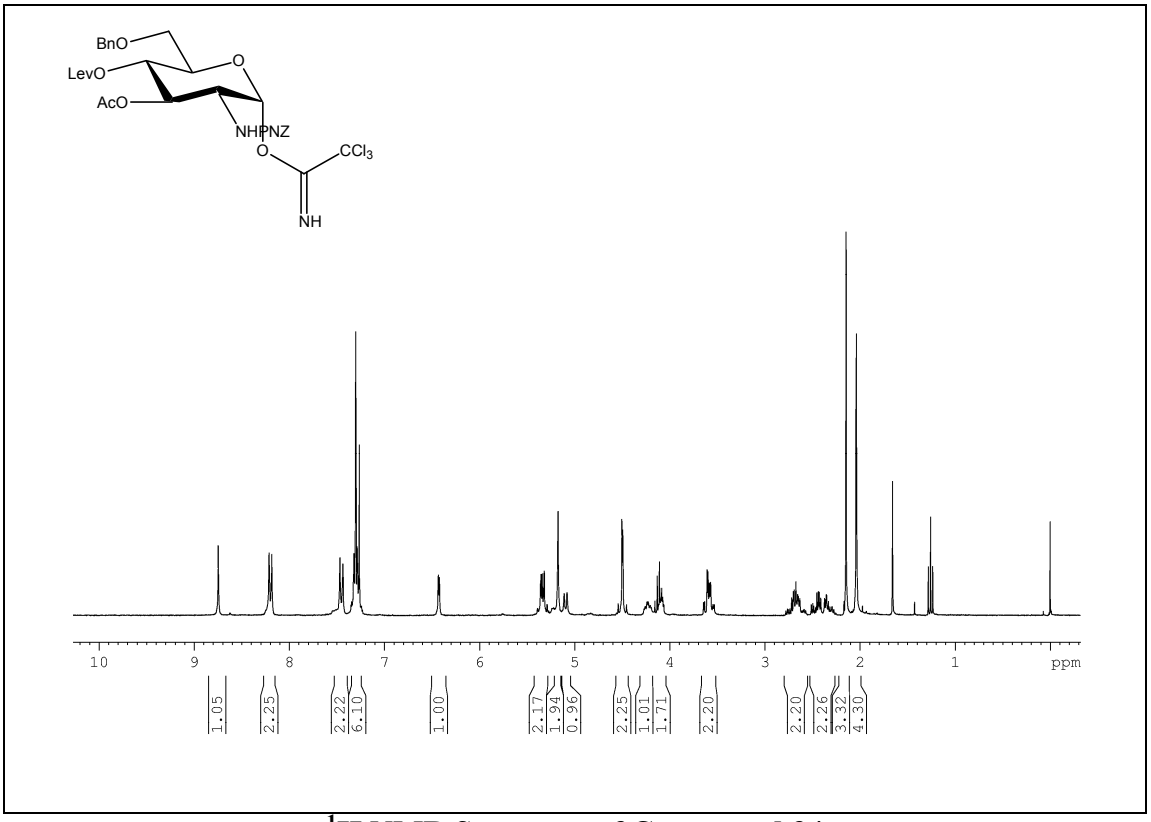
^{13}C NMR Spectrum of Compound 22



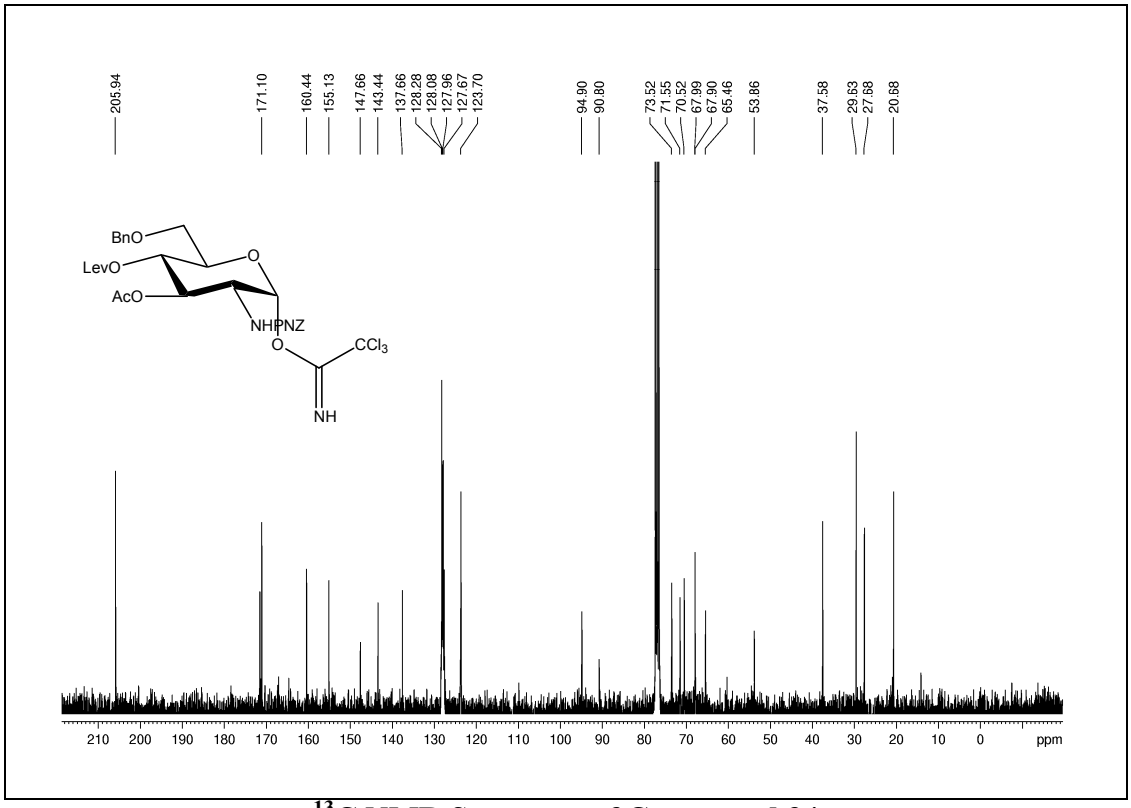
^{1}H NMR Spectrum of Compound 32



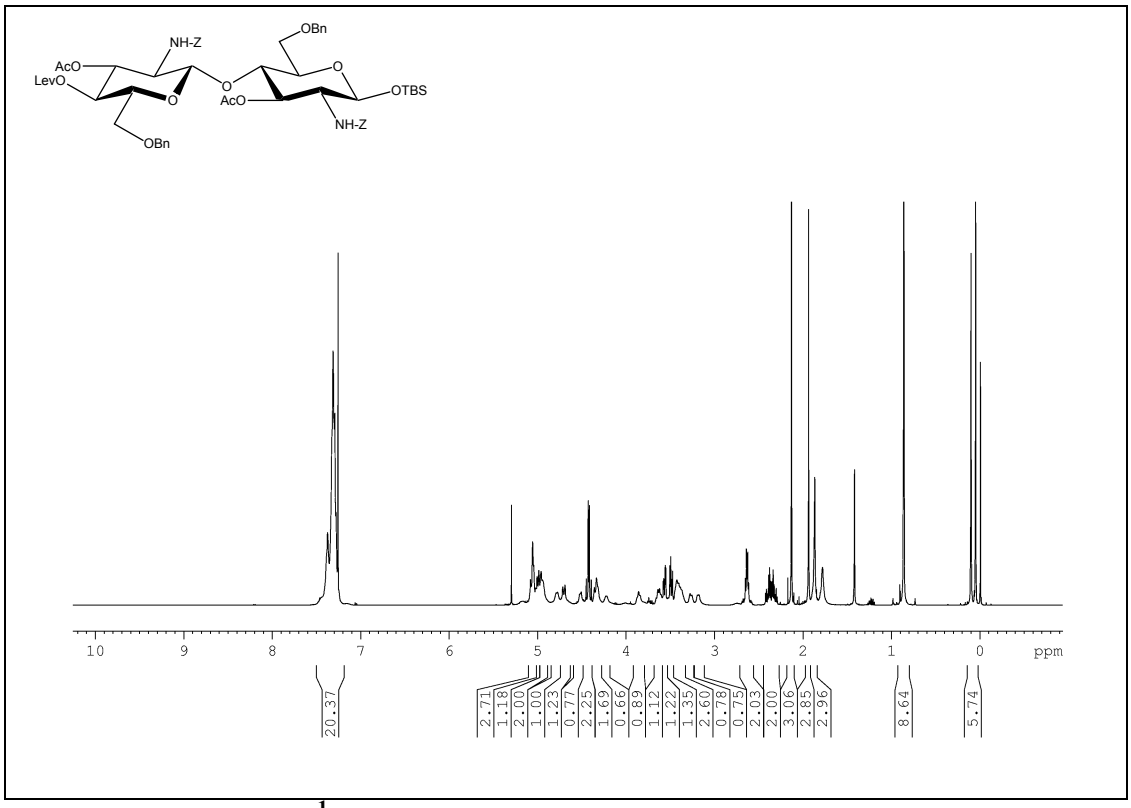
^{13}C NMR Spectrum of Compound 32



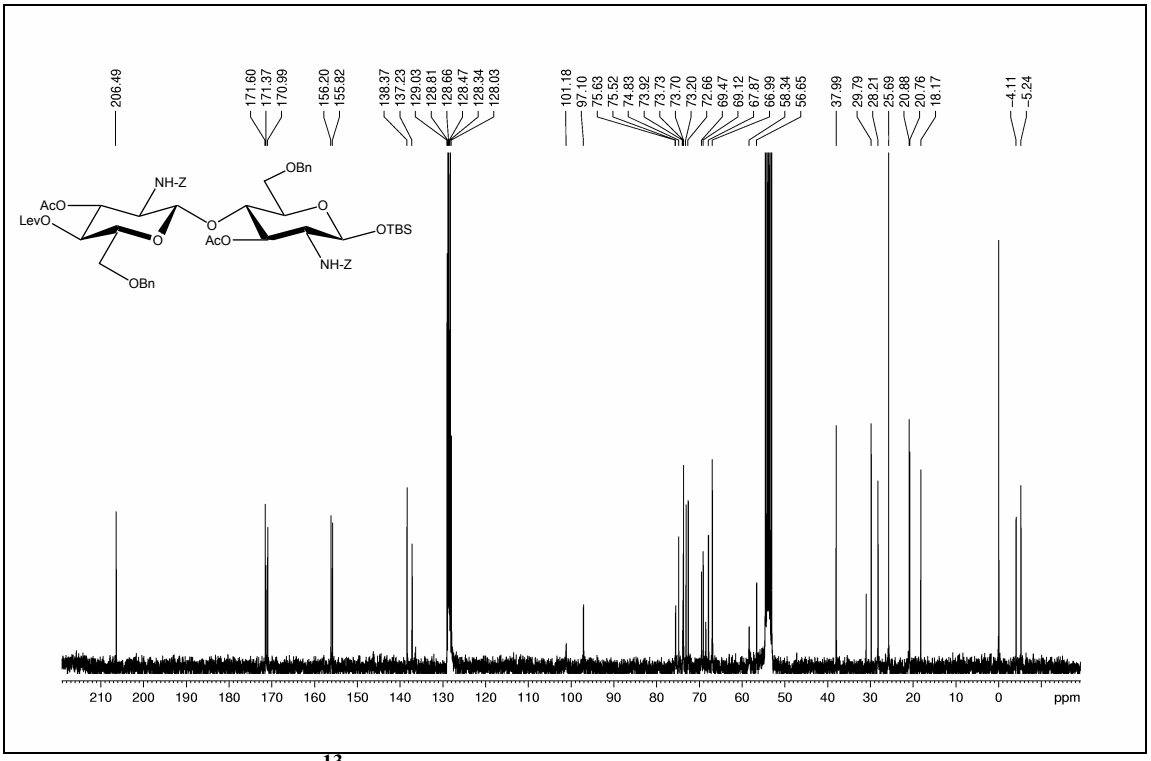
^{1}H NMR Spectrum of Compound 34



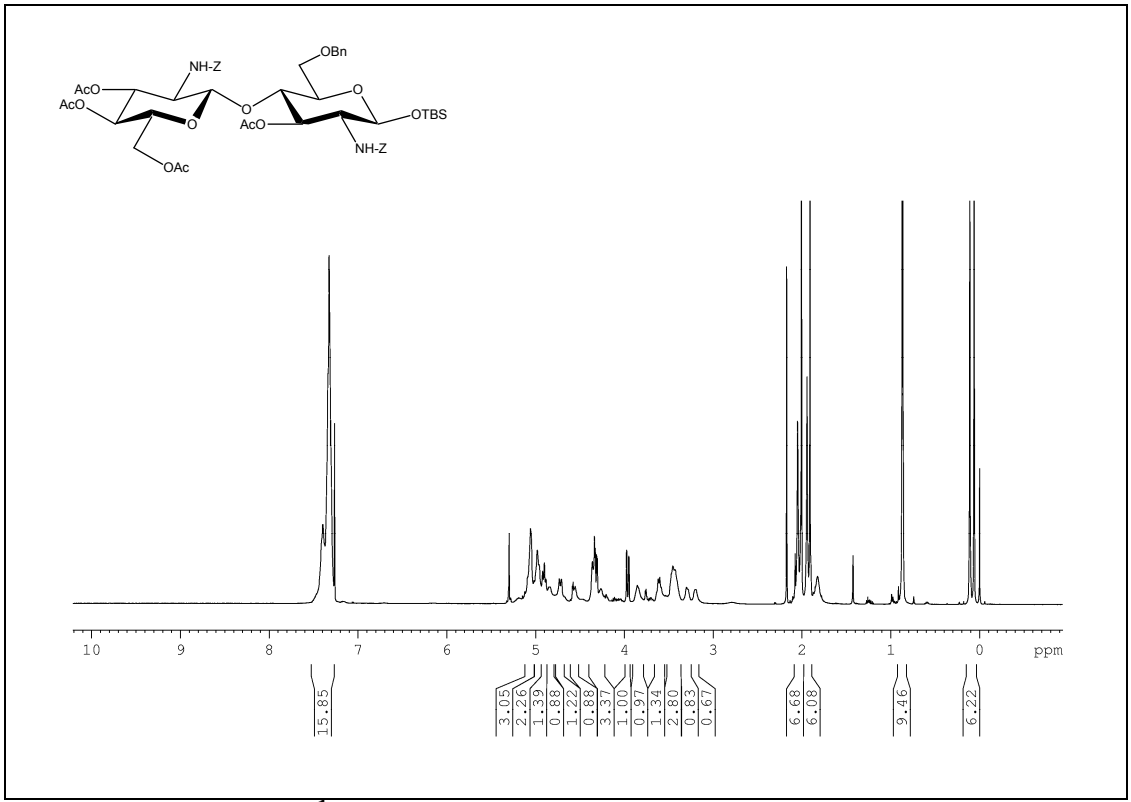
^{13}C NMR Spectrum of Compound 34



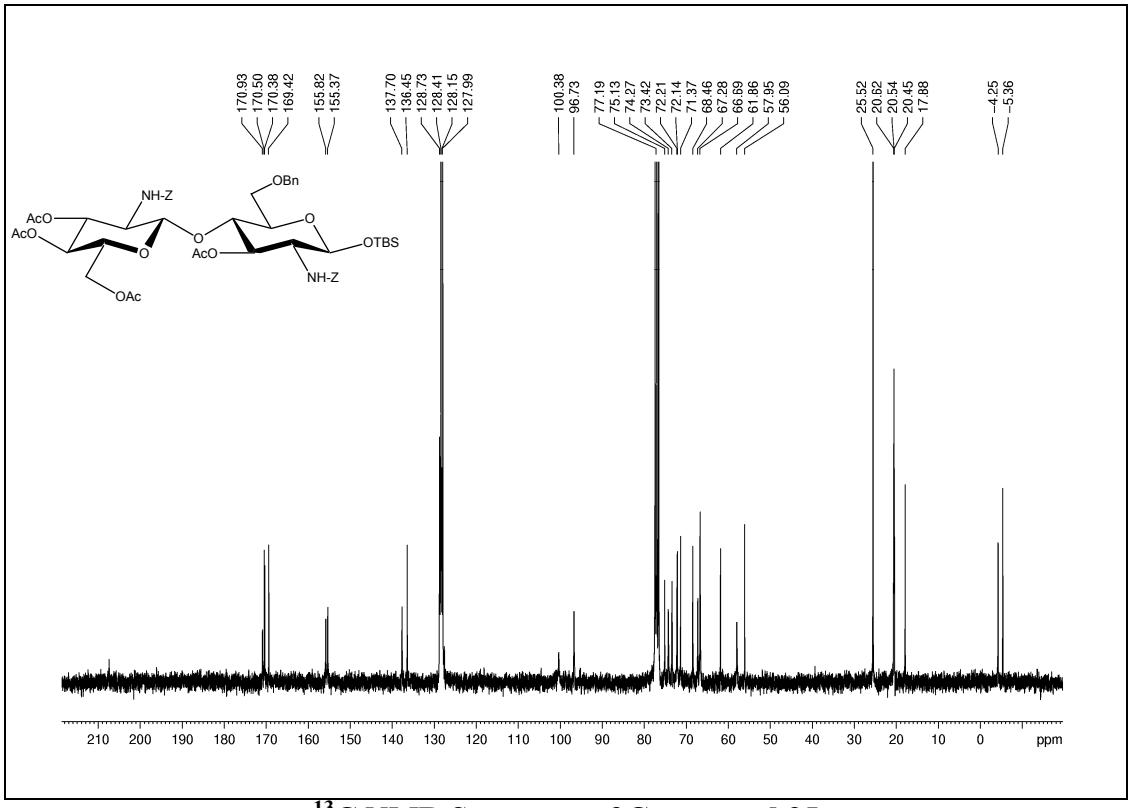
^{1}H NMR Spectrum of Compound 23



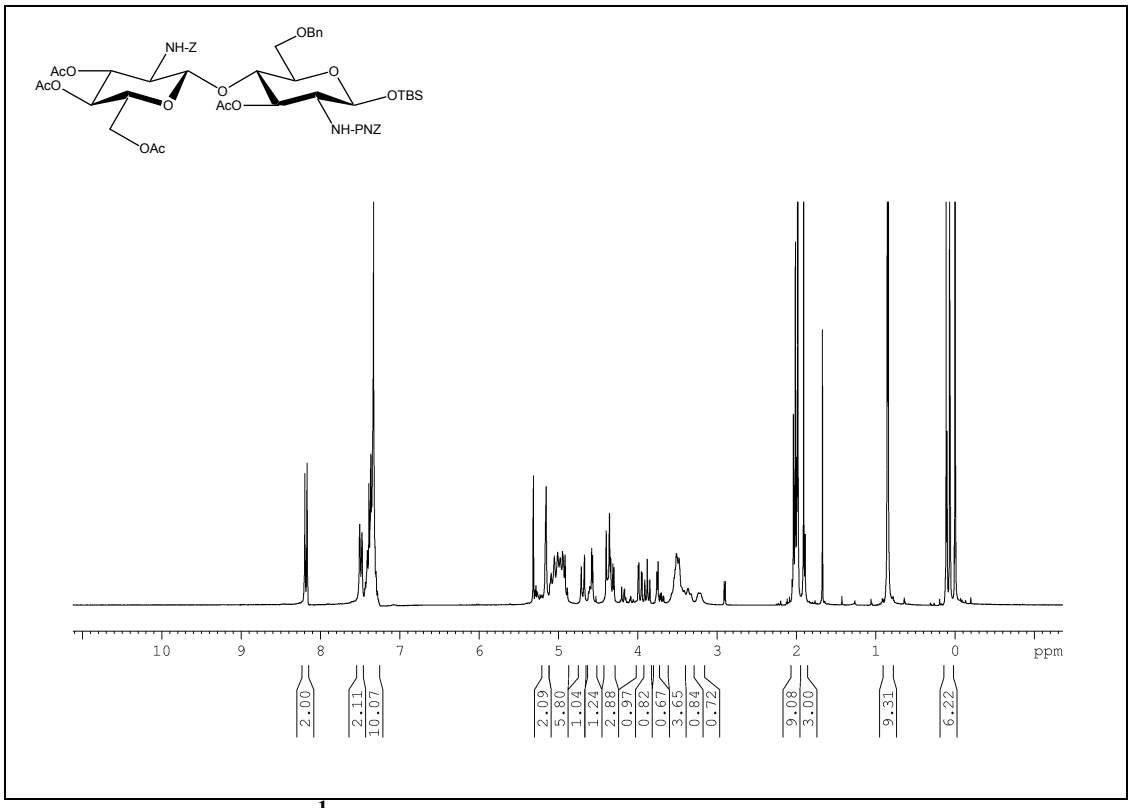
^{13}C NMR Spectrum of Compound 23



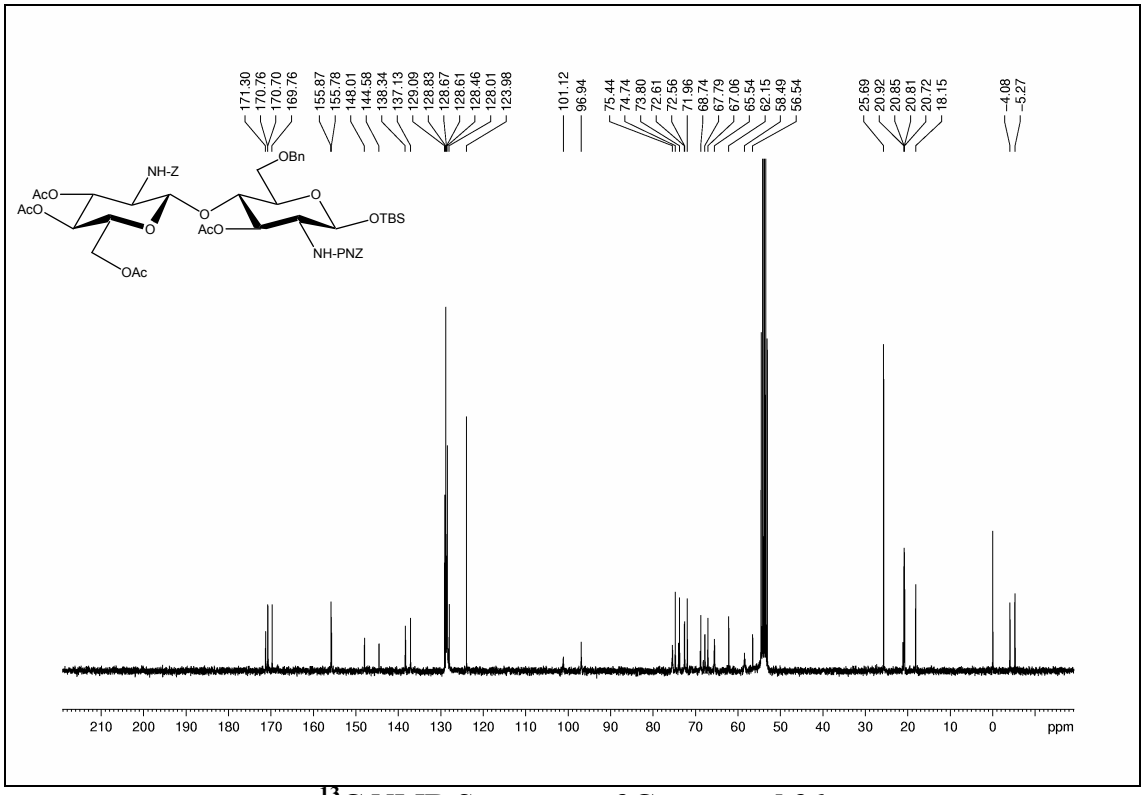
^{1}H NMR Spectrum of Compound 35



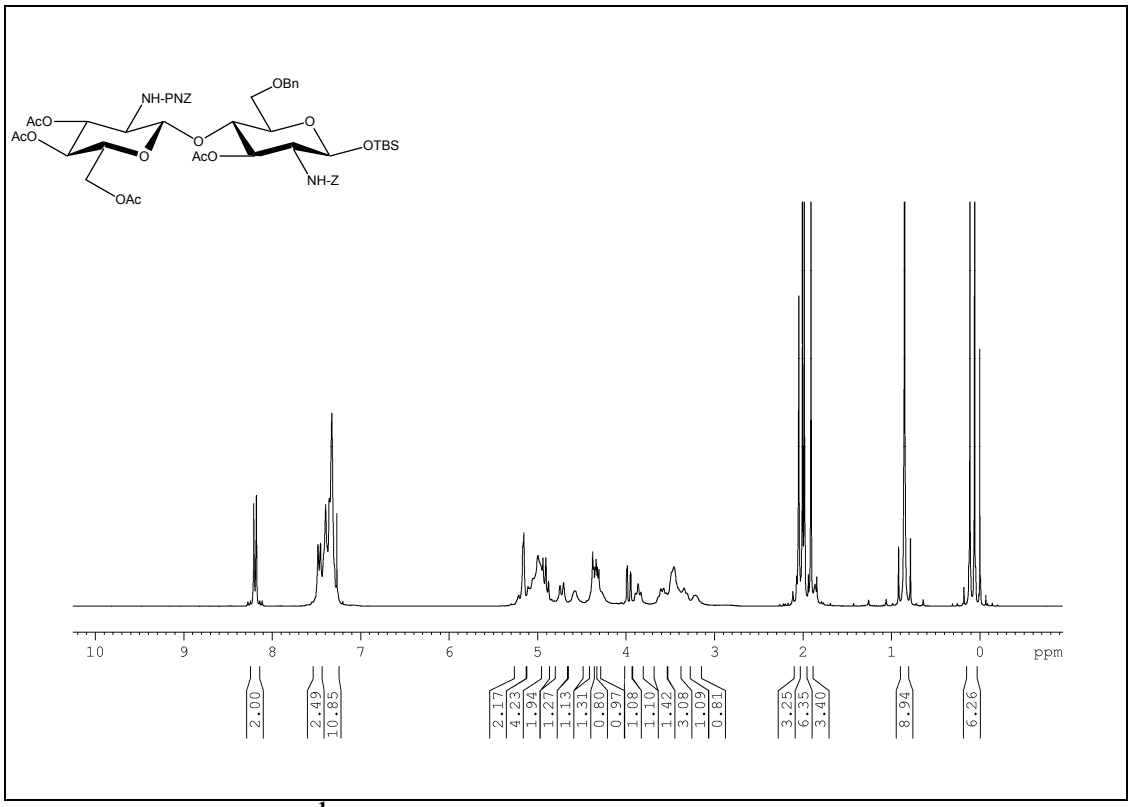
^{13}C NMR Spectrum of Compound 35



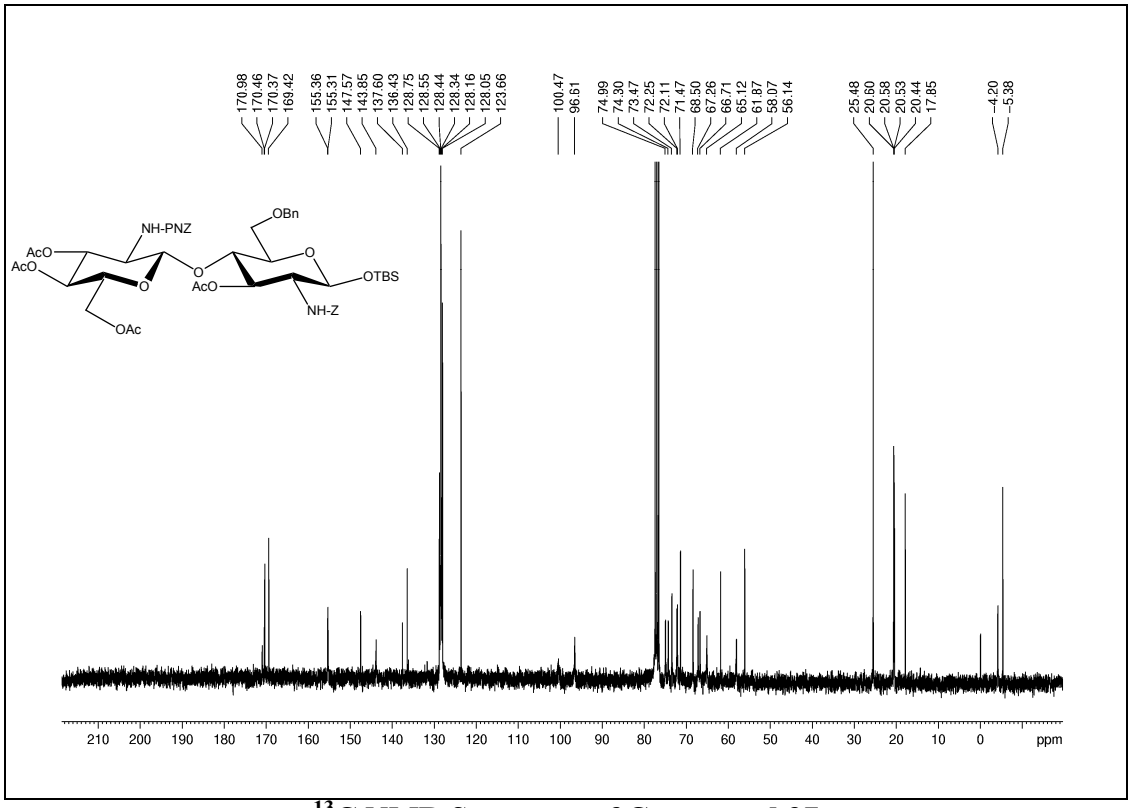
^{1}H NMR Spectrum of Compound 36



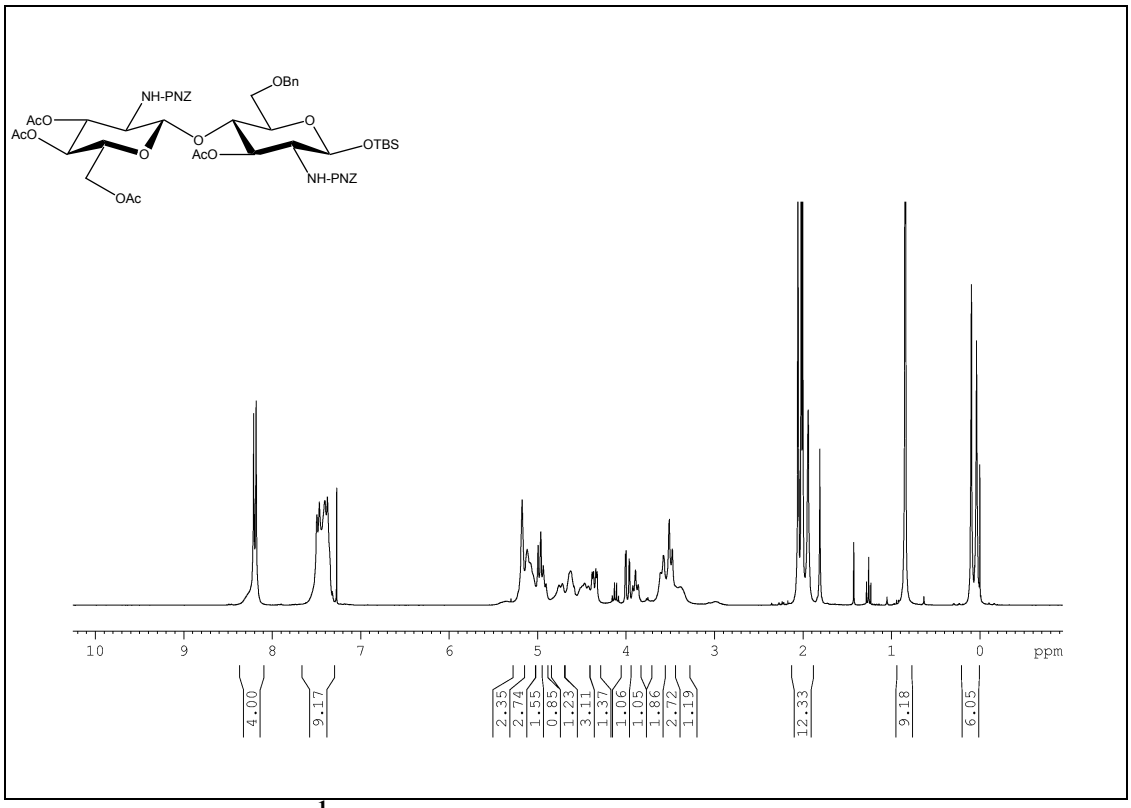
^{13}C NMR Spectrum of Compound 36



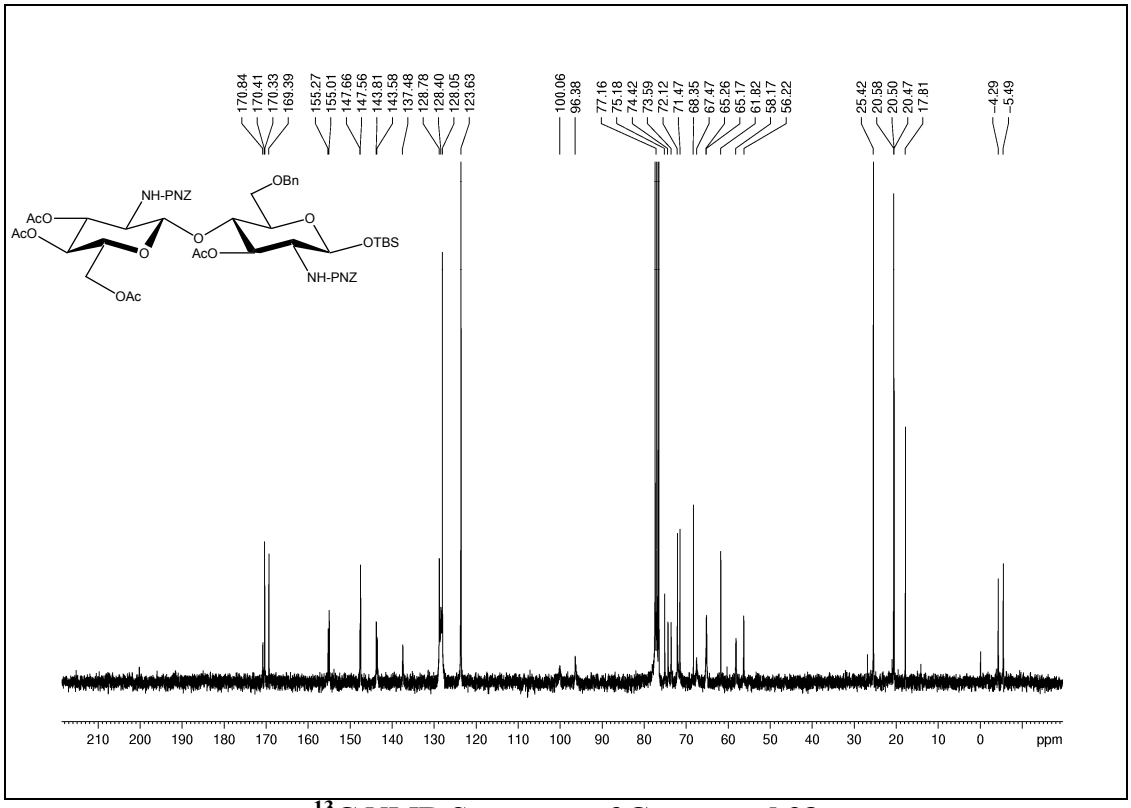
^{1}H NMR Spectrum of Compound 37



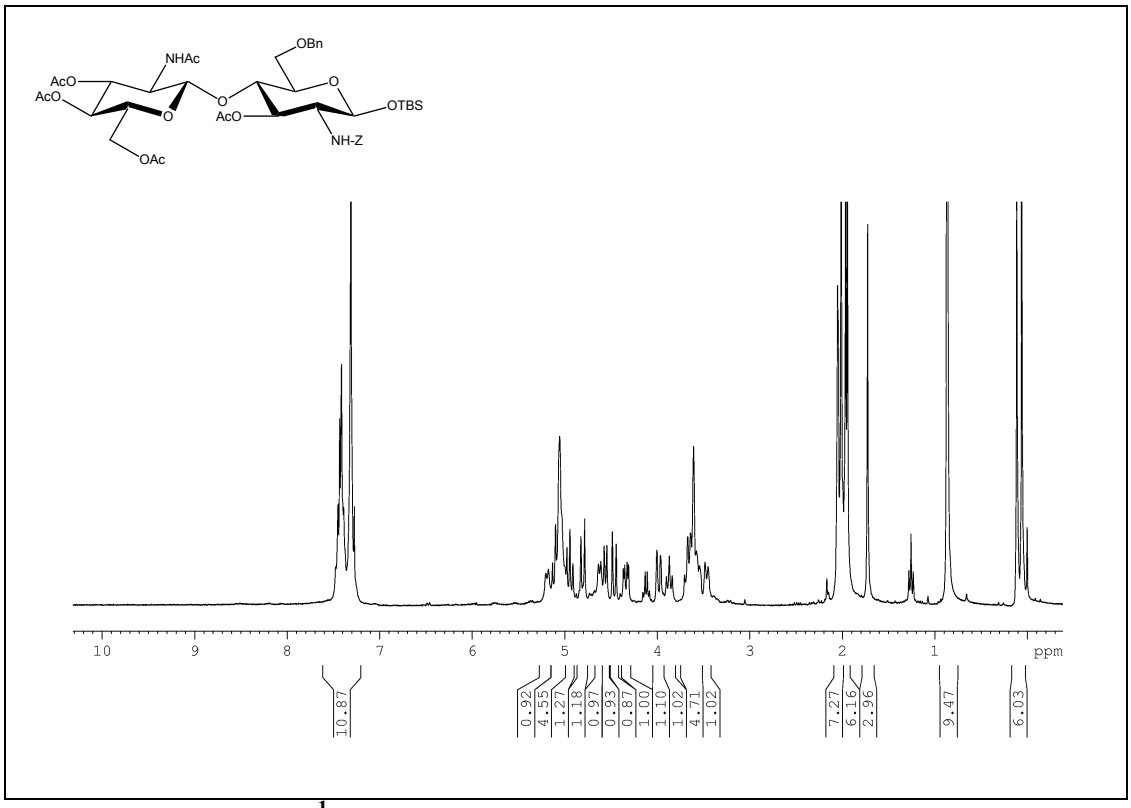
^{13}C NMR Spectrum of Compound 37



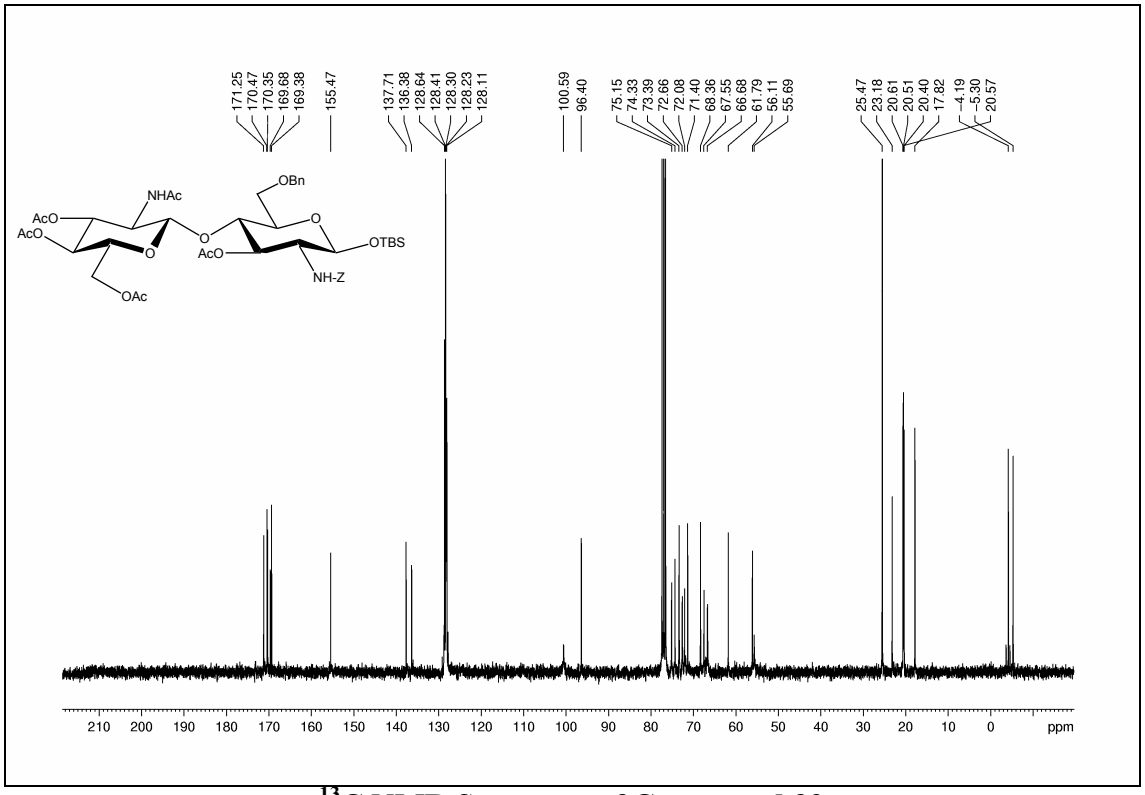
^{1}H NMR Spectrum of Compound 38



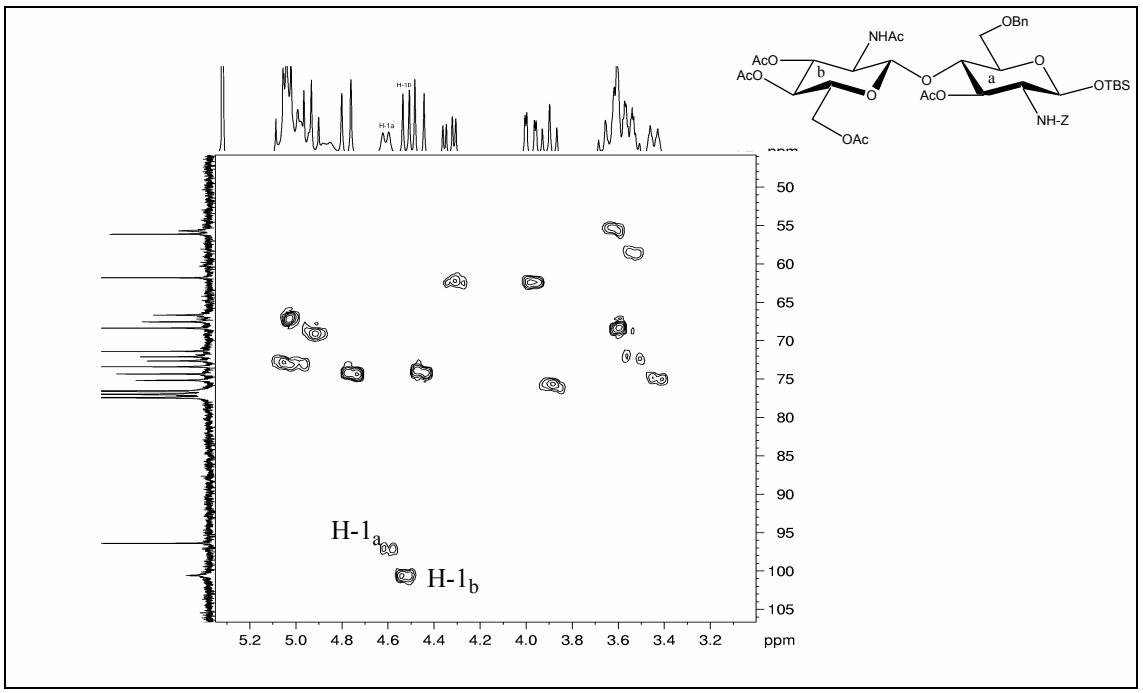
^{13}C NMR Spectrum of Compound 38



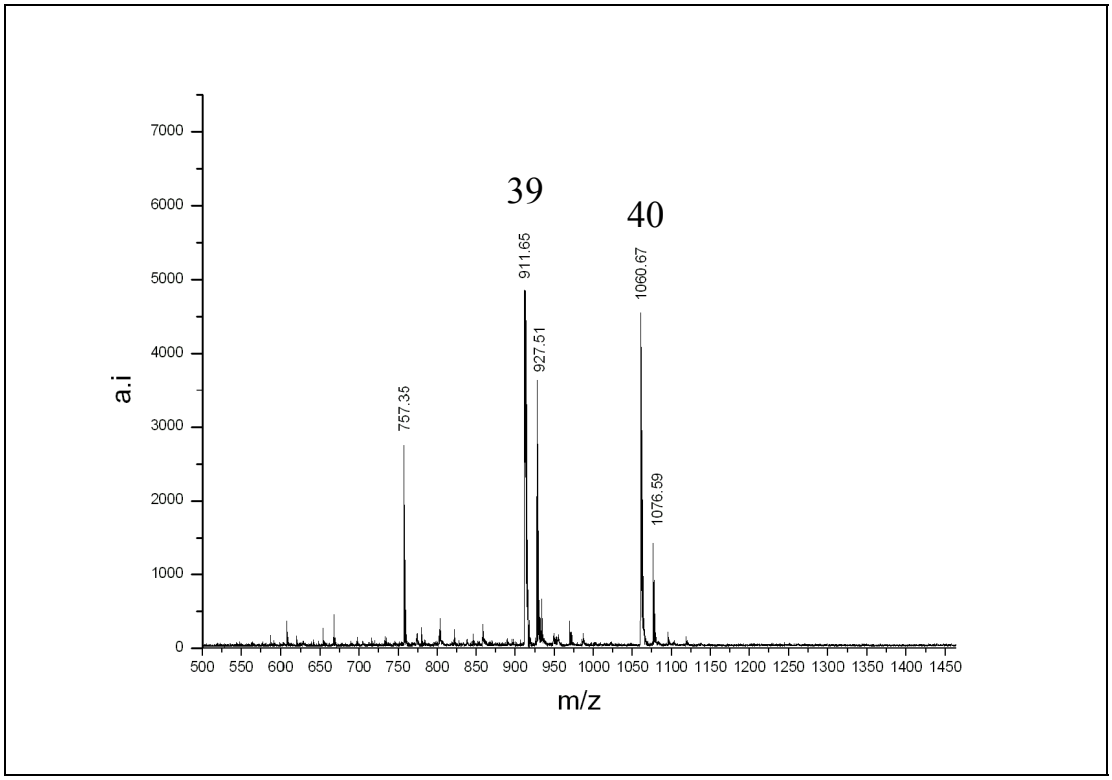
^{1}H NMR Spectrum of Compound 39



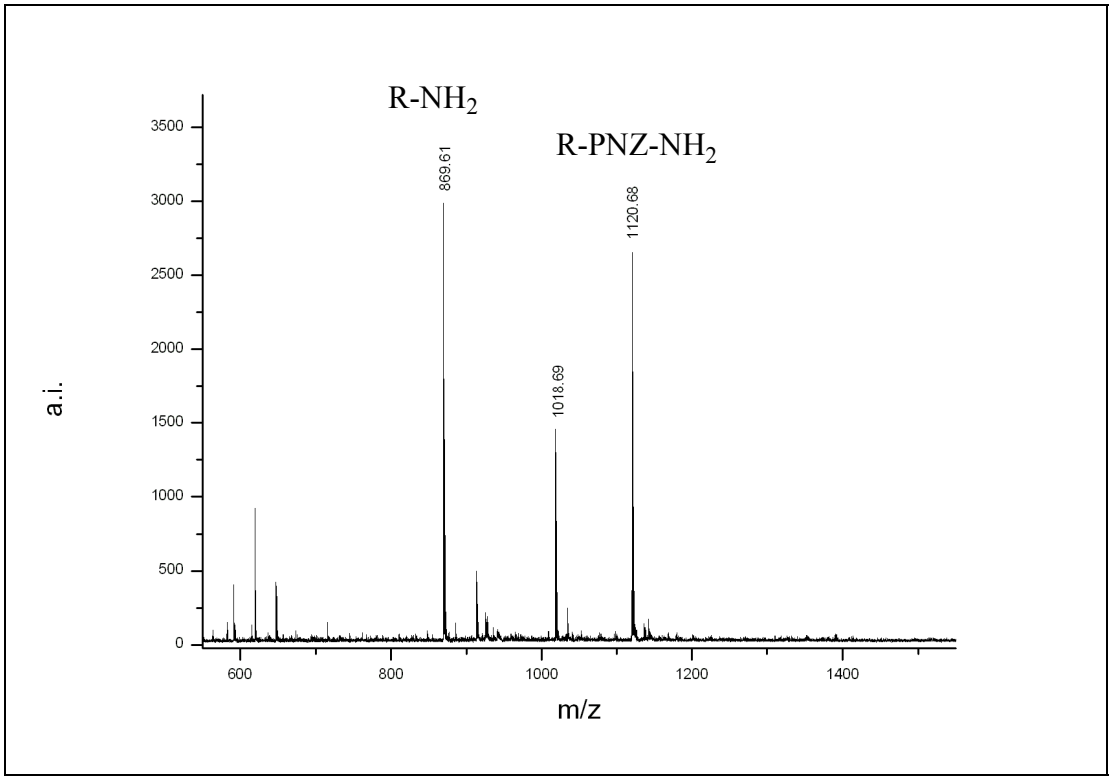
^{13}C NMR Spectrum of Compound 39



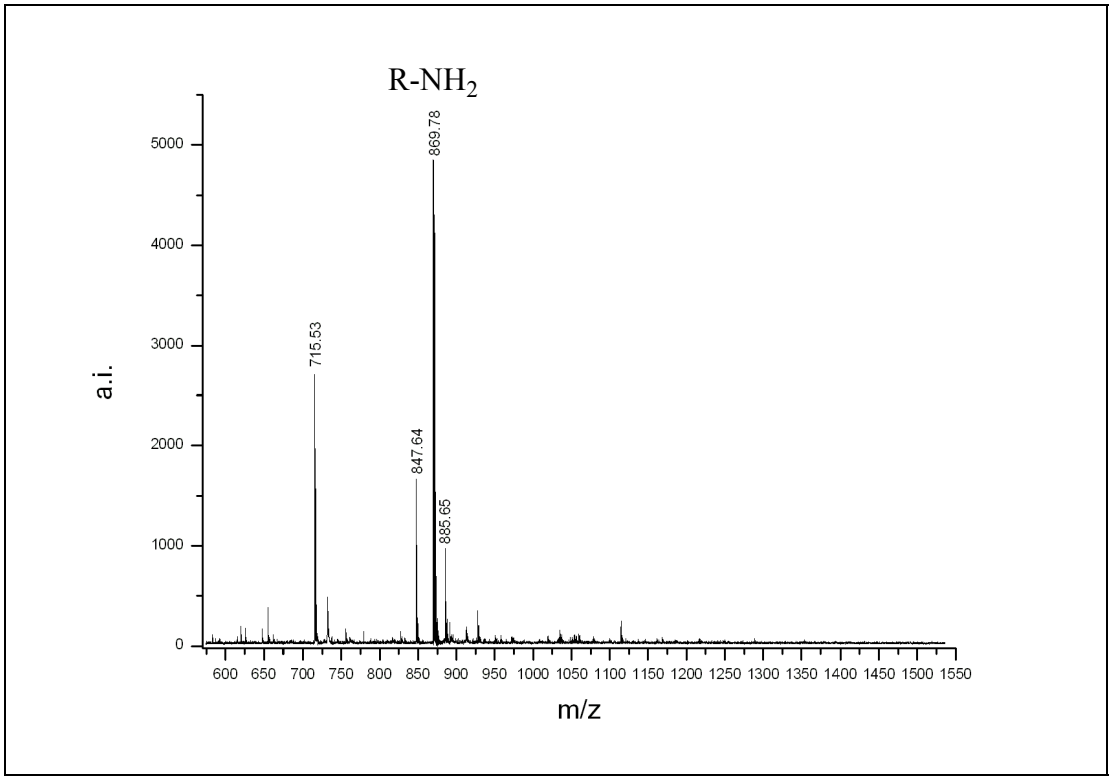
HMQC Spectrum of Compound 39



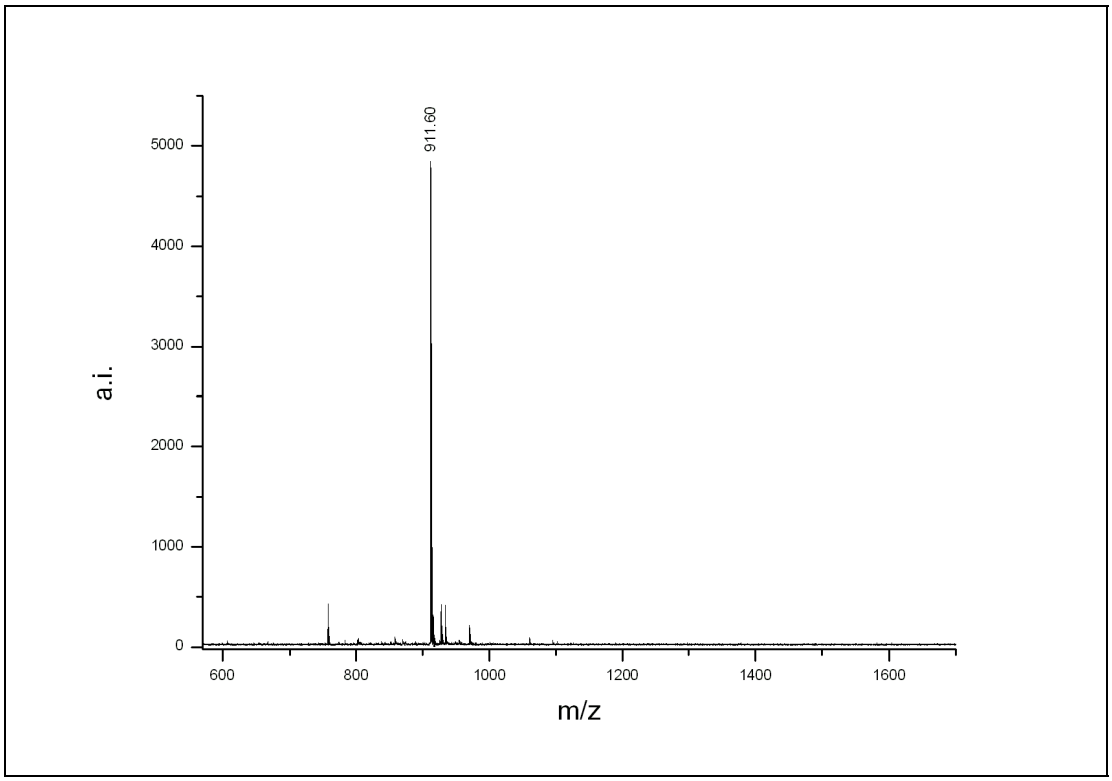
MALDI-TOF MS of mixture of products 39 and 40 (Scheme 3.1.14)



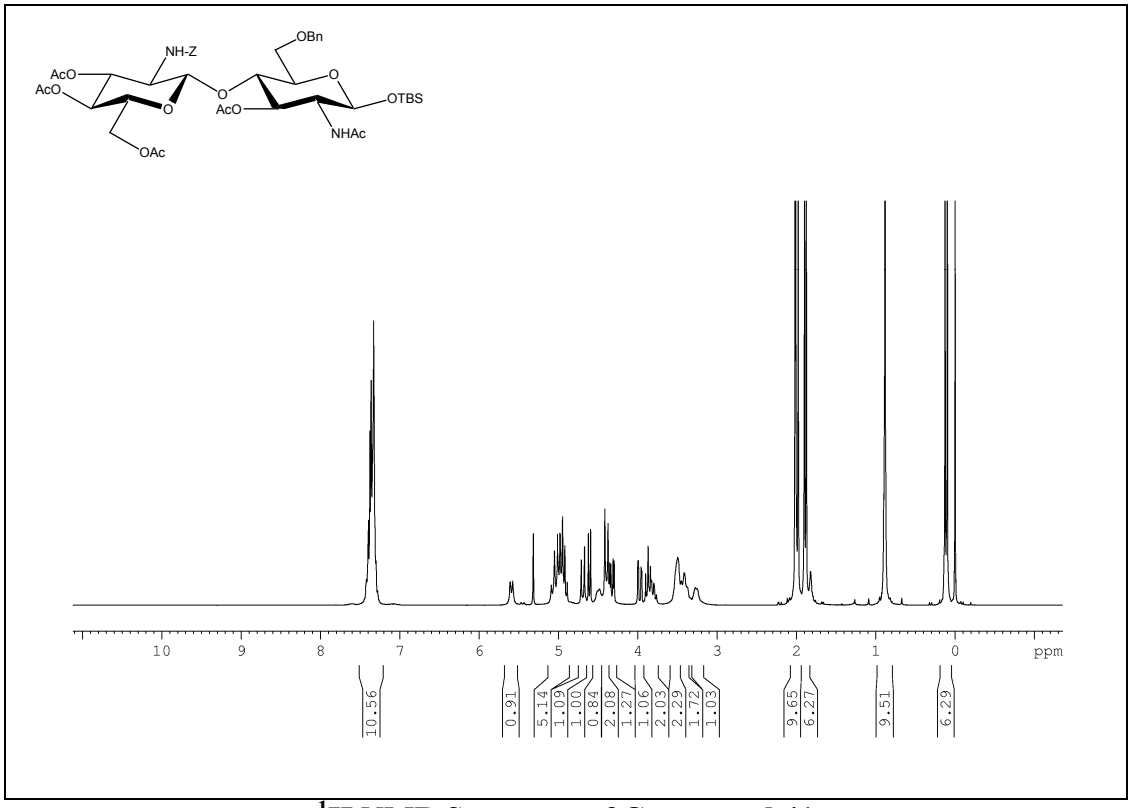
MALDI-TOF MS of crude product after sodium dithionite treatment (Scheme 3.1.16)



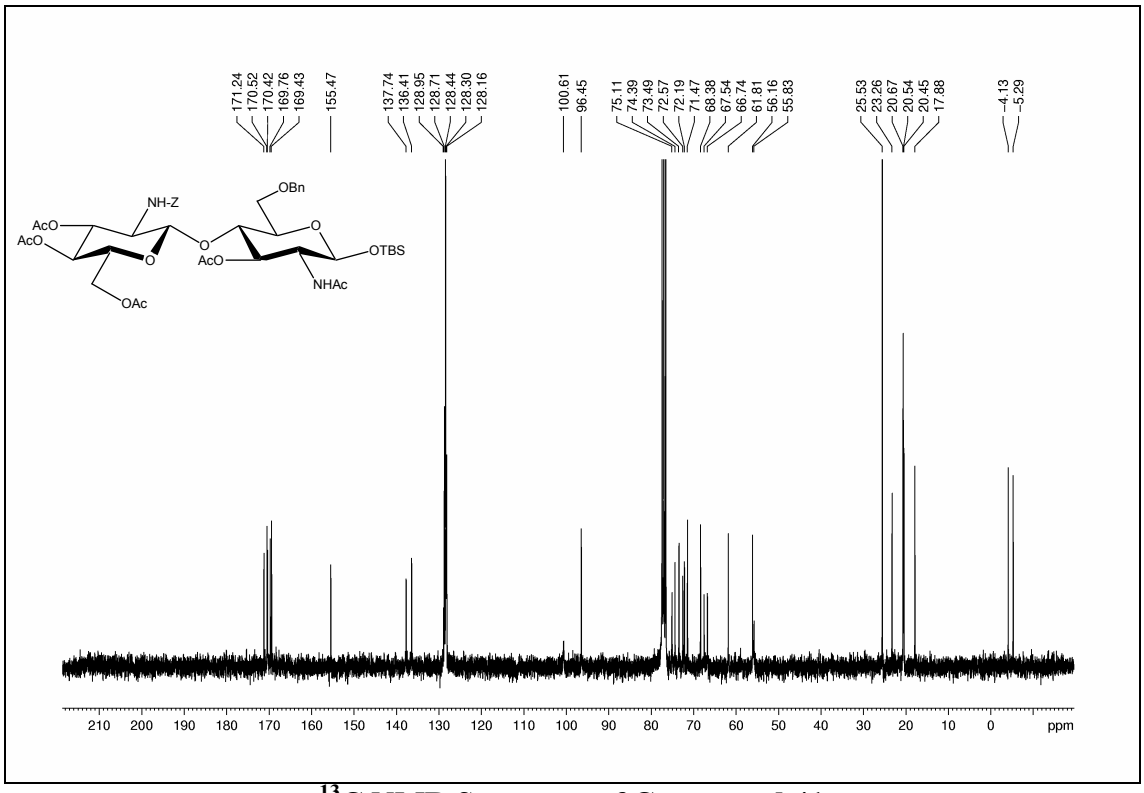
MALDI-TOF MS of crude product after treatment with TFA (Scheme 3.1.16)



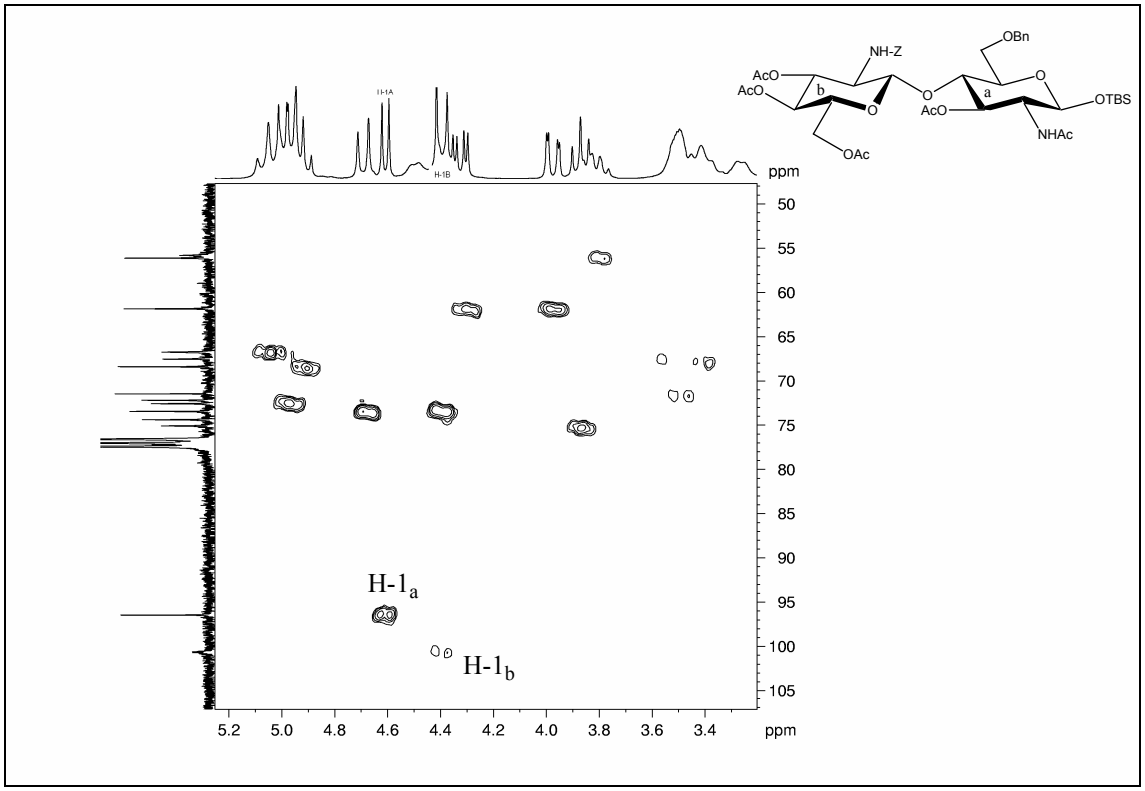
MALDI-TOF MS of Compound 39 (Scheme 3.1.16)



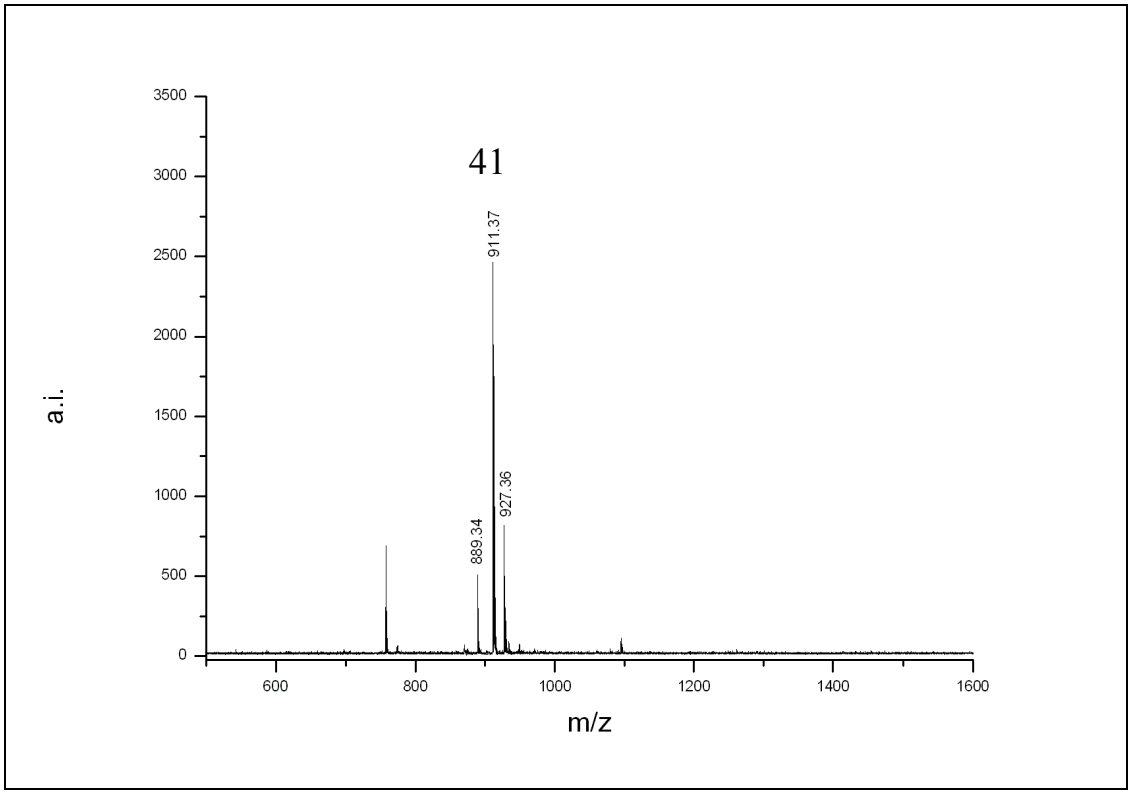
^{1}H NMR Spectrum of Compound 41



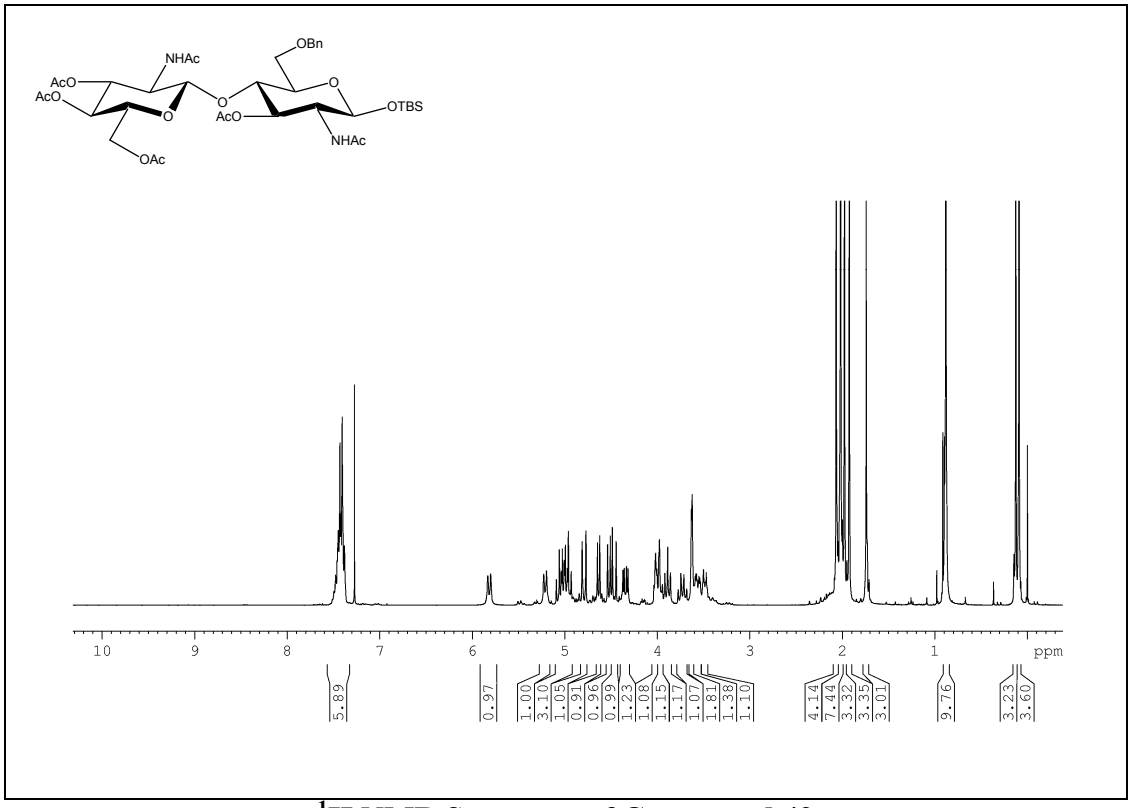
^{13}C NMR Spectrum of Compound 41



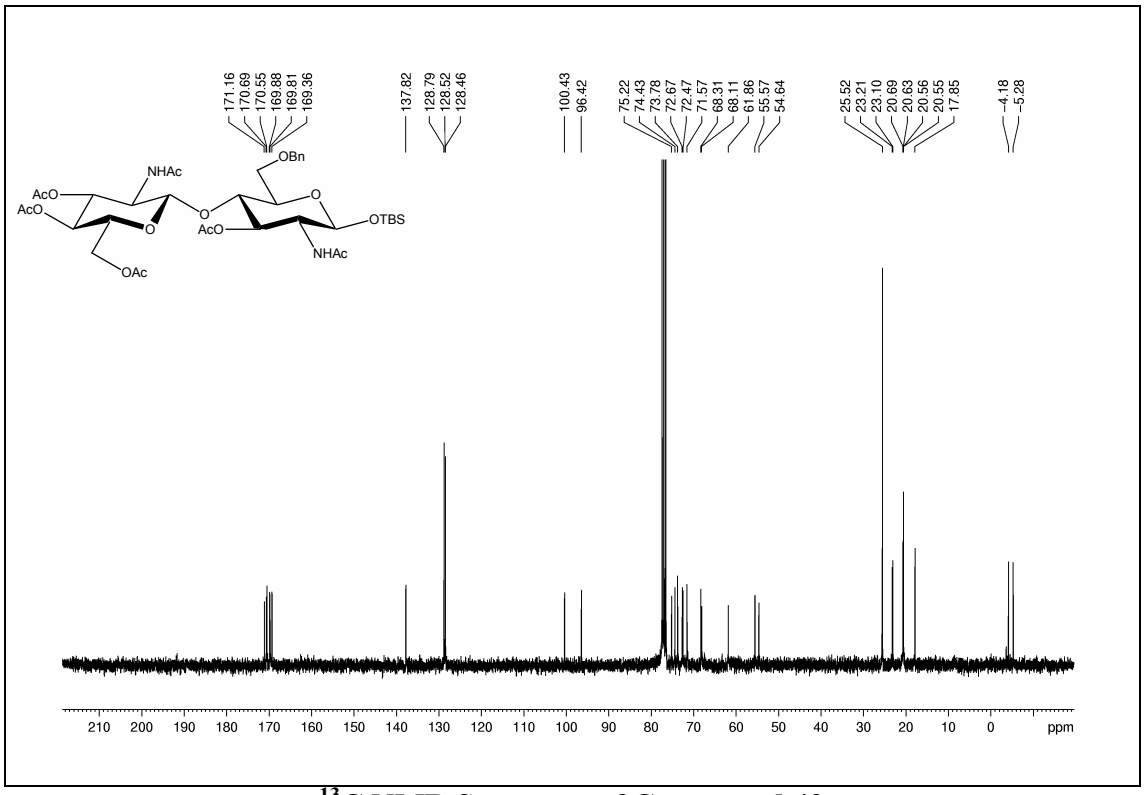
HMQC Spectrum of Compound 41



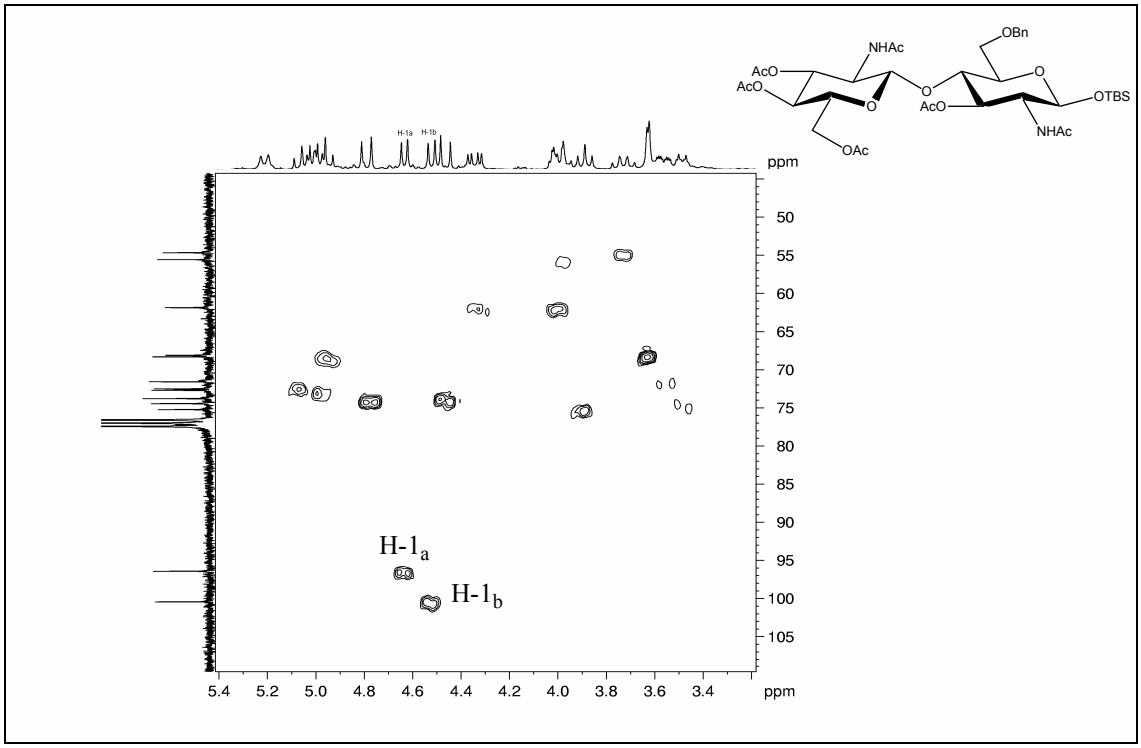
MALDI-TOF MS of Compound 41



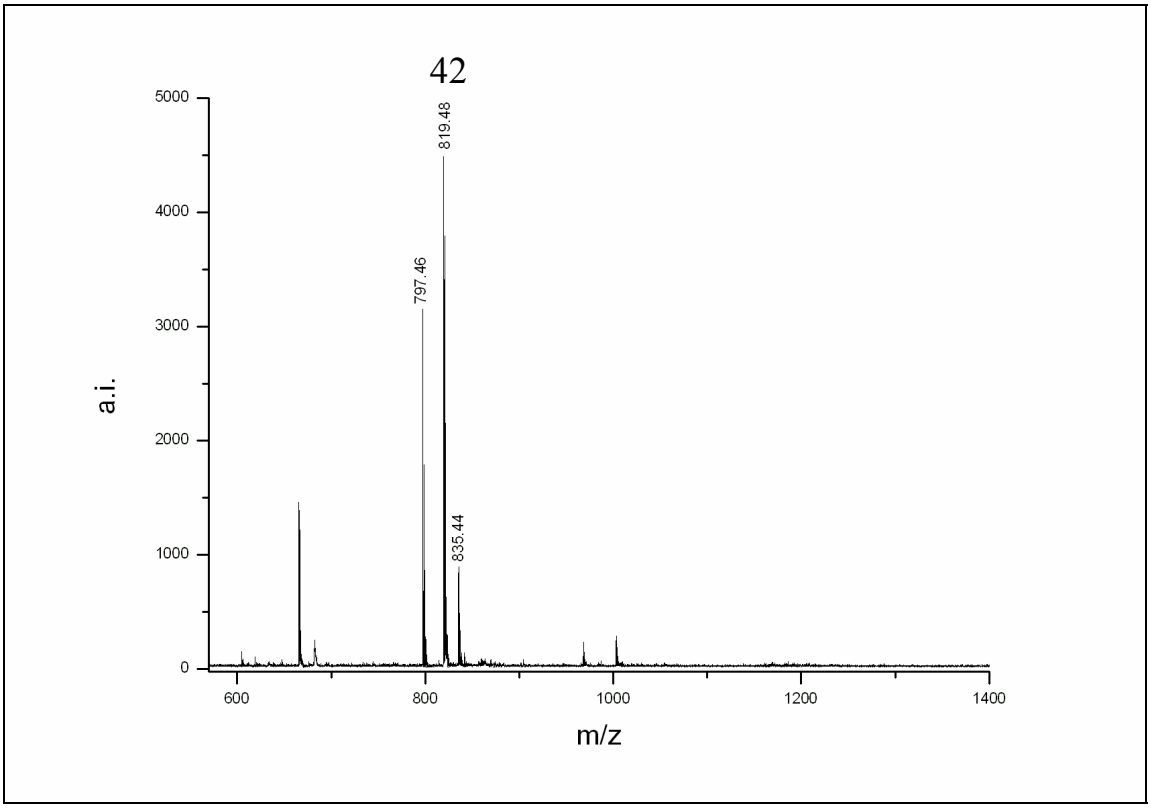
^{1}H NMR Spectrum of Compound 42



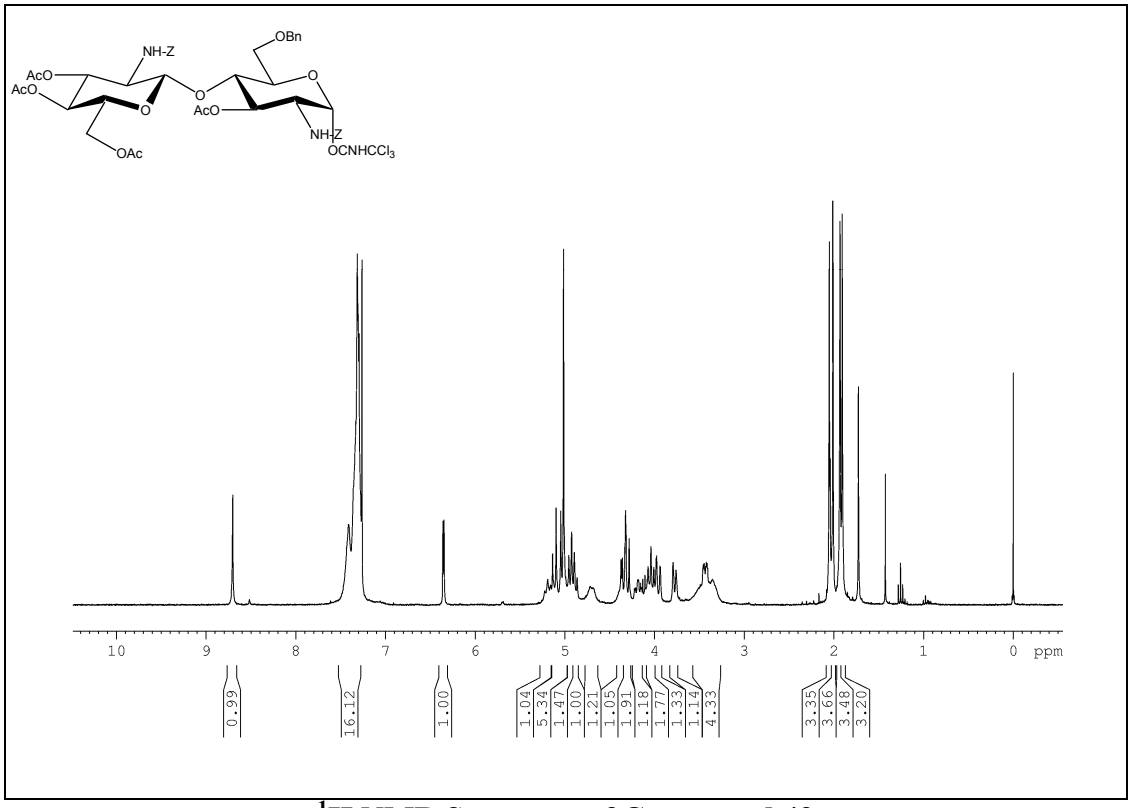
^{13}C NMR Spectrum of Compound 42



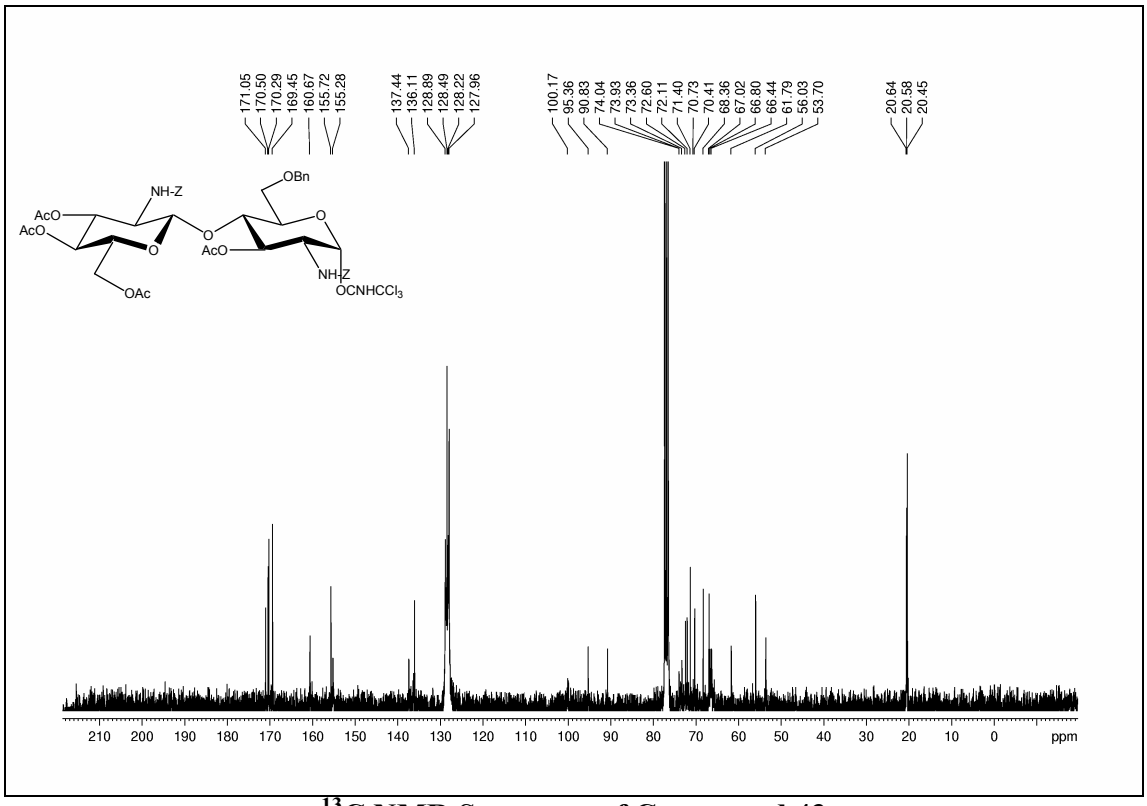
HMQC Spectrum of Compound 42



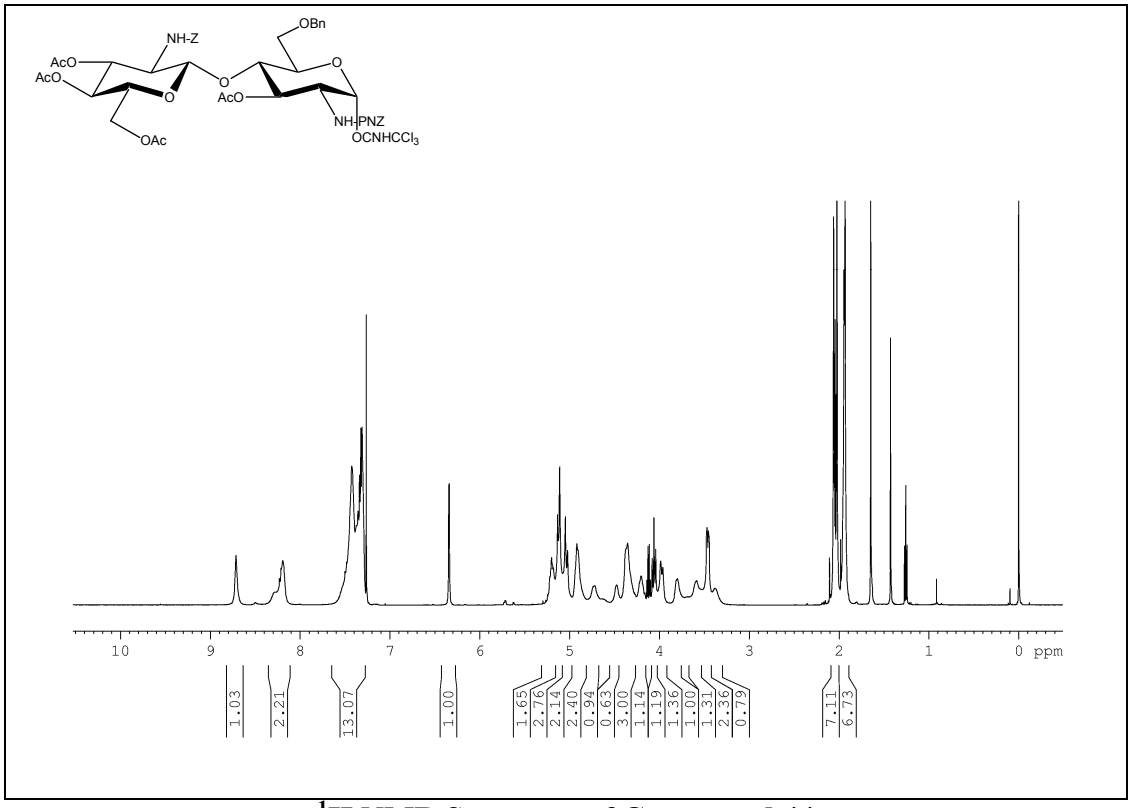
MALDI-TOF MS of Compound 42



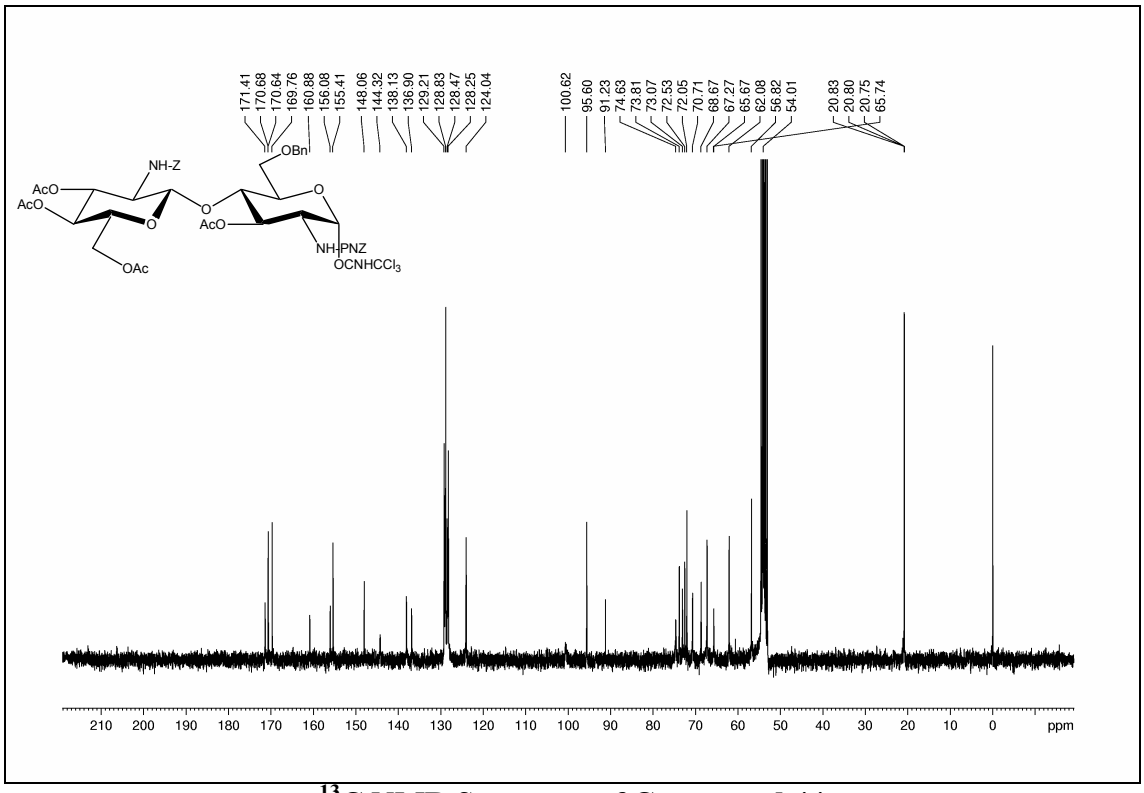
^{1}H NMR Spectrum of Compound 43



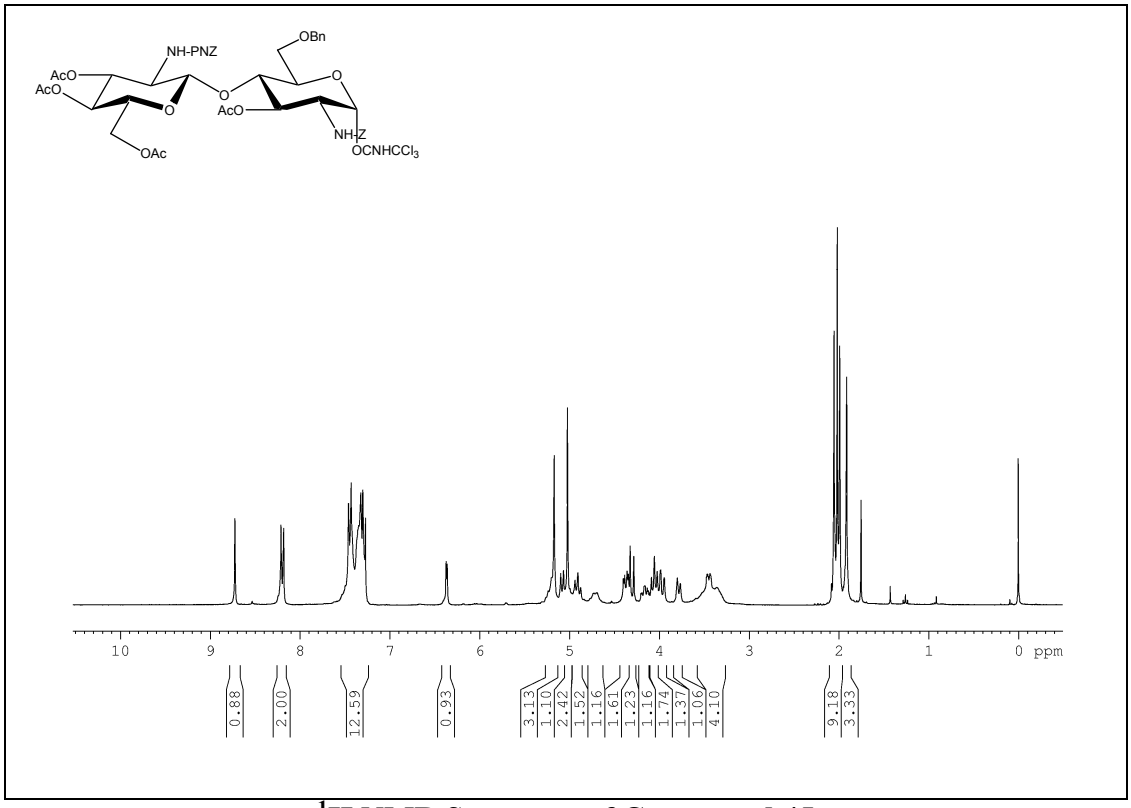
^{13}C NMR Spectrum of Compound 43



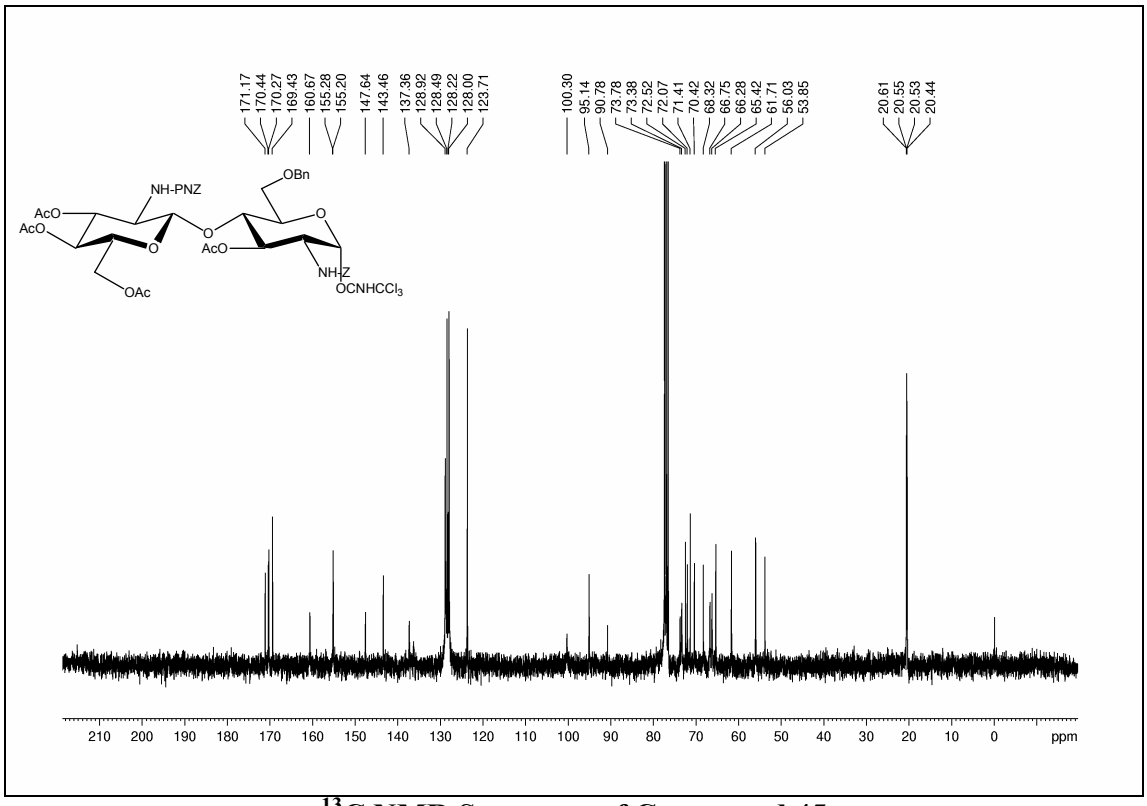
^{1}H NMR Spectrum of Compound 44



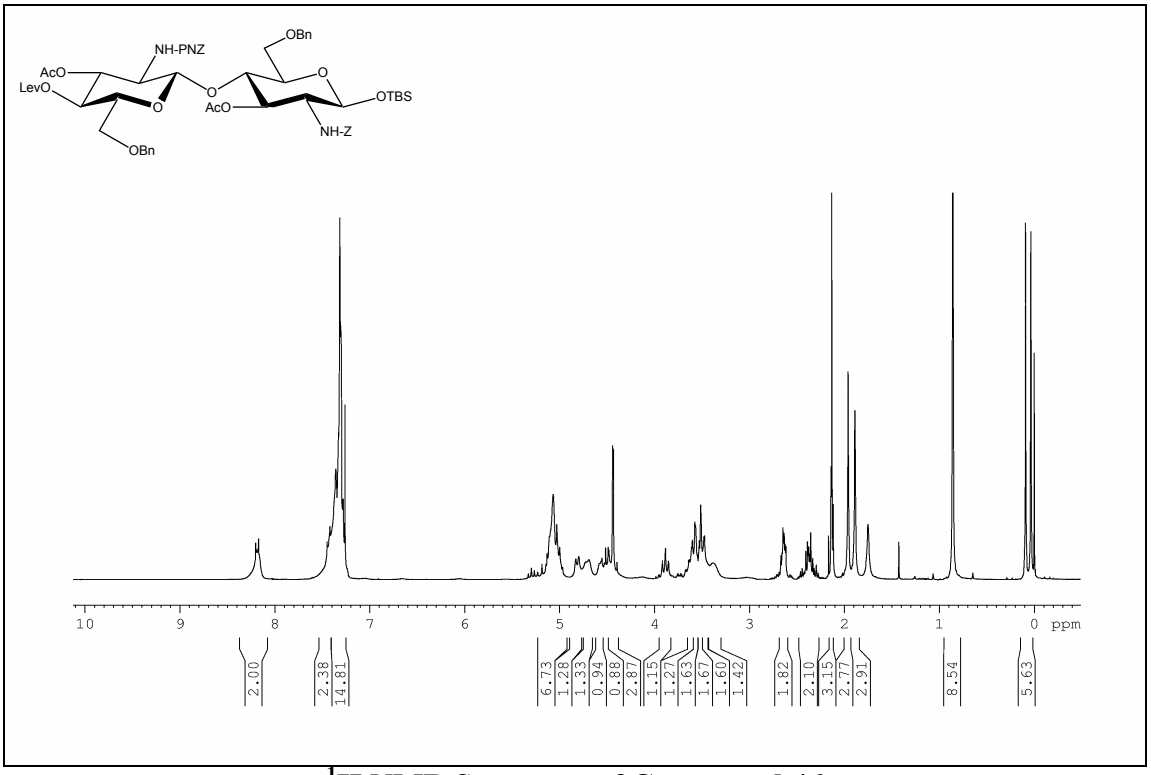
^{13}C NMR Spectrum of Compound 44



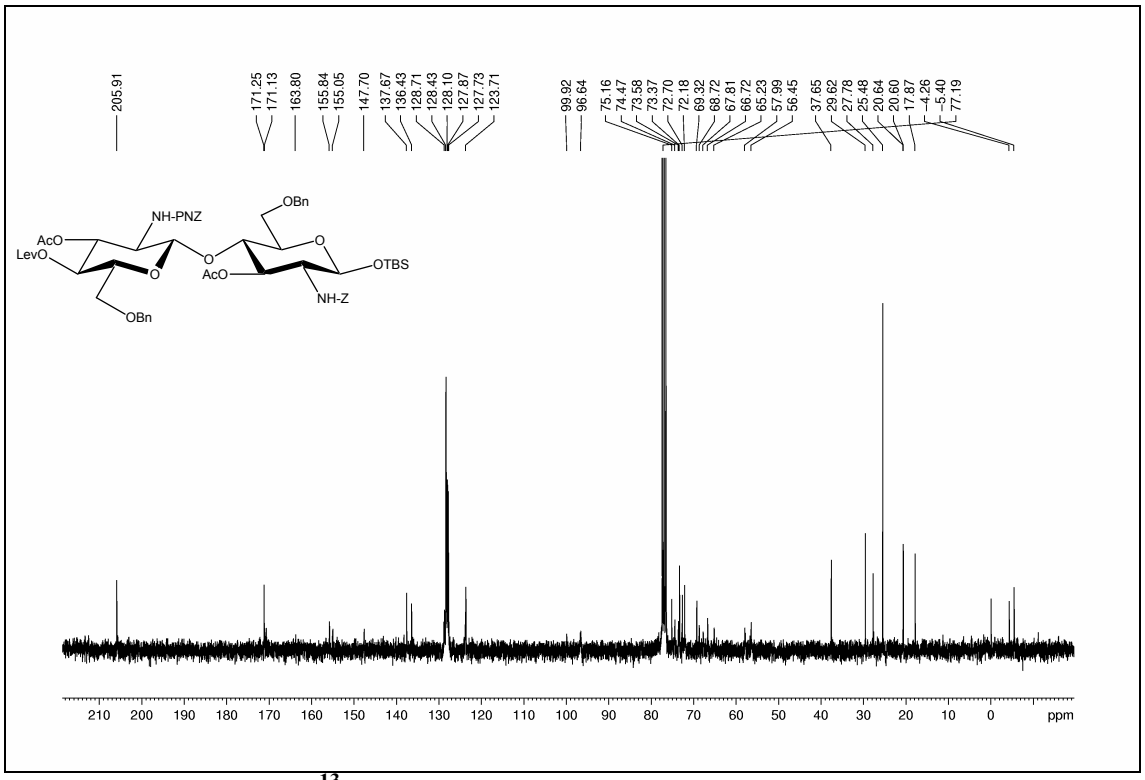
^{1}H NMR Spectrum of Compound 45



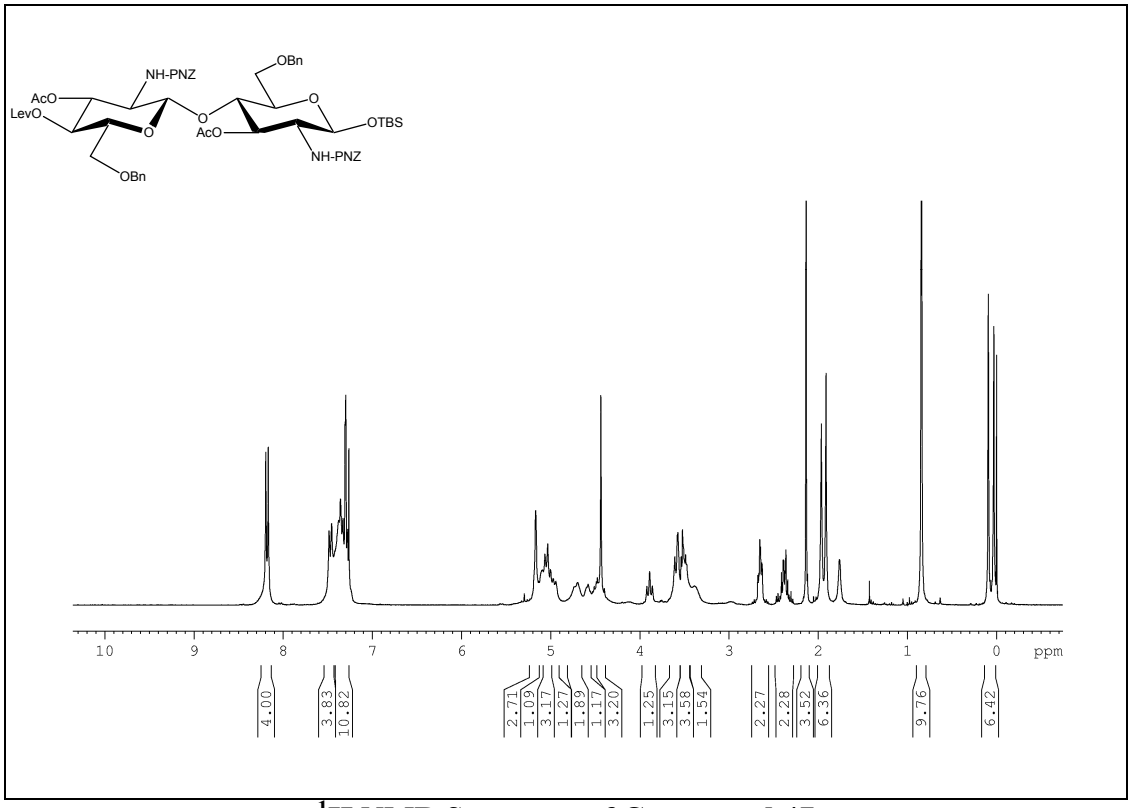
^{13}C NMR Spectrum of Compound 45



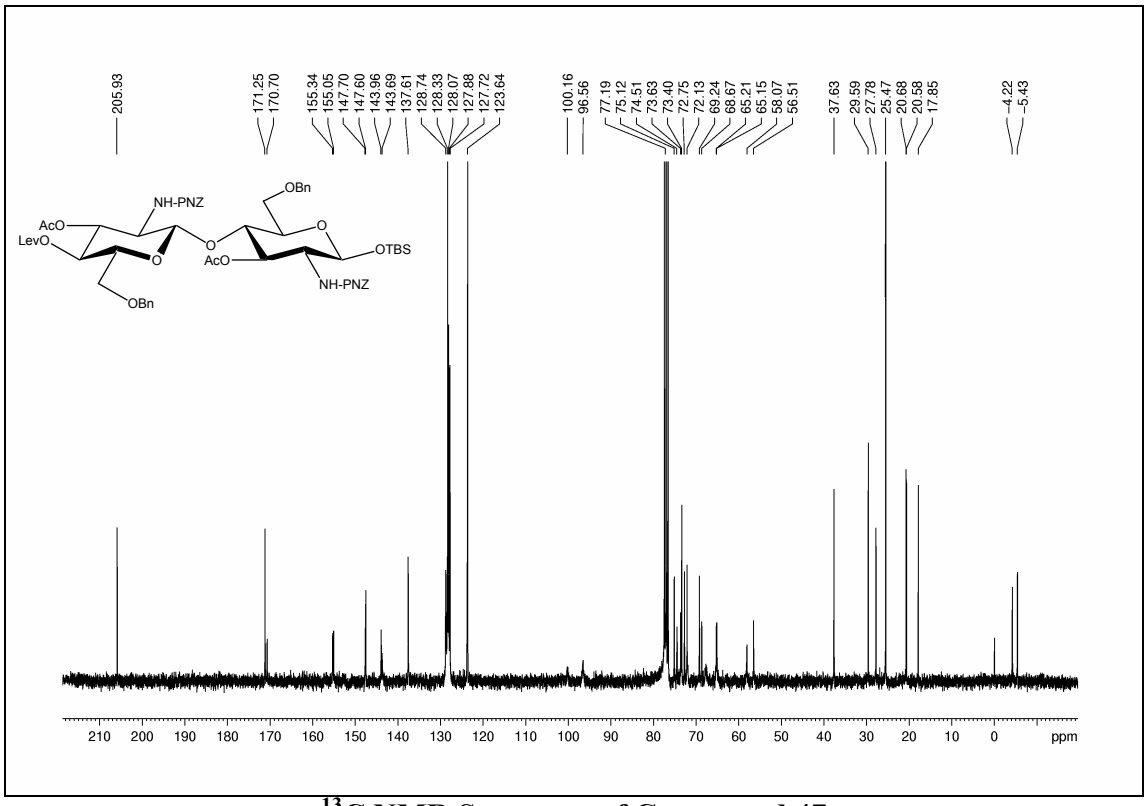
^{1}H NMR Spectrum of Compound 46



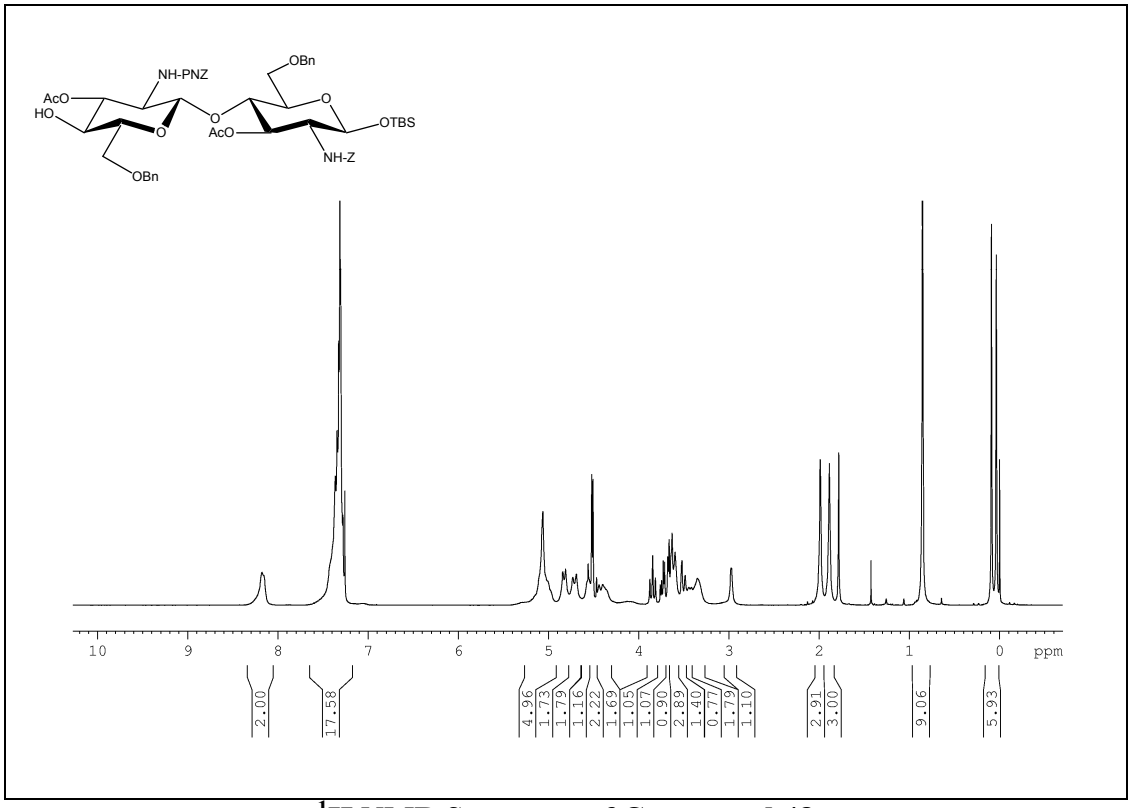
^{13}C NMR Spectrum of Compound 46



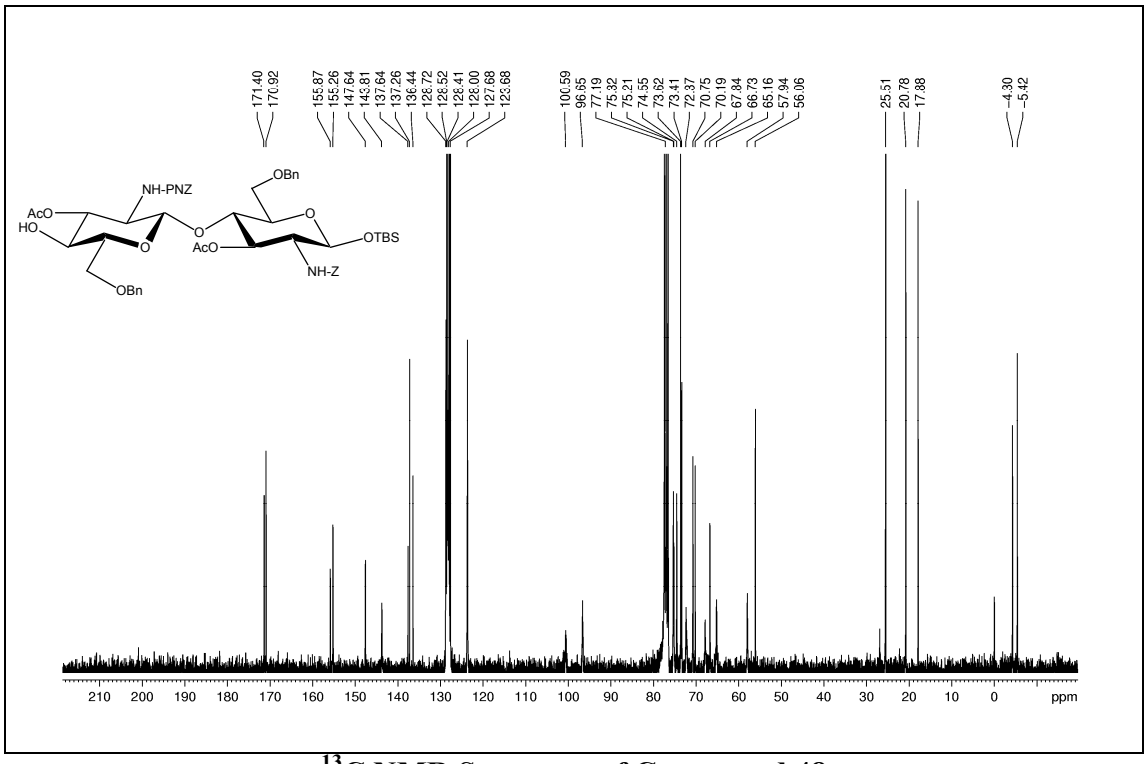
^{1}H NMR Spectrum of Compound 47



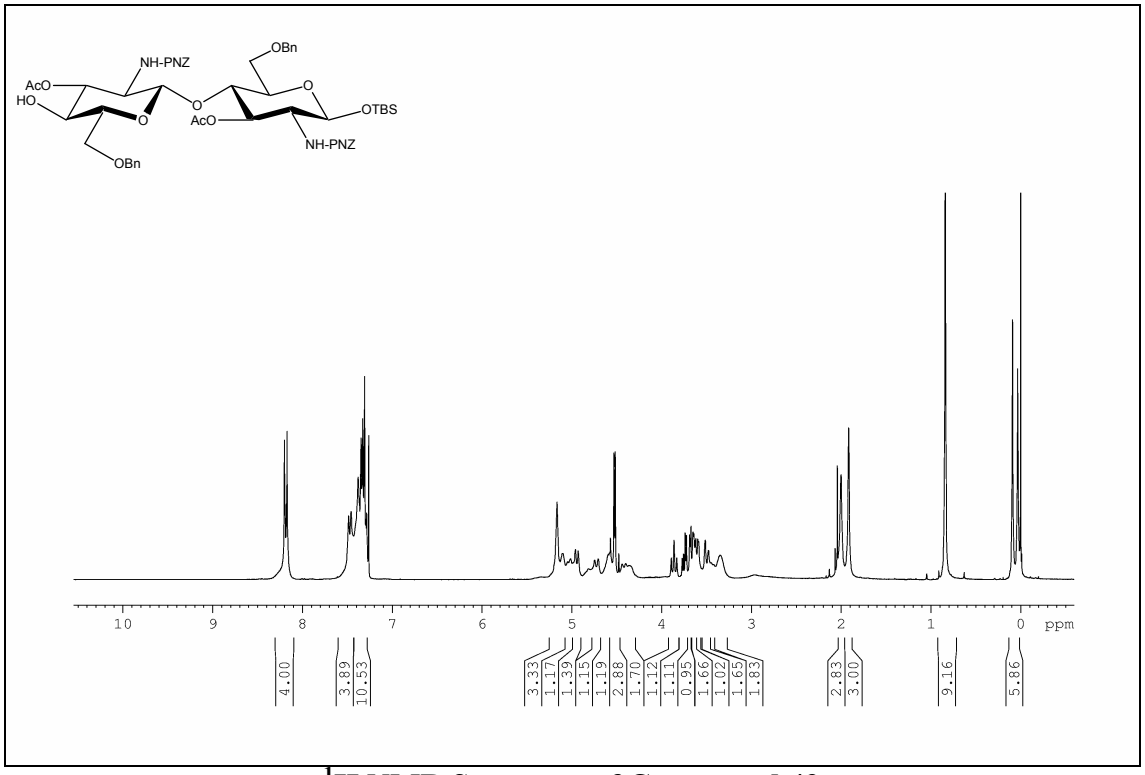
^{13}C NMR Spectrum of Compound 47



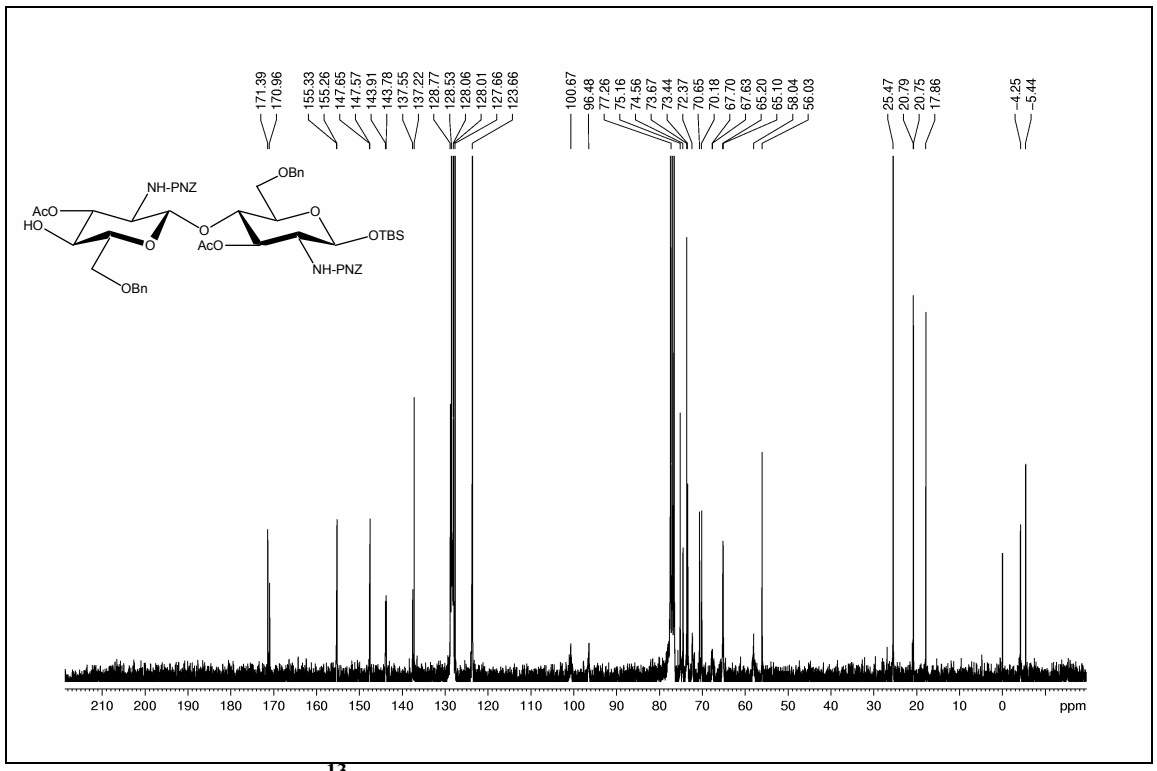
^{1}H NMR Spectrum of Compound 48



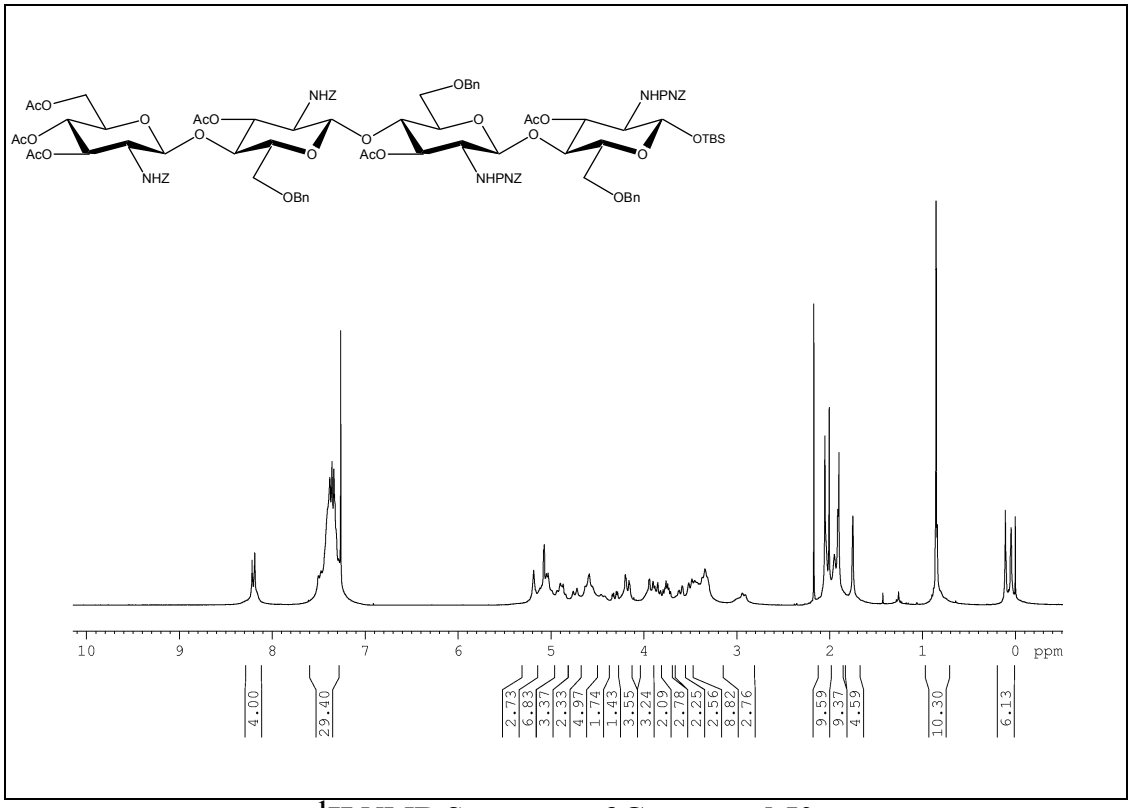
^{13}C NMR Spectrum of Compound 48



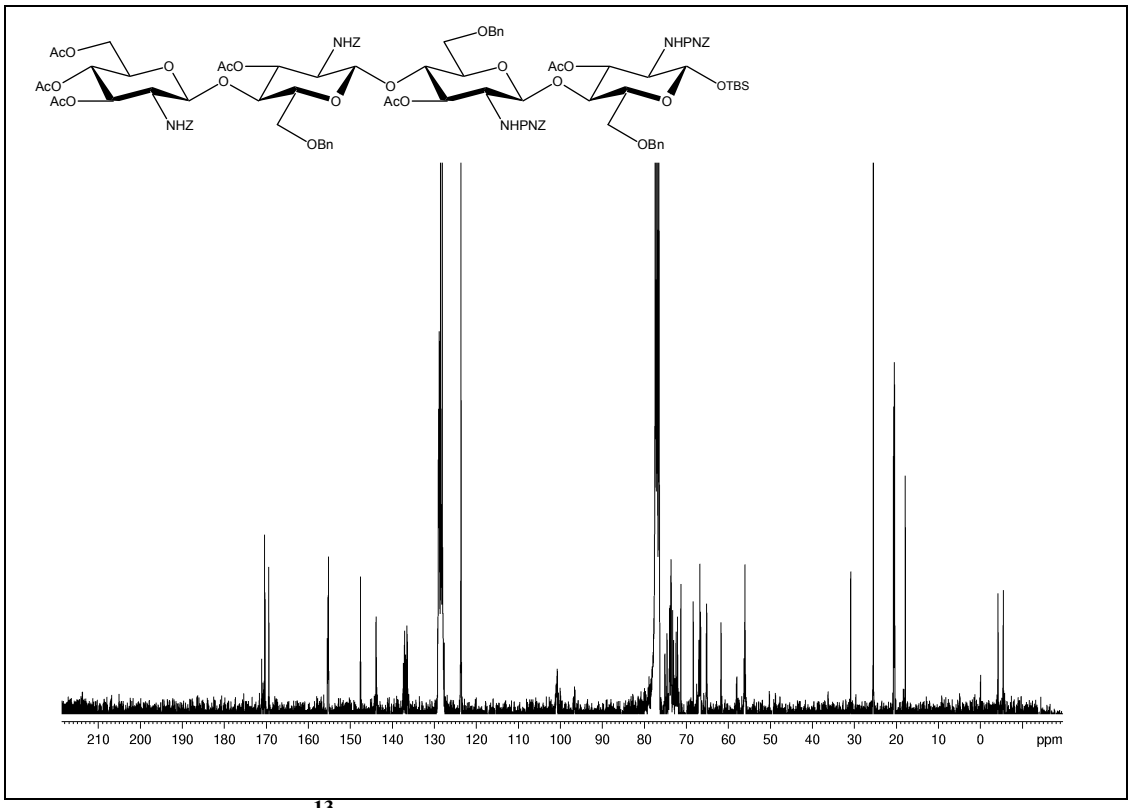
^{1}H NMR Spectrum of Compound 49



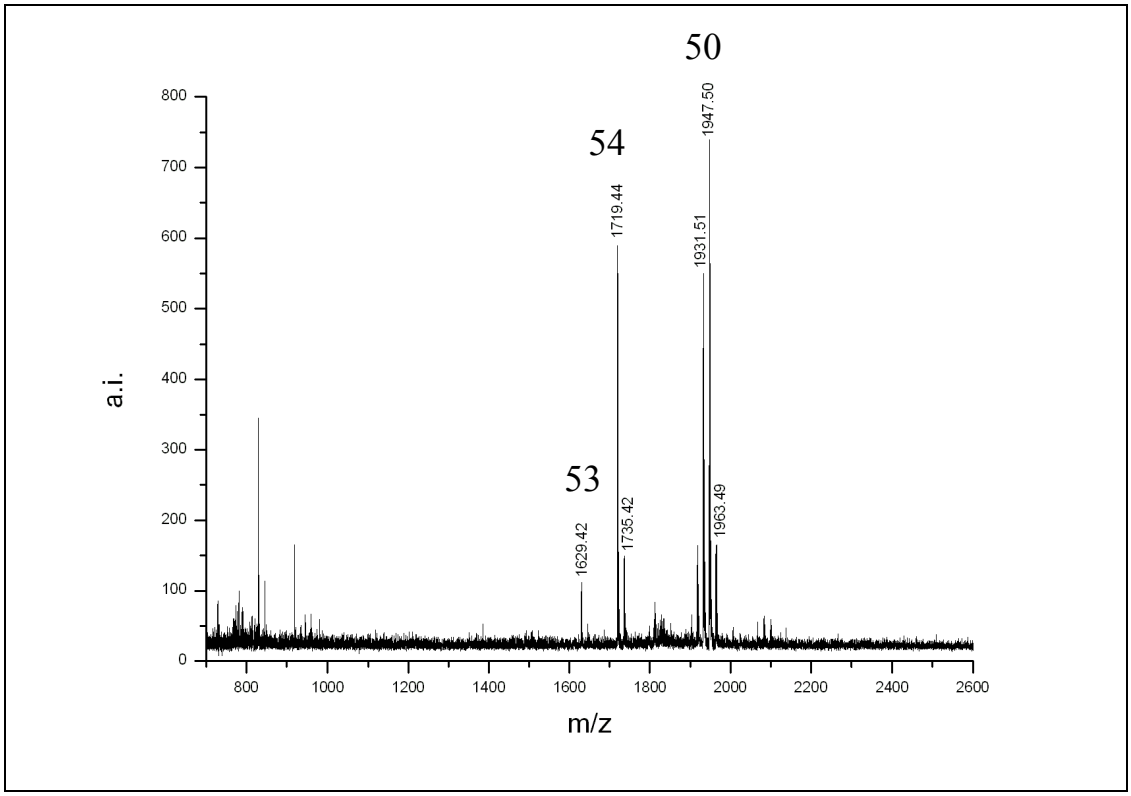
^{13}C NMR Spectrum of Compound 49



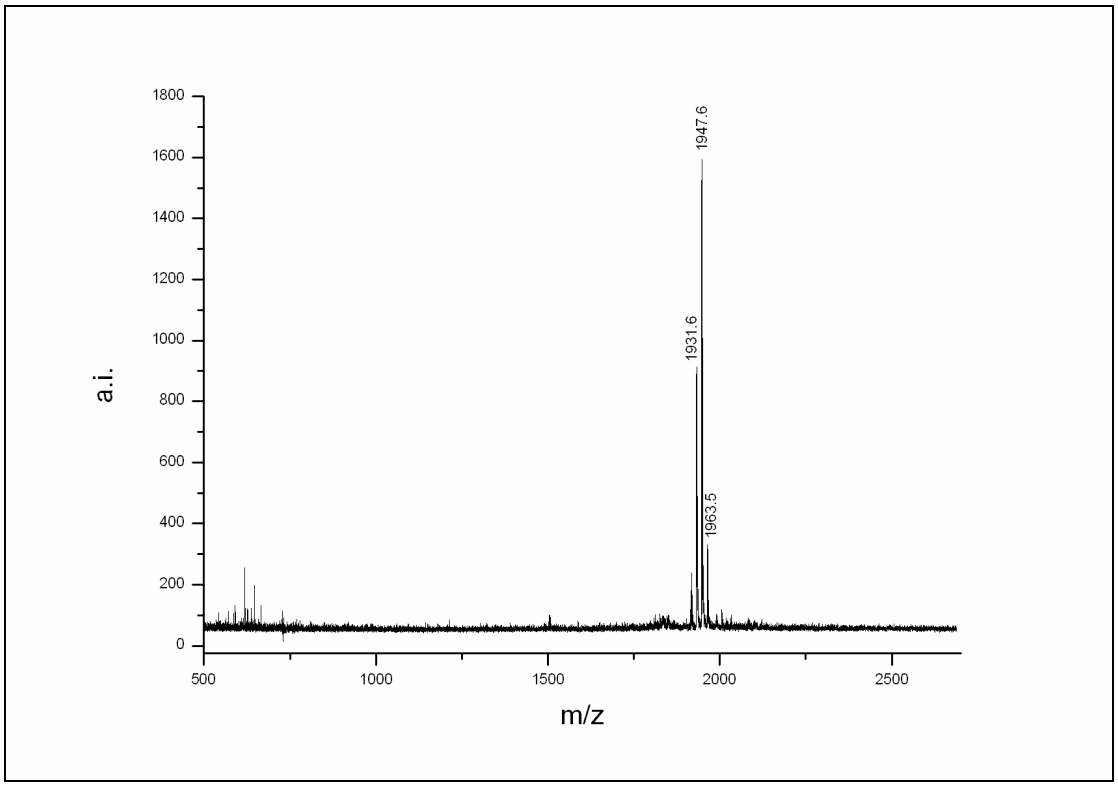
^{1}H NMR Spectrum of Compound 50



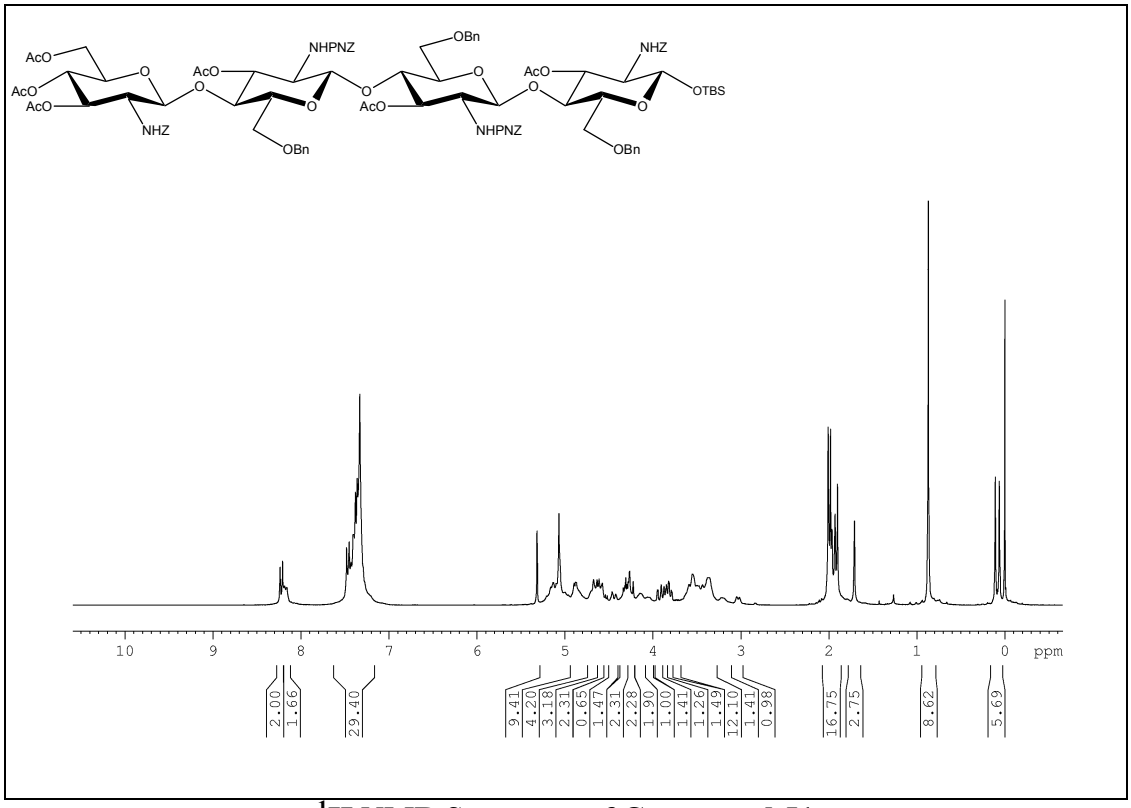
^{13}C NMR Spectrum of Compound 50



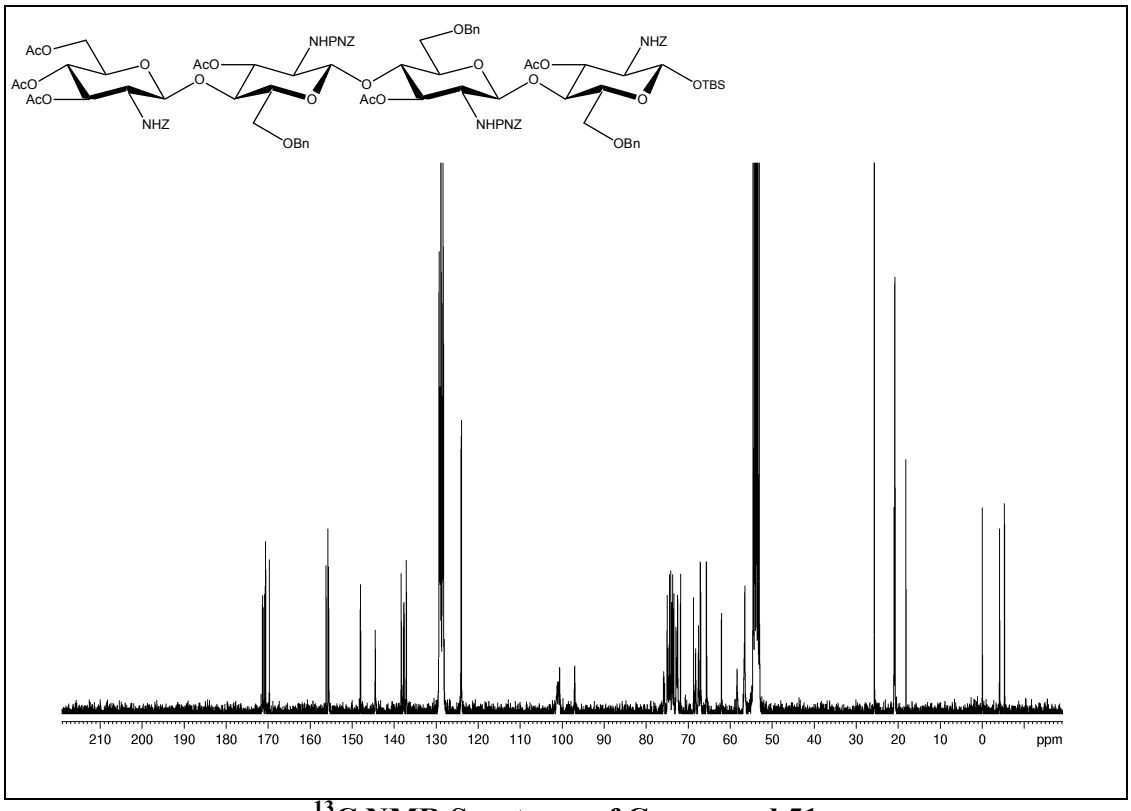
MALDI-TOF MS of Chromatographed protected tetrasaccharide 50



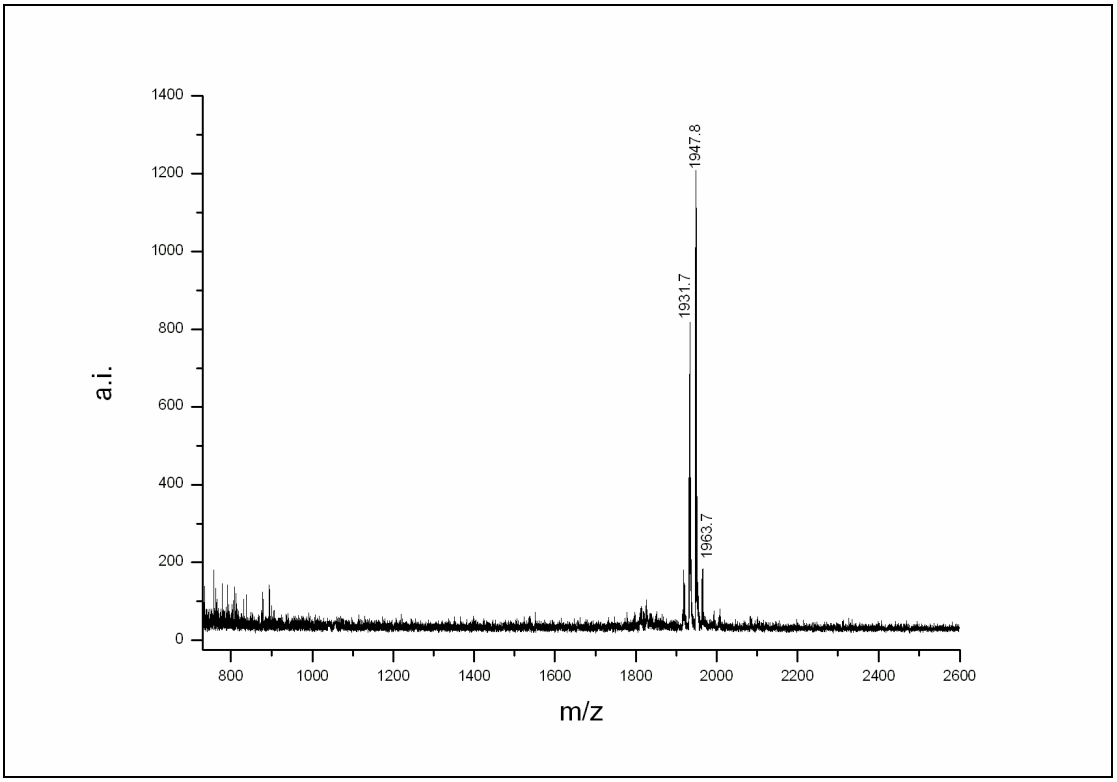
MALDI-TOF MS of Compound 50 (Scheme 3.1.26)



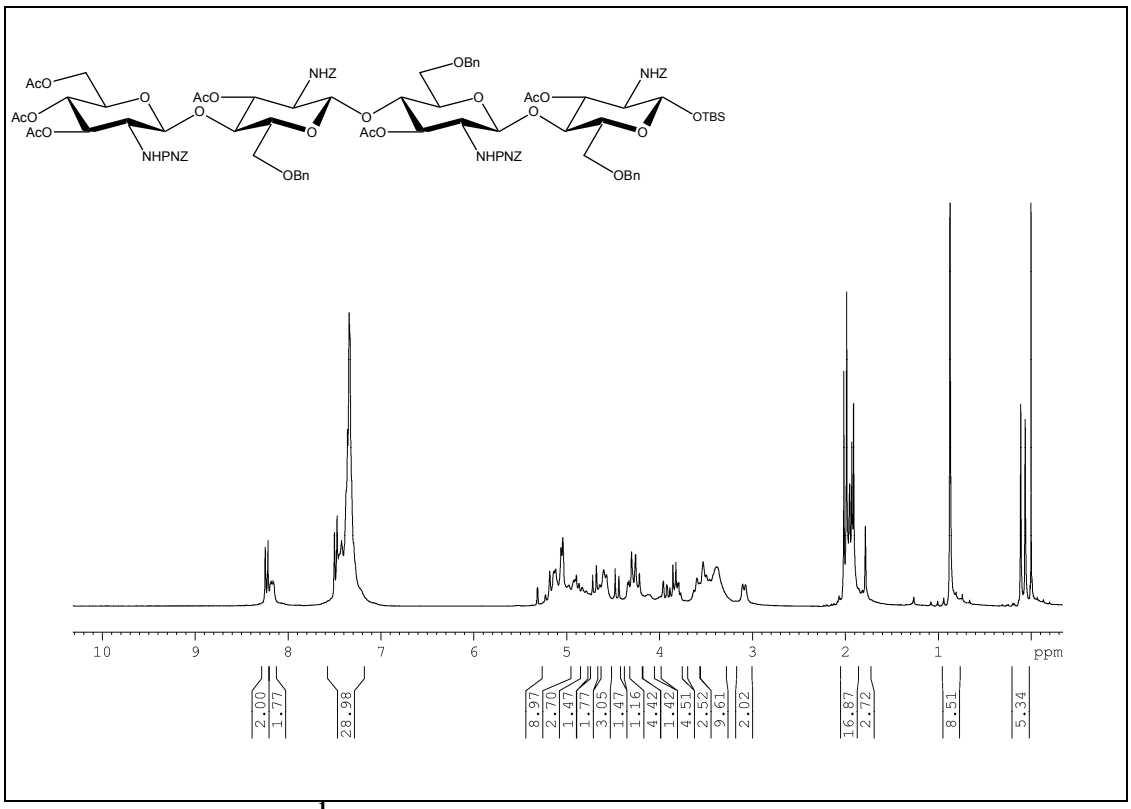
^{1}H NMR Spectrum of Compound 51



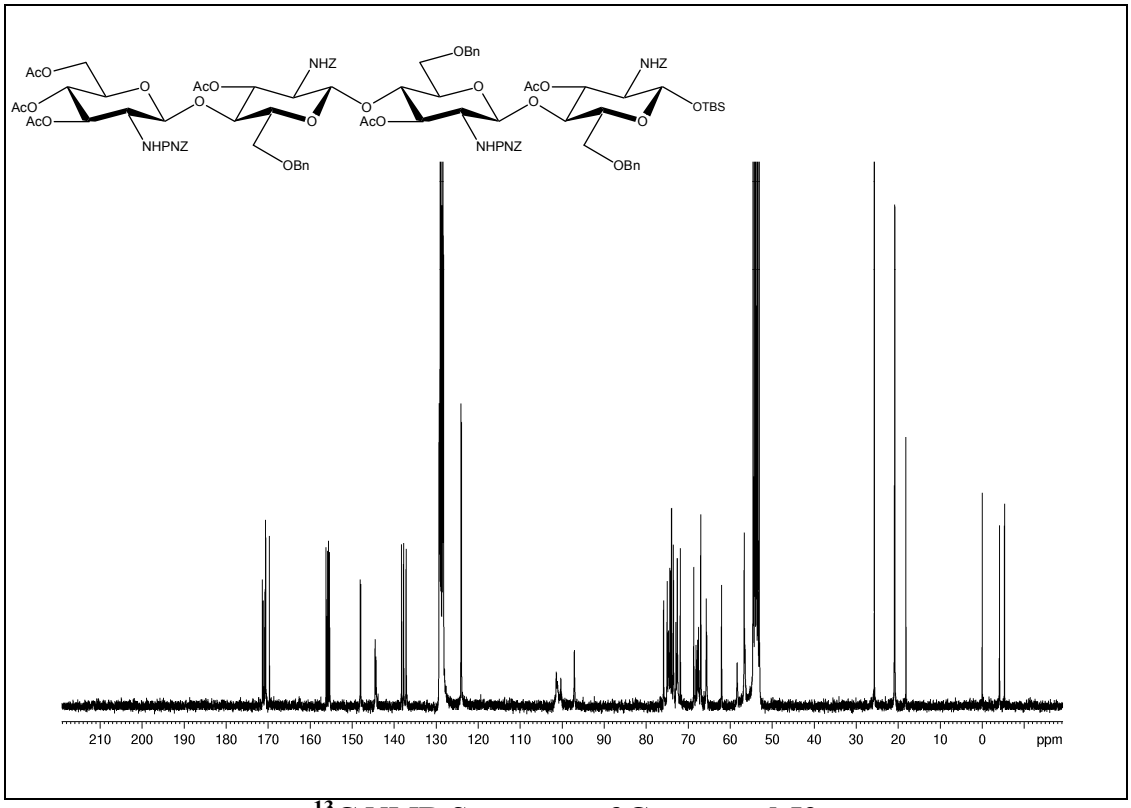
^{13}C NMR Spectrum of Compound 51



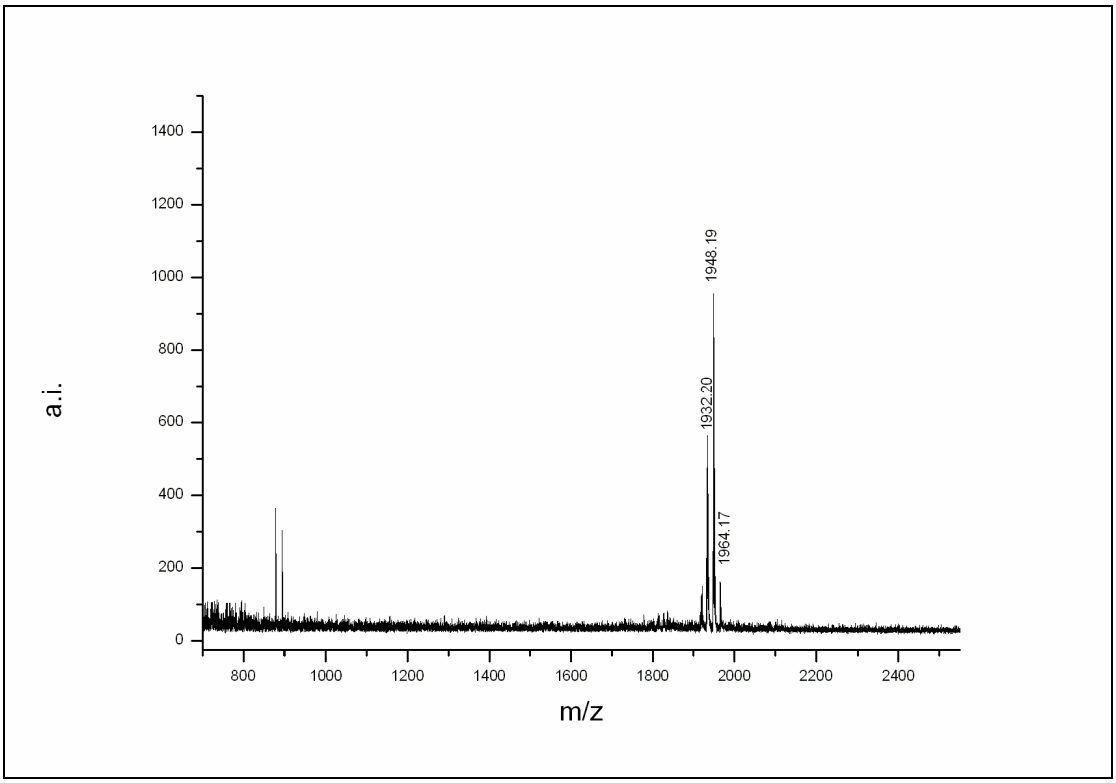
MALDI-TOF MS of Compound 51



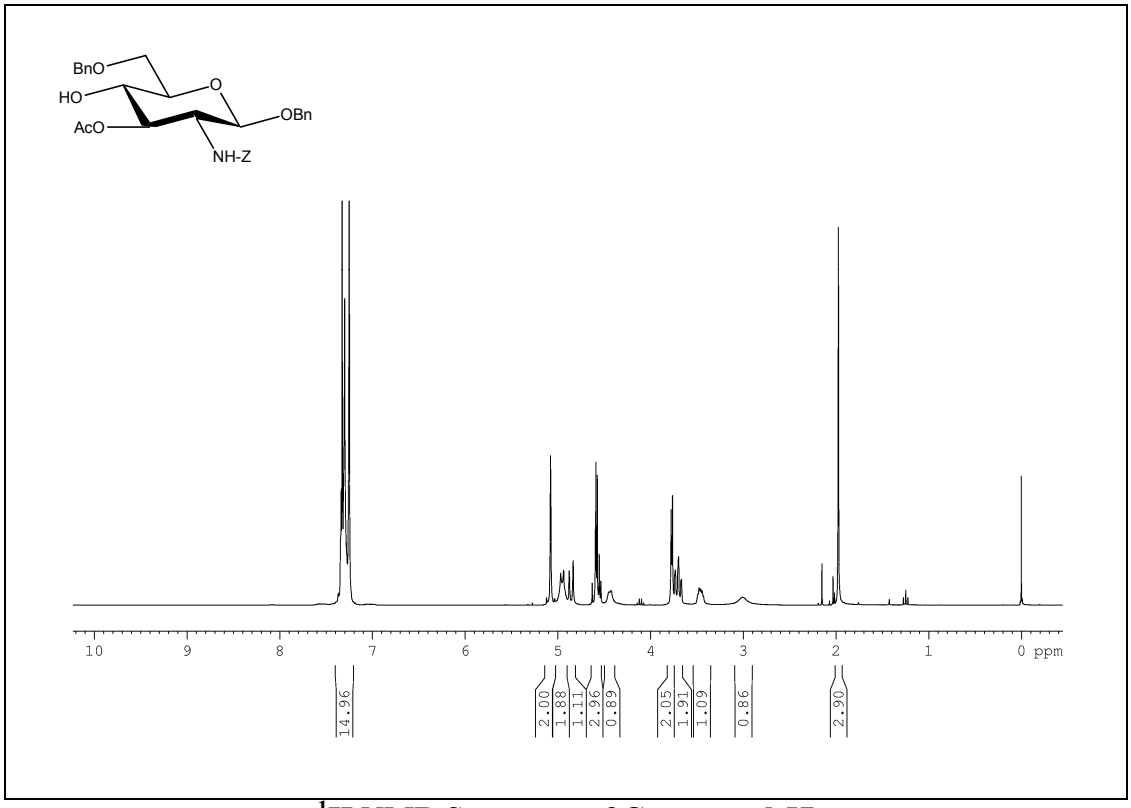
^{1}H NMR Spectrum of Compound 52



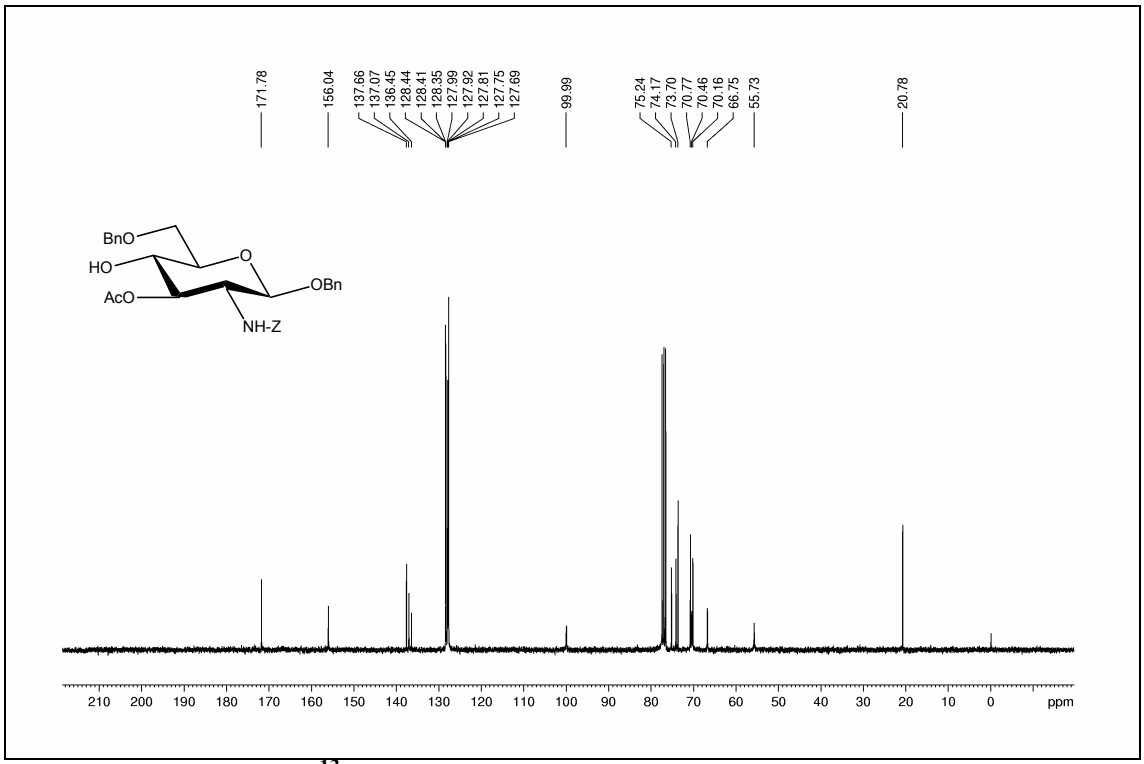
^{13}C NMR Spectrum of Compound 52



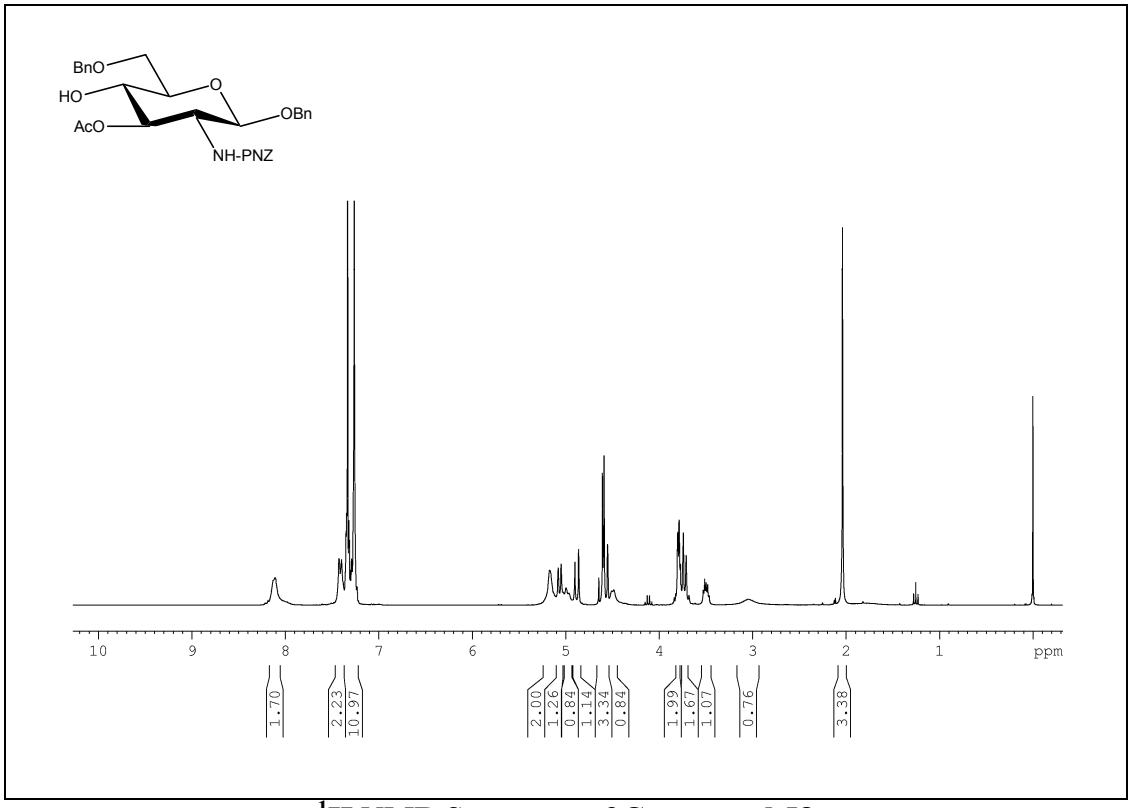
MALDI-TOF MS of Compound 52



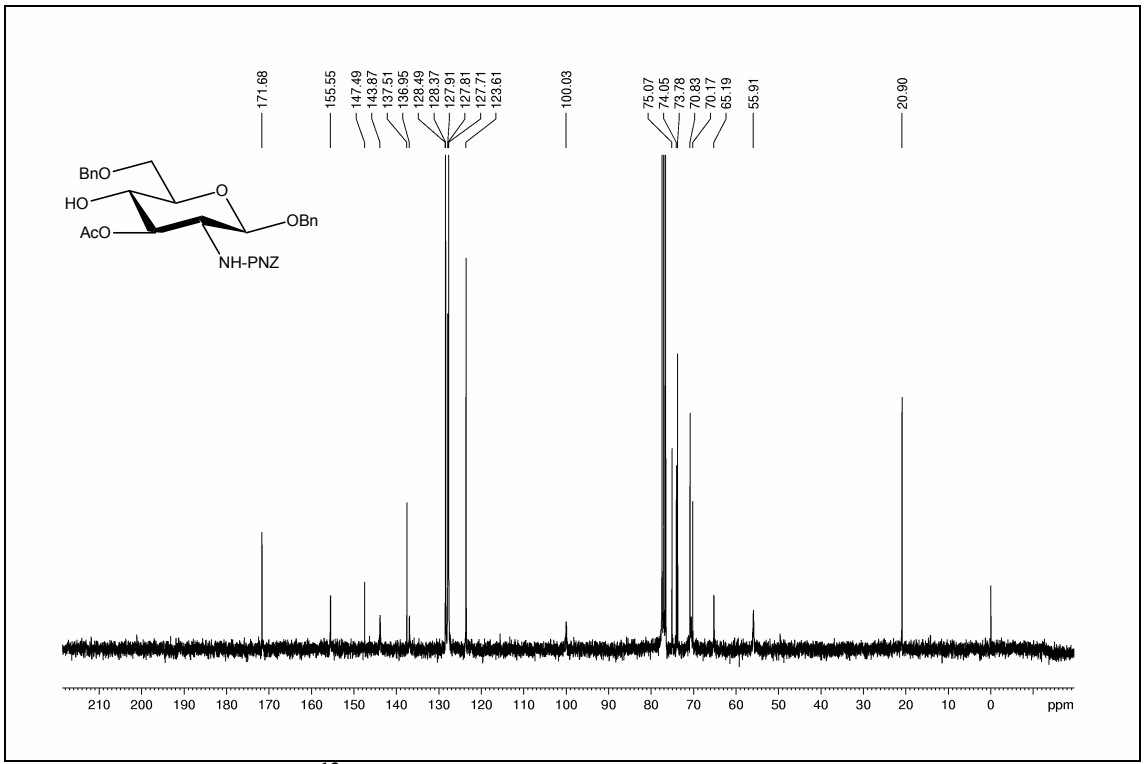
^{1}H NMR Spectrum of Compound 57



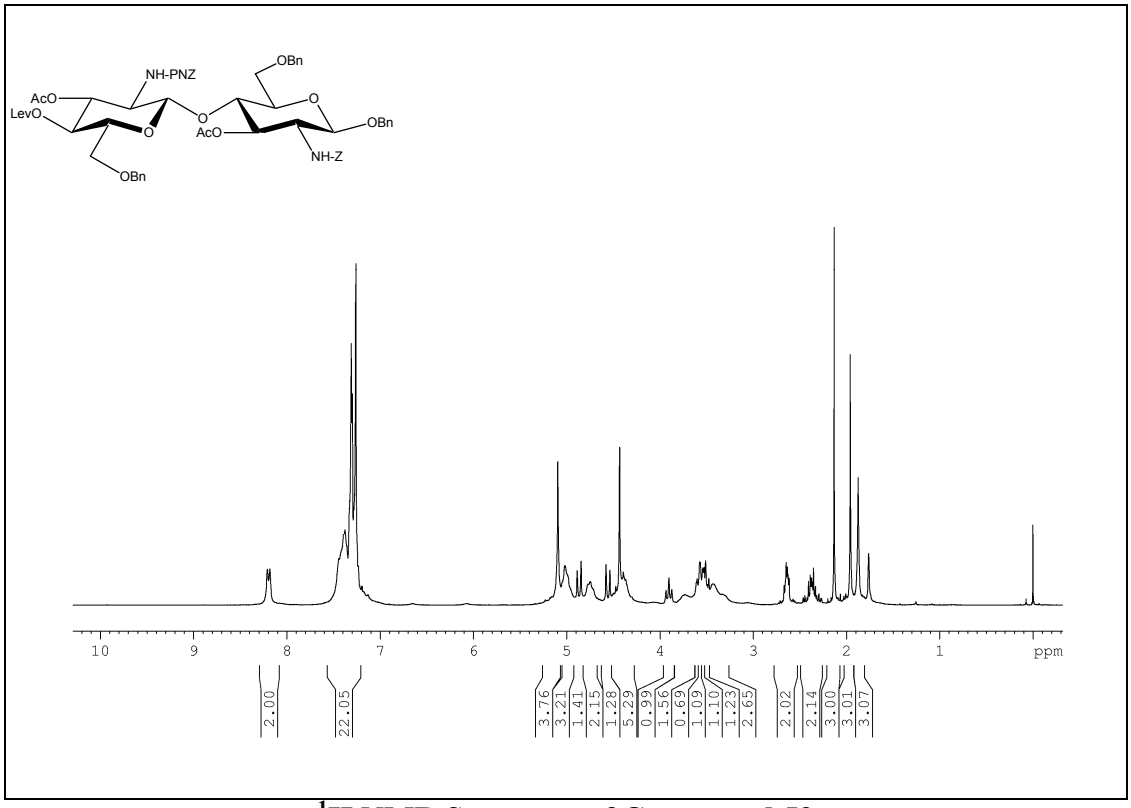
^{13}C NMR Spectrum of Compound 57



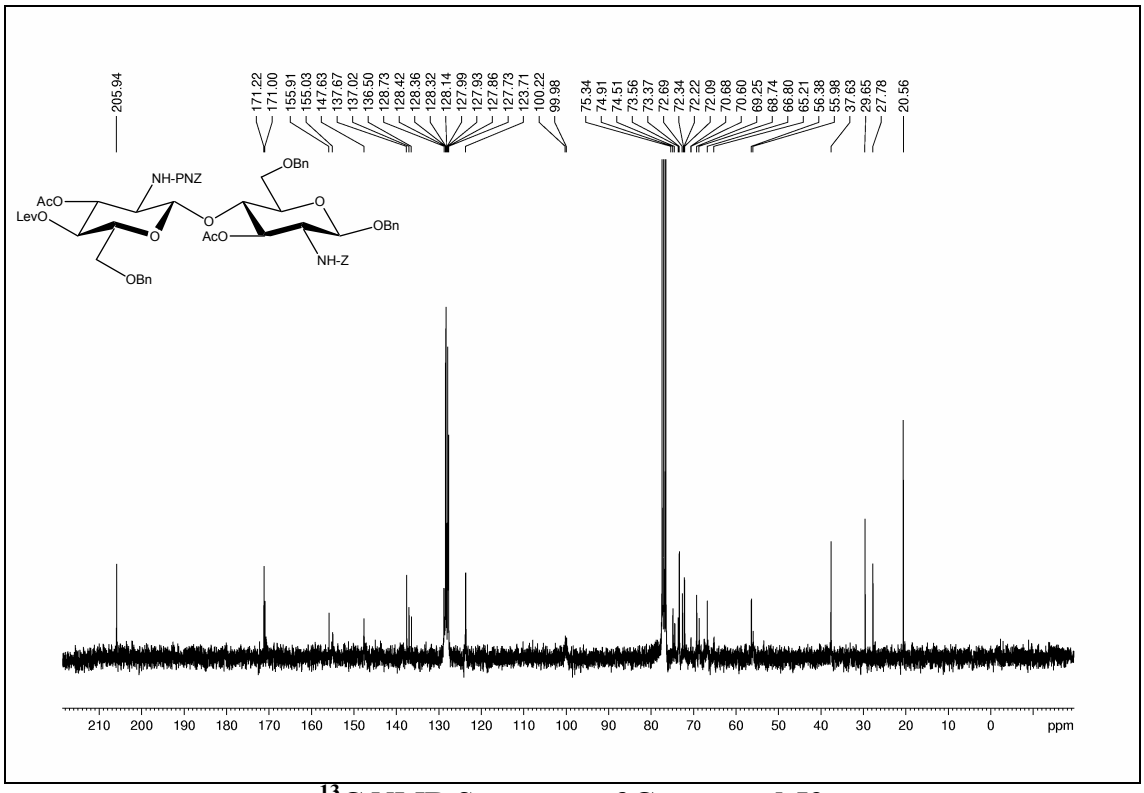
^{1}H NMR Spectrum of Compound 58



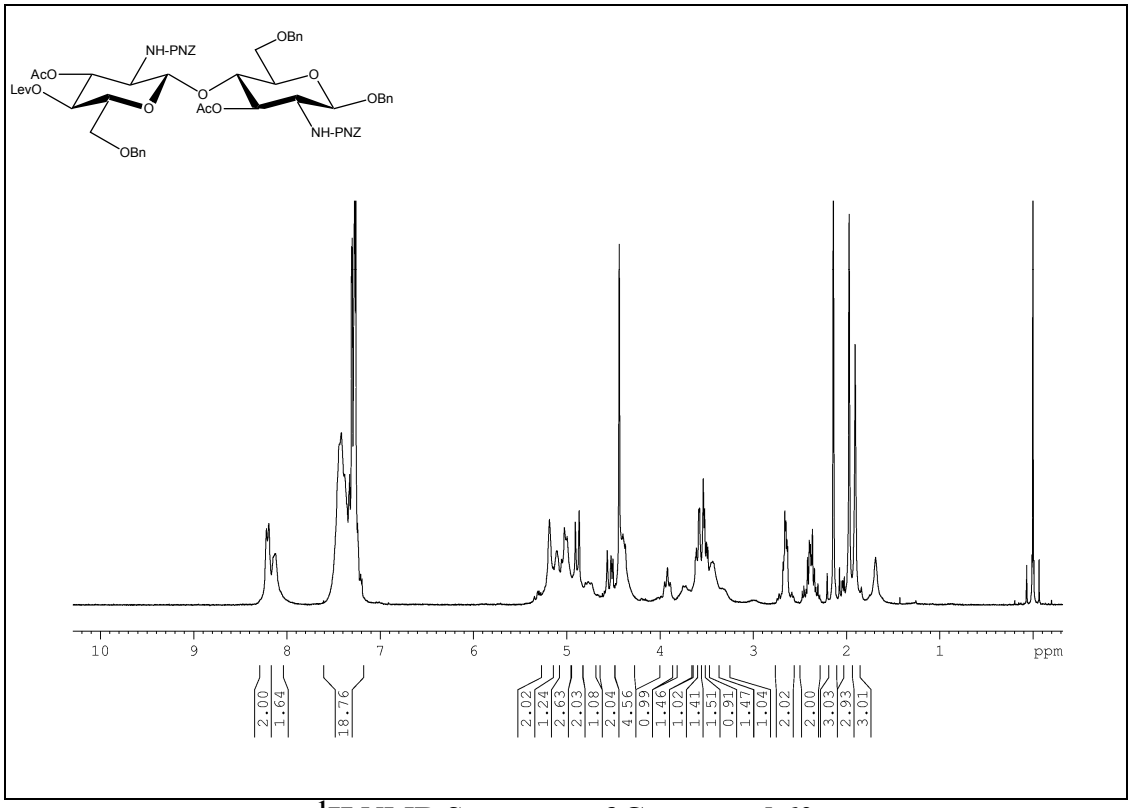
^{13}C NMR Spectrum of Compound 58



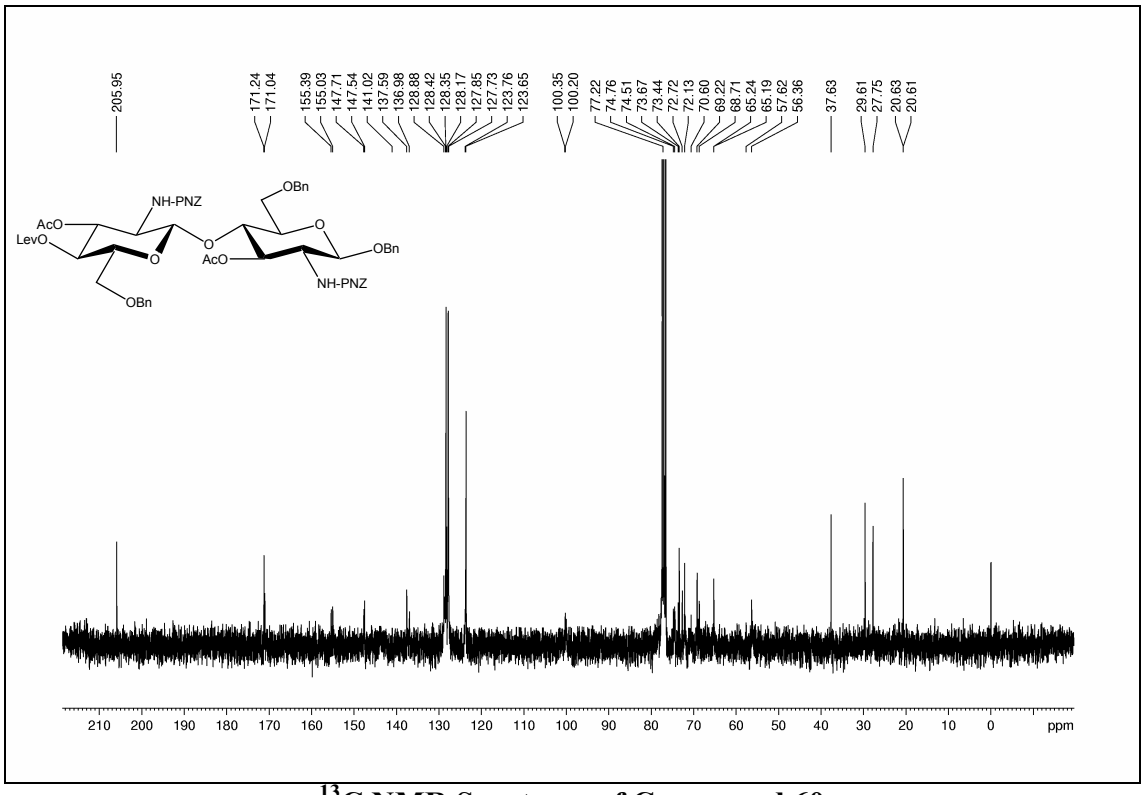
^{1}H NMR Spectrum of Compound 59



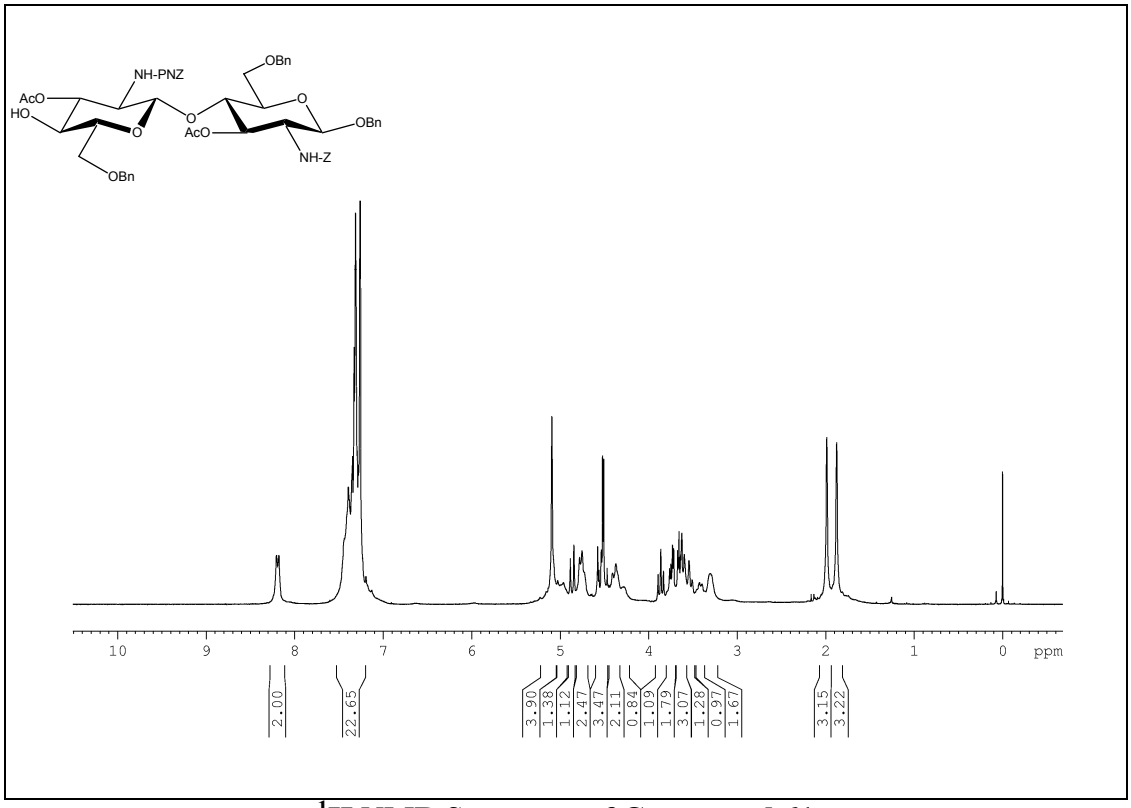
^{13}C NMR Spectrum of Compound 59



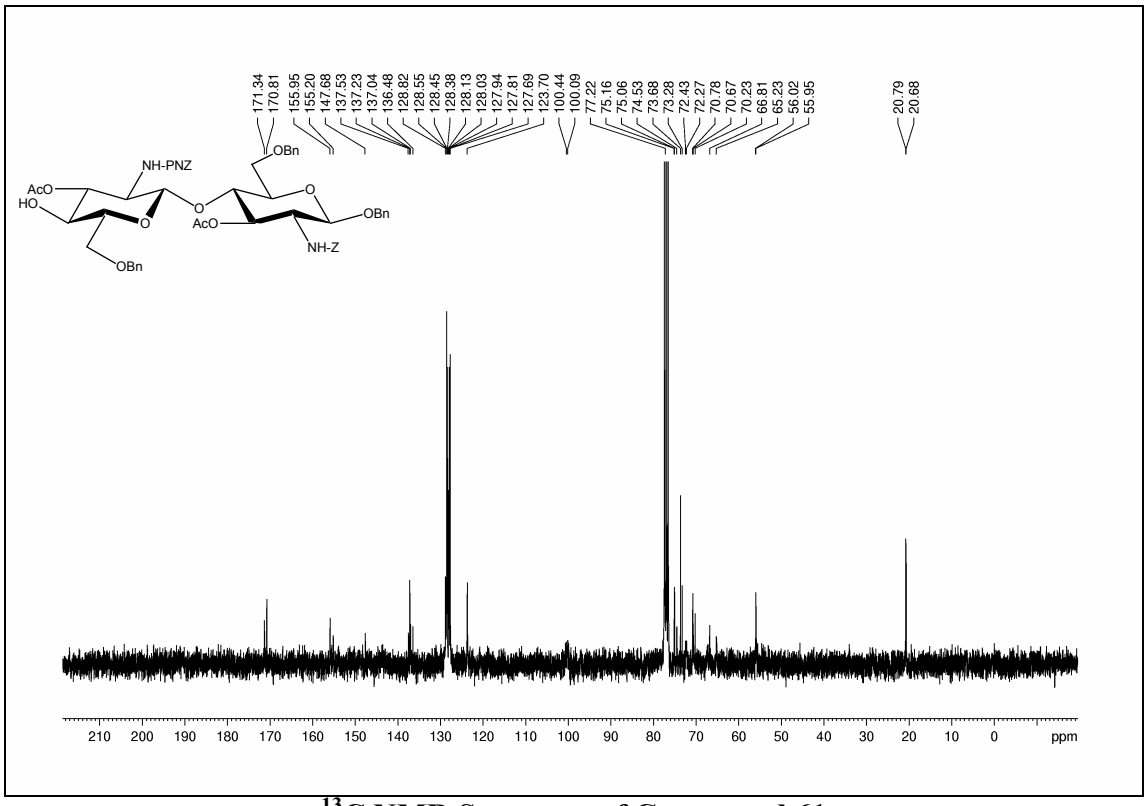
^{1}H NMR Spectrum of Compound 60



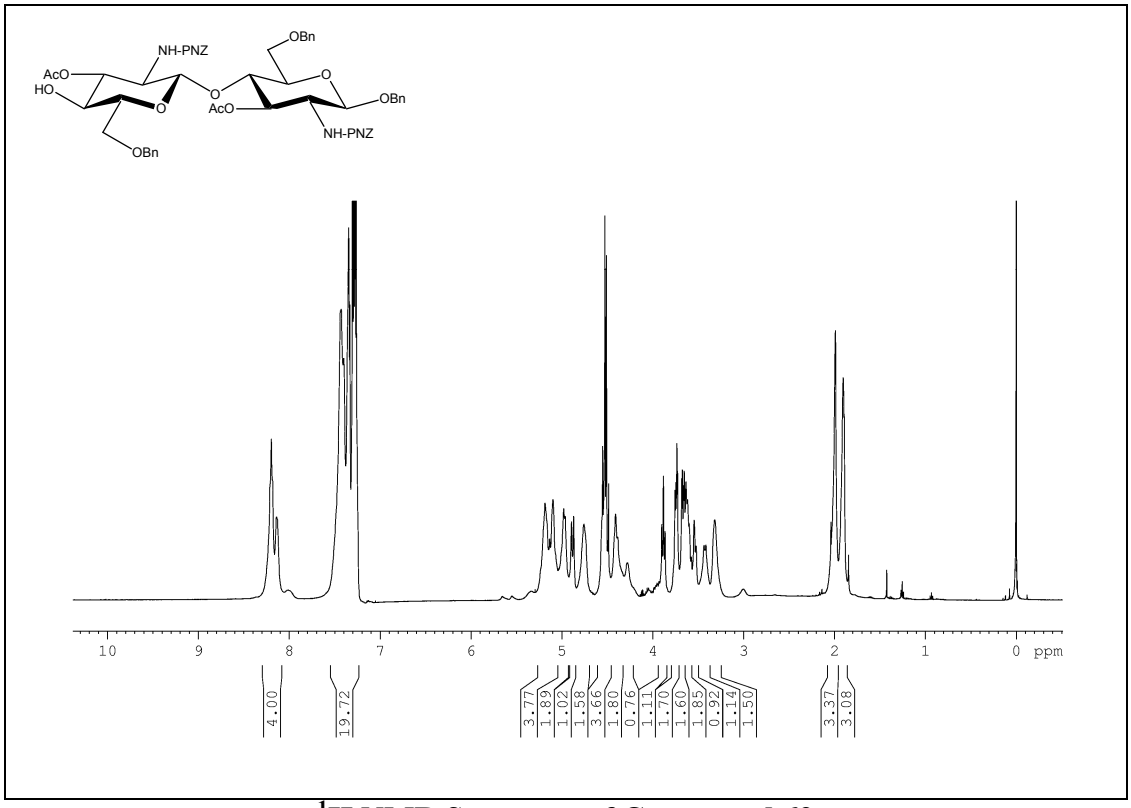
^{13}C NMR Spectrum of Compound 60



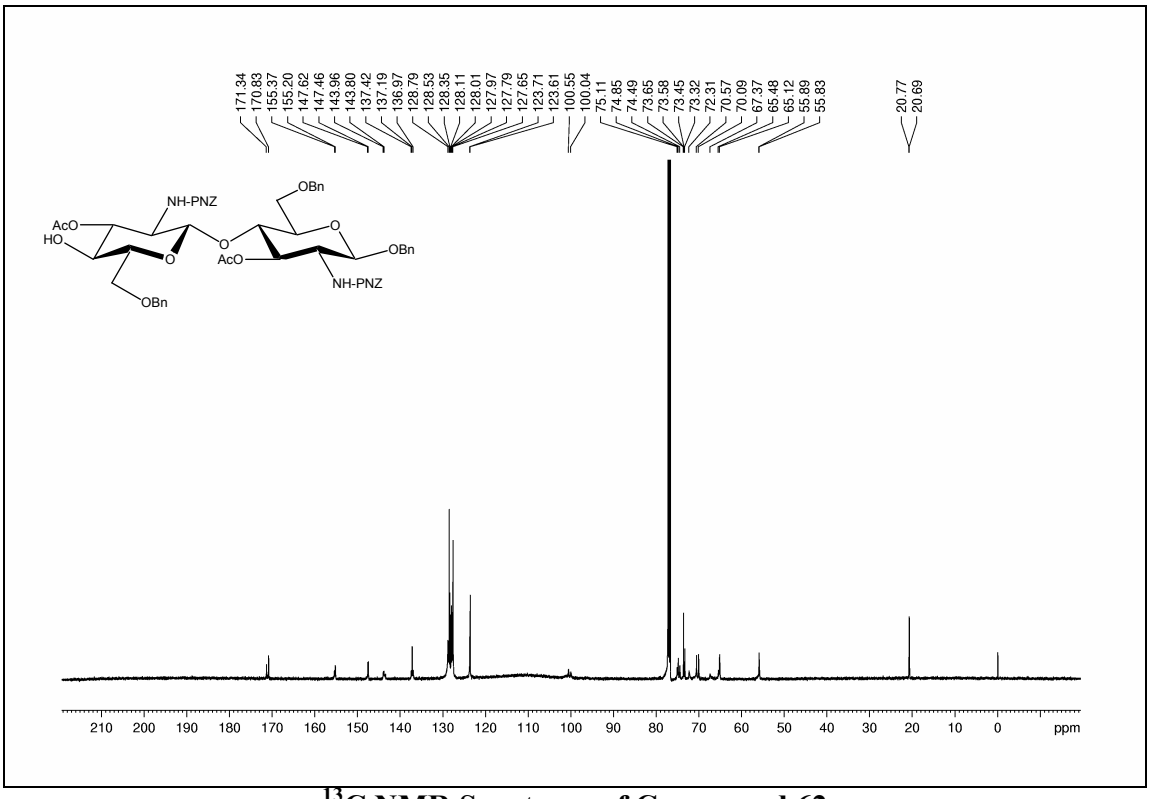
^{1}H NMR Spectrum of Compound 61



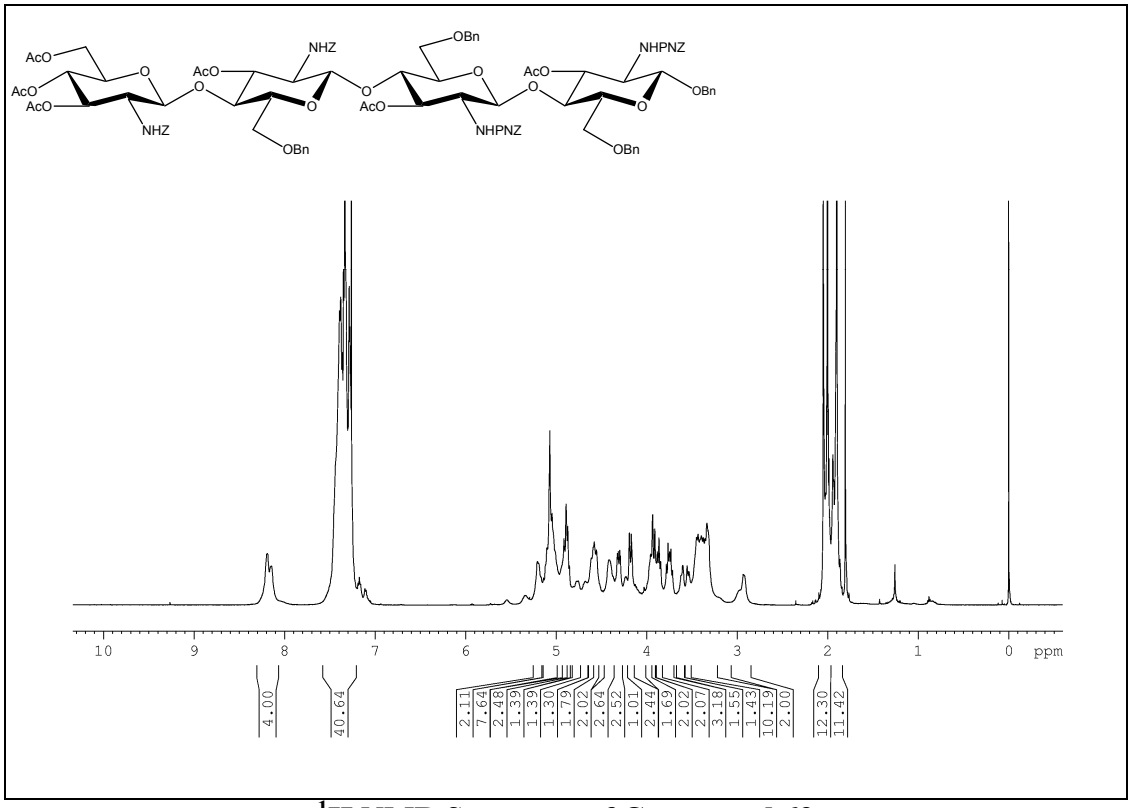
^{13}C NMR Spectrum of Compound 61



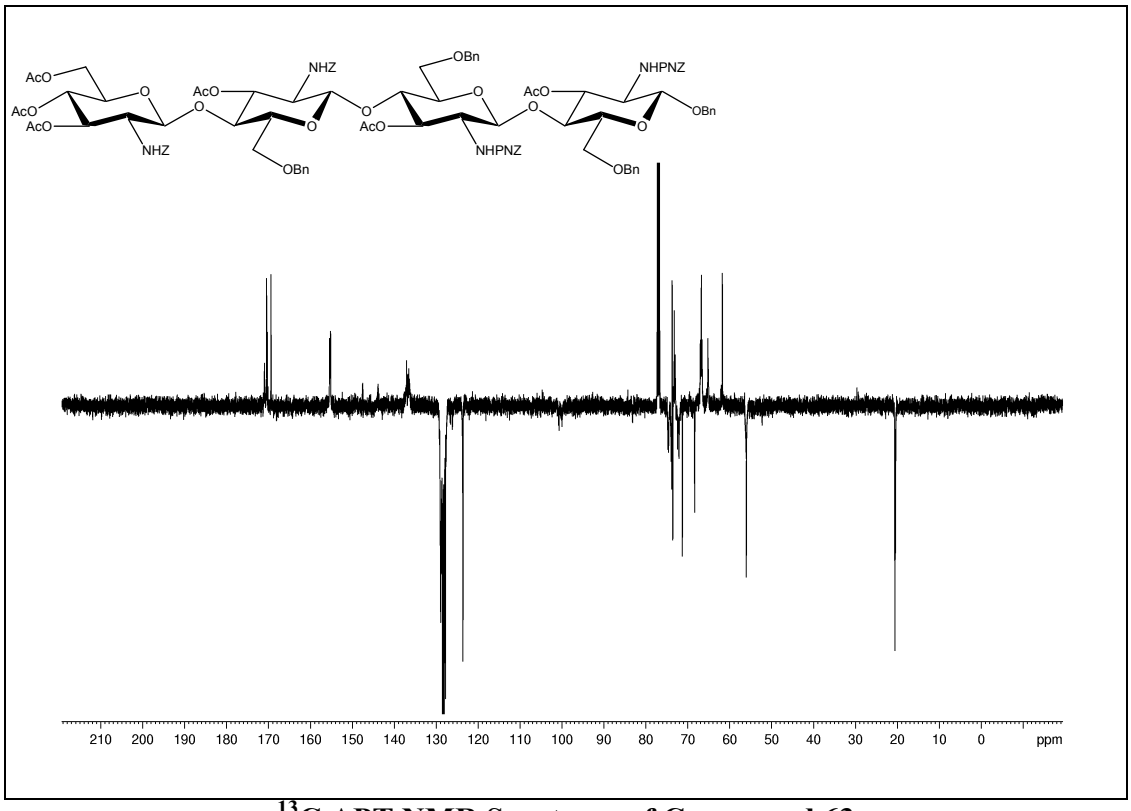
^{1}H NMR Spectrum of Compound 62



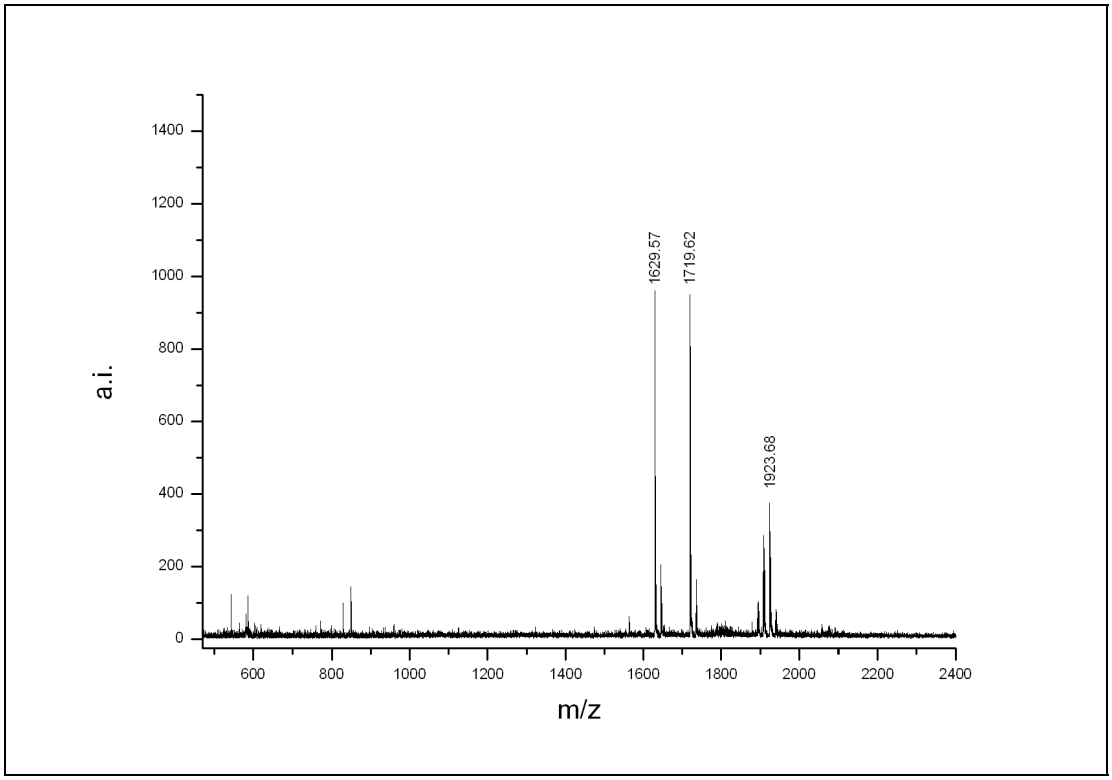
^{13}C NMR Spectrum of Compound 62



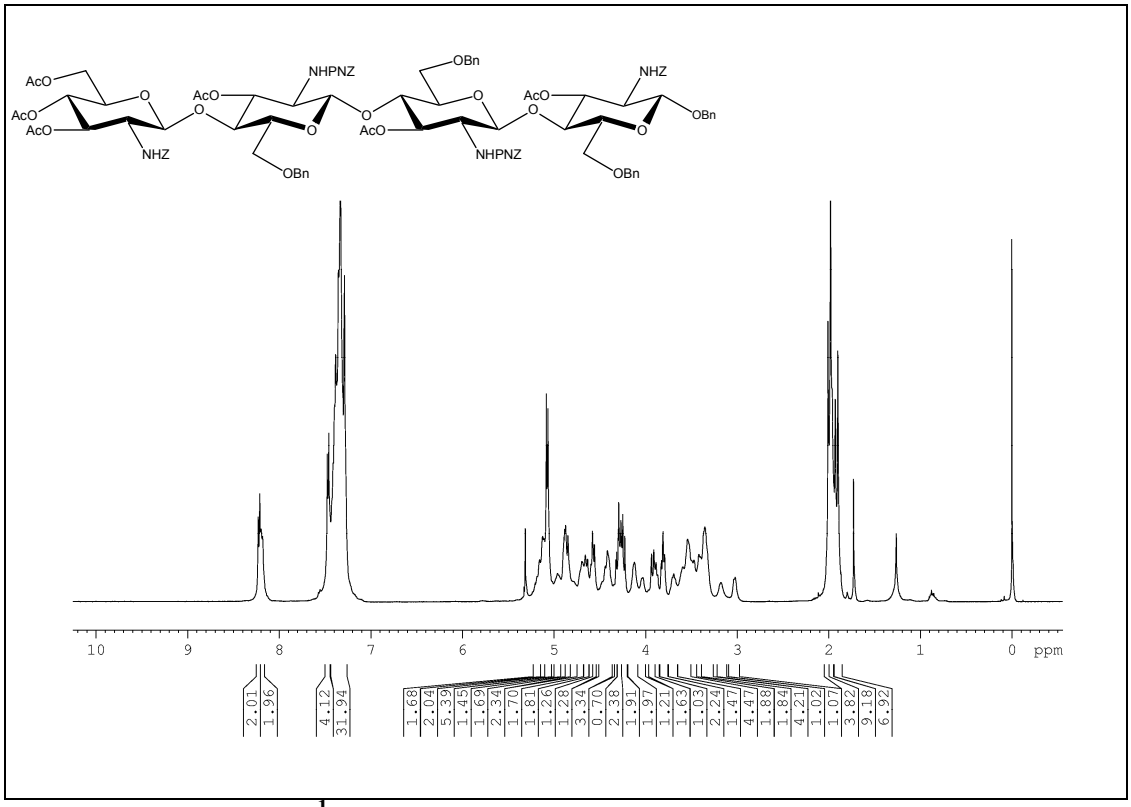
^{1}H NMR Spectrum of Compound 63



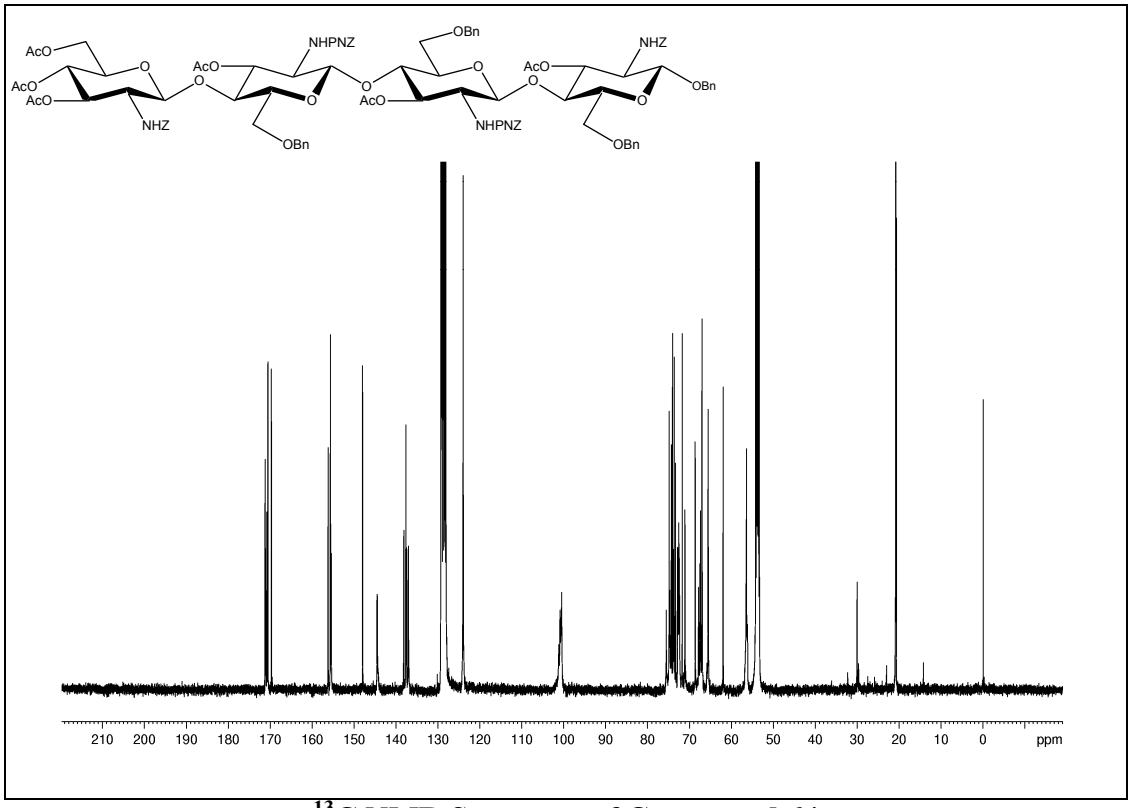
^{13}C APT NMR Spectrum of Compound 63



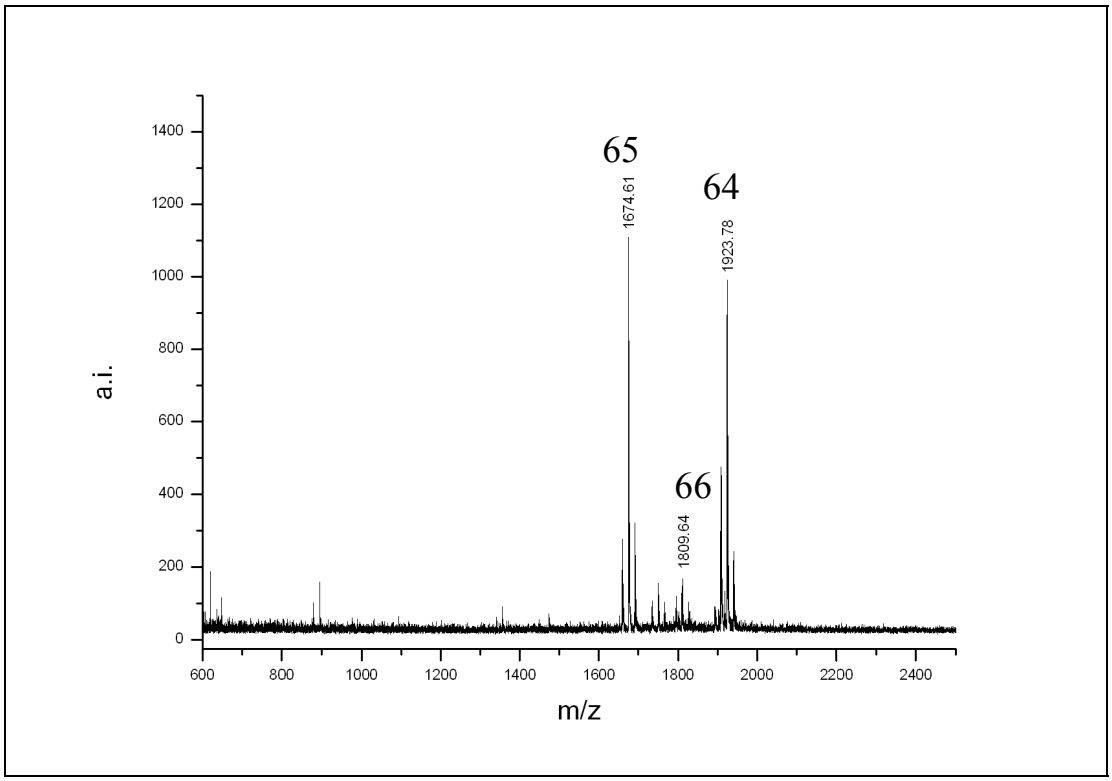
MALDI-TOF MS of Compound 63 (including byproducts 53 and 54)



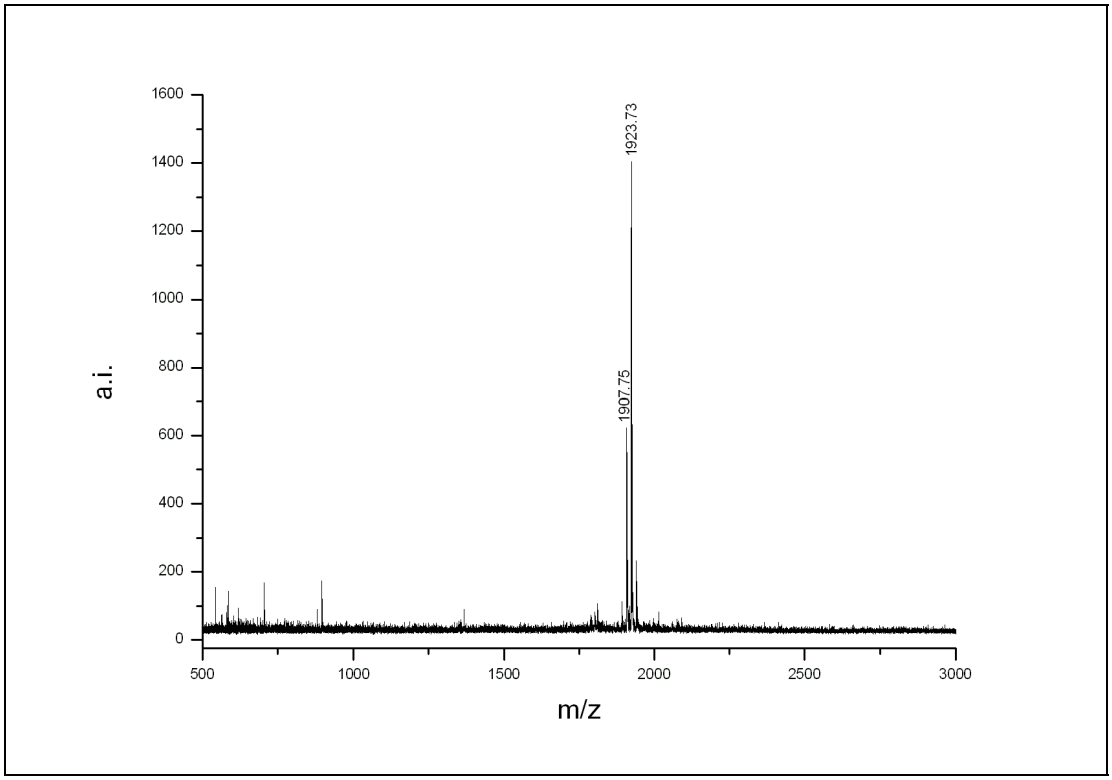
^{1}H NMR Spectrum of Compound 64



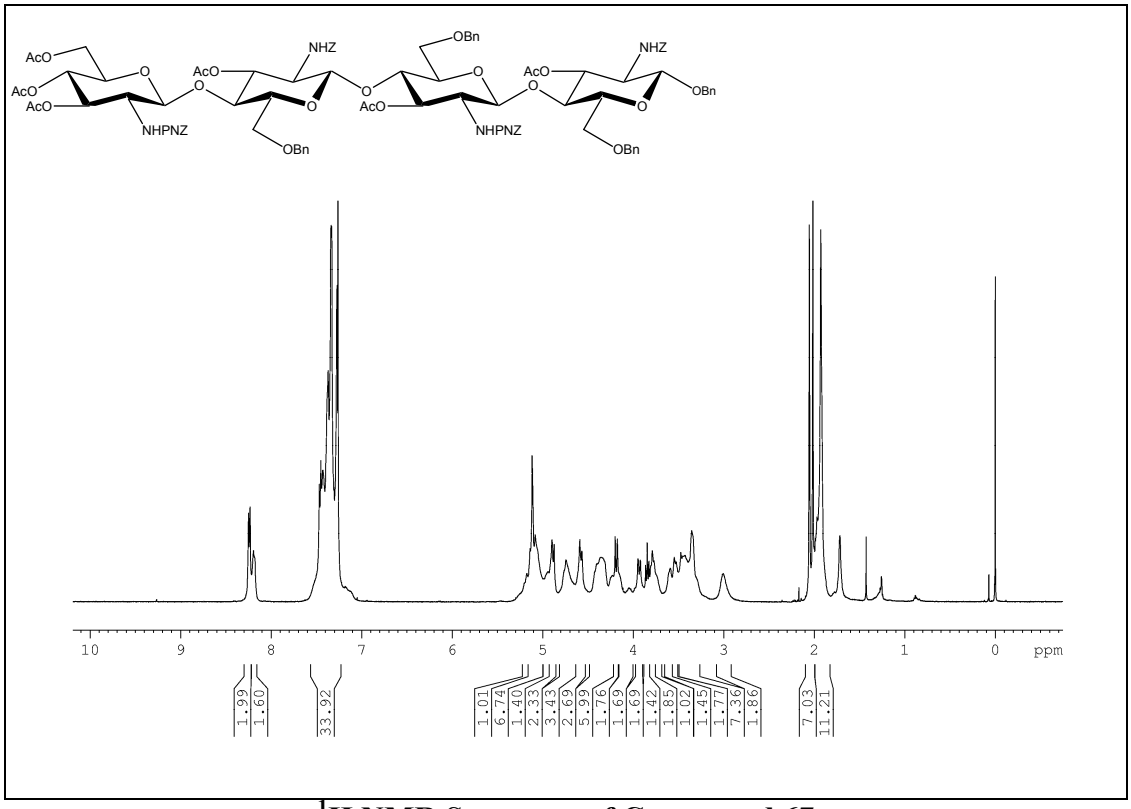
^{13}C NMR Spectrum of Compound 64



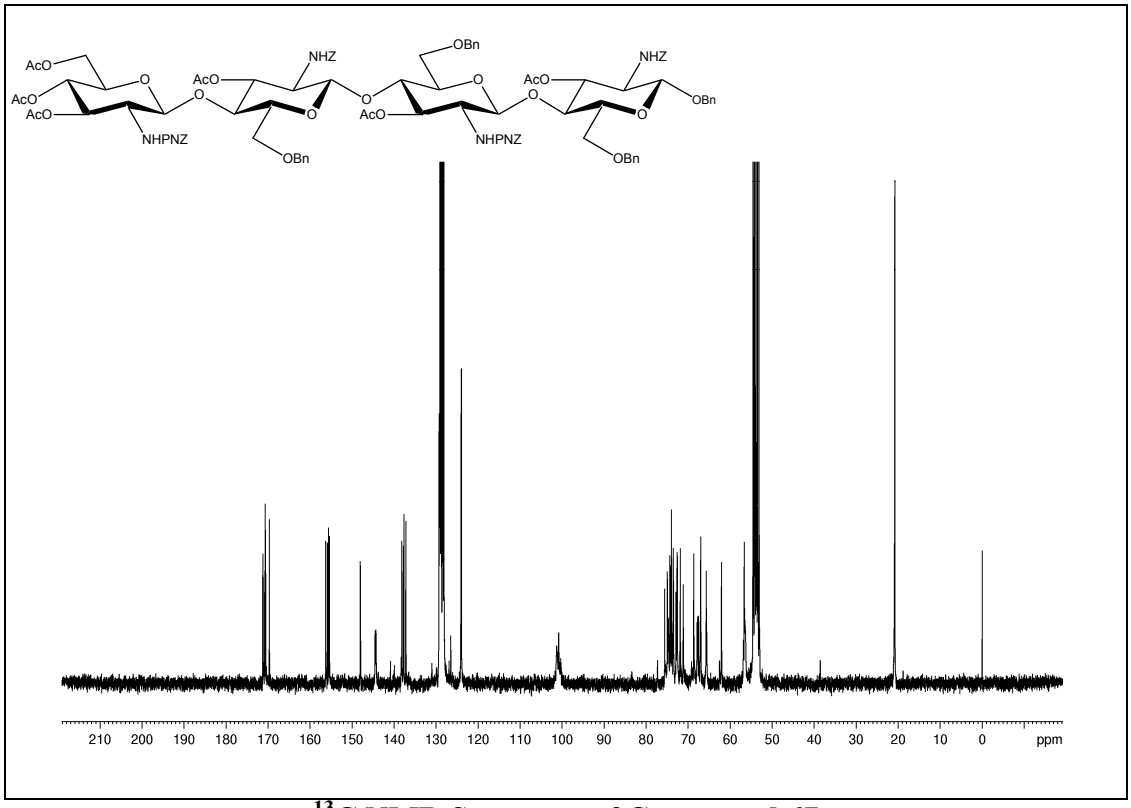
MALDI-TOF MS of chromatographed protected tetrasaccharide 64



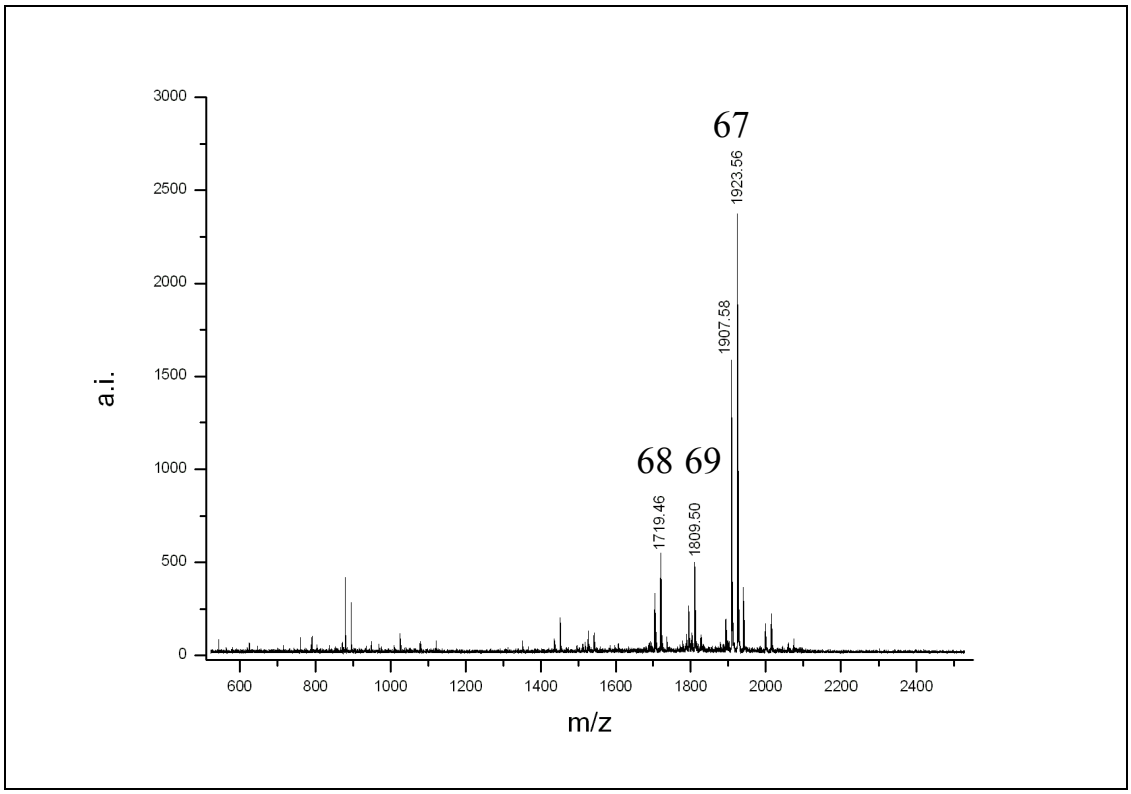
MALDI-TOF MS of Compound 64



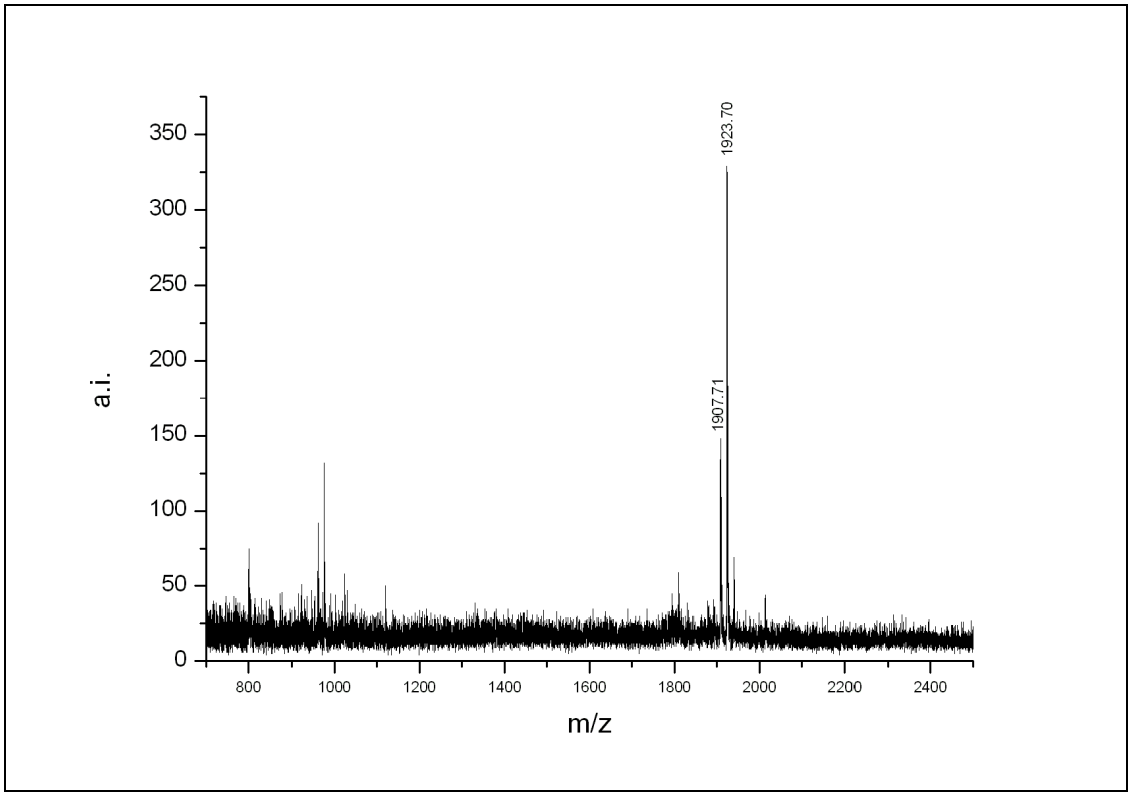
^{1}H NMR Spectrum of Compound 67



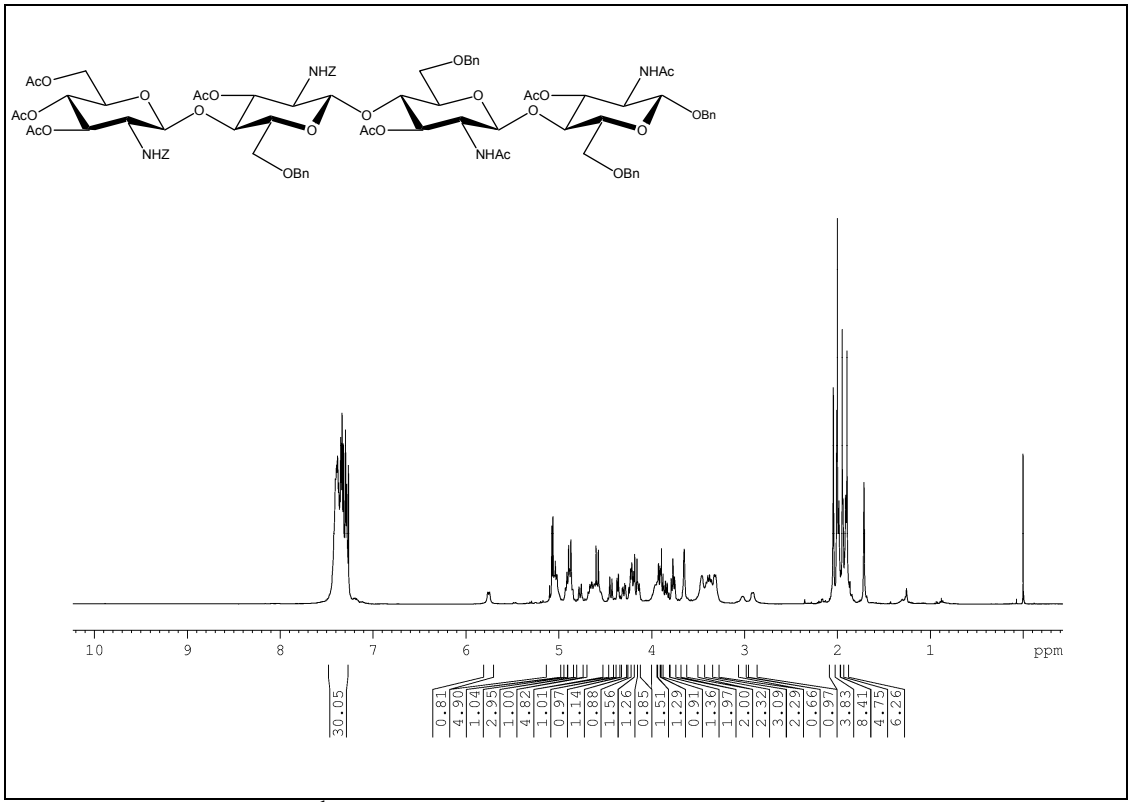
^{13}C NMR Spectrum of Compound 67



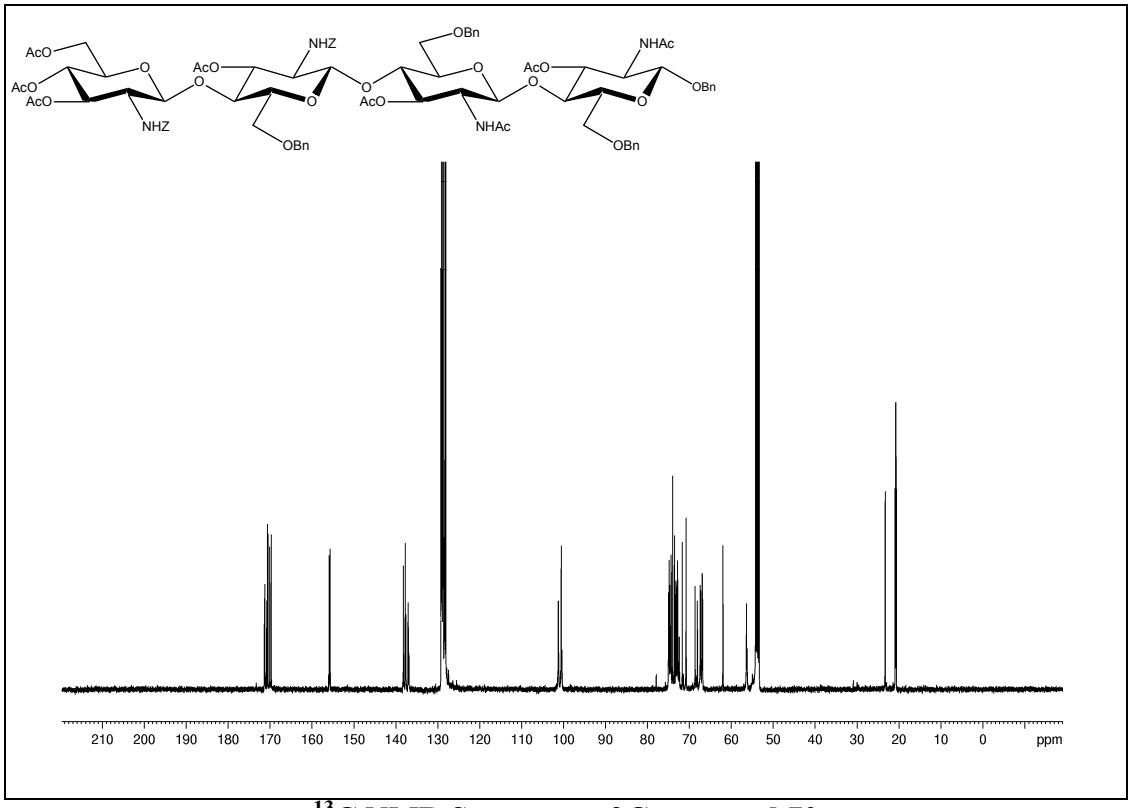
MALDI-TOF MS of chromatographed protected tetrasaccharide 67



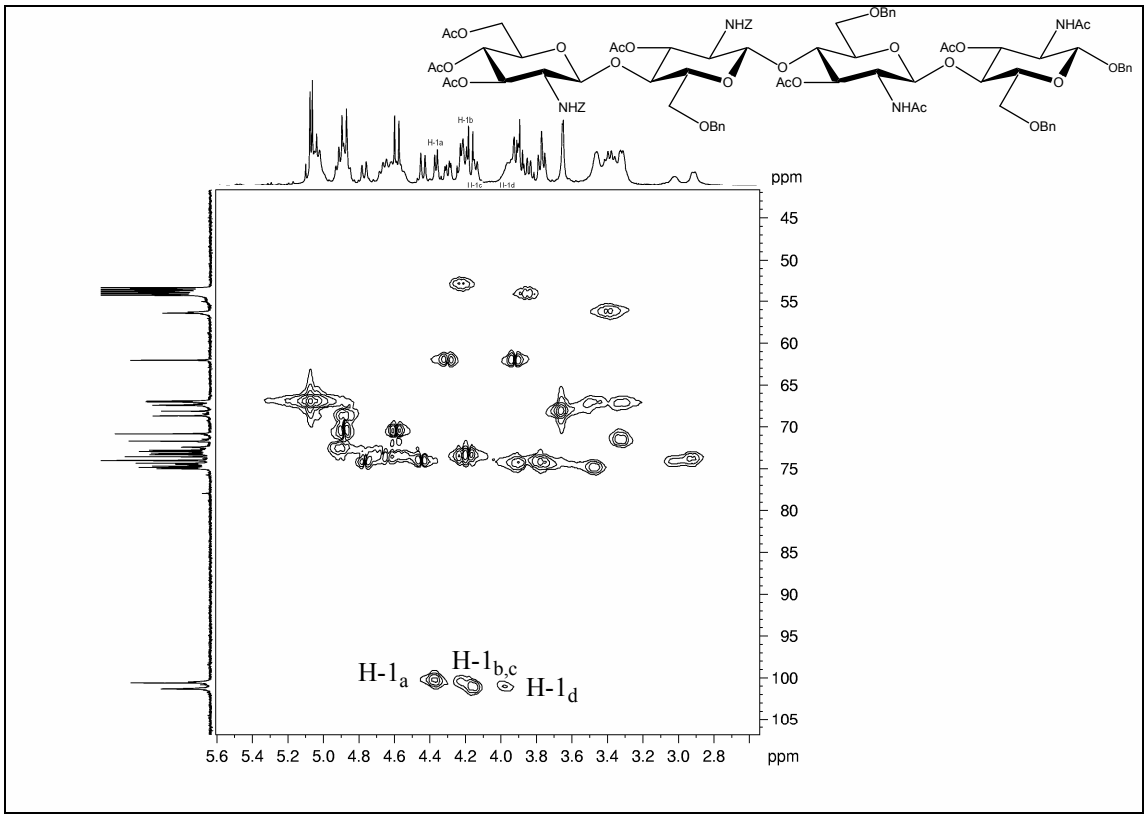
MALDI-TOF MS of Compound 67



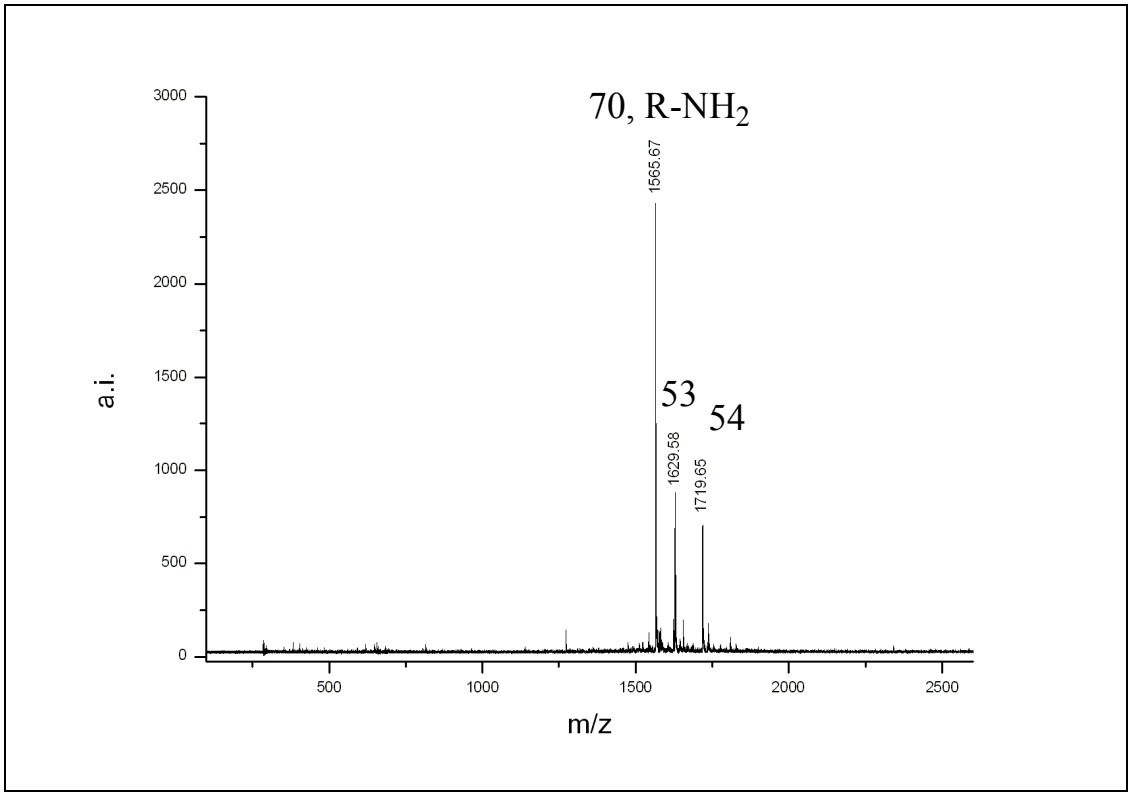
^{1}H NMR Spectrum of Compound 70



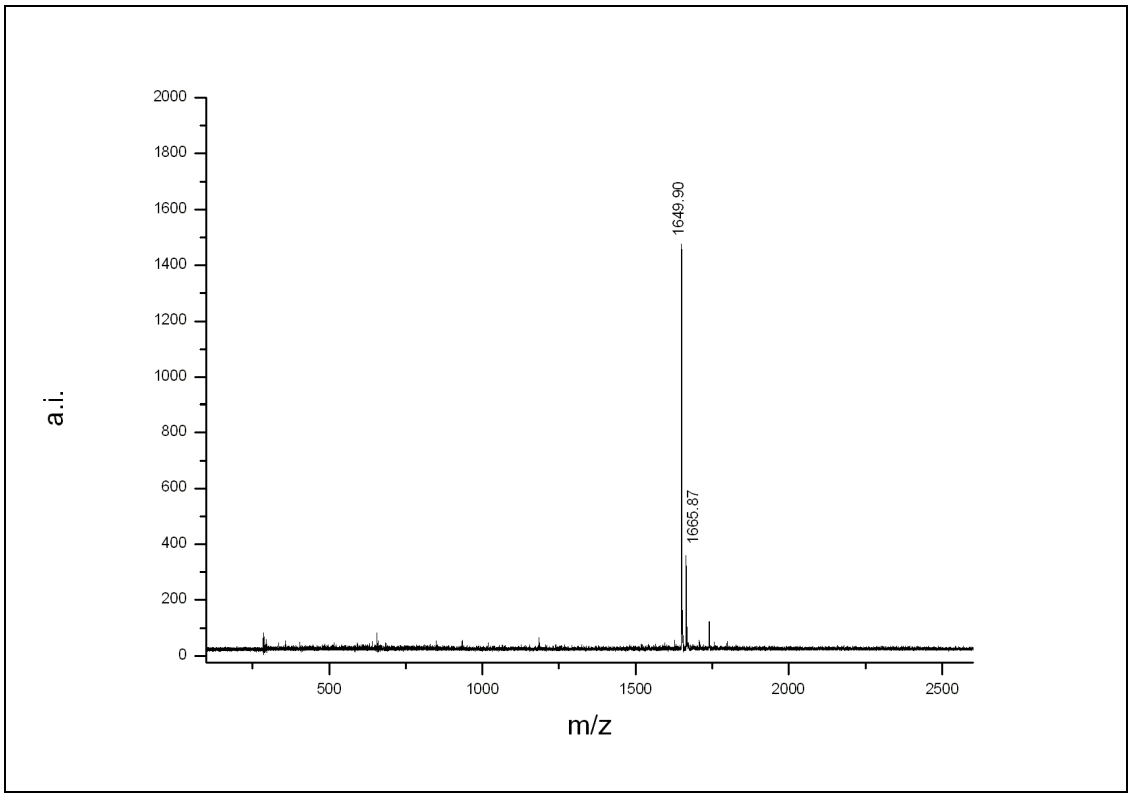
^{13}C NMR Spectrum of Compound 70



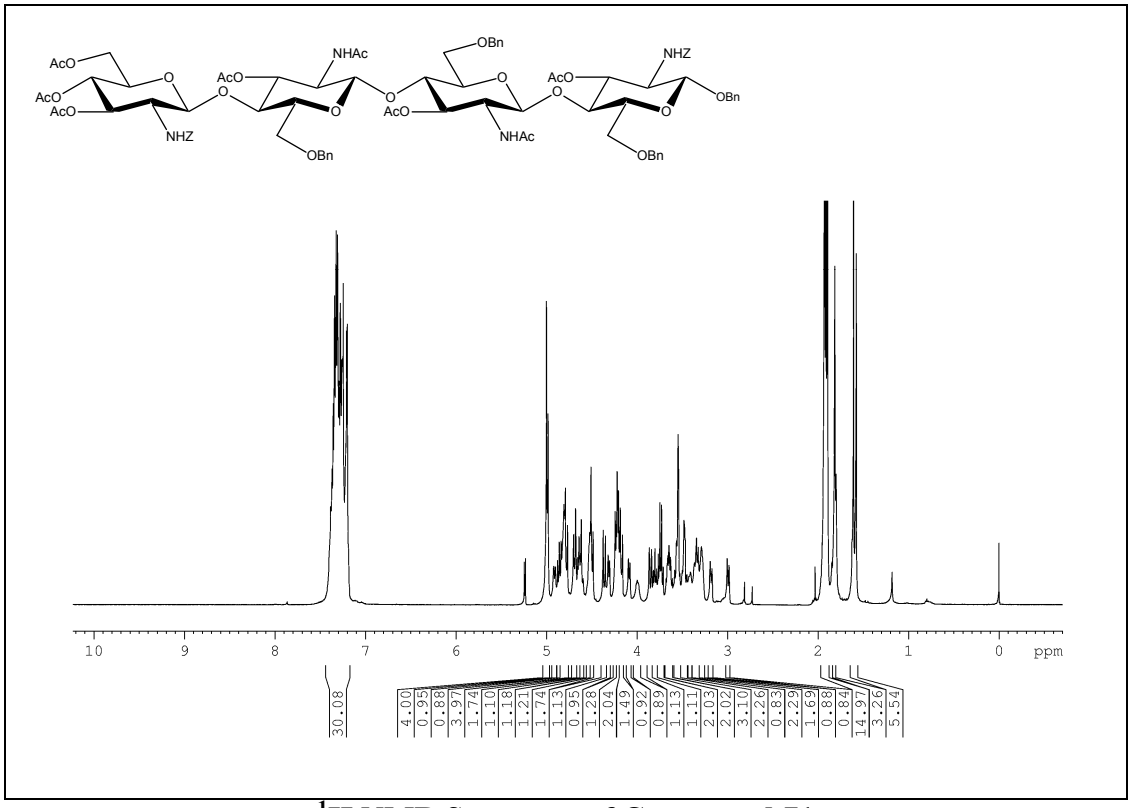
HMQC NMR Spectrum of Compound 70



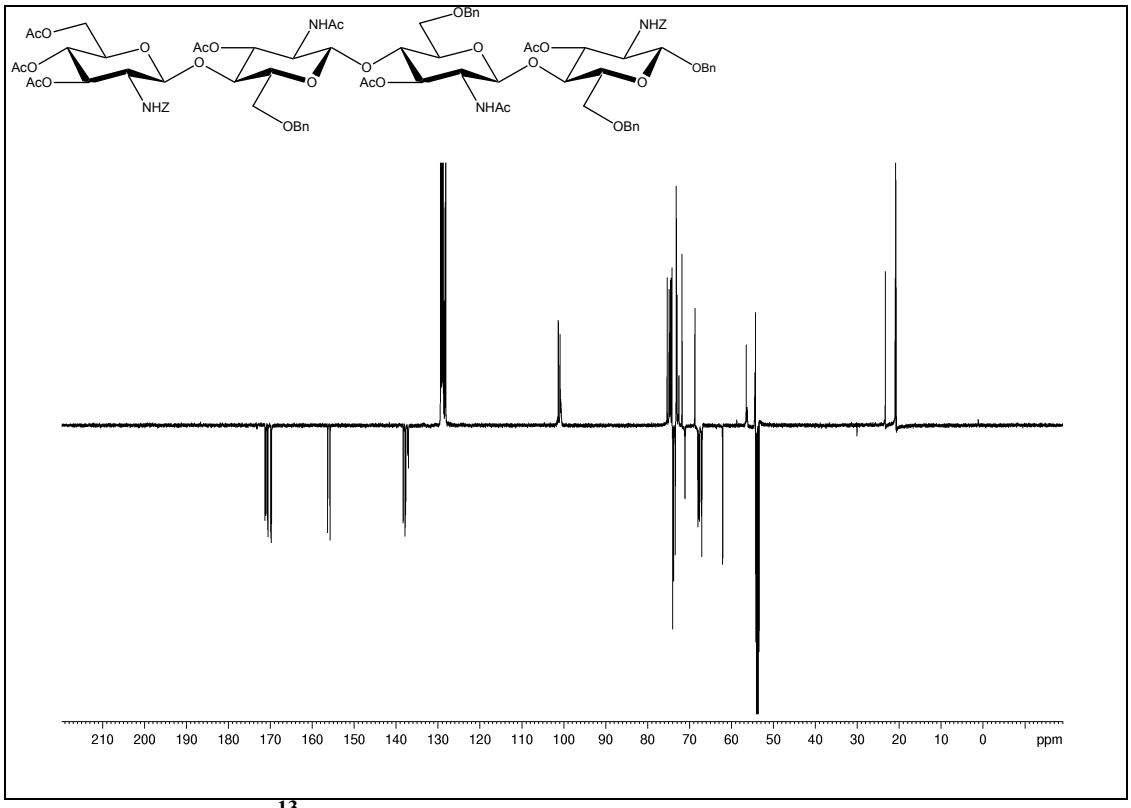
MALDI-TOF MS of reaction mixture of Compound 70 after treatment with TFA



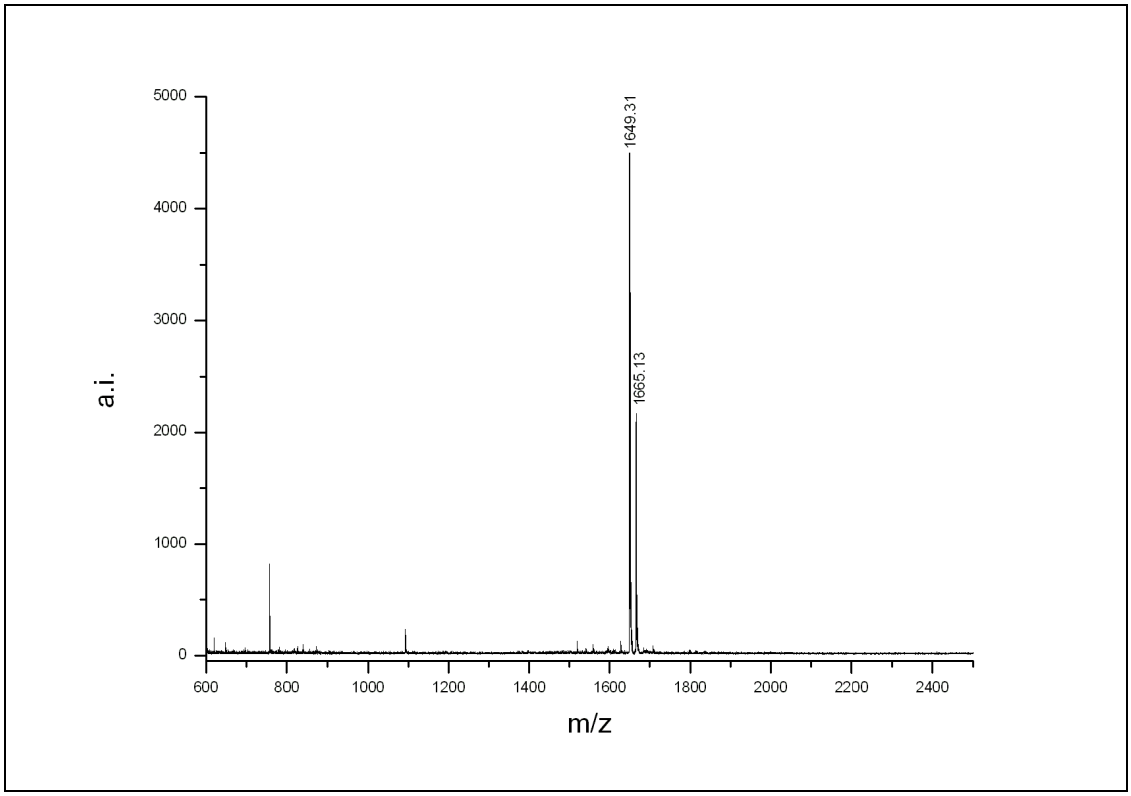
MALDI-TOF MS of Compound 70



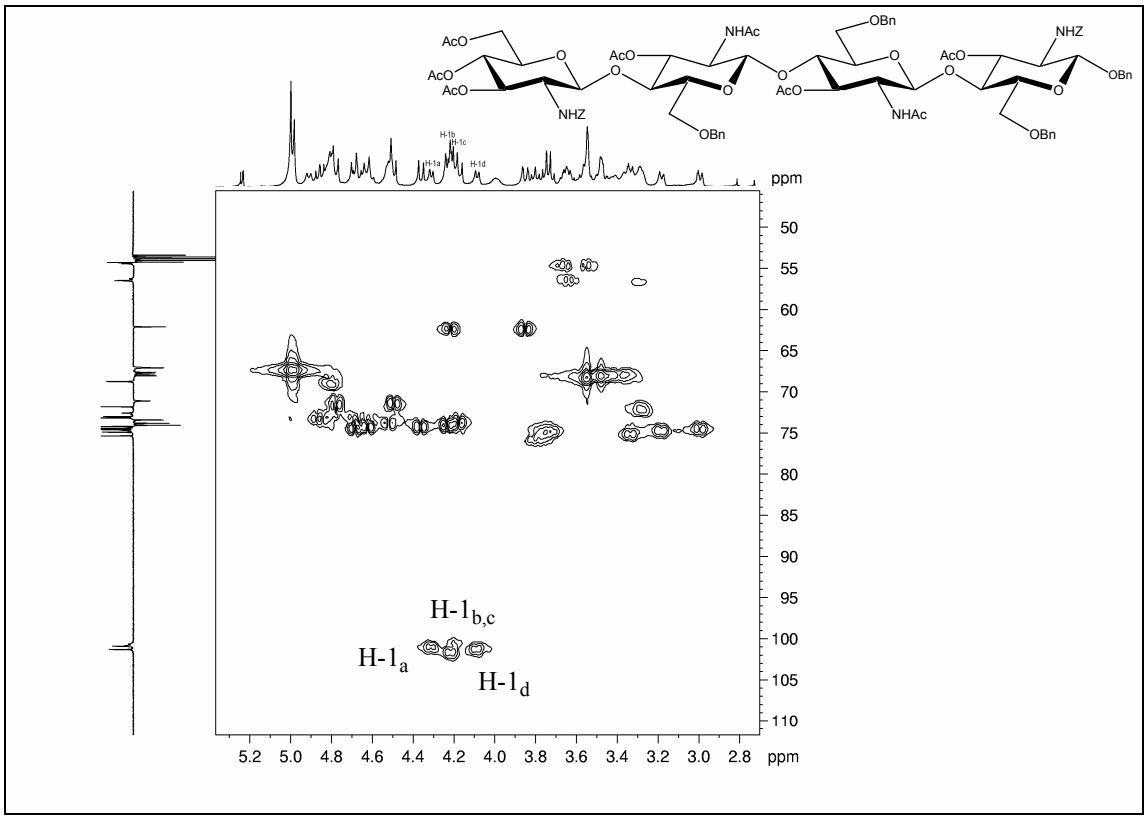
^{1}H NMR Spectrum of Compound 71



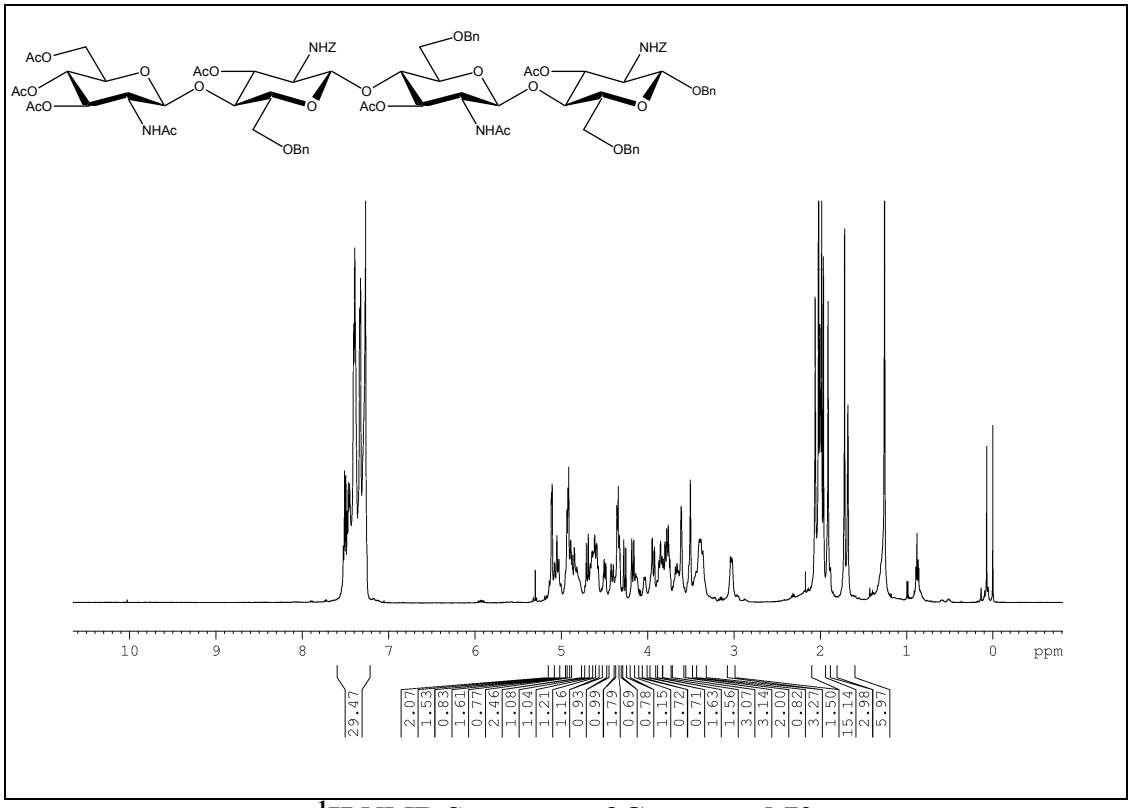
^{13}C APT NMR Spectrum of Compound 71



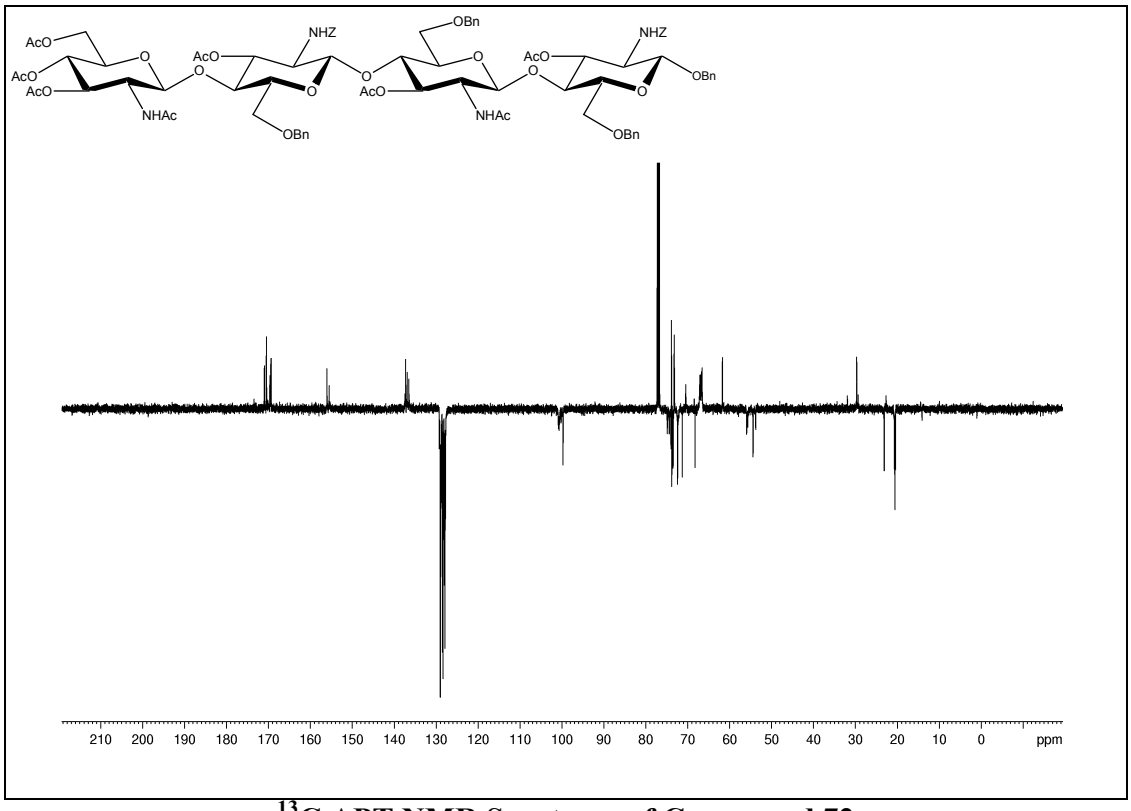
MALDI-TOF MS of Compound 71



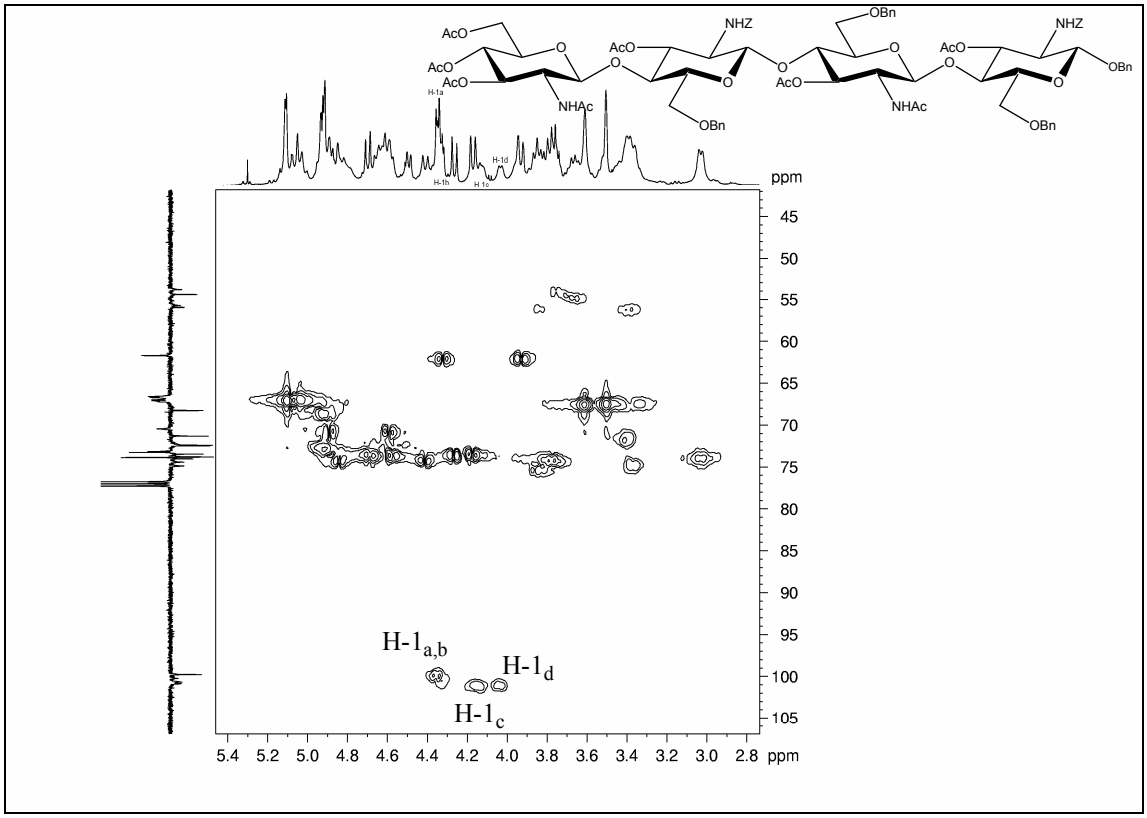
HMQC Spectrum of Compound 71



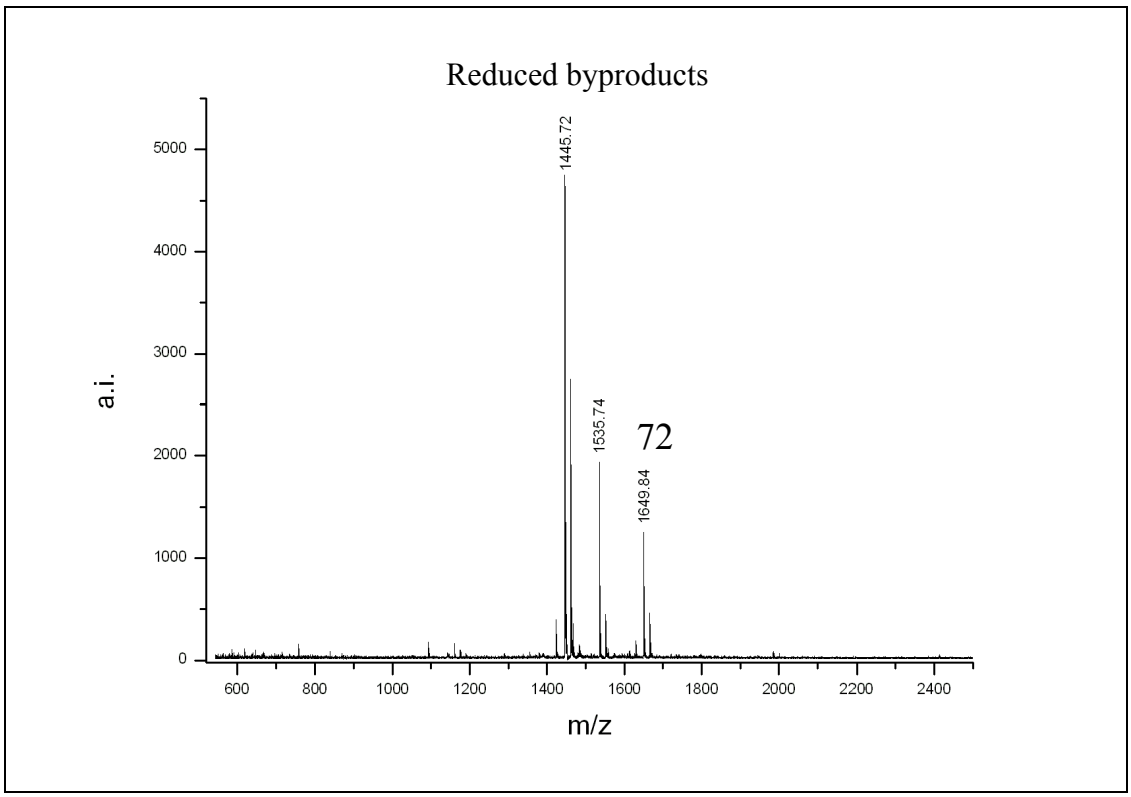
^{1}H NMR Spectrum of Compound 72



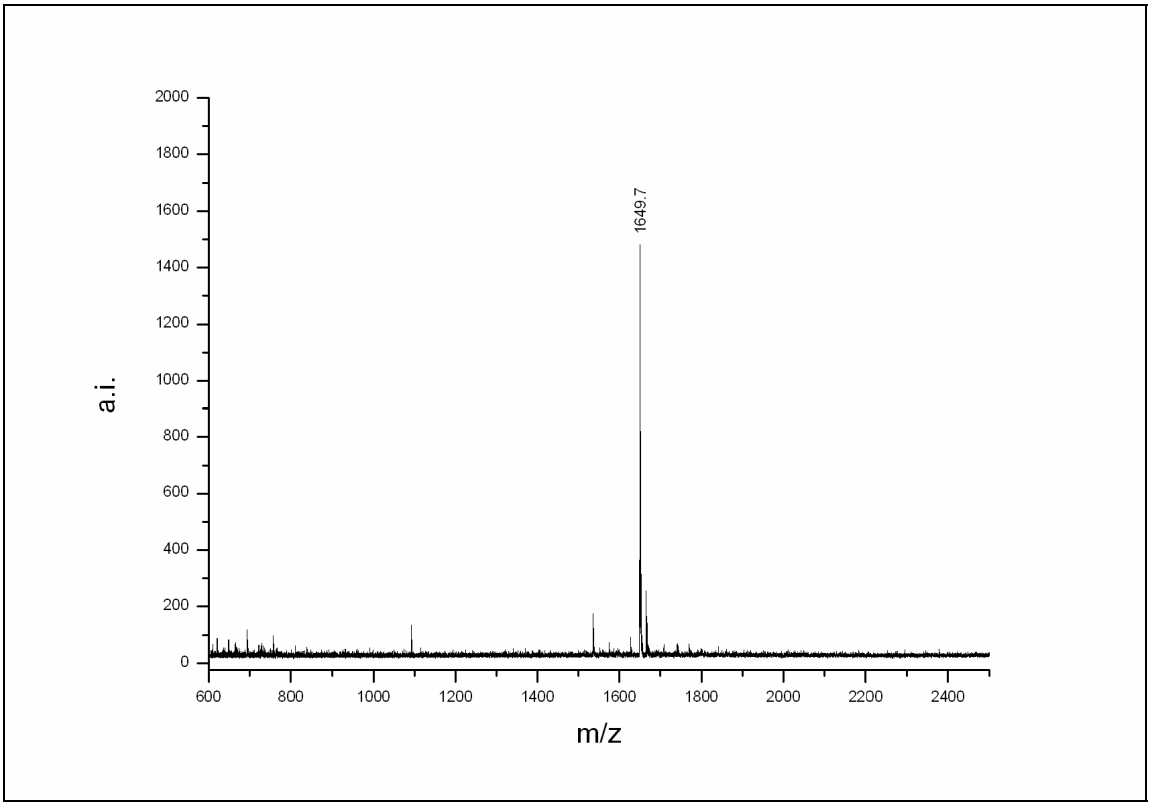
^{13}C APT NMR Spectrum of Compound 72



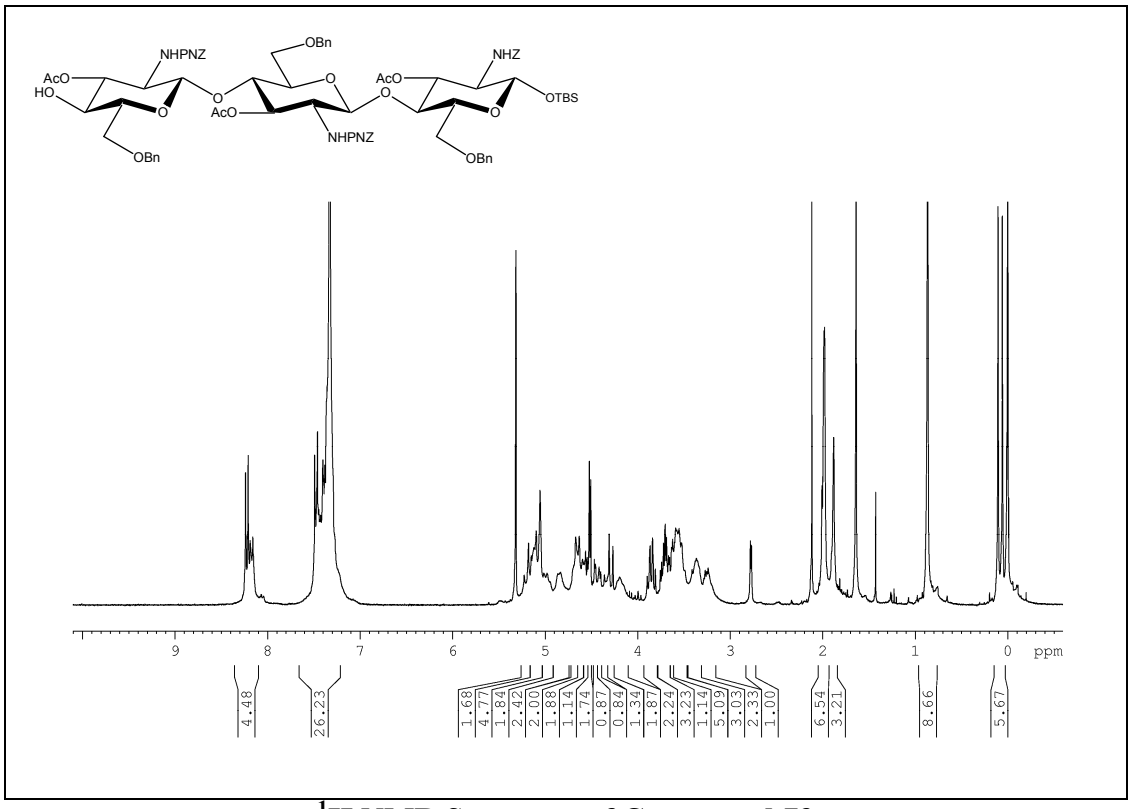
HMQC Spectrum of Compound 72



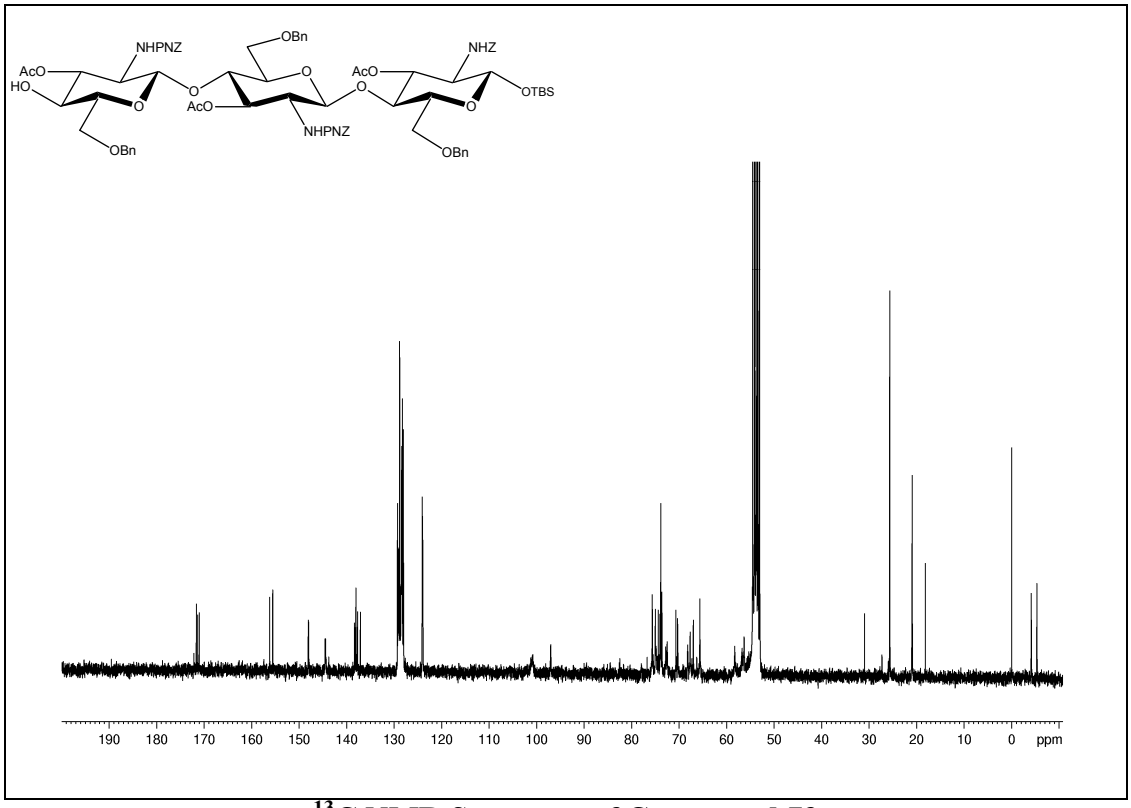
MALDI-TOF MS of Compound 72 (Reduction of byproducts to the corresponding acetate derivatives)



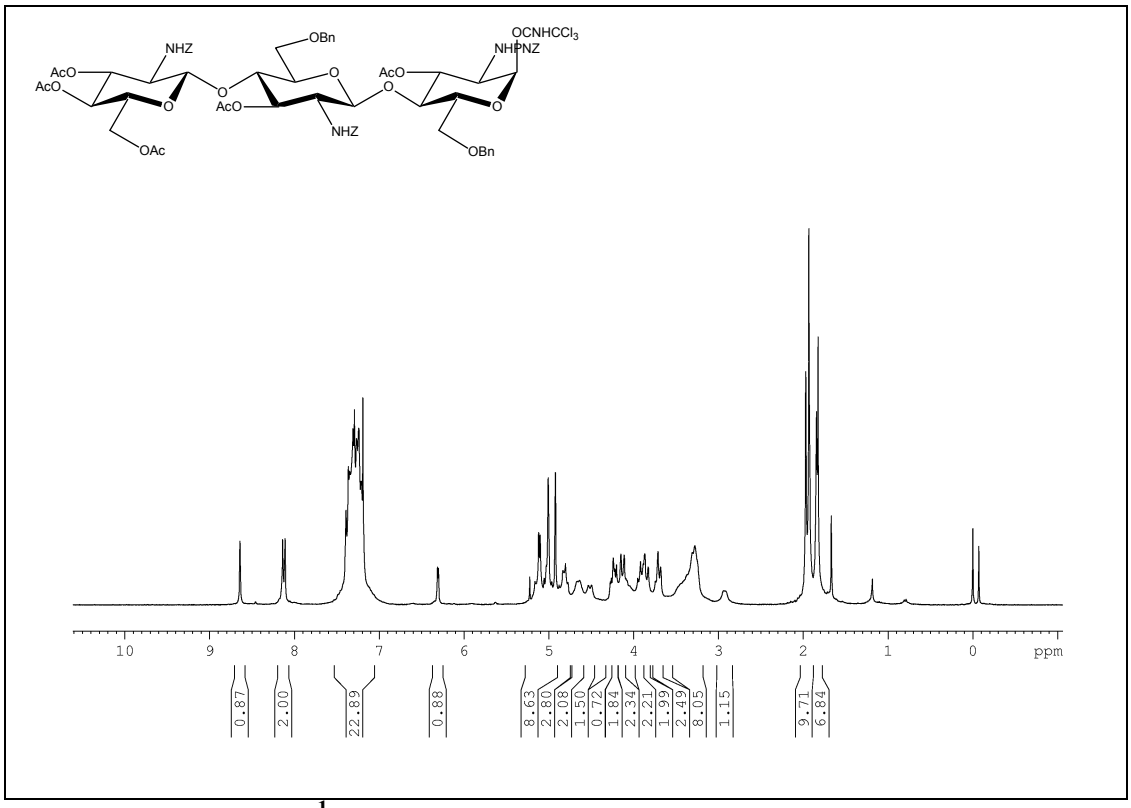
MALDI-TOF MS of Compound 72 (After Purification using RP-18 Preparative TLC)



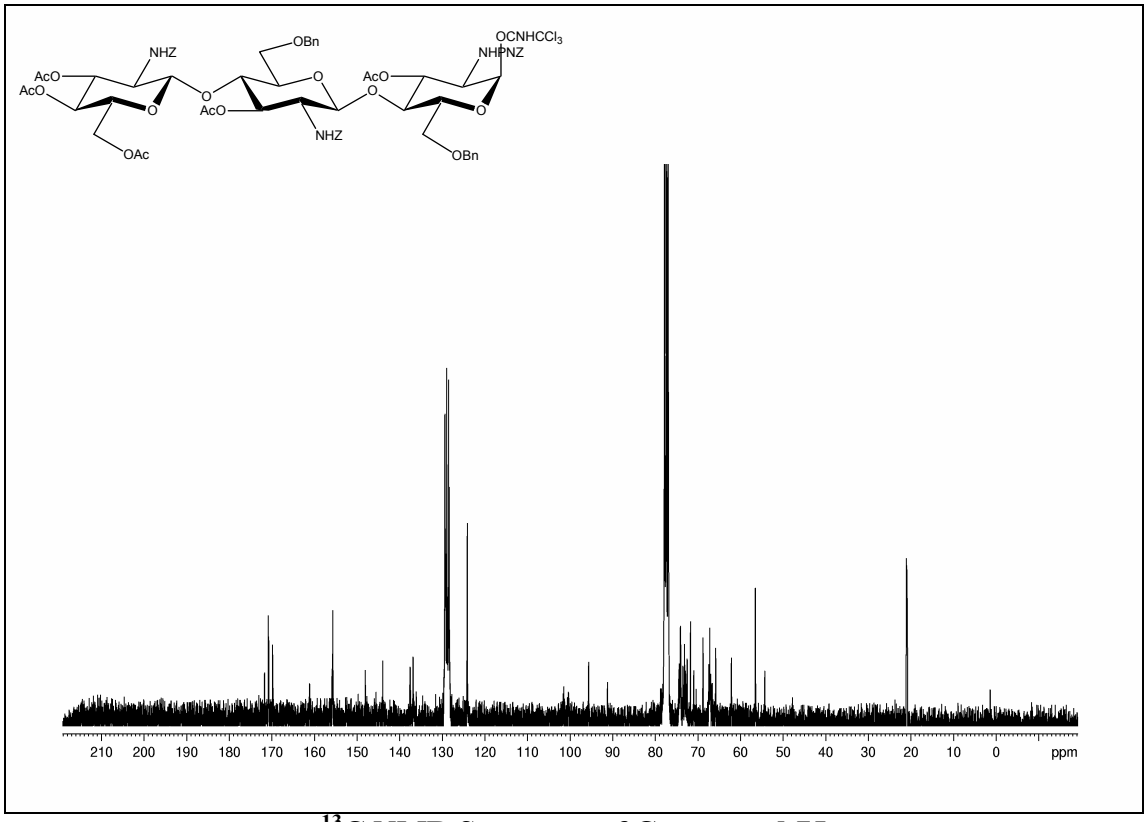
^{1}H NMR Spectrum of Compound 73



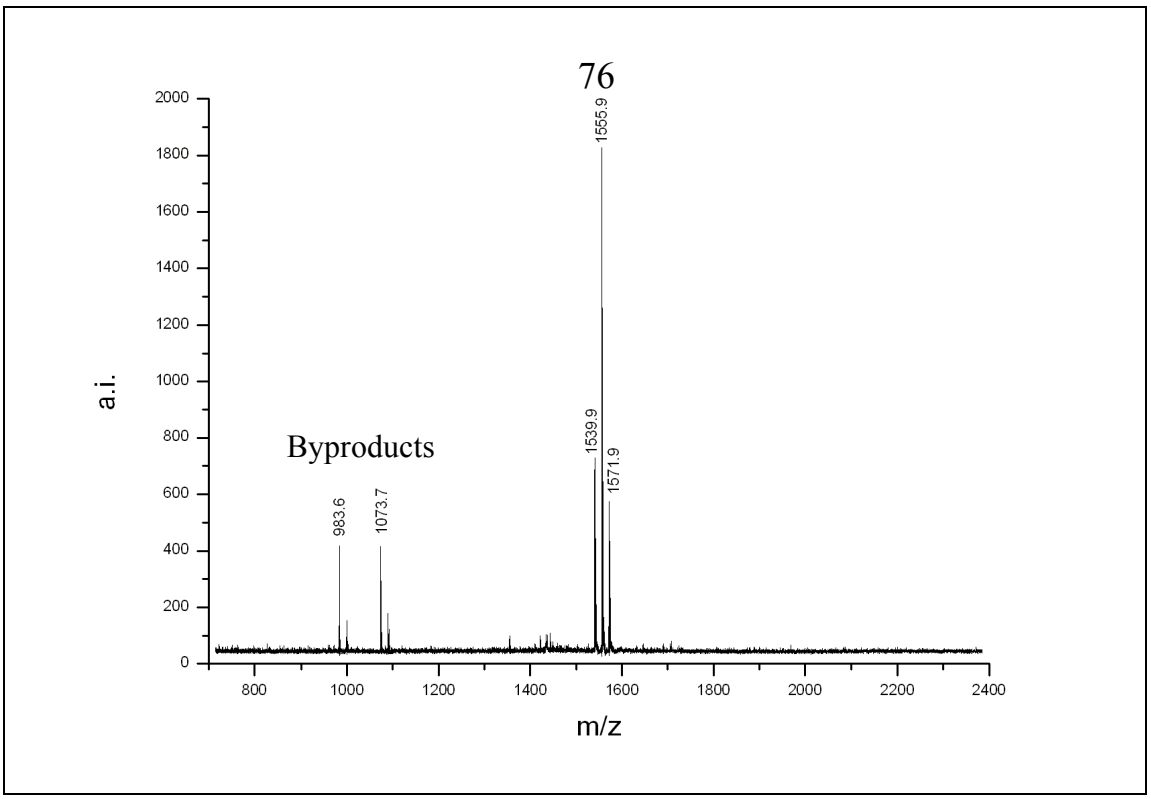
^{13}C NMR Spectrum of Compound 73



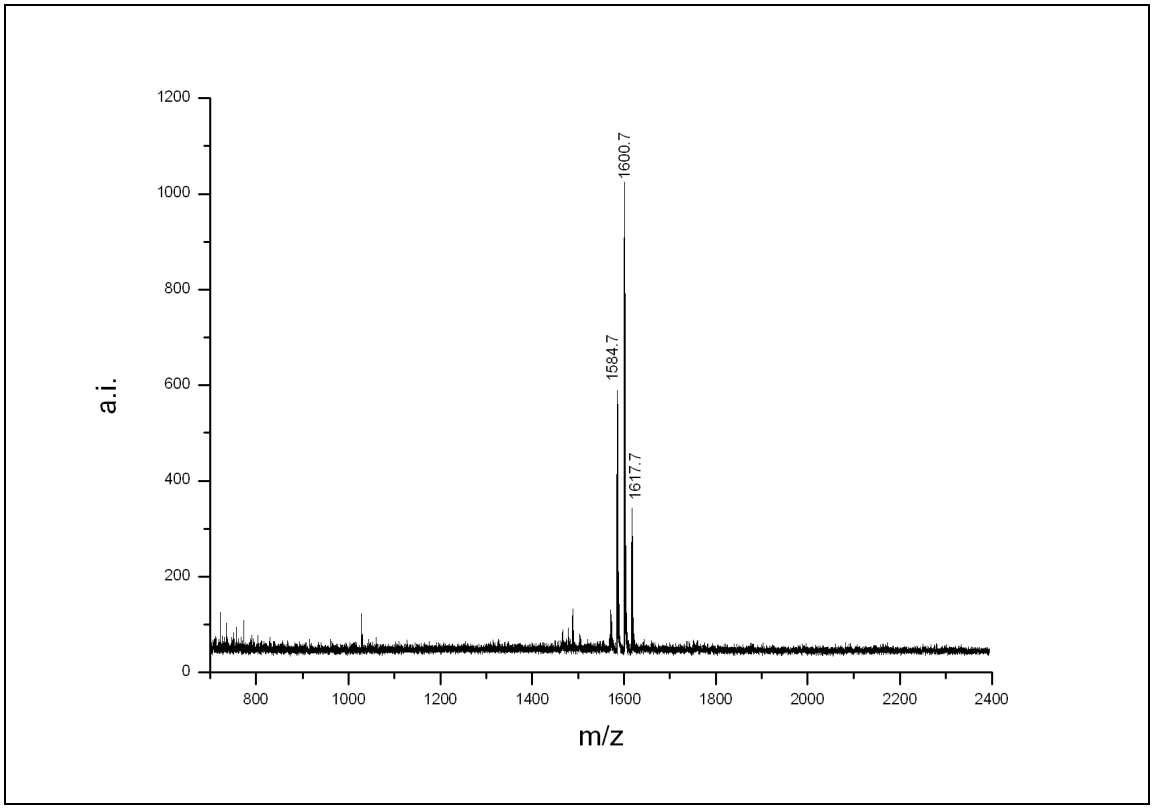
^{1}H NMR Spectrum of Compound 75



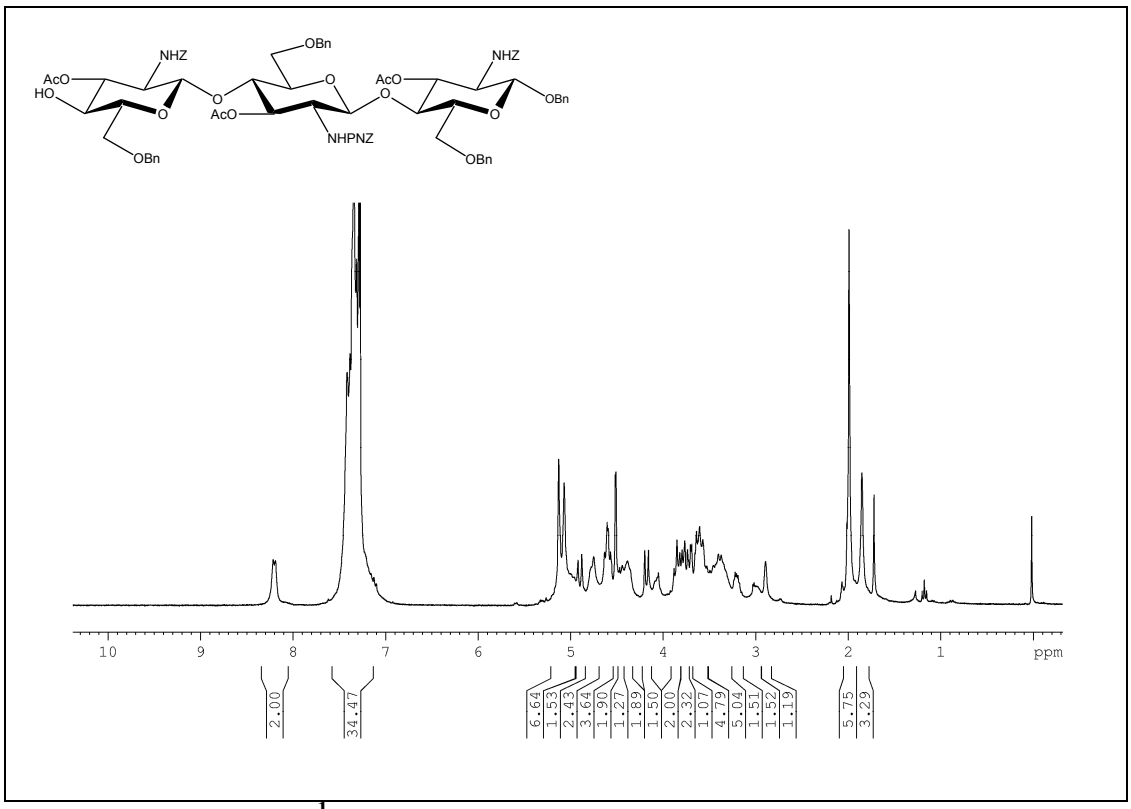
^{13}C NMR Spectrum of Compound 75



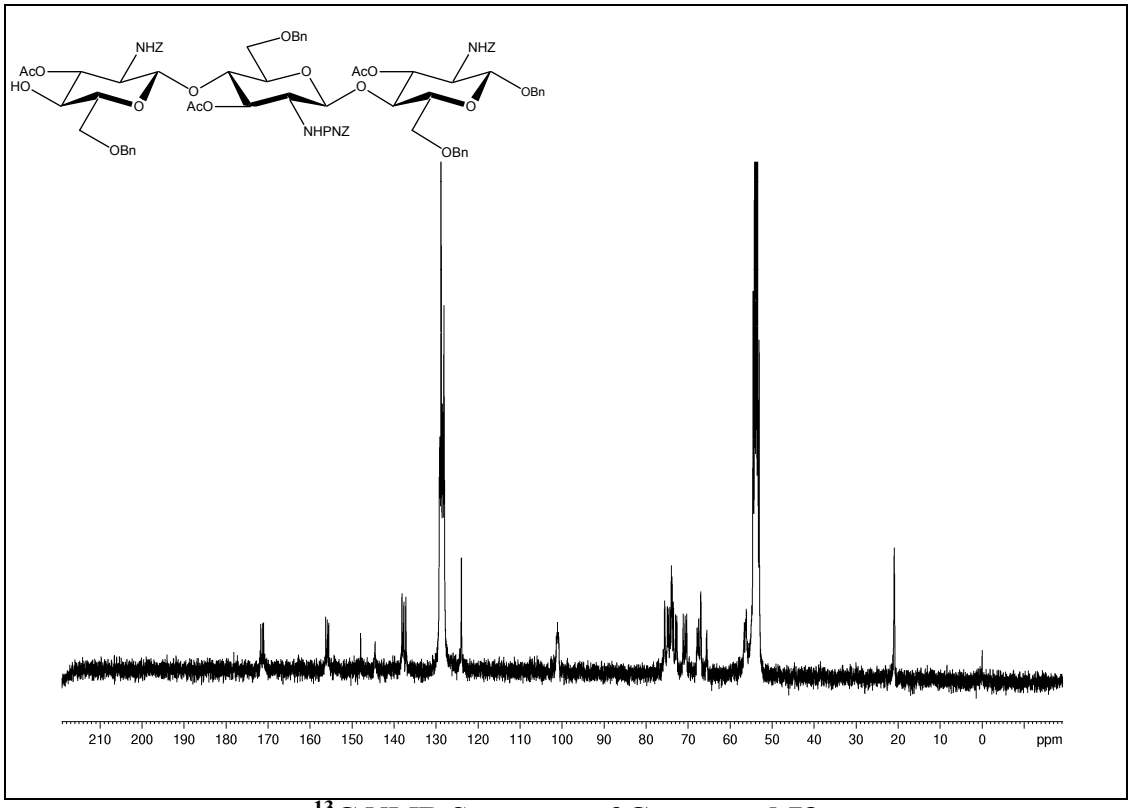
MALDI-TOF MS of Chromatographed Compound 76 (presence of byproducts)



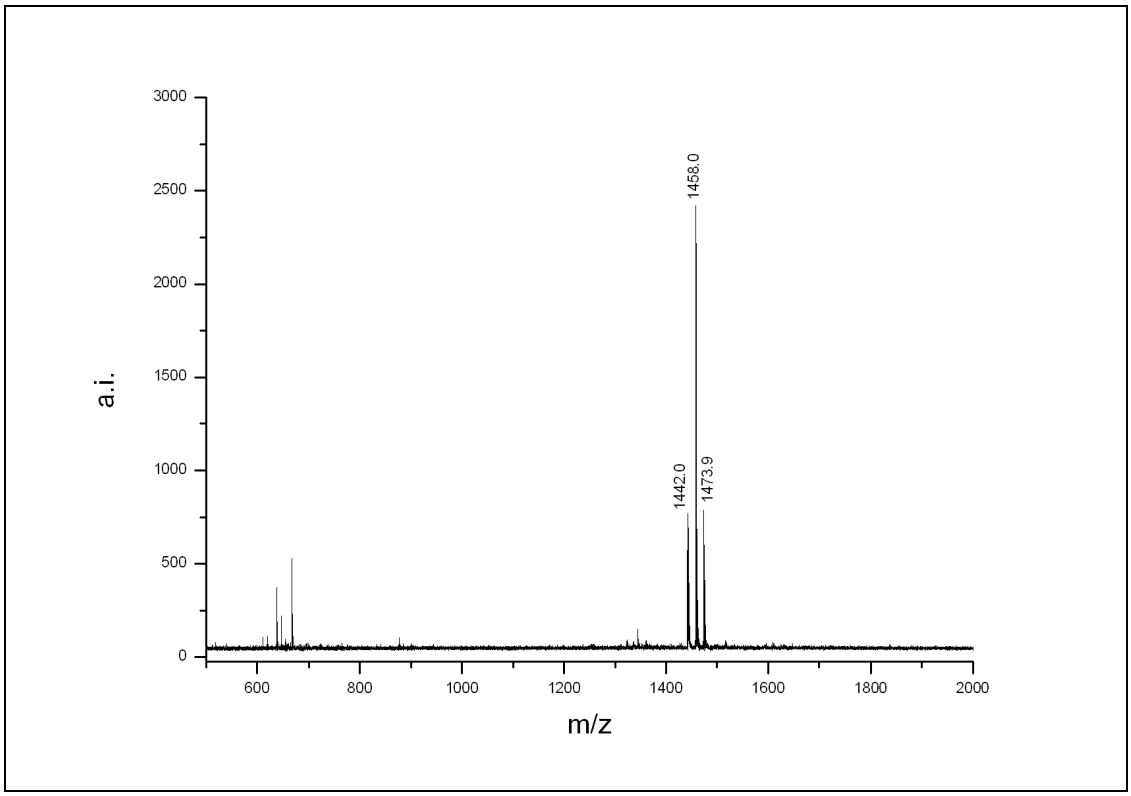
MALDI-TOF MS of Compound 77



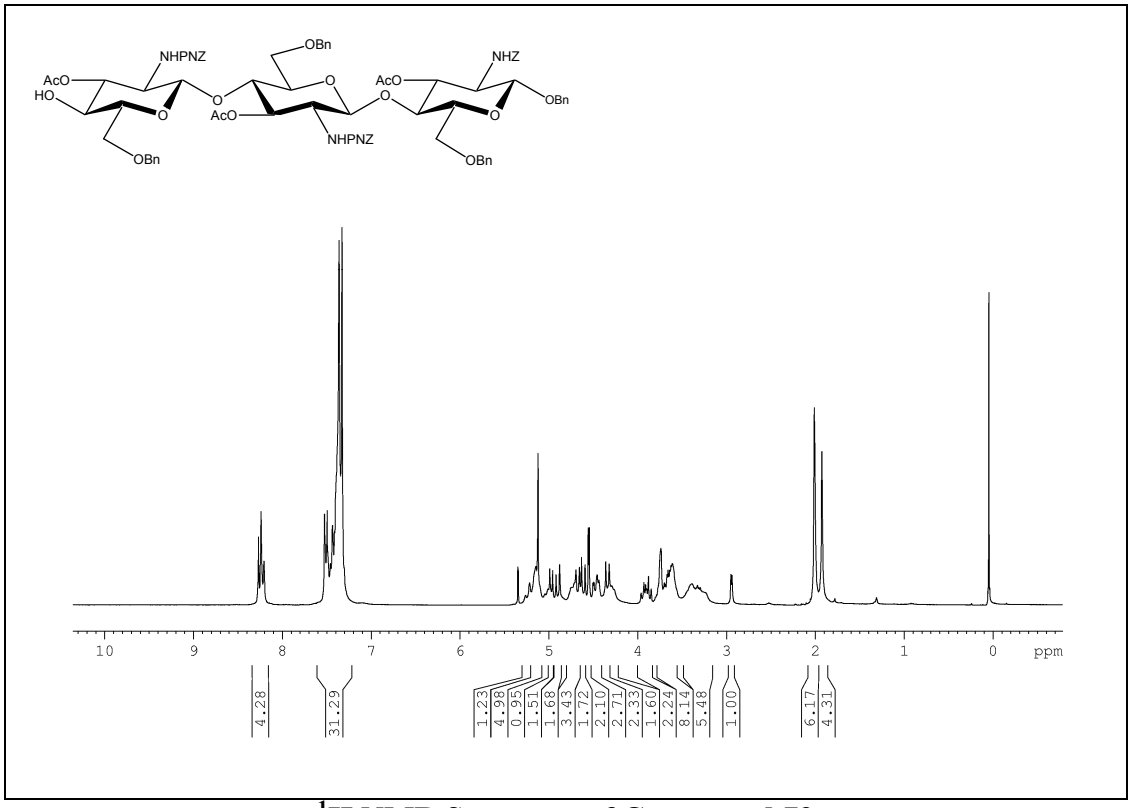
^{1}H NMR Spectrum of Compound 78



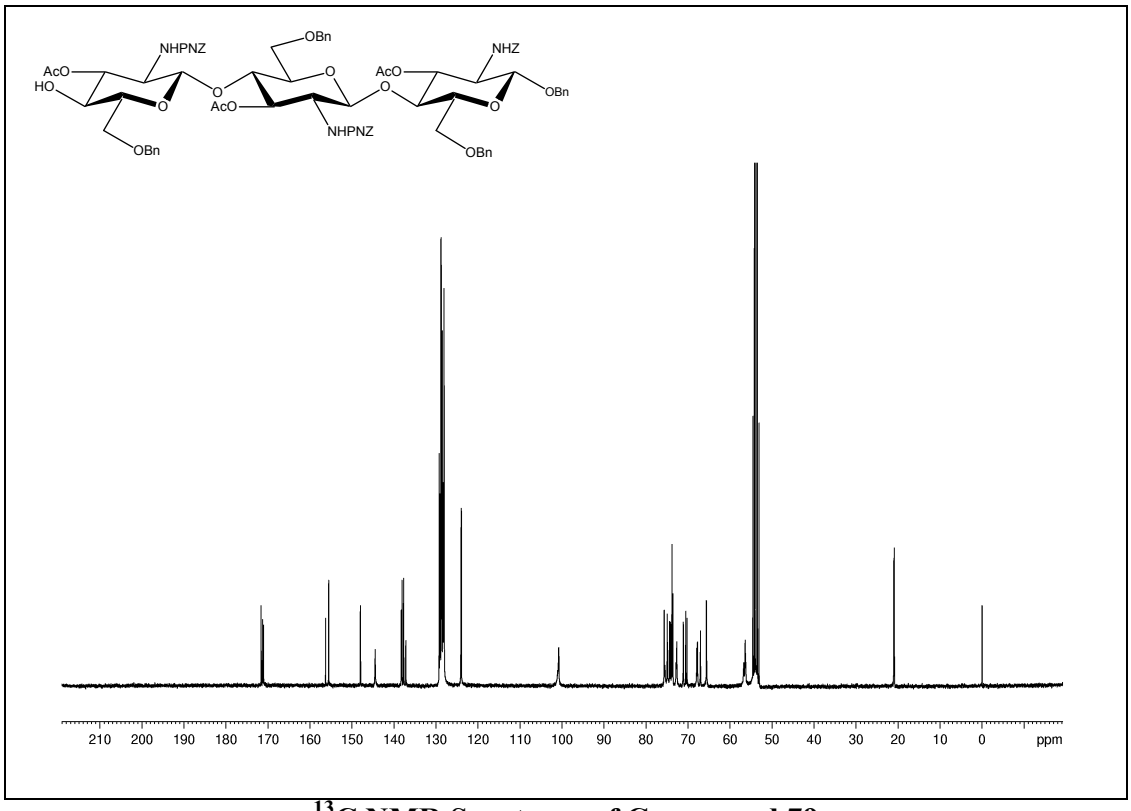
^{13}C NMR Spectrum of Compound 78



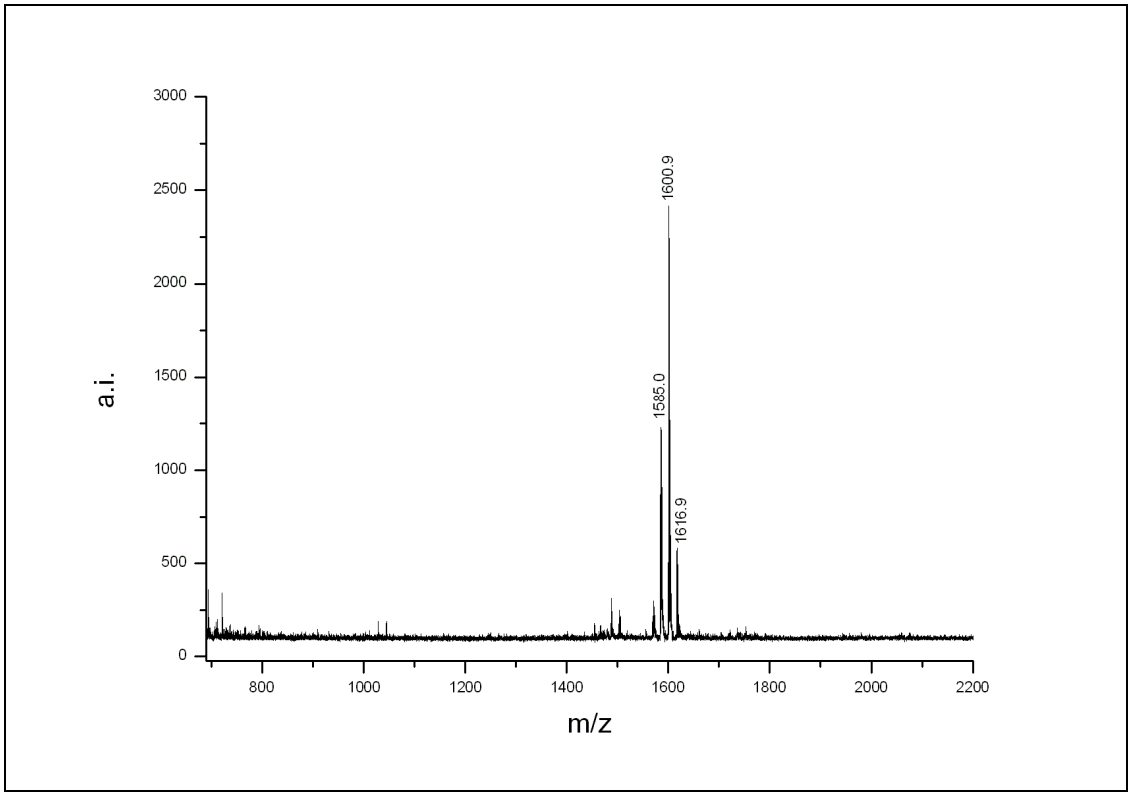
MALDI-TOF MS of Compound 78



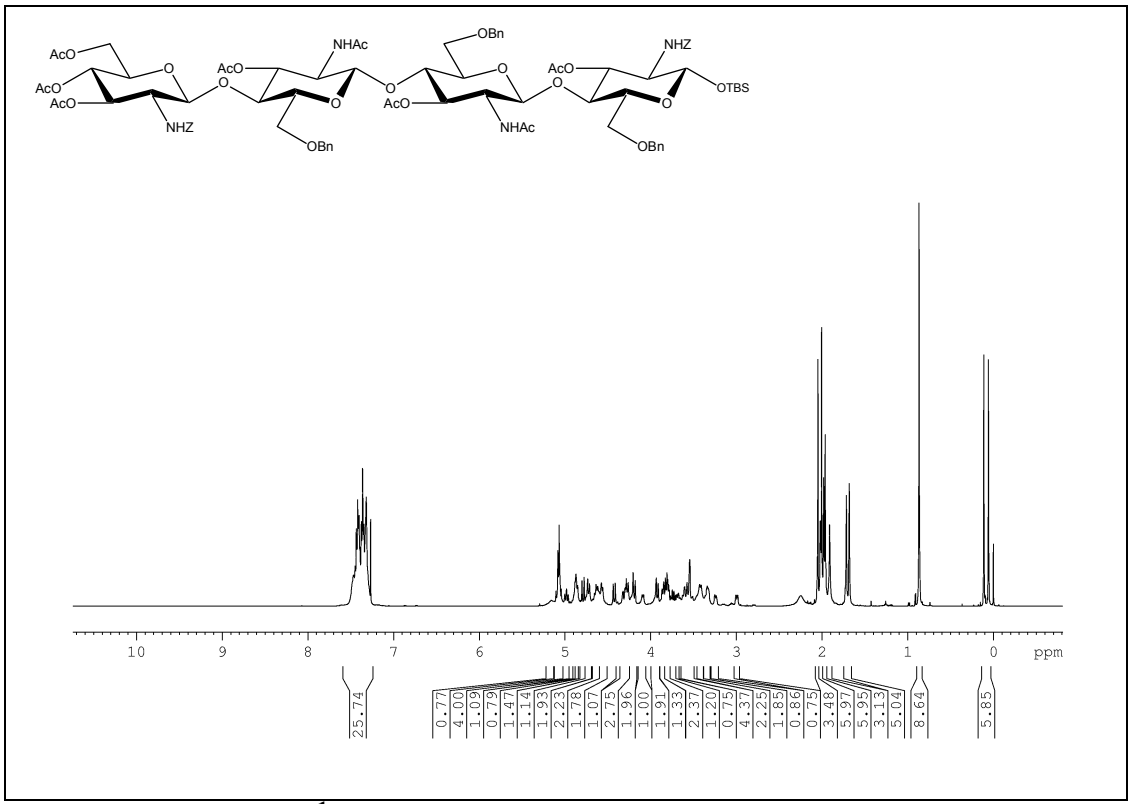
^{1}H NMR Spectrum of Compound 79



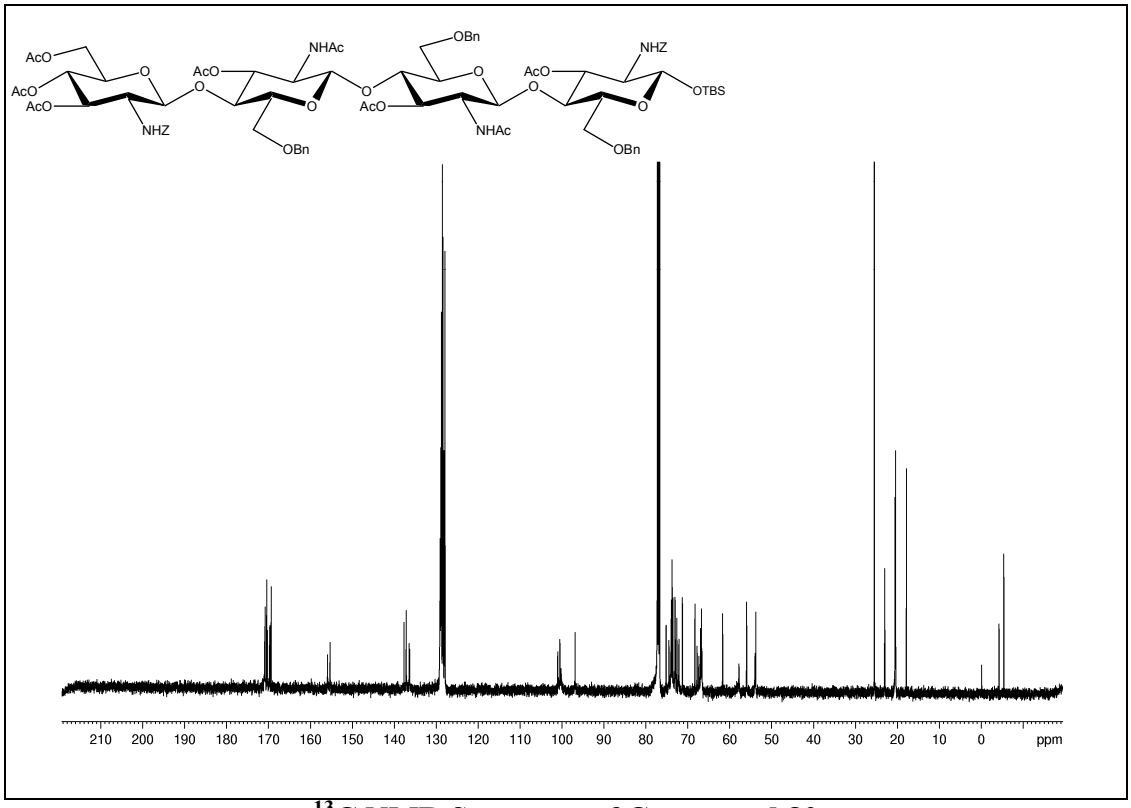
^{13}C NMR Spectrum of Compound 79



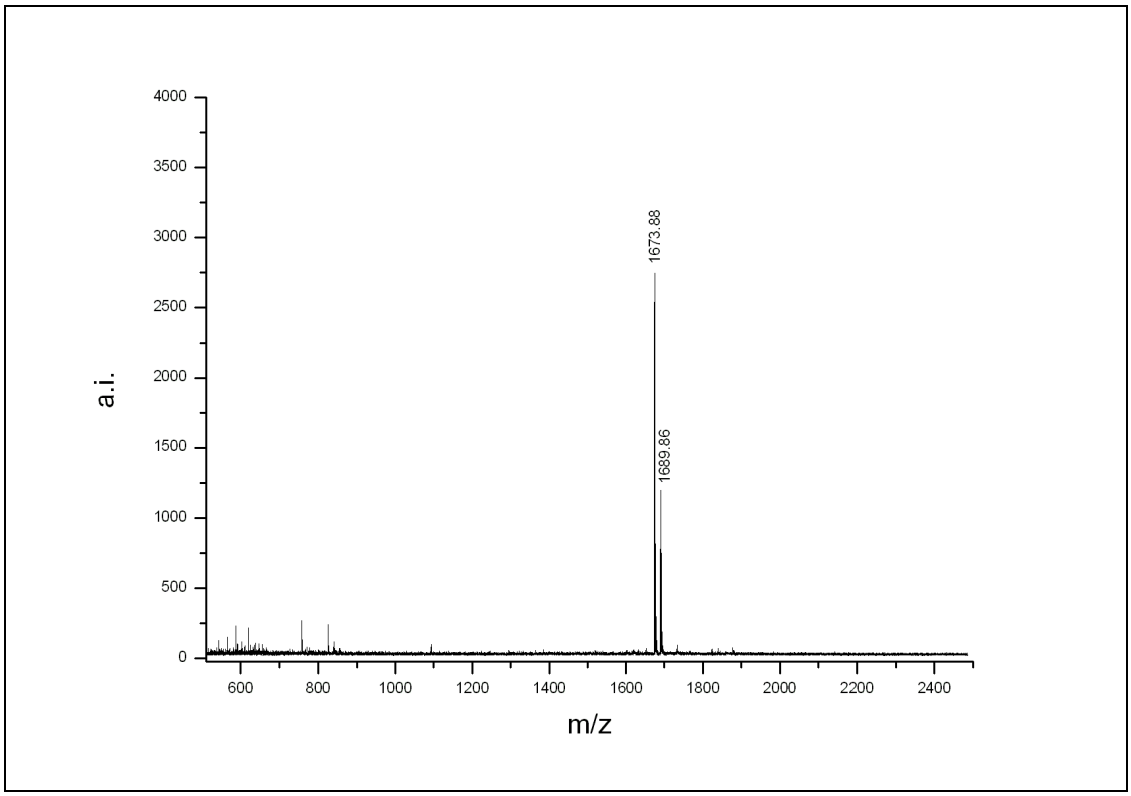
MALDI-TOF MS of Compound 79



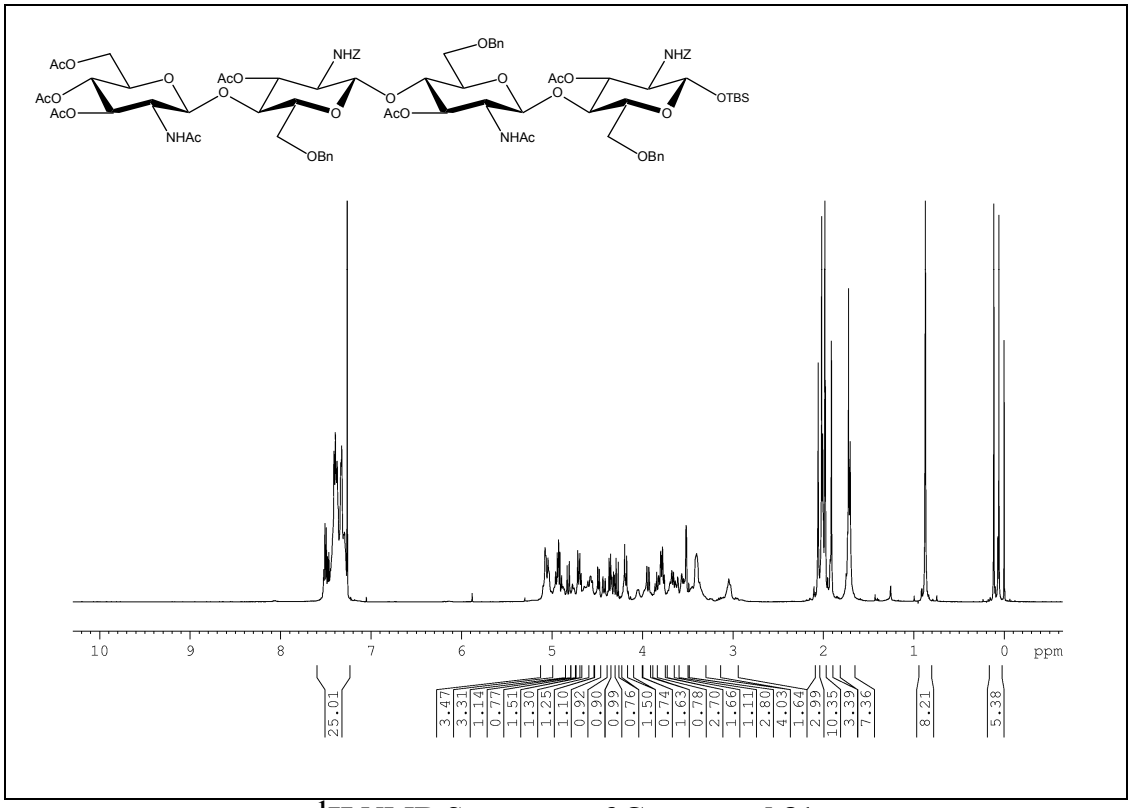
^{1}H NMR Spectrum of Compound 80



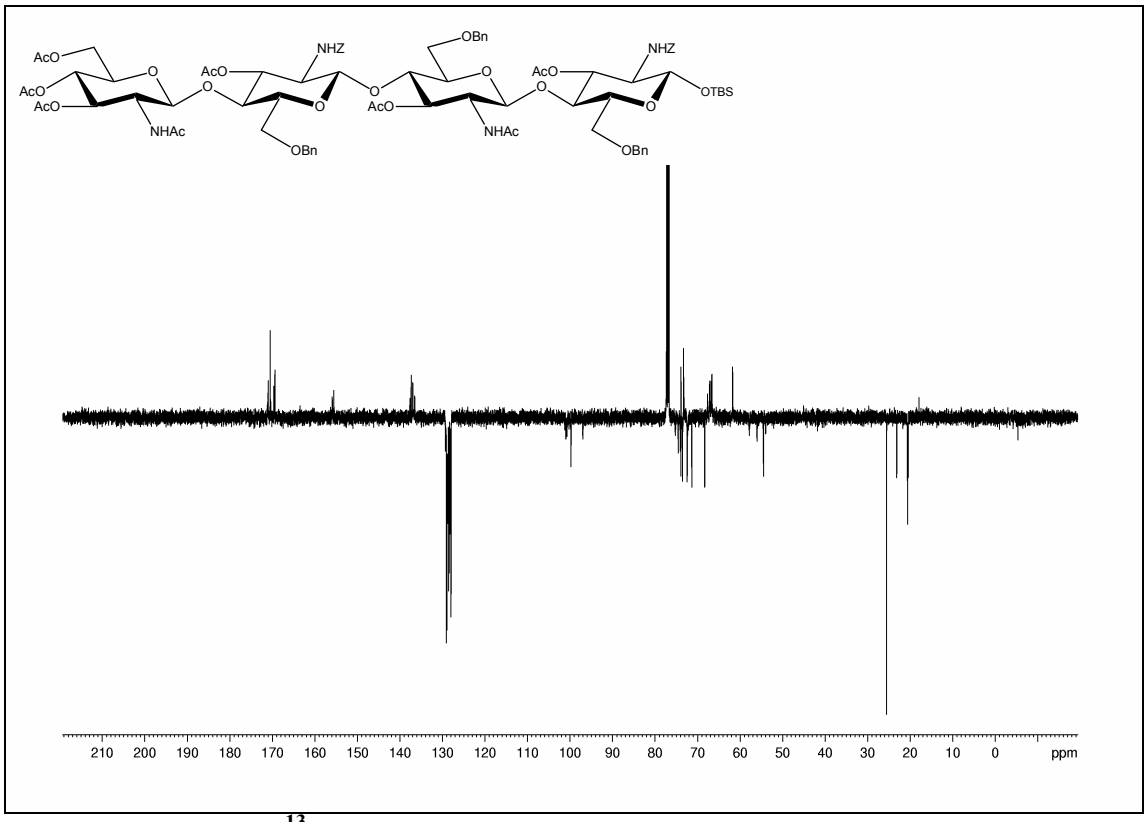
^{13}C NMR Spectrum of Compound 80



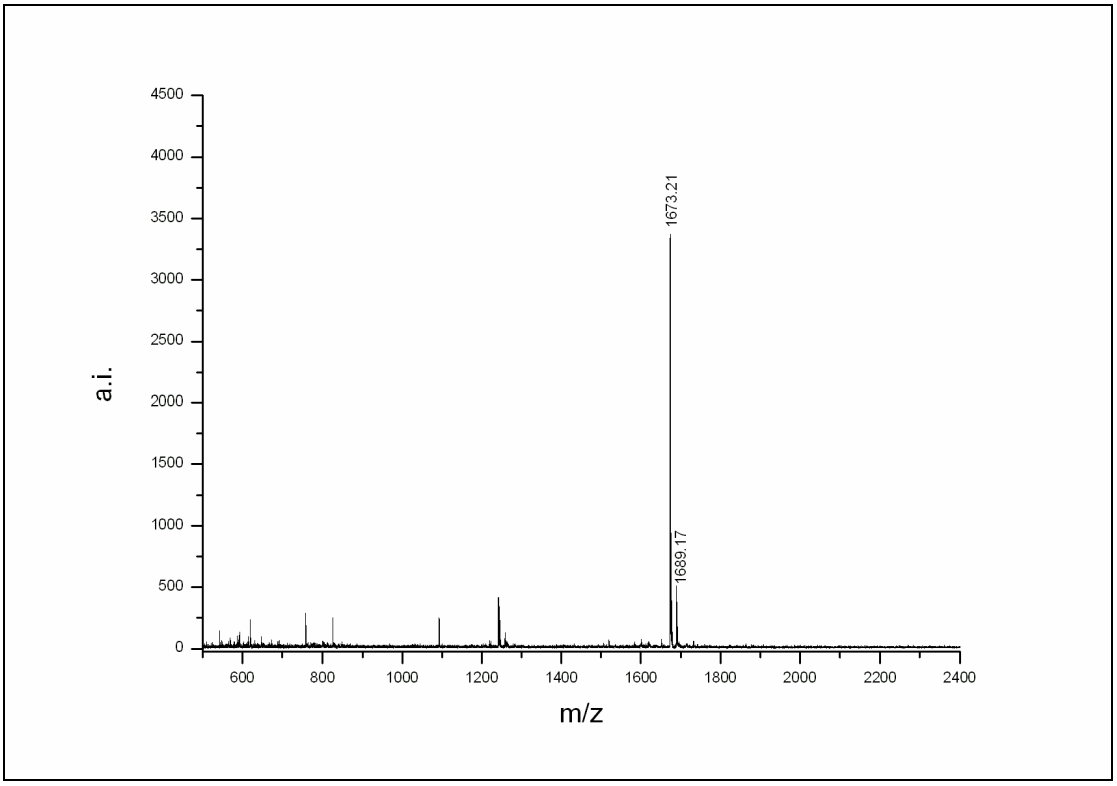
MALDI-TOF MS of Compound 80



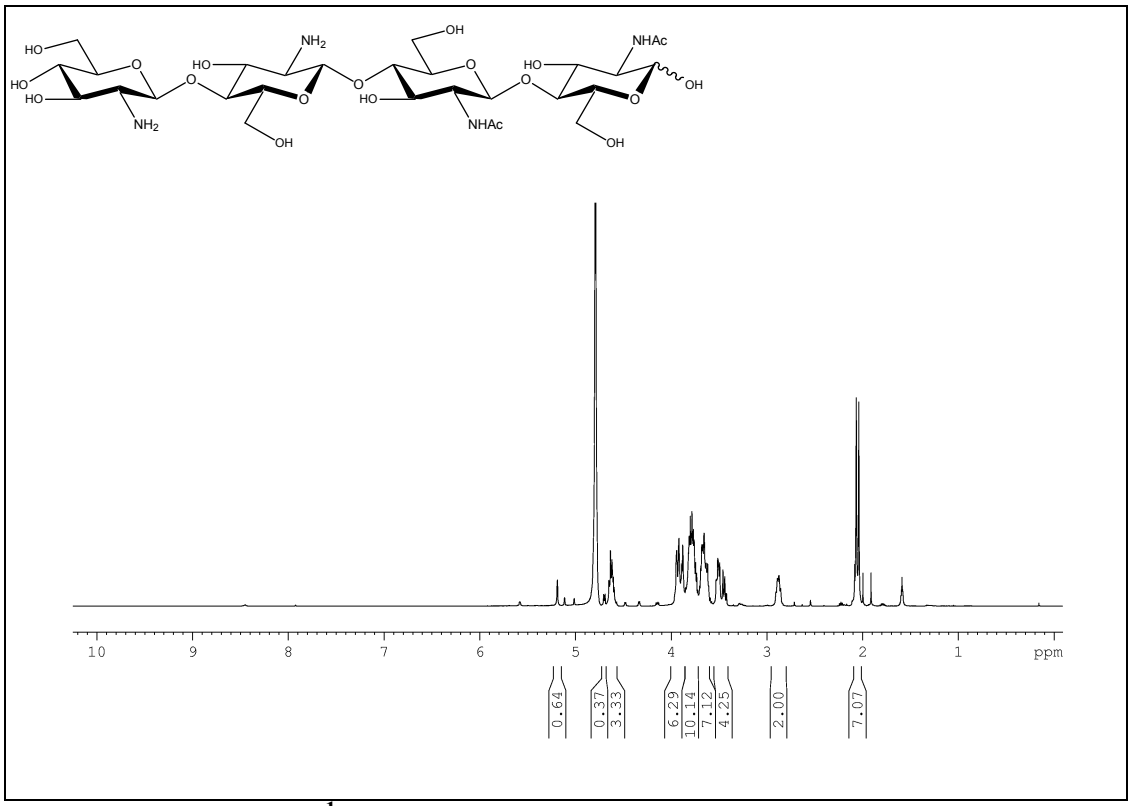
^{1}H NMR Spectrum of Compound 81



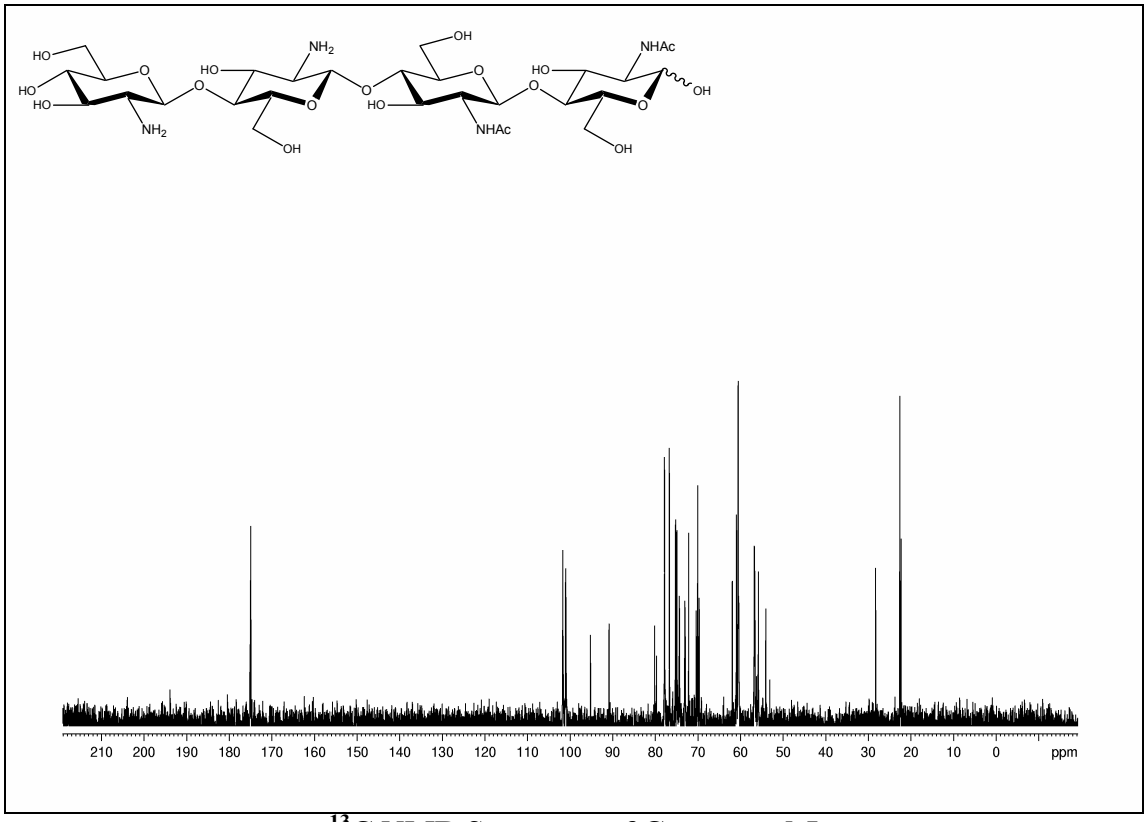
^{13}C APT NMR Spectrum of Compound 81



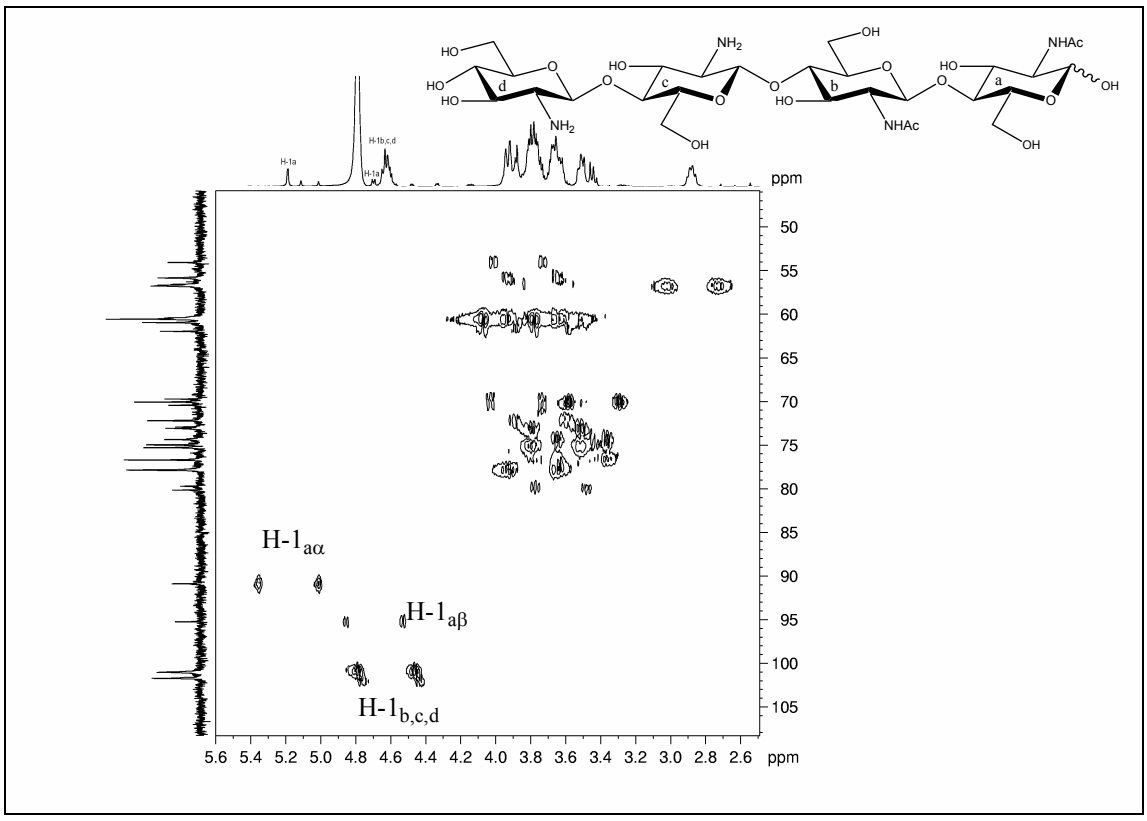
MALDI-TOF MS of Compound 81



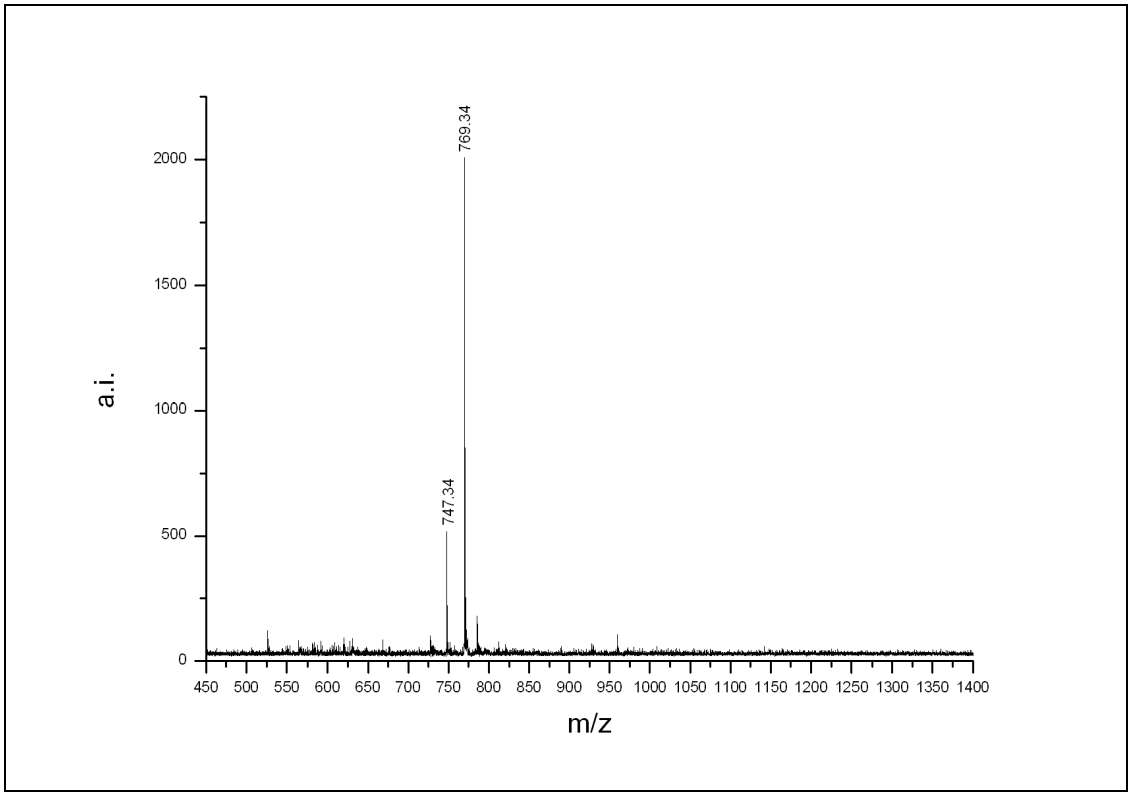
^{1}H NMR Spectrum of Compound 5



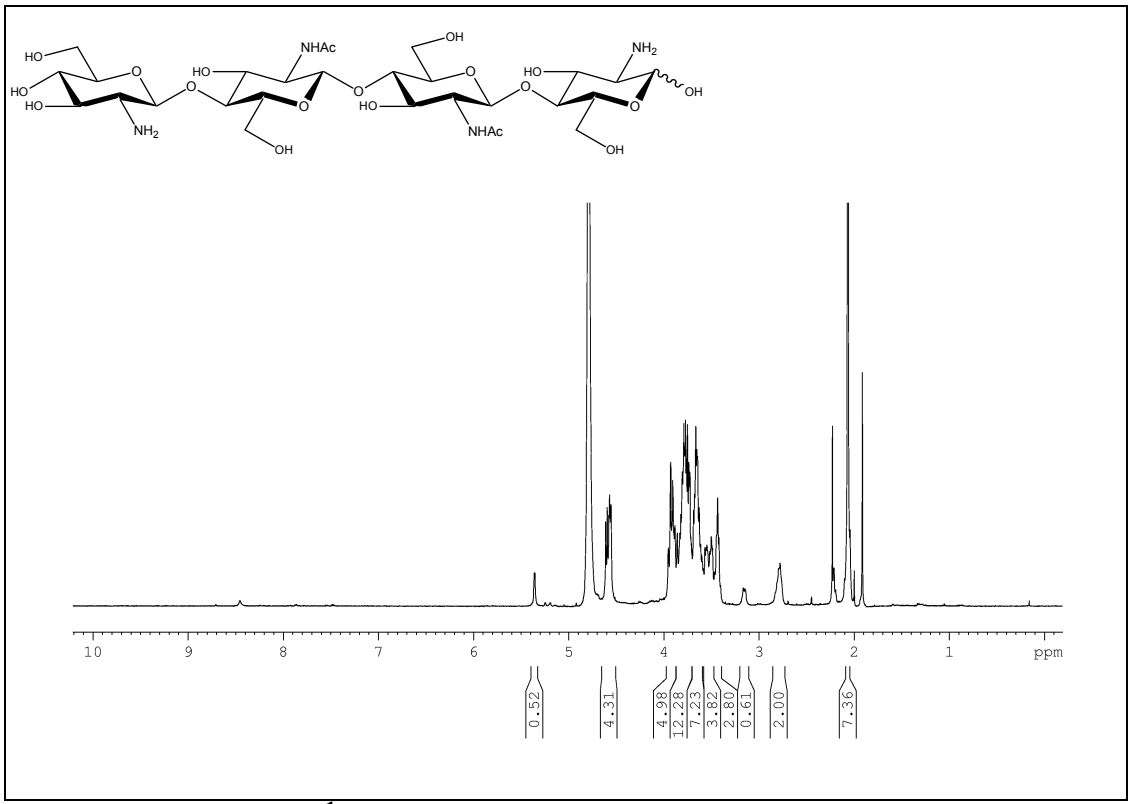
^{13}C NMR Spectrum of Compound 5



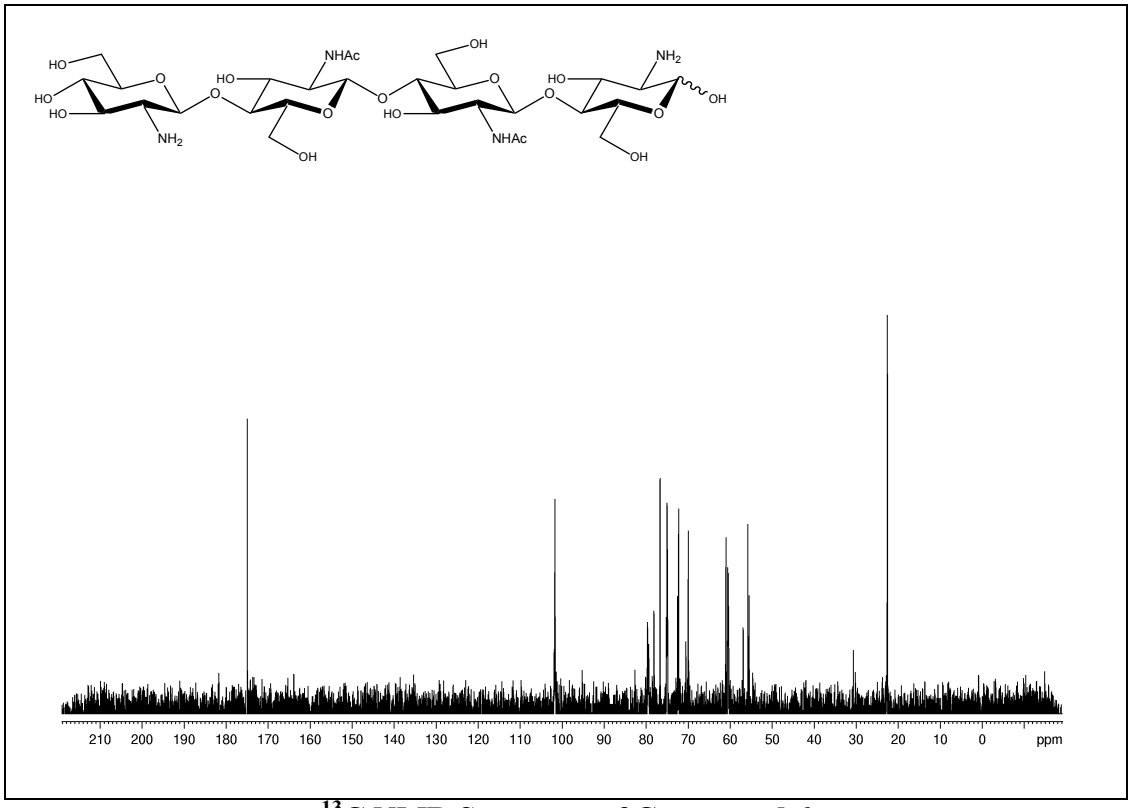
HMQC (Coupled) Spectrum of Compound 5



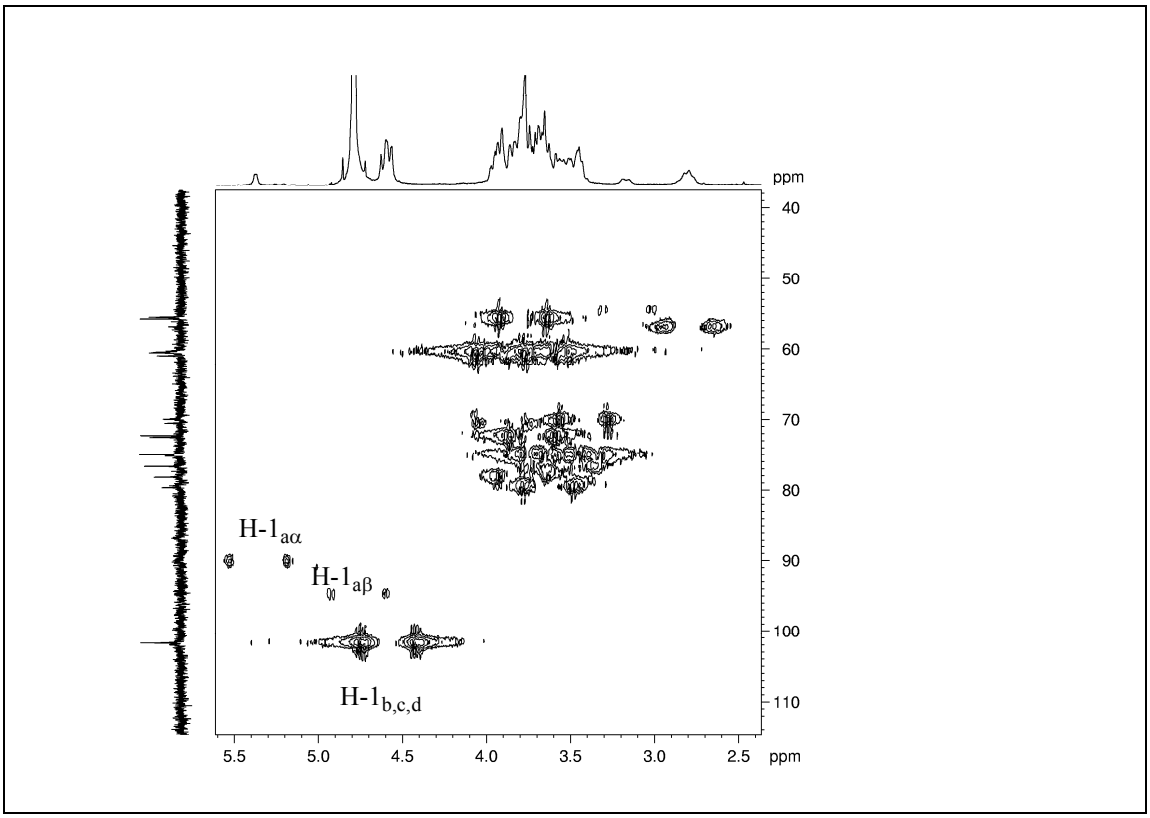
MALDI-TOF MS of Compound 5



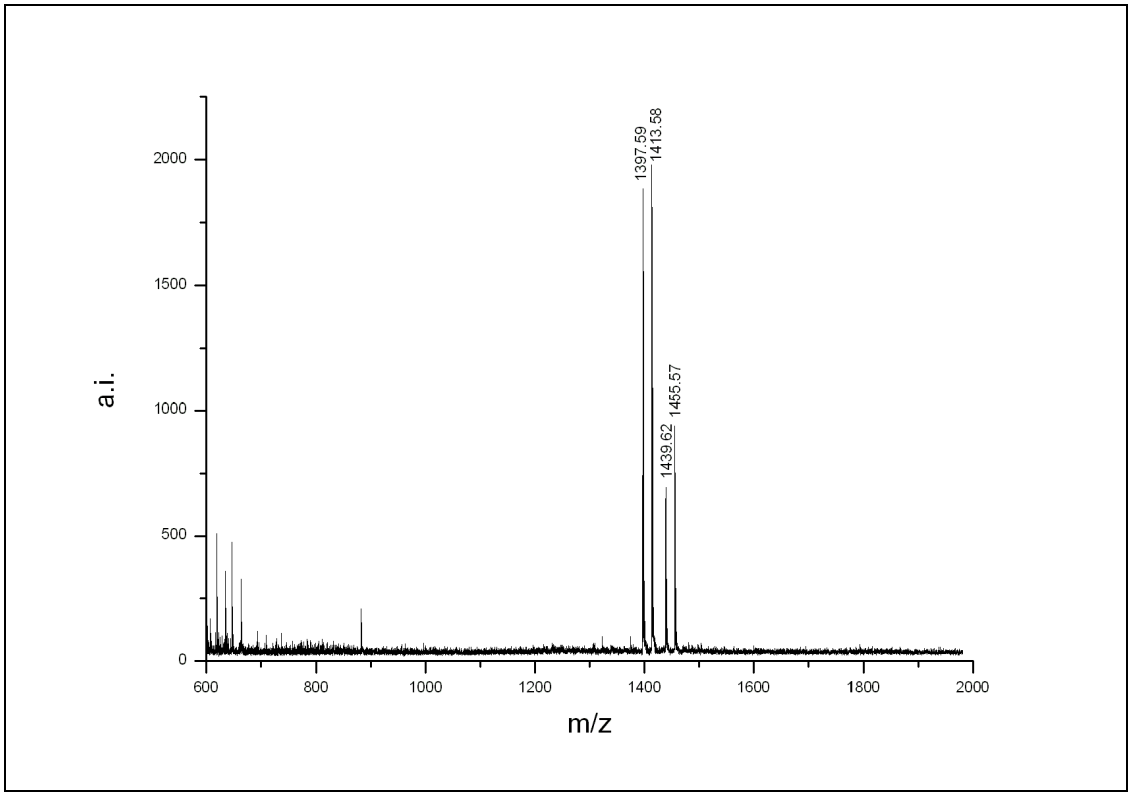
^{1}H NMR Spectrum of Compound 6



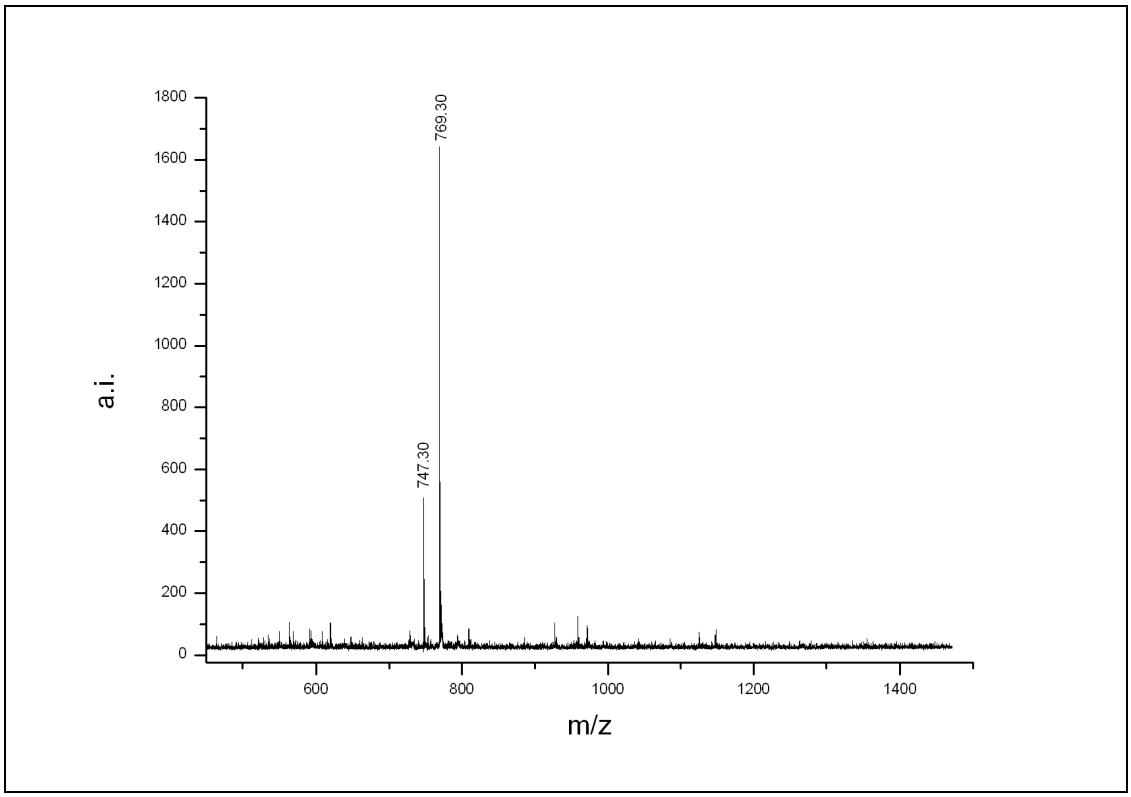
^{13}C NMR Spectrum of Compound 6



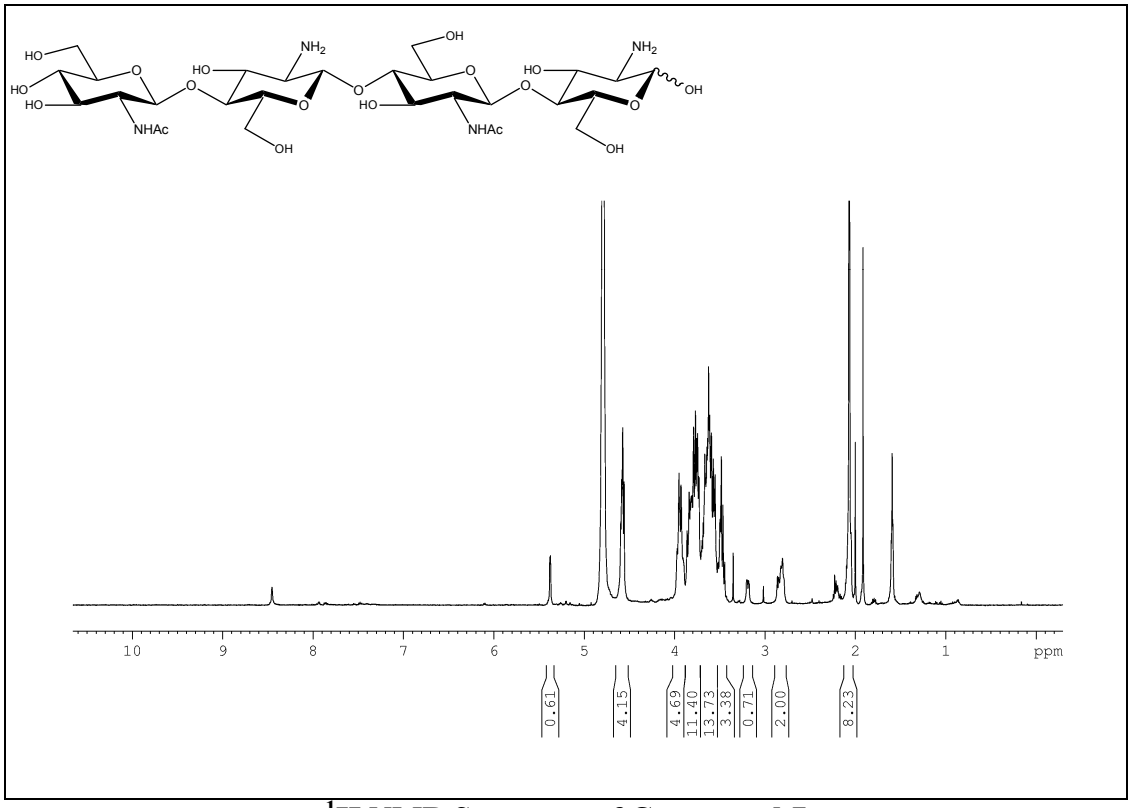
HMQC (Coupled) NMR Spectrum of Compound 6



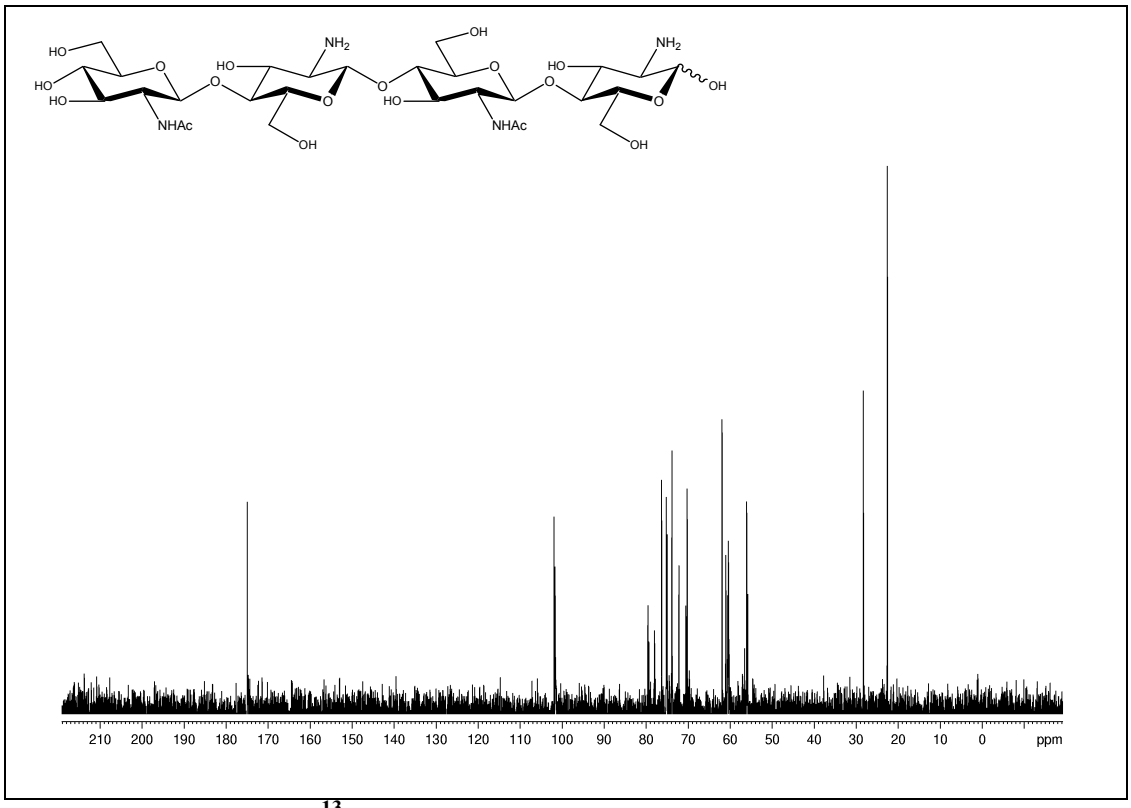
MALDI-TOF MS of Compound 6 (After treatment with MeOH/NH_3)



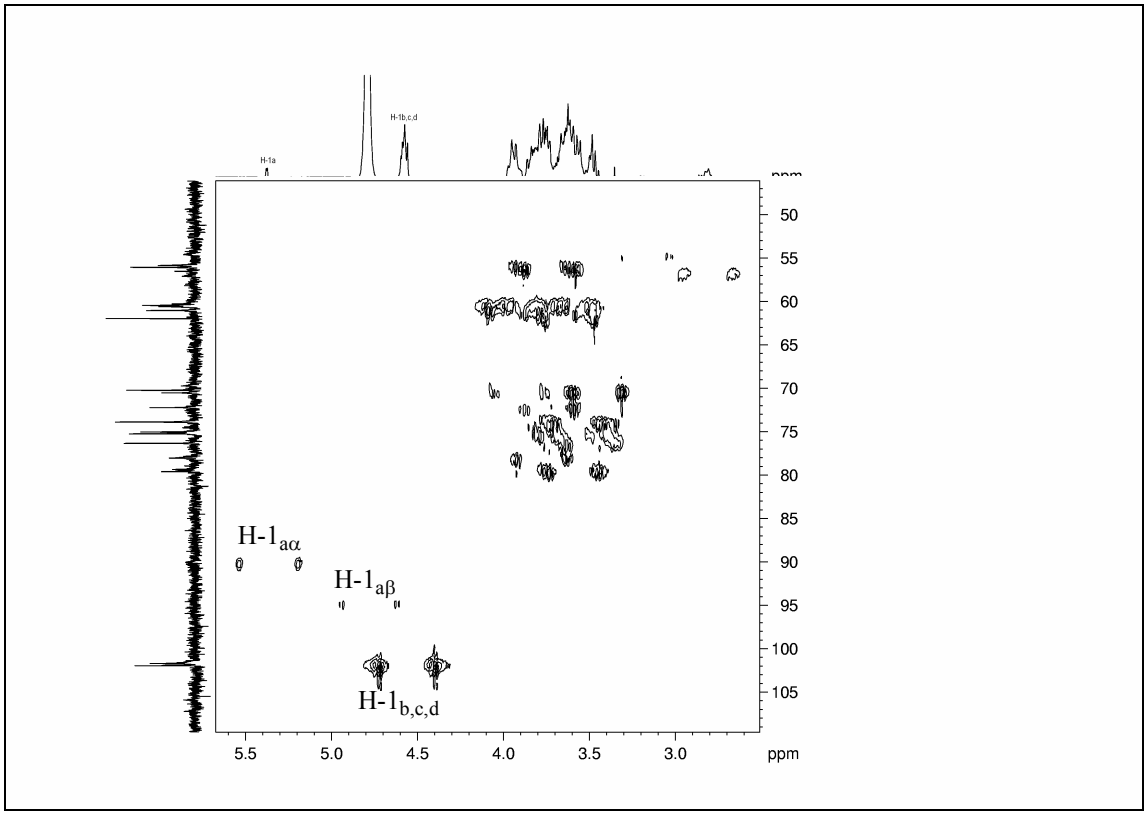
MALDI-TOF MS of Compound 6



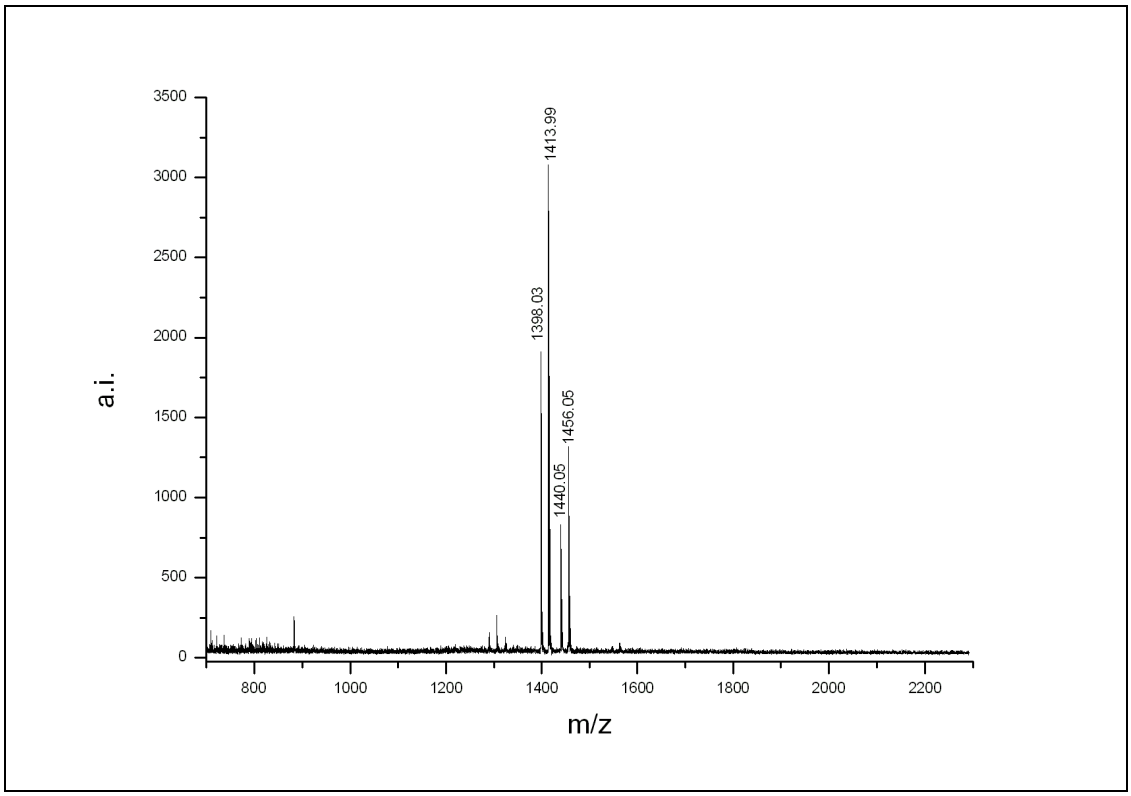
^{1}H NMR Spectrum of Compound 7



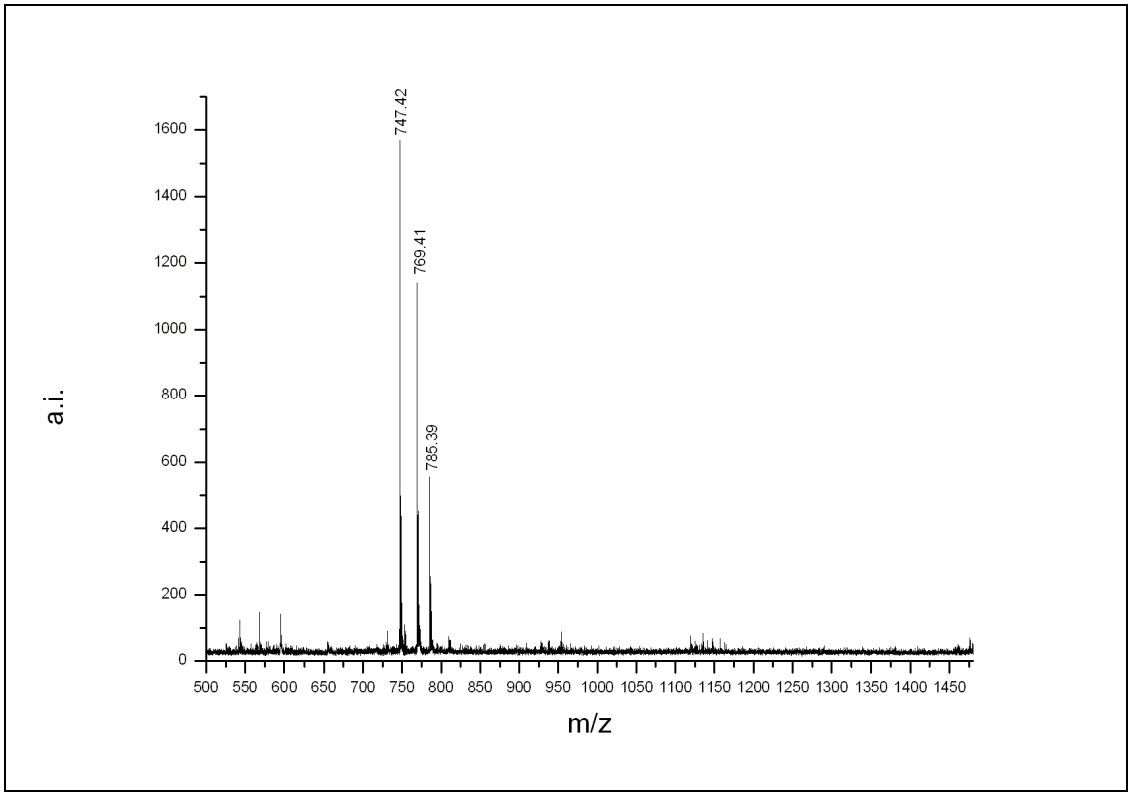
^{13}C NMR Spectrum of Compound 7



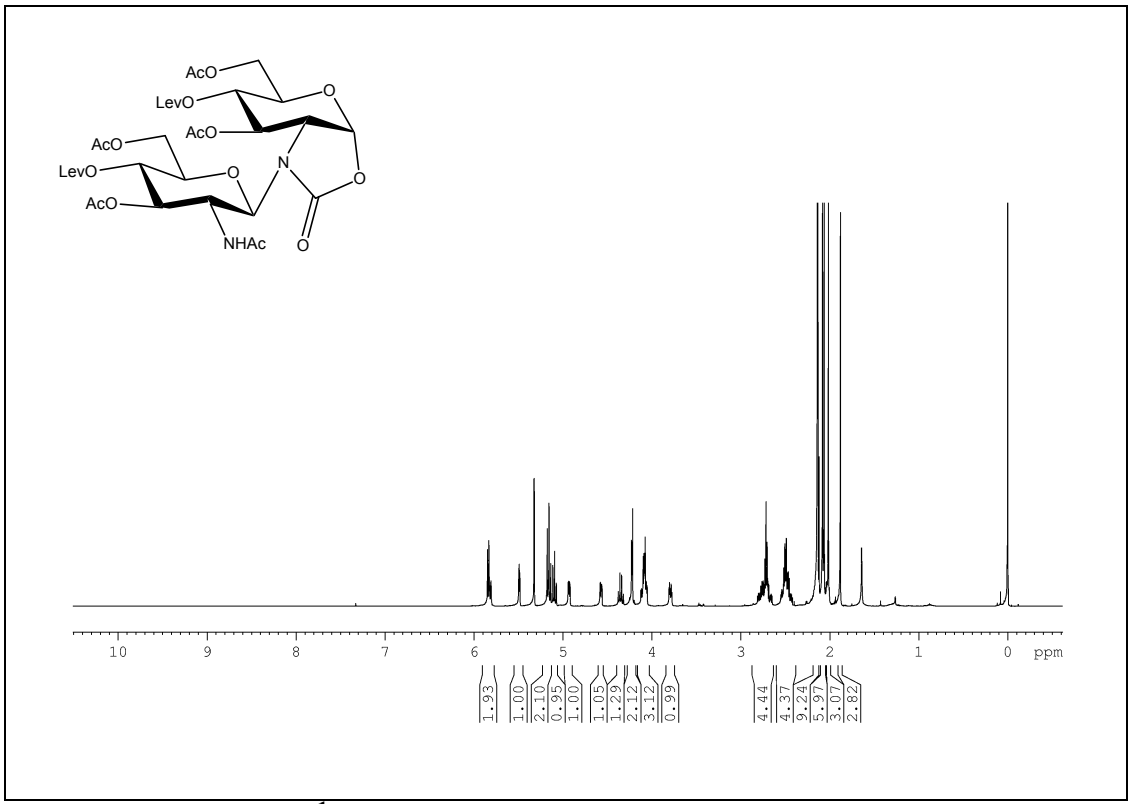
HMQC (Coupled) NMR Spectrum of Compound 7



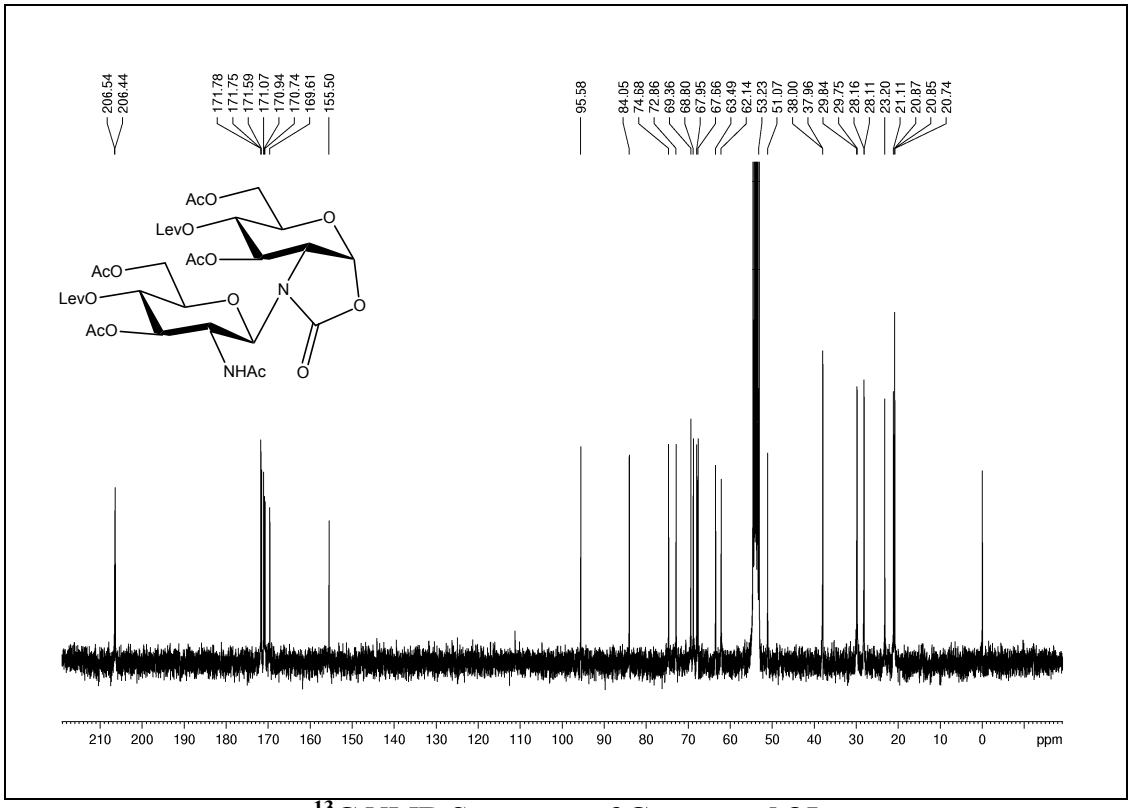
MALDI-TOF MS of Compound 7 (After treatment with MeOH/NH_3)



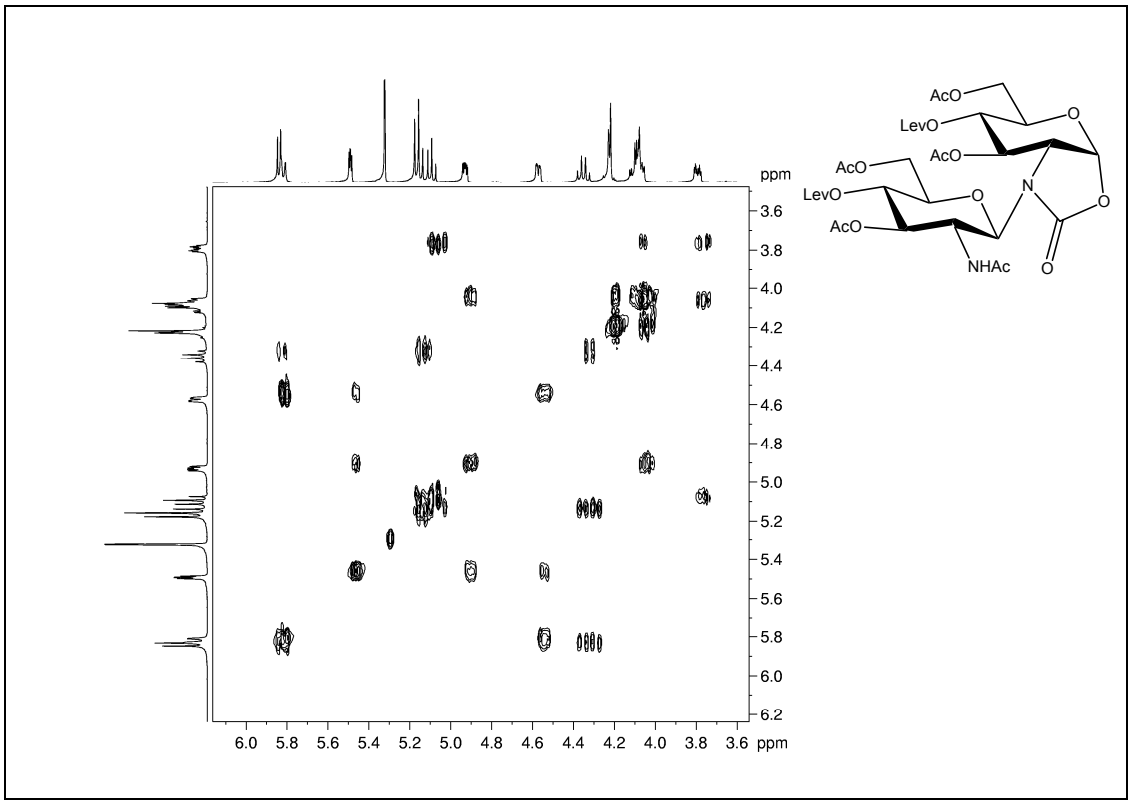
MALDI-TOF MS of Compound 7



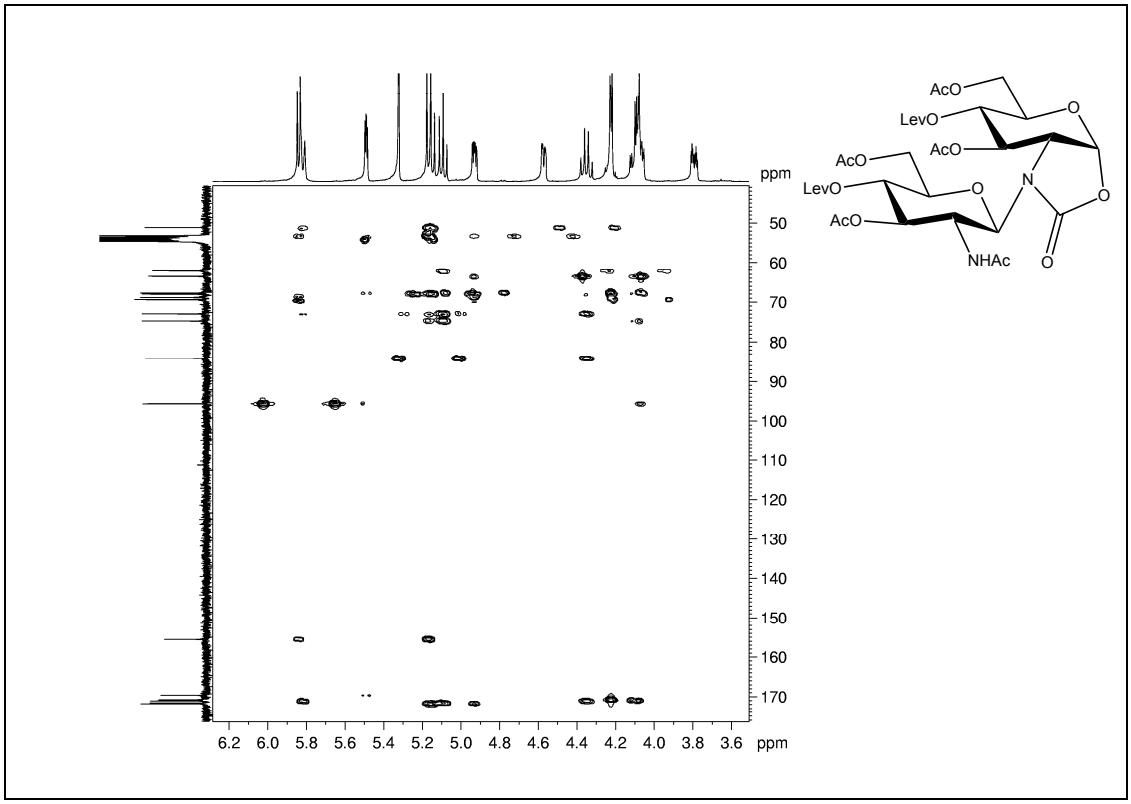
^{1}H NMR Spectrum of Compound 85



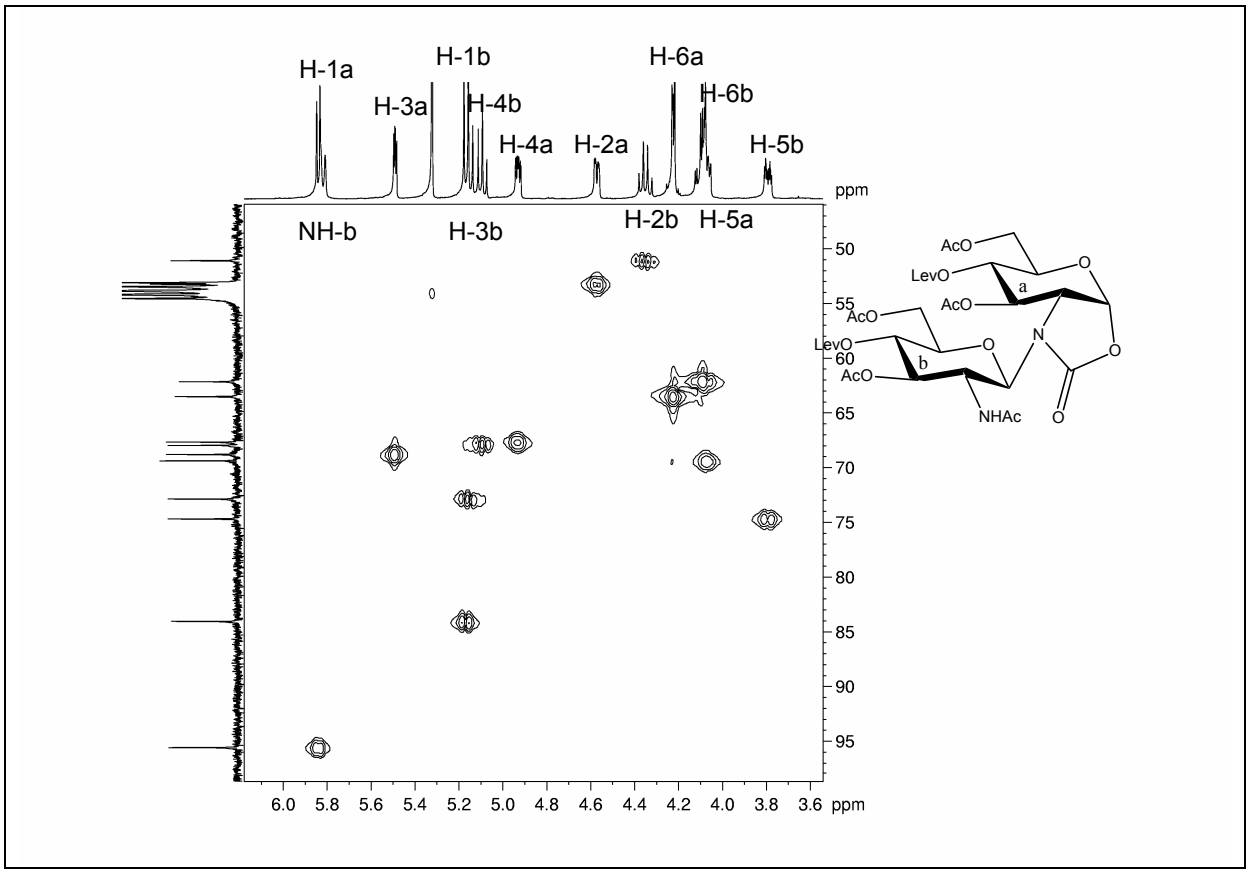
^{13}C NMR Spectrum of Compound 85



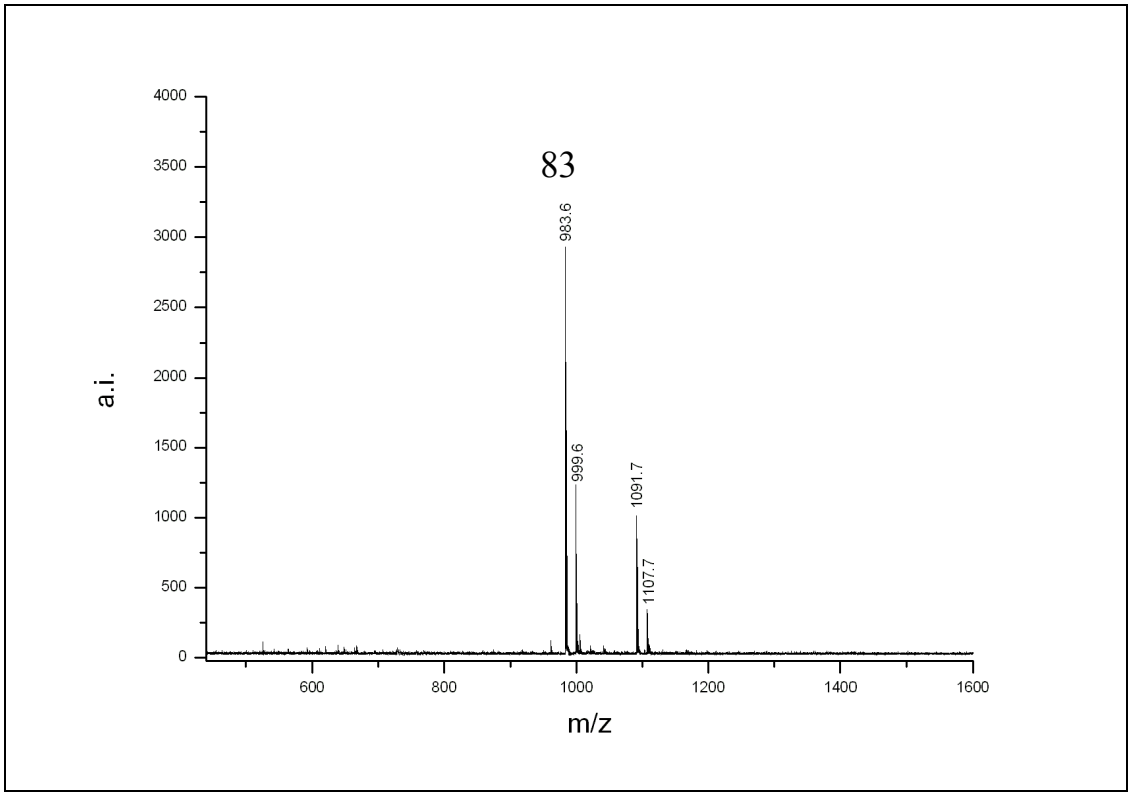
H-H COSY Spectrum of Compound 85



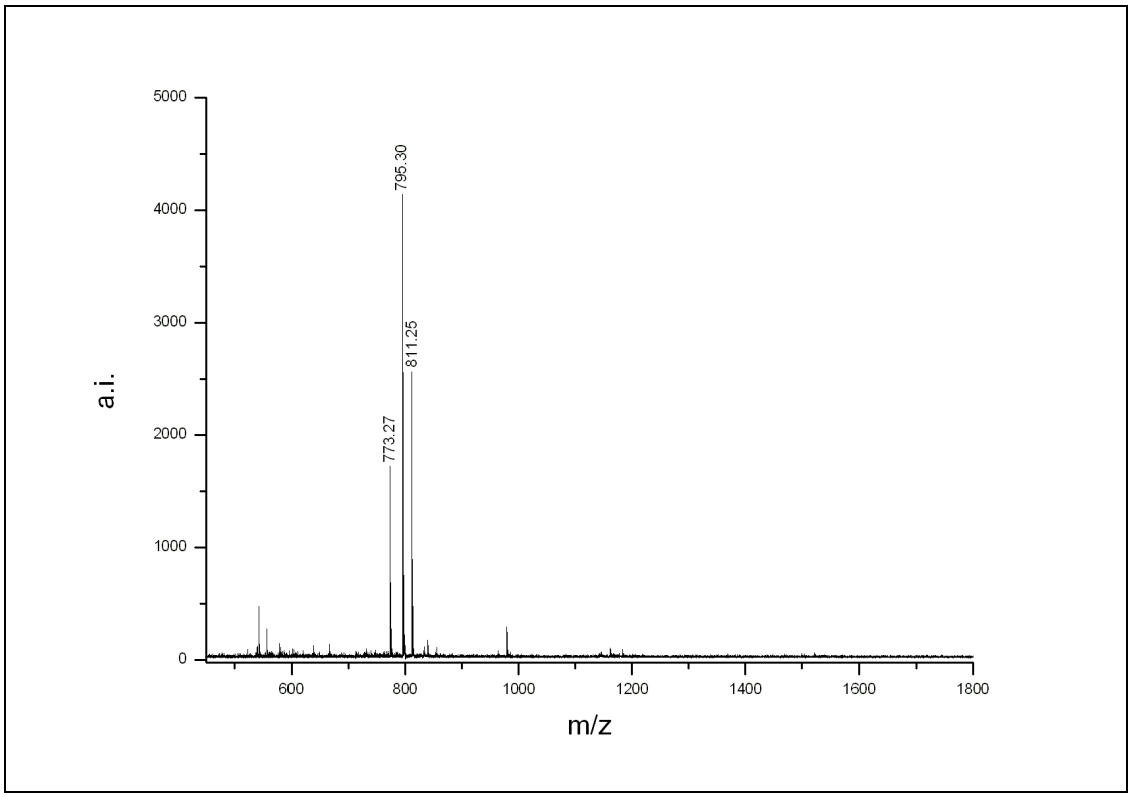
HMBC Spectrum of Compound 85



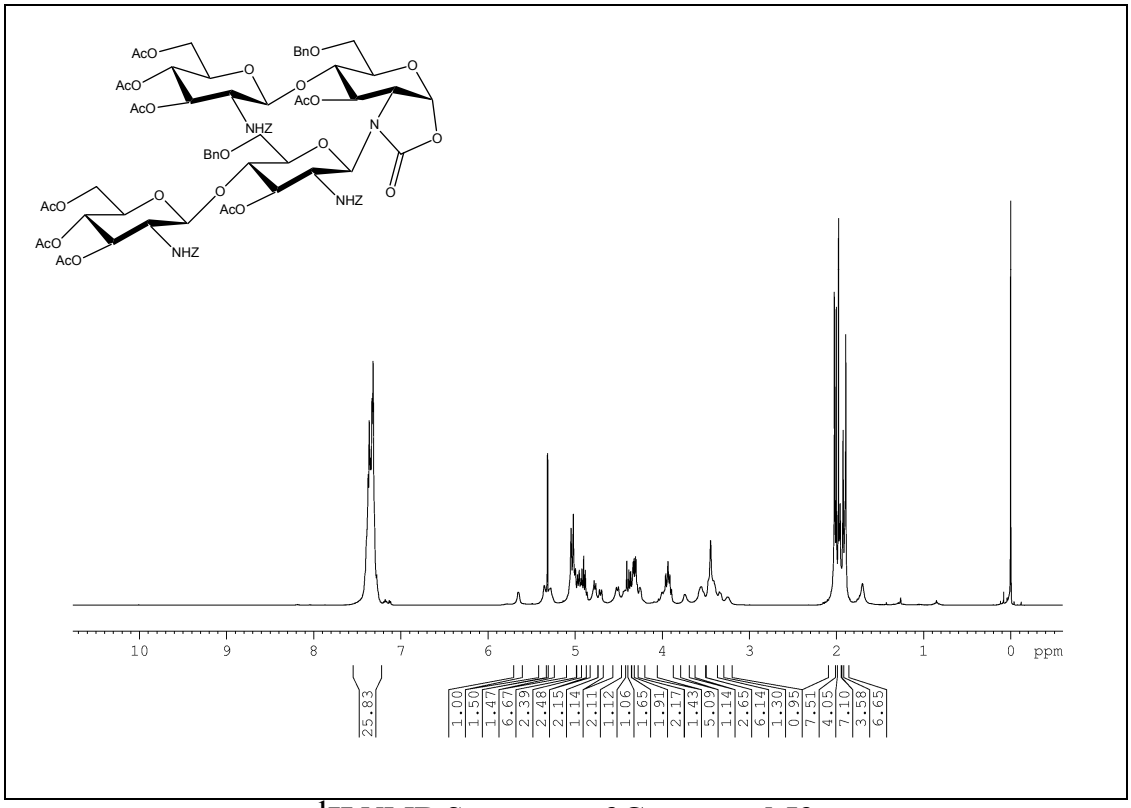
HMQC Spectrum of Compound 85



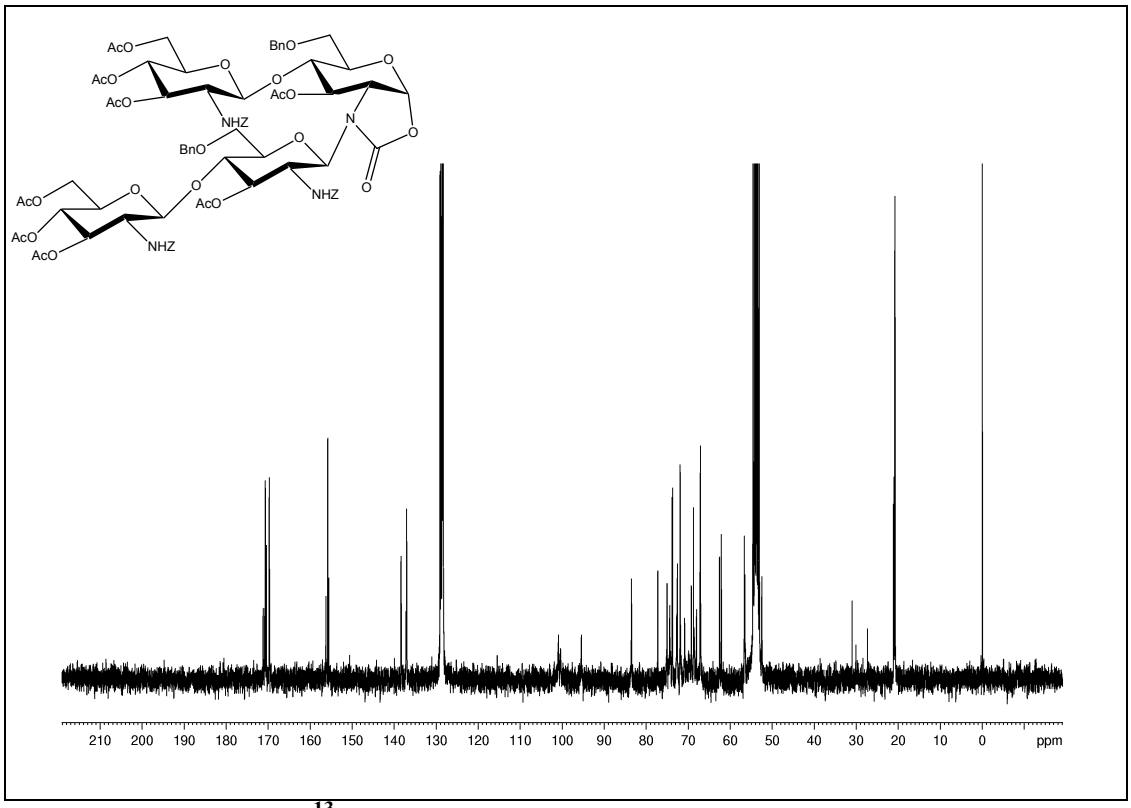
MALDI-TOF MS of Compound 83



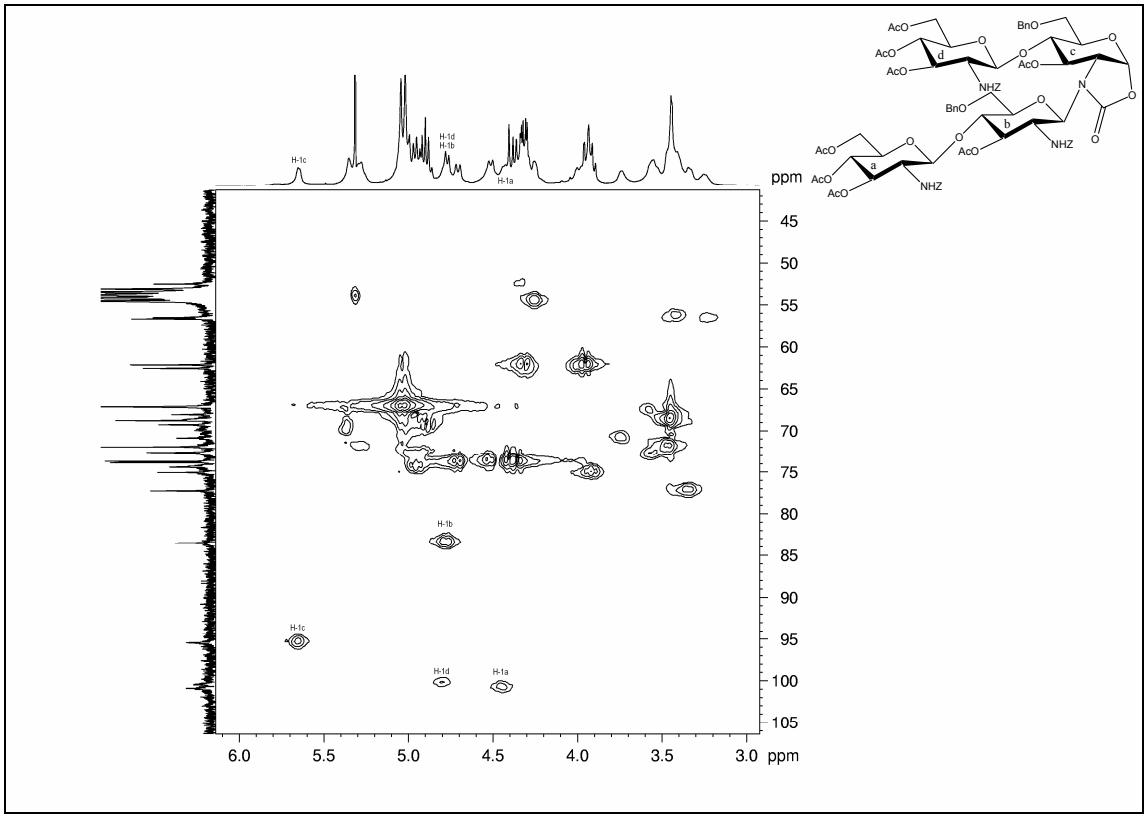
MALDI-TOF MS of Compound 85



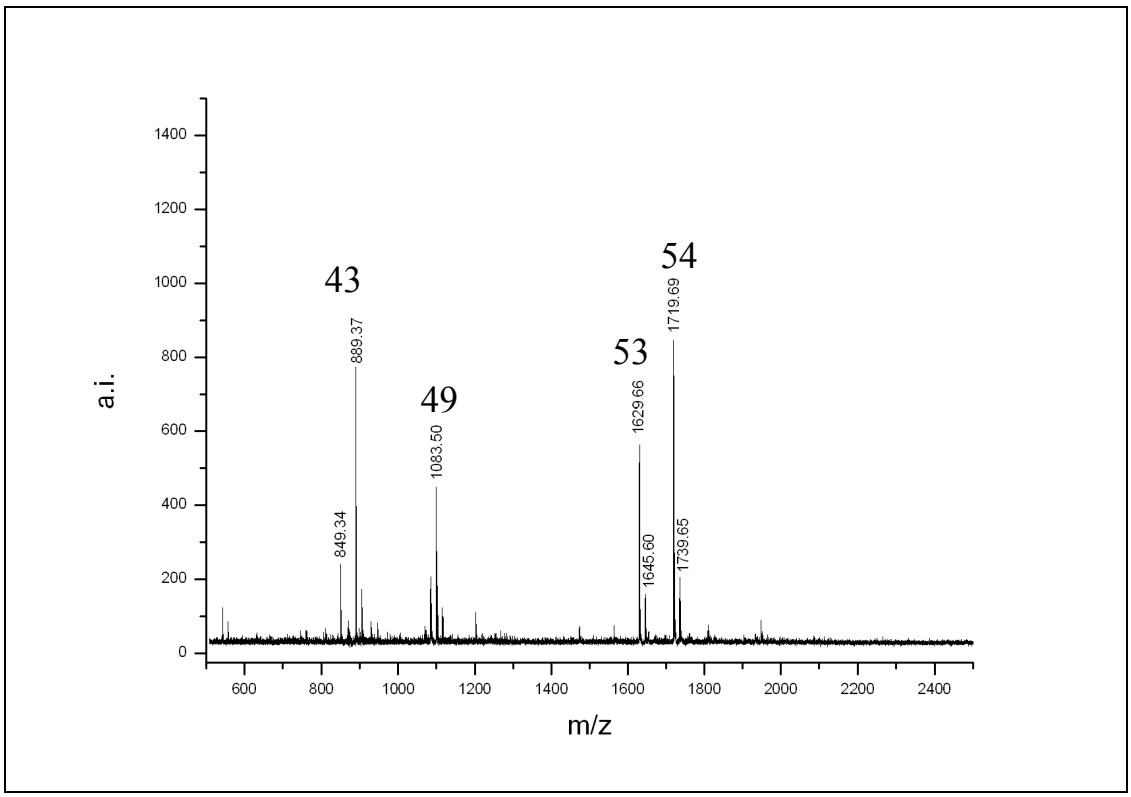
^{1}H NMR Spectrum of Compound 53



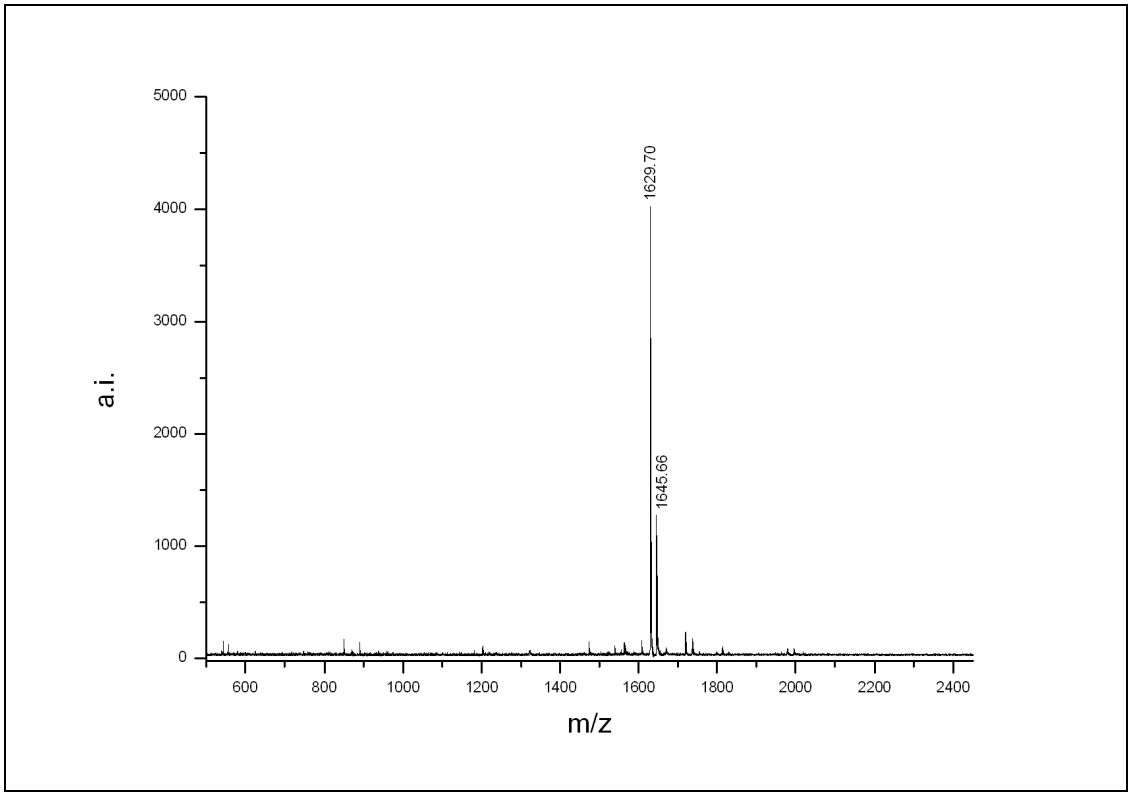
^{13}C NMR Spectrum of Compound 53



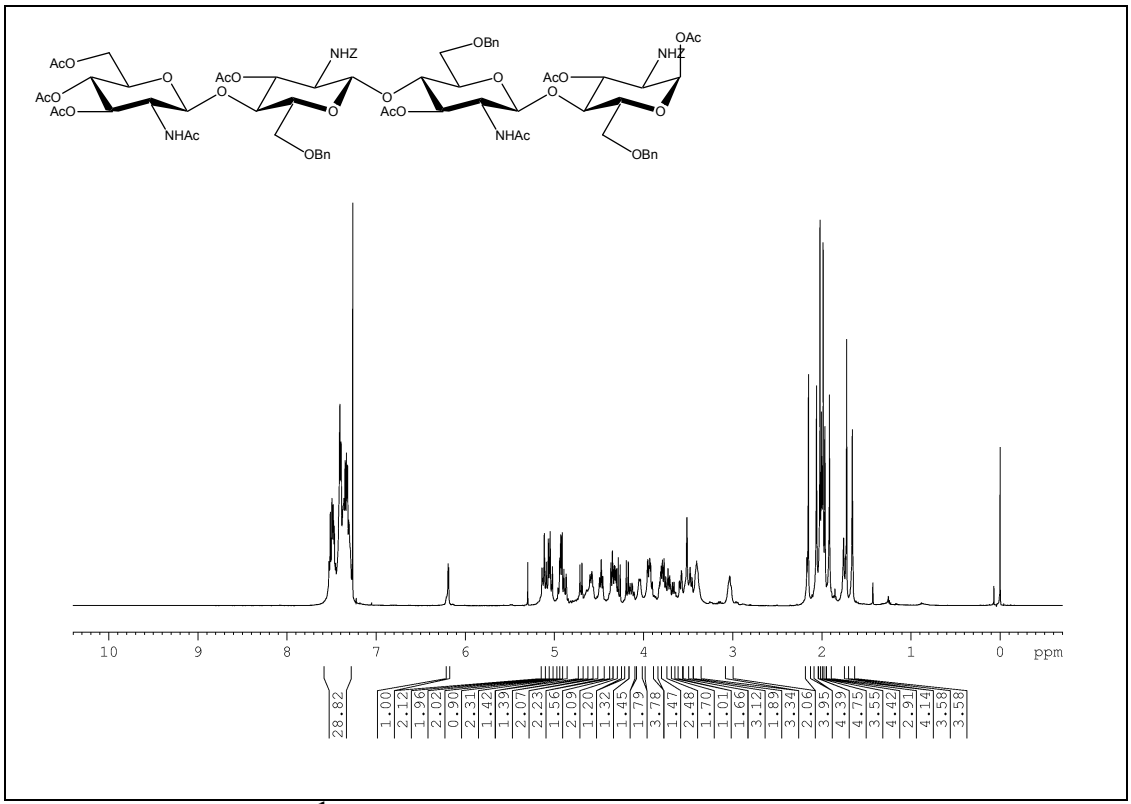
HMQC Spectrum of Compound 53



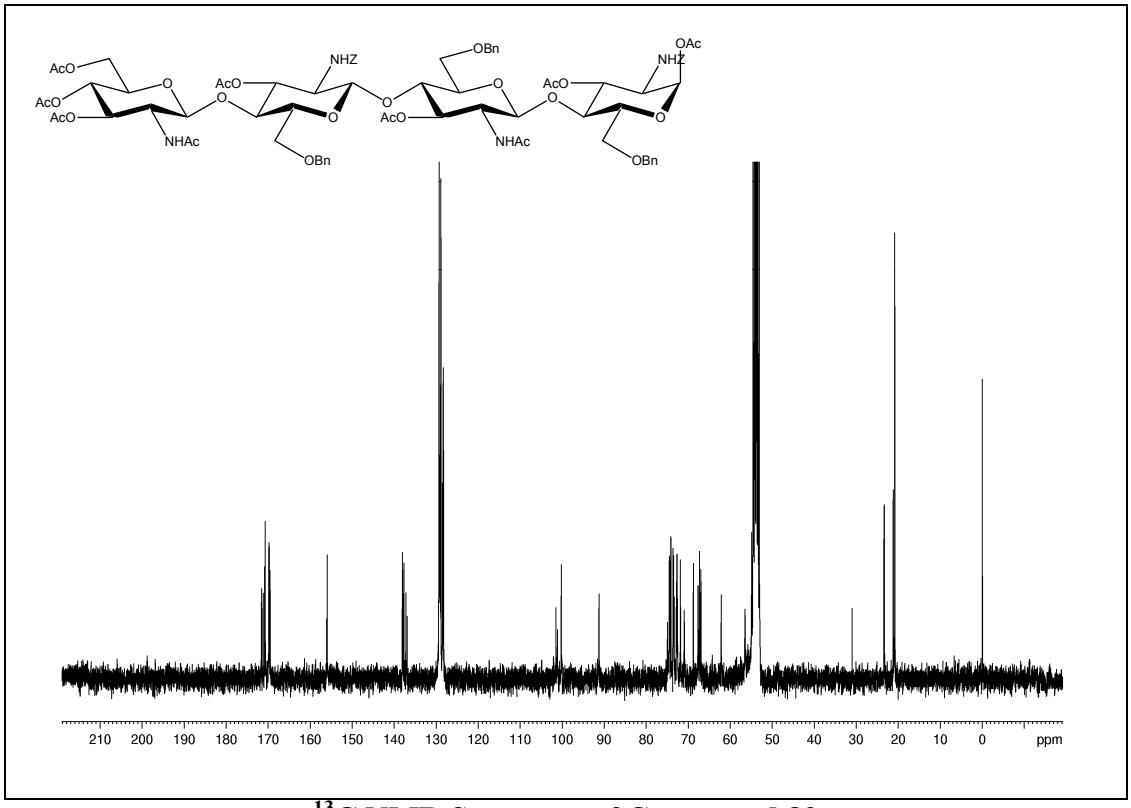
MALDI-TOF MS of crude product (Reaction between acceptor 49 and donor 43 under TMSOTf activation, Table 3.1.3)



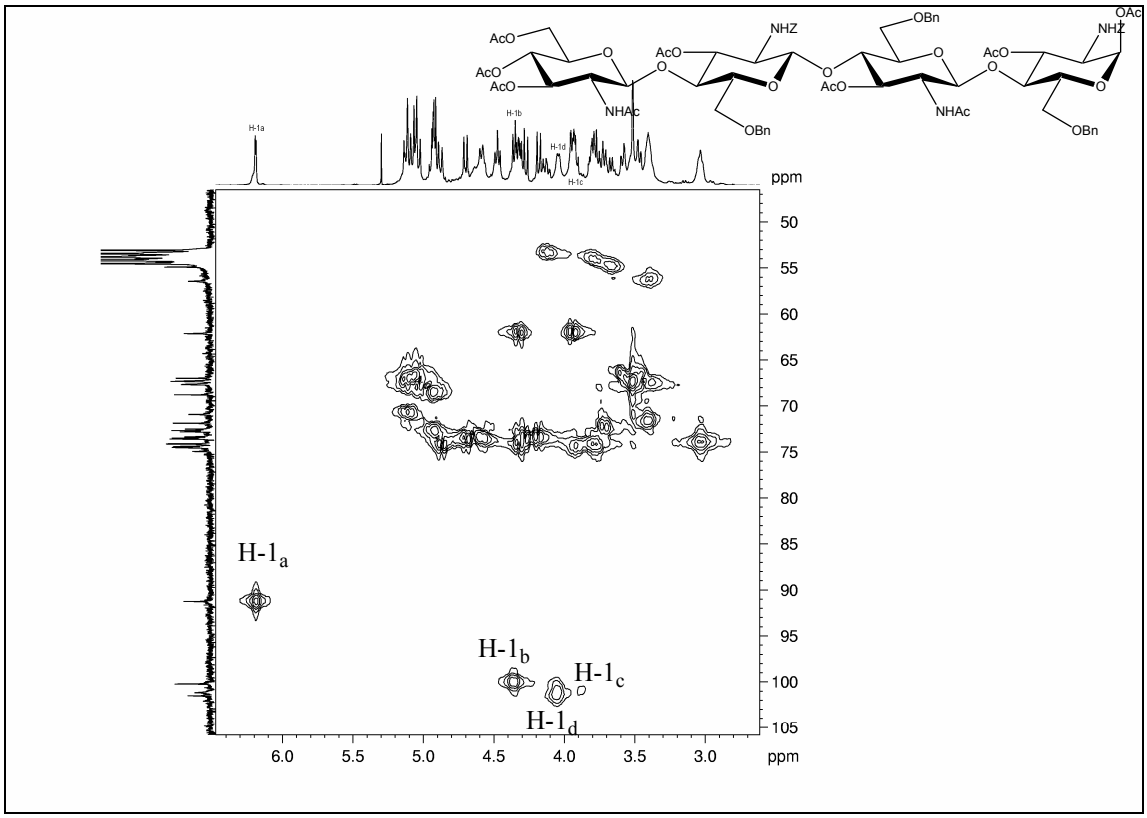
MALDI-TOF MS of byproduct 53



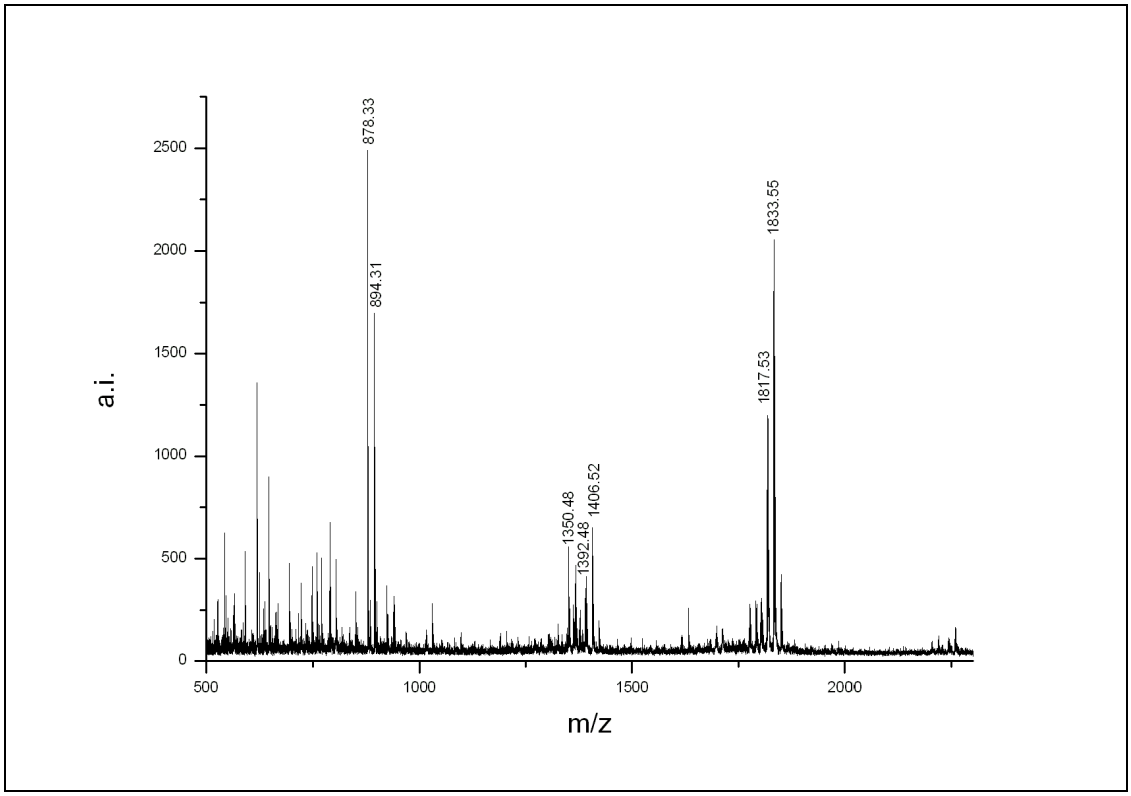
^{1}H NMR Spectrum of Compound 89



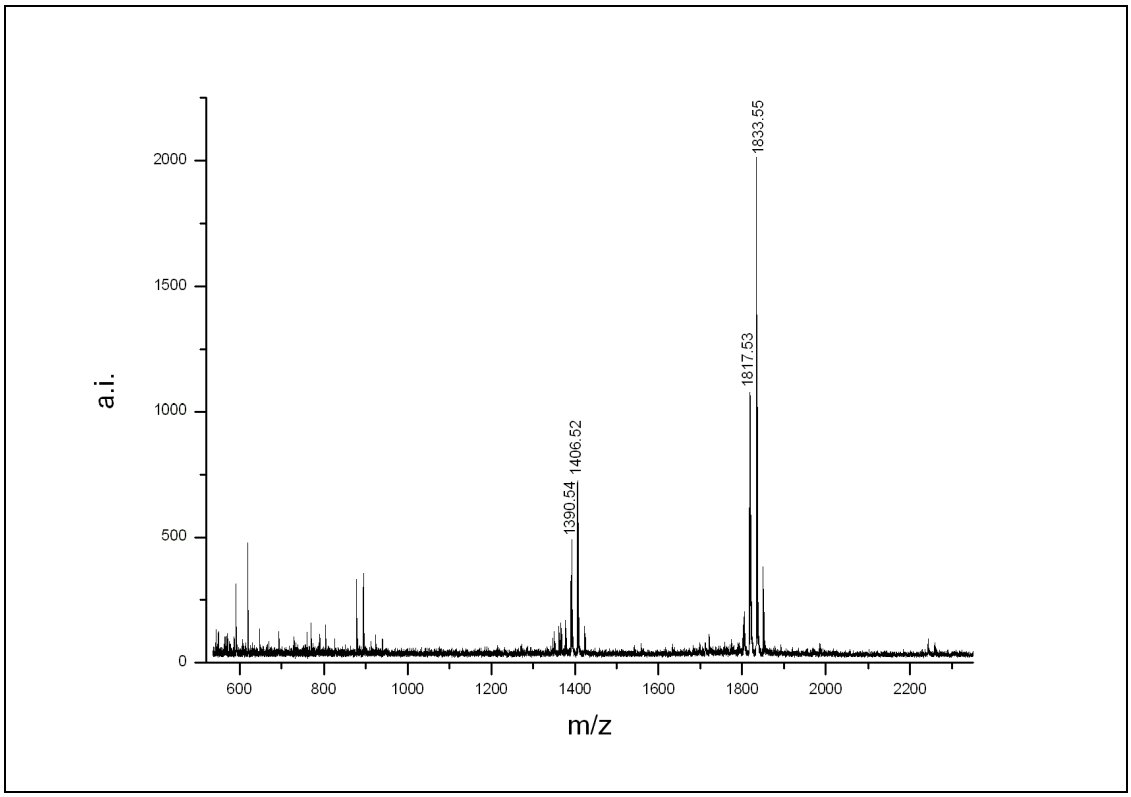
^{13}C NMR Spectrum of Compound 89



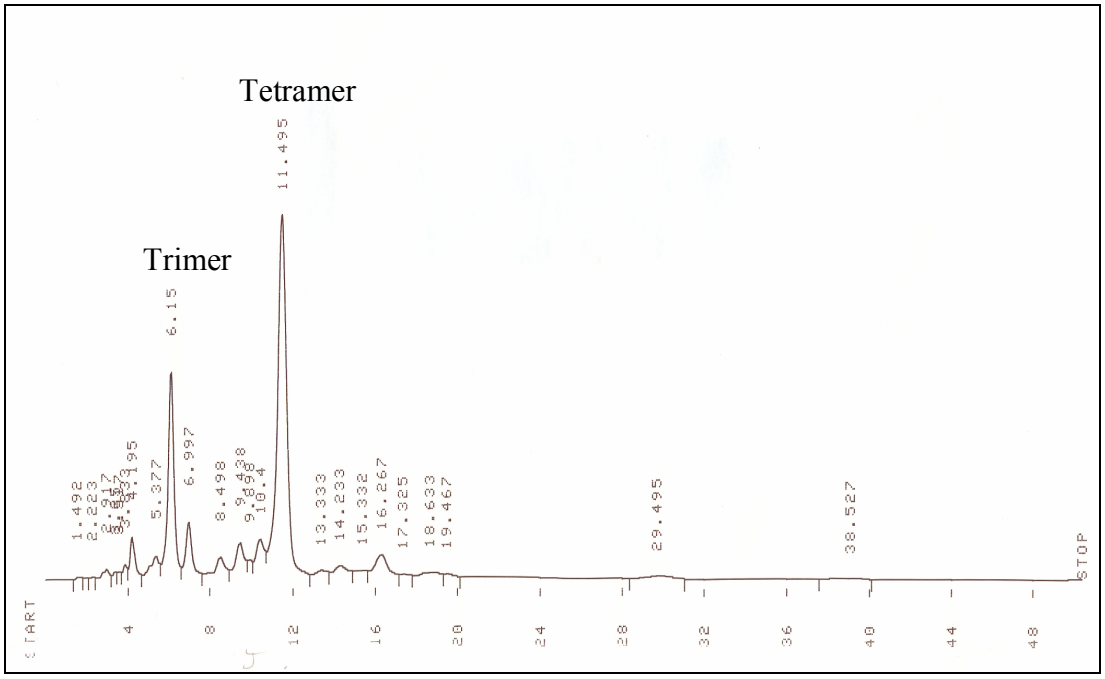
HMQC Spectrum of Compound 89



MALDI-TOF MS of crude product after cleavage from the resin (86)

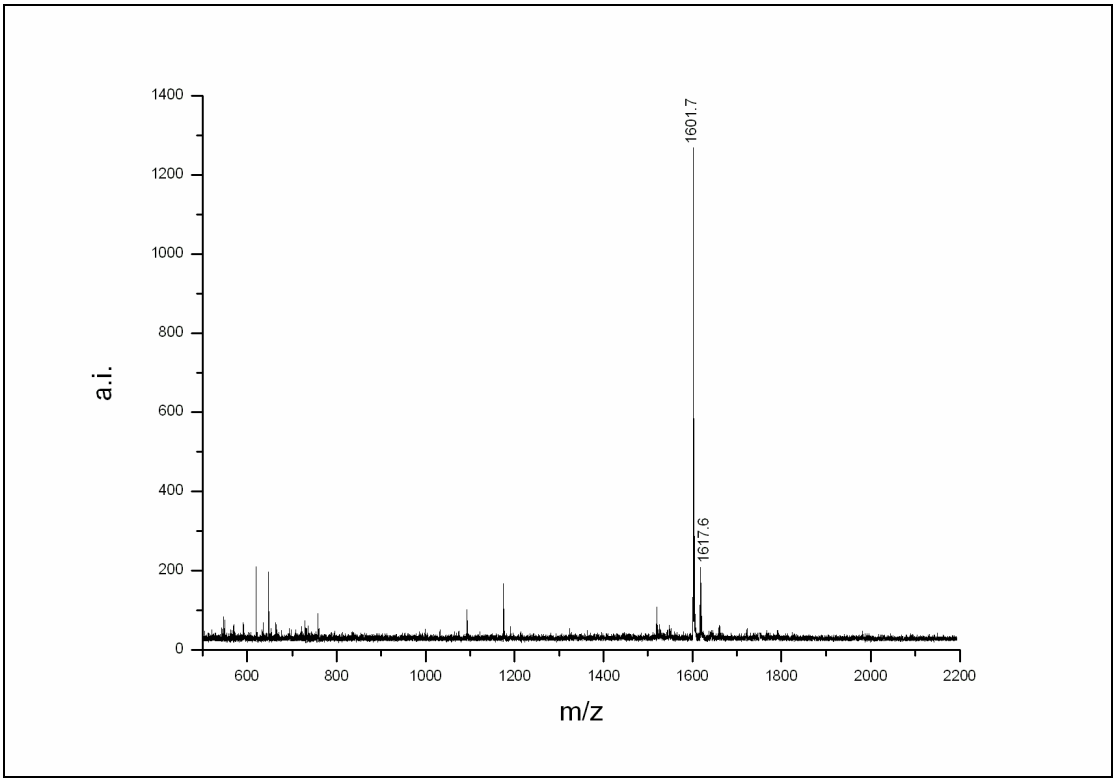


MALDI-TOF MS of fraction containing compound 86 after purification

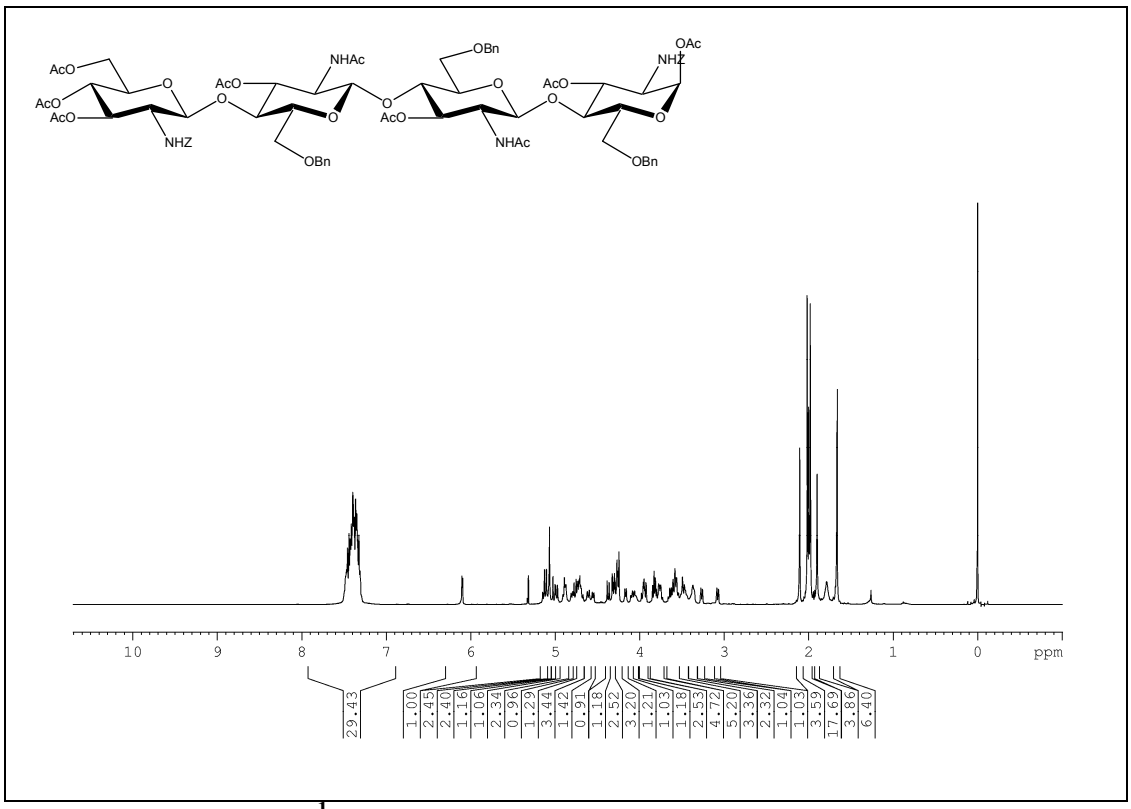


Chromatogram of crude product (86, protected tetrasaccharide ADAD)

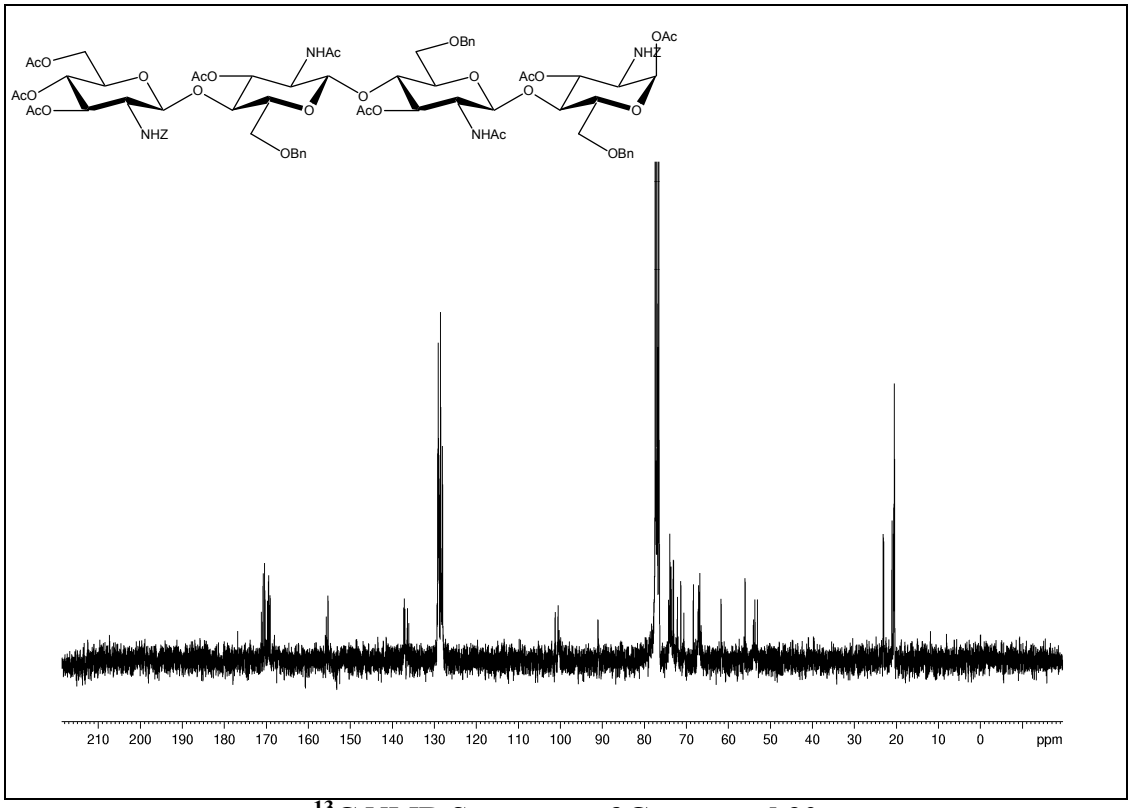
PKNO	TIME	AREA	MK	IDNO	CONC
1	1.492	285			0.0961
2	2.223	206			0.0695
3	2.917	2629	V		0.8859
4	3.35	1121	V		0.3778
5	3.517	1094	V		0.3686
6	3.833	2532	V		0.8534
7	4.195	8910	V		3.0026
8	5.377	8845	V		2.9807
9	6.15	49509	V		16.685
10	6.997	15175	V		5.1141
11	8.498	9702	V		3.2696
12	9.438	12854	V		4.3318
13	9.898	3233	V		1.0897
14	10.4	13072	V		4.4052
15	11.495	132839	V		44.7677
16	13.333	3876	V		1.3061
17	14.233	6557	V		2.2099
18	15.332	3580	V		1.2063
19	16.267	11936	V		4.0226
20	17.325	791	V		0.2666
21	18.633	3882	V		1.3084
22	19.467	521	V		0.1755
23	29.495	2181			0.7351
24	38.527	1400			0.4717
	TOTAL	296729			100



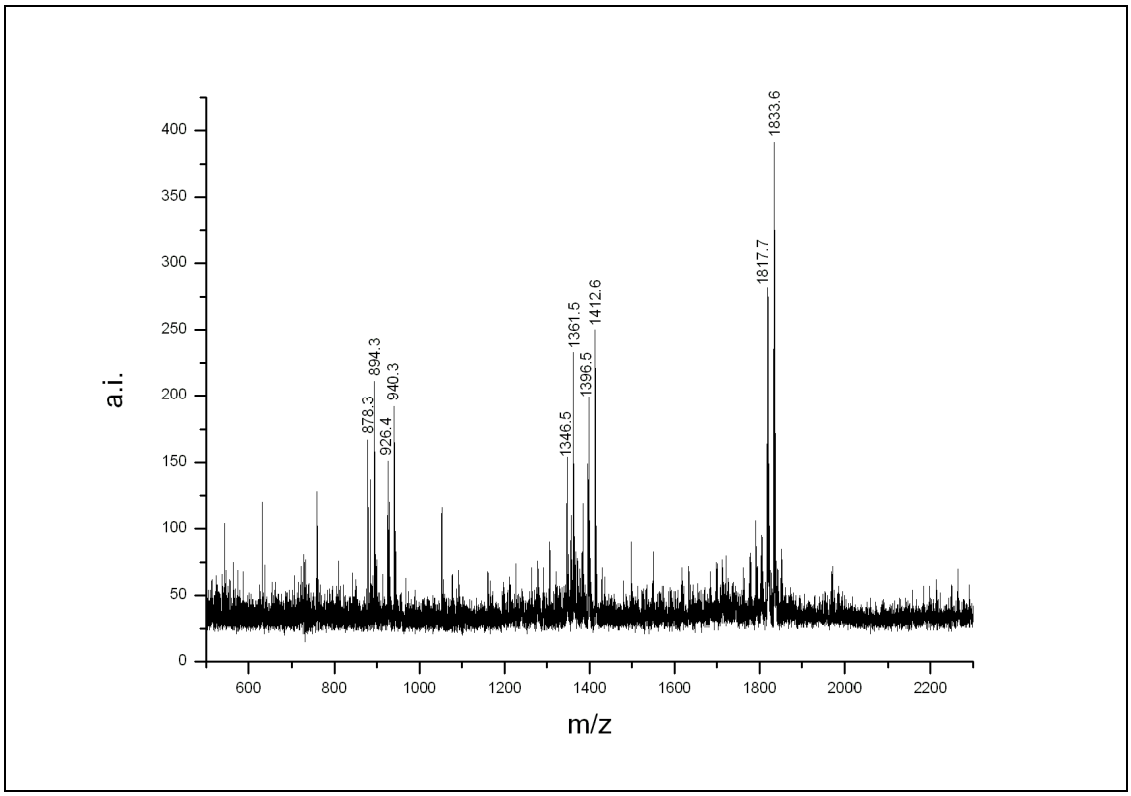
MALDI-TOF MS of Compound 89



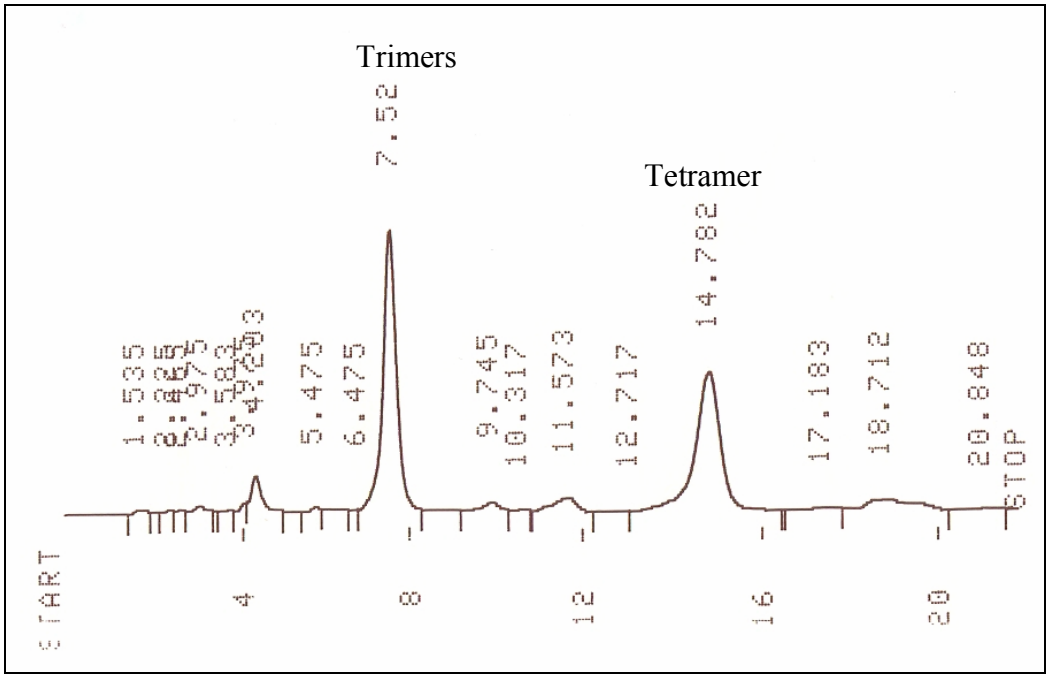
^{1}H NMR Spectrum of Compound 90



^{13}C NMR Spectrum of Compound 90

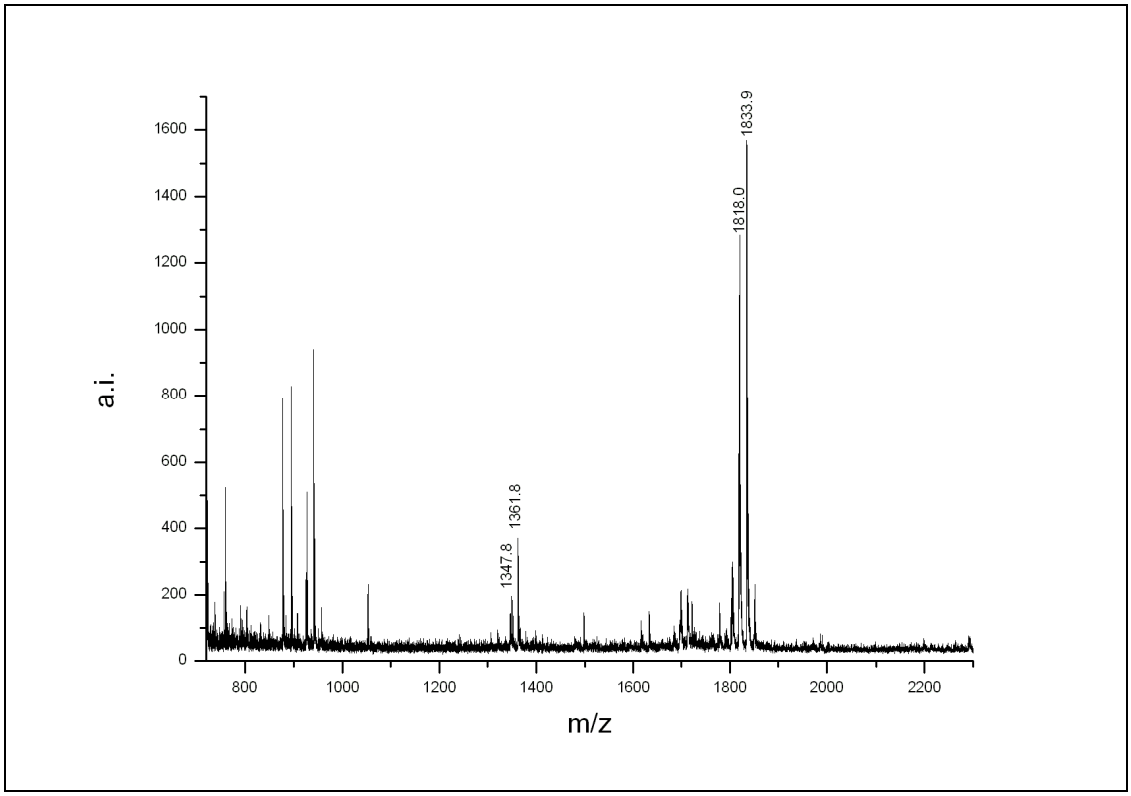


MALDI-TOF MS of crude product after cleavage from the resin (87)

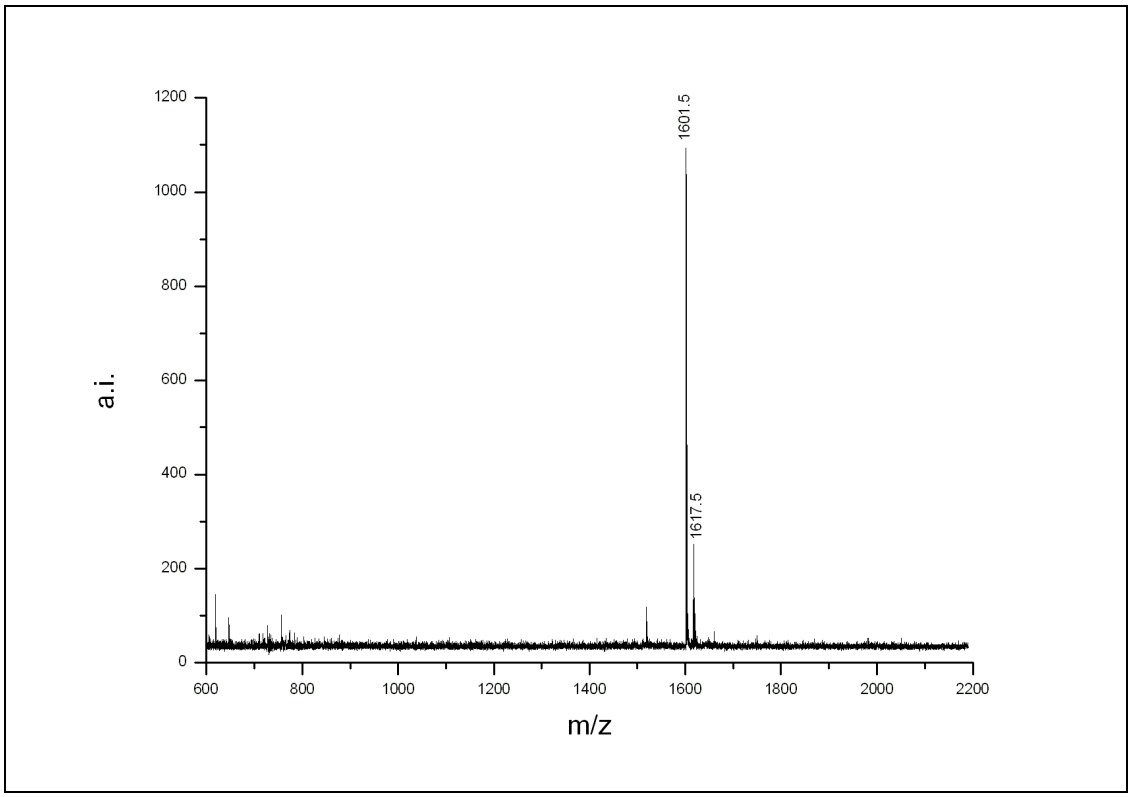


Chromatogram of crude product (87, protected tetrasaccharide DAAD)

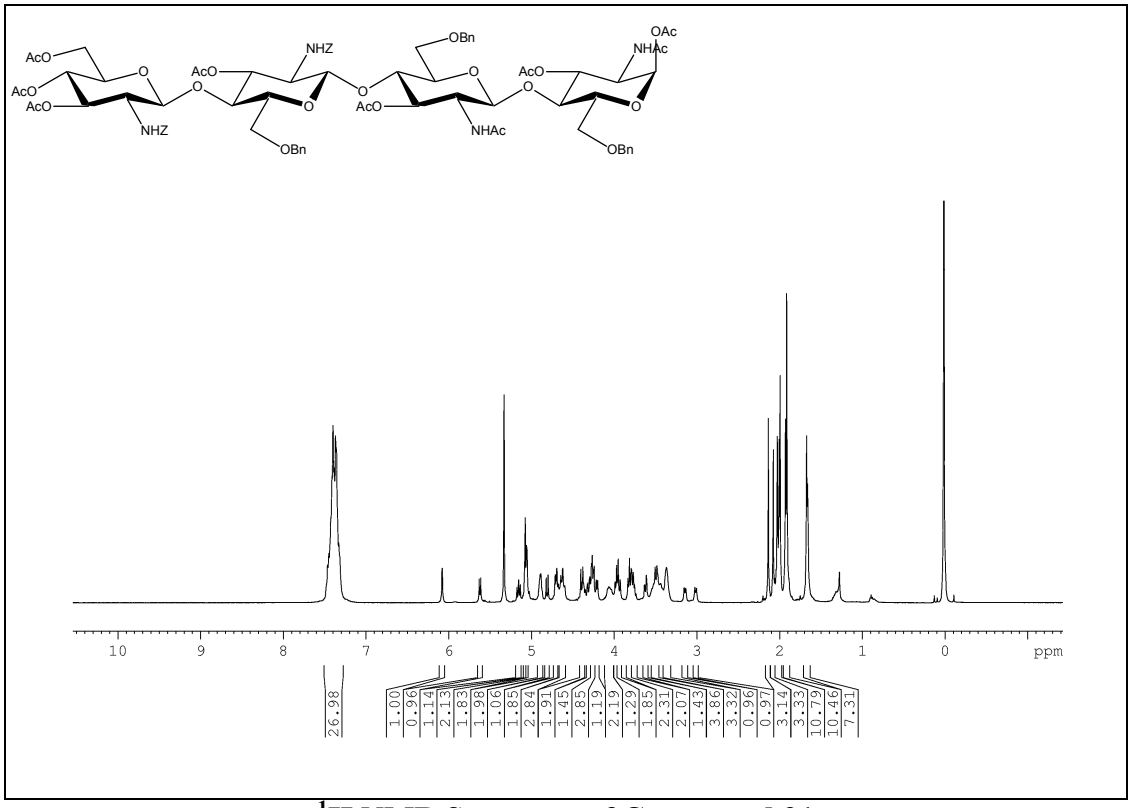
PKNO	TIME	AREA	MK	IDNO	CONC
1	1.535	347			0.37
2	2.225	251			0.2679
3	2.465	230	V		0.2454
4	2.975	745	V		0.7953
5	3.583	166			0.1768
6	3.975	678	V		0.7237
7	4.203	3950	V		4.2186
8	5.475	379			0.4047
9	6.475	240			0.2559
10	7.52	38930	V		41.5743
11	9.745	1426			1.523
12	10.317	110	V		0.1178
13	11.573	3524			3.7638
14	12.717	196	V		0.2093
15	14.782	35929	V		38.3691
16	17.183	900			0.961
17	18.712	5176	V		5.5271
18	20.848	465			0.4963
	TOTAL	93640			100



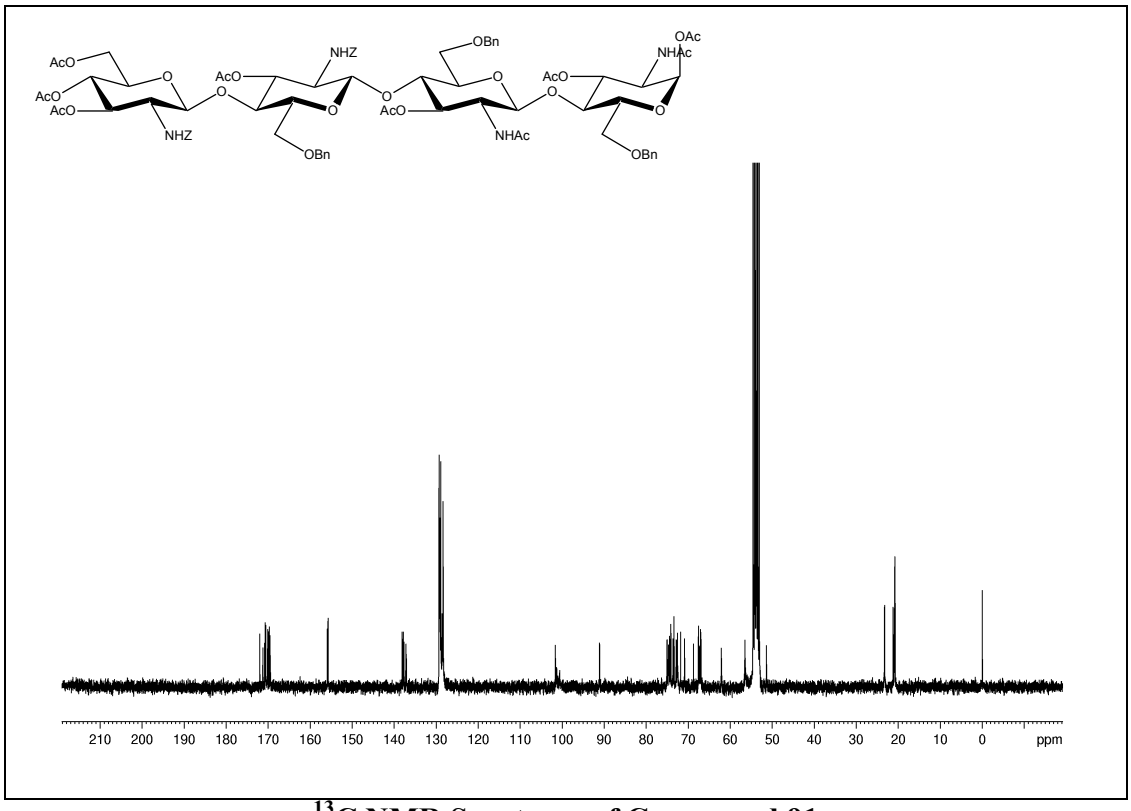
MALDI-TOF MS of fraction containing compound 87 after purification



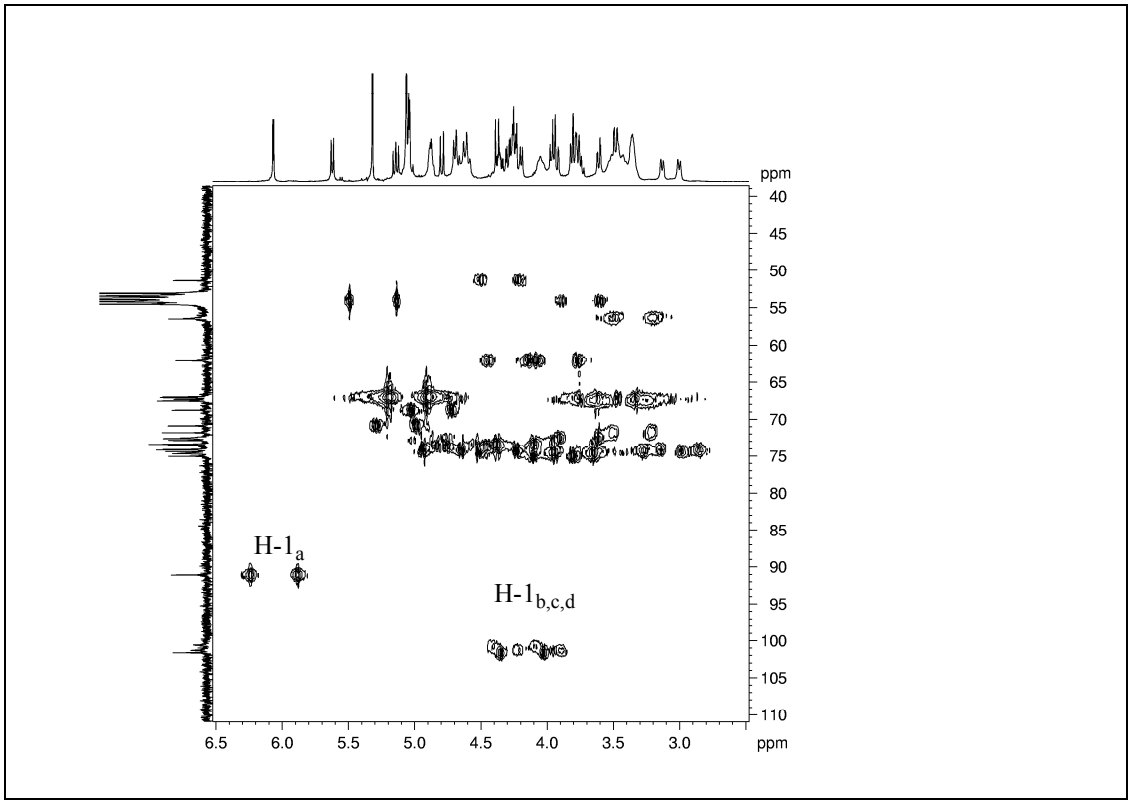
MALDI-TOF MS of Compound 90



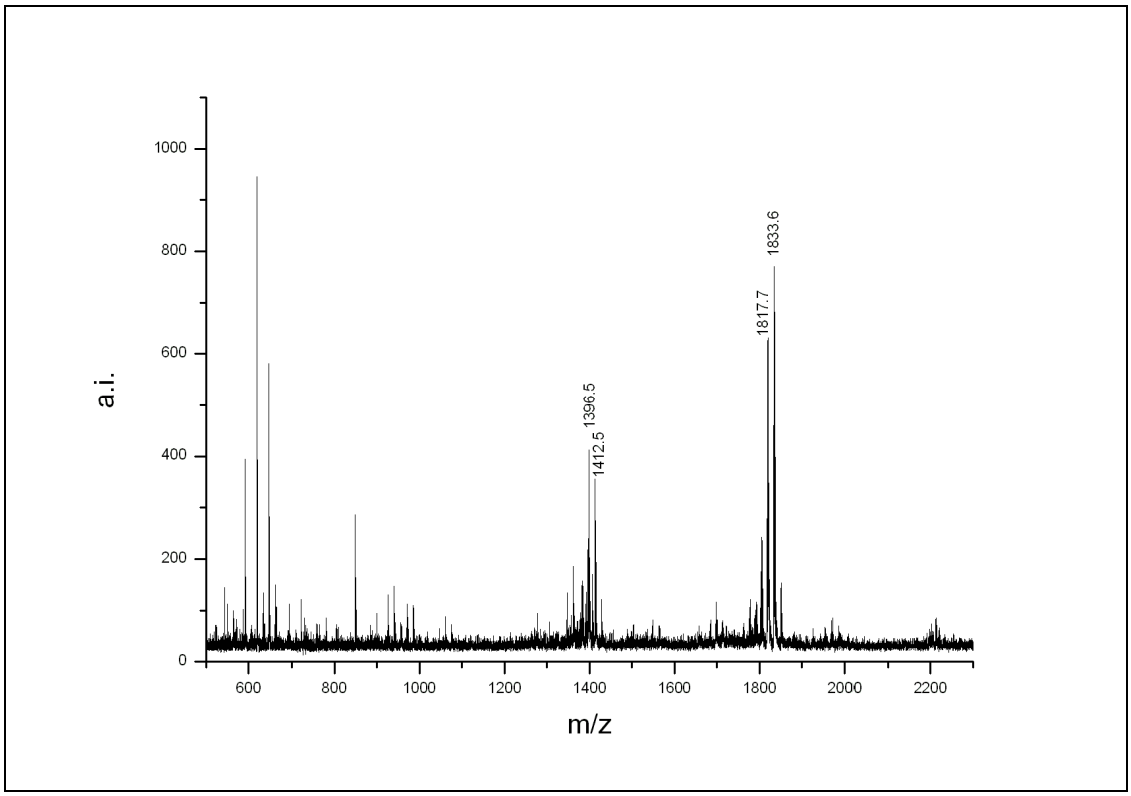
^{1}H NMR Spectrum of Compound 91



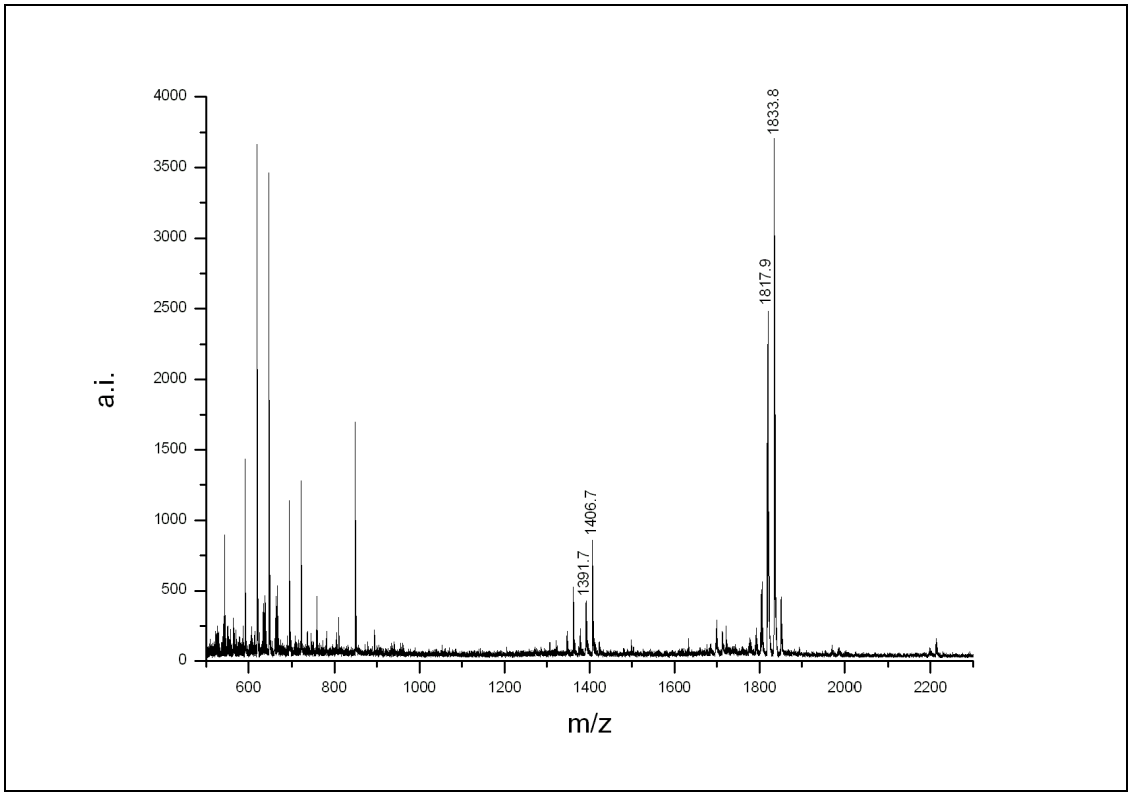
^{13}C NMR Spectrum of Compound 91



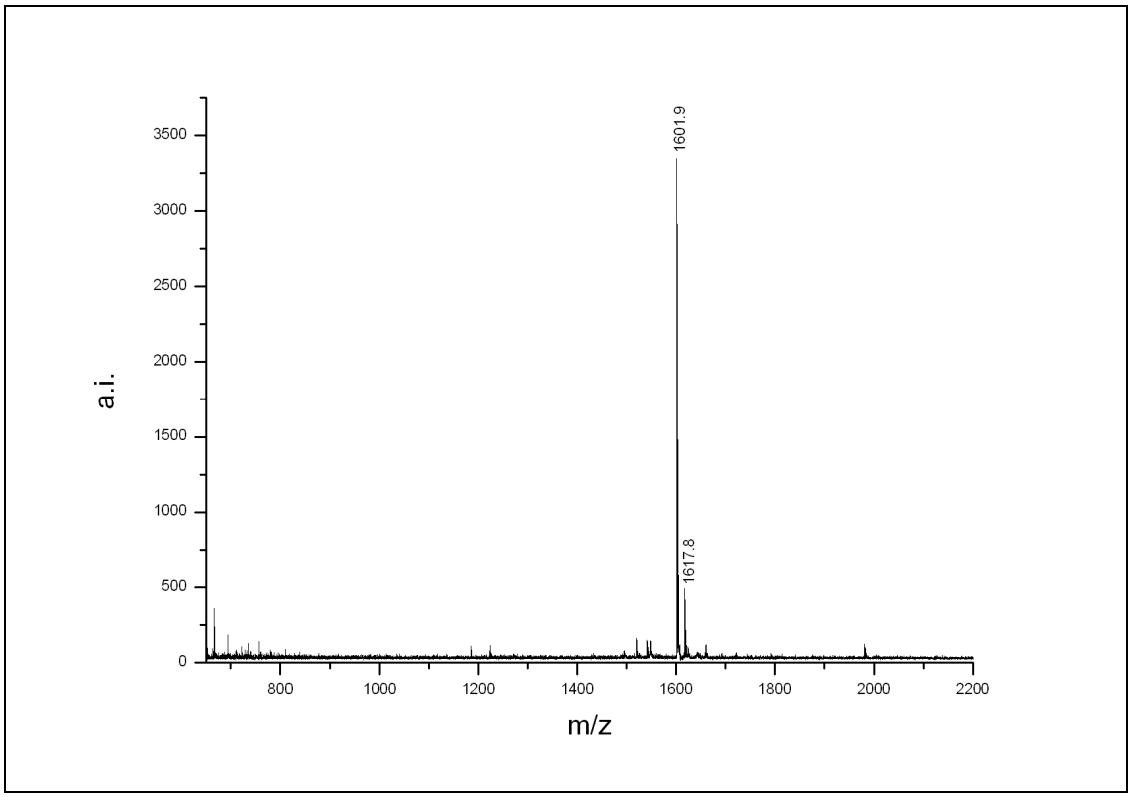
HMQC (Coupled) Spectrum of Compound 91



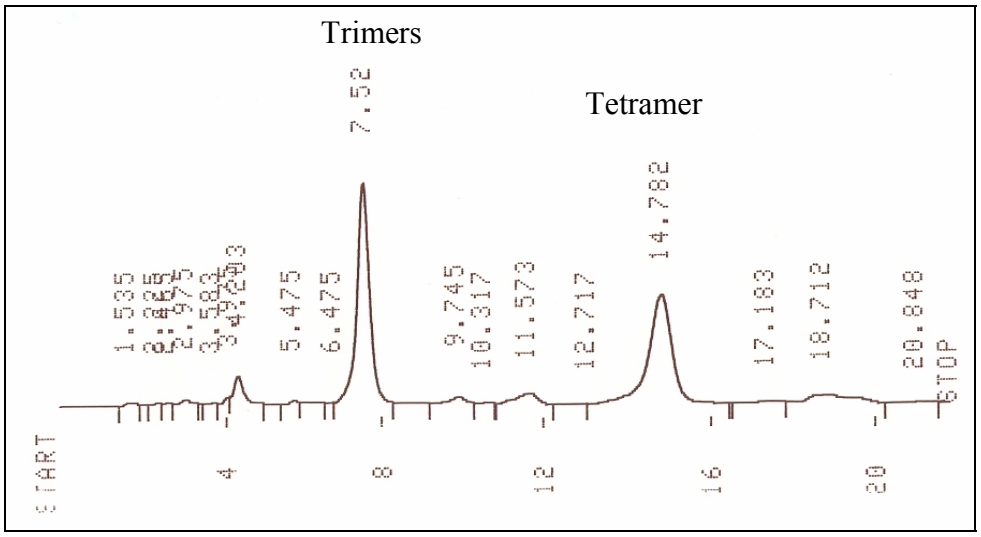
MALDI-TOF MS of crude product after cleavage from the resin (88)



MALDI-TOF MS of fraction containing compound 88 after purification



MALDI-TOF MS of Compound 91



Chromatogram of purified fraction (88, protected tetrasaccharide DDAA)

PKNO	TIME	AREA	MK	IDNO	CONC
1	1.535	347			0.37
2	2.225	251			0.2679
3	2.465	230	V		0.2454
4	2.975	745	V		0.7953
5	3.583	166			0.1768
6	3.975	678	V		0.7237
7	4.203	3950	V		4.2186
8	5.475	379			0.4047
9	6.475	240			0.2559
10	7.52	38930	V		41.5743
11	9.745	1426			1.523
12	10.317	110	V		0.1178
13	11.573	3524			3.7638
14	12.717	196	V		0.2093
15	14.782	35929	V		38.3691
16	17.183	900			0.961
17	18.712	5176	V		5.5271
18	20.848	465			0.4963
	TOTAL	93640			100

CURRICULUM VITAE

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Nationality : Indian

Marital Status : Single

Education Details:

Course	Year of Study	Institute
Ph.D	Since Sep 2004	Institute for Chemistry, University of Potsdam, Germany
M.Sc (Chemistry)	2001-2003	Indian Institute of Technology - Madras, Chennai, India
B.Sc (Chemistry)	1998-2001	Bishop Heber College, Trichy, Tamilnadu, India

Work Experience:

Position	Period	Organization
Project Associate	Mar 2004 – Aug 2004	Indian Institute of Technology – Madras, Chennai, India
Project Assistant	Jun 2003 – Feb 2004	Indian Institute of Technology – Madras, Chennai, India

Publication

V. Balakumar, A. Aravind and S. Baskaran, "A Highly Regio and Chemoselective Reductive Cleavage of Benzylidene Acetals with $EtAlCl_2$-Et_3SiH", *Synlett*, **2004**, 04, 647-650.

Balakumar Vijayakrishnan and Martin G. Peter, "Solution and solid phase synthesis of *N,N'*-diacetylated chitotetraoses" (Manuscript under preparation).

Poster presented in Conferences

Balakumar Vijayakrishnan and Martin G. Peter, "Synthesis of Hetero Chitooligosaccharides", **3rd Glycan Forum**, Berlin, *Germany*, 22-23 February **2007**.

Balakumar Vijayakrishnan and Martin G. Peter, "Synthesis of Hetero Chitooligosaccharides", **8th Tetrahedron Symposium**, Berlin, *Germany*, 27-29 June **2007**.

Balakumar Vijayakrishnan and Martin G. Peter, "Synthesis of Hetero Chitooligosaccharides", **234th ACS National Meeting & Exposition**, Boston, MA, *USA*, 19-23 August **2007**.