Institut für Chemie
Arbeitsgruppe Naturstoffchemie

Synthesis of Hetero-chitooligosaccharides

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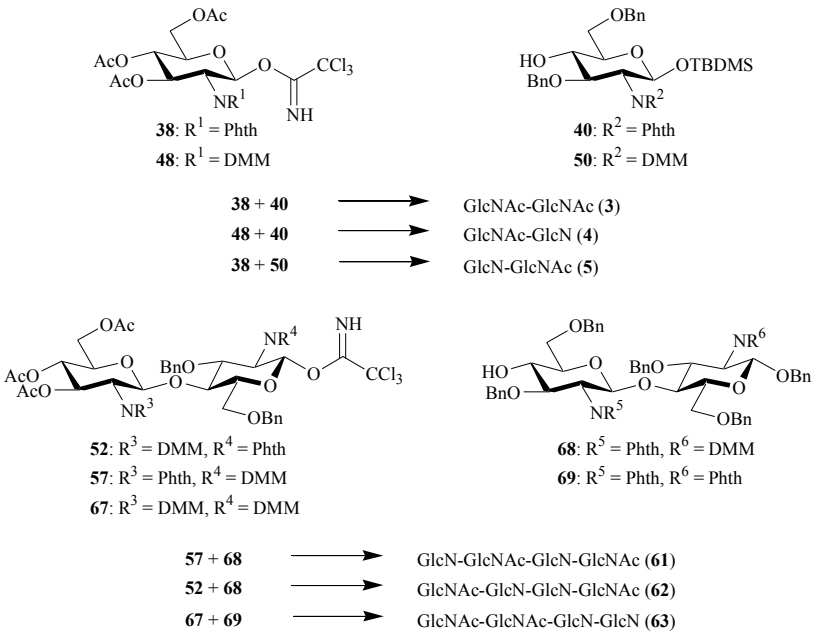
Abstract - Deutsch

Schlüsselwörter: Kohlenhydrate / Chitooligosaccharide / Glycosylierung / Synthesemethoden / Trichloracetimidate

Chitooligosacchride bestehen aus linear β-(1→4)-verknüpften 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and/or 2-amino-2-deoxy-β-D-glucopyranose (GlcN) Einheiten. Sie beanspruchen aufgrund ihrer bemerkenswerten biologischen Eigenschaften – u.a. antibakterielle, antitumor, antimykotische und Elicitor Aktivität - grosses Interesse. Sie sind durch chemischen oder enzymatischen Abbau von Chitosan zugänglich, wobei diese Methoden unausweichlich zu komplexen, sehr heterogenen Mischungen von Chiooligosacchariden führen. Chemische Synthesen von Chitooligosacchariden mit definierter Sequenz von GlcNAc und GlcN Einheiten sind daher von erheblichem Interesse.

In der vorliegenden Arbeit werden Synthesen von partiell acetylierten Chitobiosen und –tetraosen beschrieben. Die Aminogruppen wurden als *N*-Dimethylmaleoyl- bzw. Phthaloylimide geschützt. Die Donoren wurden als Trichloacetimidate aktiviert, wobei aufgrund von Nachbargruppeneffekten ausschliesslich die β-Glycoside entstehen. Die Trimethylsilyltrifluoromethansulfonat-promovierte Glycosidierung geeigneter Akzeptoren lieferte schliesslich die Chitobiosen **3-5** und die Chitotetraosen **61-63** in guten Ausbeuten (siehe nachfolgendes Schema).

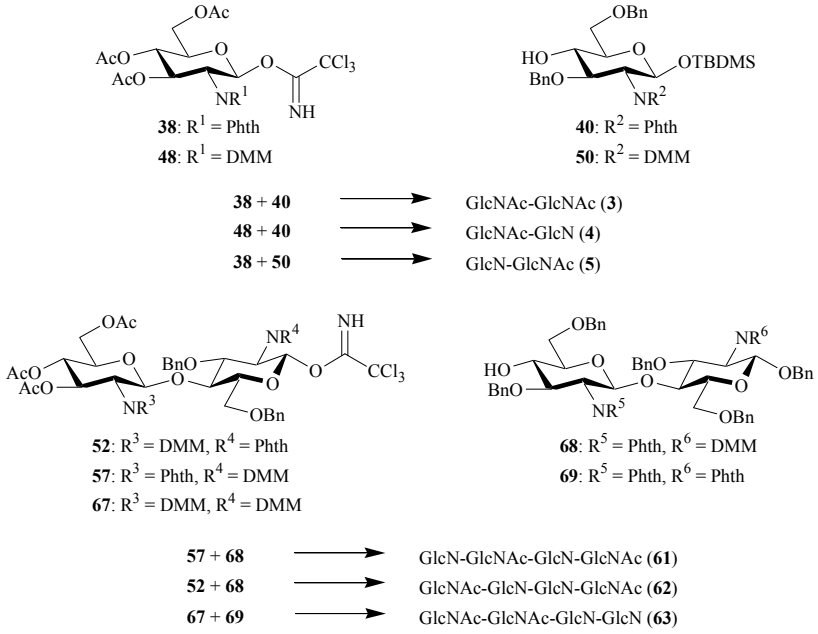


Abstract

Keywords: Carbohydrates / Chitooligosaccharides / Glycosylation / Synthetic methods / Trichloroacetimidates

Chitooligosaccharides are composed of linear β-(1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and/or 2-amino-2-deoxy-β-D-glucopyranose (GlcN). They are of interest due to their remarkable biological properties including antibacterial, antitumor, antifungal and elicitor activities. They can be obtained from the aminoglucan chitosan by chemical or enzymatic degradation which obviously affords rather heterogenous mixtures. On the other hand, chemical synthesis provides pure compounds with defined sequences of GlcNAc and GlcN monomers.

The synthesis of homo- and hetero-chitobioses and hetero-chitotetraoses is described in this thesis. Dimethylmaleoyl and phthaloyl groups were used for protection of the amines. The donor was activated as the trichloroacetimidate in order to form the β-linkages. Glycosylation in the presence of trimethylsilyl trifluoromethanesulfonate, followed by *N*- and *O*-deprotection furnished chitobioses **3**-**5** and chitotetraoses **61**-**63**, as shown below, in good yields.

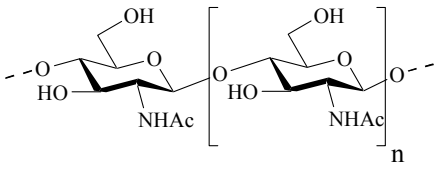


Contents

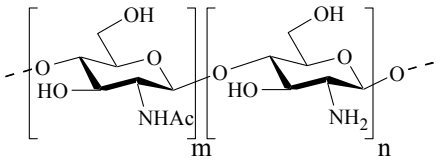
1. Introduction

1.1 Chitin and chitosan

Chitin is one of the most abundant polysaccharides in nature. It is one of the main component of the cell walls of fungi, the exoskeletons of arthropods, such as crustaceans (e.g. crab, lobster and shrimp) and insects (e.g. ants, beetles and butterflies), the radular of molluscs and the beaks of cephalopods (e.g. squid and octopuses).[1,2] Chitin is mostly obtained from the exoskeleton of industrially processed crustaceans which contains between 20 to 40% of chitin.[3] It can be described as a homopolymer of a β-(1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc), which is a biologically safe, biocompatible and biodegradable polymer (Figure 1).



Chitin



Chitosan

Figure 1. Structure of chitin and chitosan.

Chitosan is a deacetylated derivative of chitin composed of randomly distributed β-(1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose (GlcN) and GlcNAc units (Figure 1). It is generally prepared by the alkaline *N*-deacetylation of chitin. The distinction between chitin and chitosan can be made either by degree of acetylation (DA) or by their solubility in 1% aqueous acetic acid. Chitosan generally has DA < 0.4 and it is soluble in 1% aqueous acetic acid, whereas chitin is insoluble.[4,5] The amino group in chitosan has a

pKa value of ~6.5, thus chitosan is positively charged and soluble in acidic to neutral solution with a formal charge dependent on pH and %DA value. Due to the presence of positive charge, it can readily interact with negatively charged substances such as protein, anionic polysaccharides (e.g. alginate, carrageenan, pectin, etc.), fatty acids, bile acids and phospholipids.[6,7]

1.2 Applications of chitosan

The poor solubility of chitin is the major limiting factor in its utilization. Chitosan is considered as a useful polysaccharide because of its free amino groups that contribute polycationic, chelating and dispersion forming properties along with the solubility in dilute acetic acid. Chitosan possesses exceptional chemical and biological qualities that can be used in a wide variety of industrial and medical applications.[8]

In medicine, chitosan shows antimicrobial, antiviral and antifungal activities which make it a favorable option for biomedical applications.[9,10] It has been proved to be useful in promoting tissue growth in tissue repair and accelerating wound-healing and bone regeneration.[11-13] Moreover, chitosan can be incorporated into hydrogels and microspheres which demonstrate large potential in delivery system for drugs, proteins or genes.[13-17]

In food industry, chitosan has been used as stabillizer and thickener. It also can be used as freshness preserver, cleaner and health food additives.[18,19]

In cosmetic, chitosan forms a protective, moisturizing, elastic film on the surface of the skin that has the ability to bind other ingredients that act on the skin. In this way, it can be use in formulating moisturizing agents to enhance their bioactivity and effectiveness. Today, chitosan is a component in some skin-care creams, shampoos and hairsprays due to its antibacterial properties.[20,21]

In environmental protection, chitosan has been used as active mud coagulant, adhesive and adsorbent of heavy metal ion and organic compounds.[22,23]

1.3 Chitooligosaccharides

Many researchers have focused chitosan as a source of bioactive material during past few decades. However, poor solubility under physiological conditions makes its chemical modification difficult and limits its application to date. Therefore, a new interest has recently been emerged on partially hydrolyzed chitosan, chitooligosaccharides.

Chitooligosaccharides are β-(1→4)-linked homo or hetero oligomers of GlcNAc and GlcN. They have a considerable potential to be utilized in number of useful applications, because they are not only water-soluble but also possess distinctive biological activities, for instance antibacterial,[24-26] antitumor[27-29] and antifungal[30] activities, elicitors of plant defence,[31,32] and protective effects against infection with some pathogens in mice.[33,34] They have been used for biological studies on lectin.[35-37] Furthermore, fully and partially *N*-acetylated chitooligosaccharides are of interest for the determination of the substrate specificities and mechanisms of chitinase[38,39] and lysozymes.[40,41]

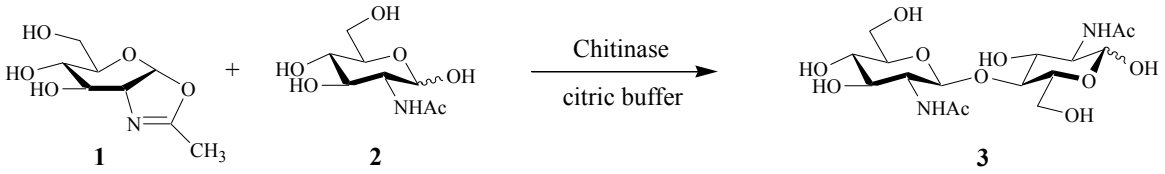
With respect to antimicrobial effect against bacteria and fungi, inhibitory activity of chitooligosaccharides are significantly higher than that of chitosan, but insignificantly lower than that of chitosanase. Among the chitooligosaccharides, the activities increase with increase of degree of deacetylation (DD), but decrease with increase of degree of polymerization (DP).[42] On the other hand, chitooligosaccharides with high DP exhibit a bactericidal effect towards *Bacillus cereus* (Gram-positive) and *Escherichia coli* (Gram-negative) more efficiently than the lower ones.[24]

In comparision of the ability to elicit resistance reactions in wheat leaves, partially *N*-acetylated chitooligosaccharides show more potent activity than homo oligomers of either GlcNAc or GlcN.[32] Akiyama and co-workers studied (+)-pisatin-inducing activities of oligomers of chitosan from dimer to heptamer and their partially/completely *N*-acetyl derivatives in pea epicotyl elicitor assay.[43] The partially *N*-acetyl chitosan pentamer to hexamer exhibited significant elicitor activity, whereas chitosan oligomers had moderate or no activity.

Due to the intriguing biological activities of chitooligosaccharides, different protocols have been developed to prepare both fully and partially *N*-acetylated chitosan

oligomers. Chemical and enzymatic depolymerization of chitin and chitosan are most frequently used.[43-49]

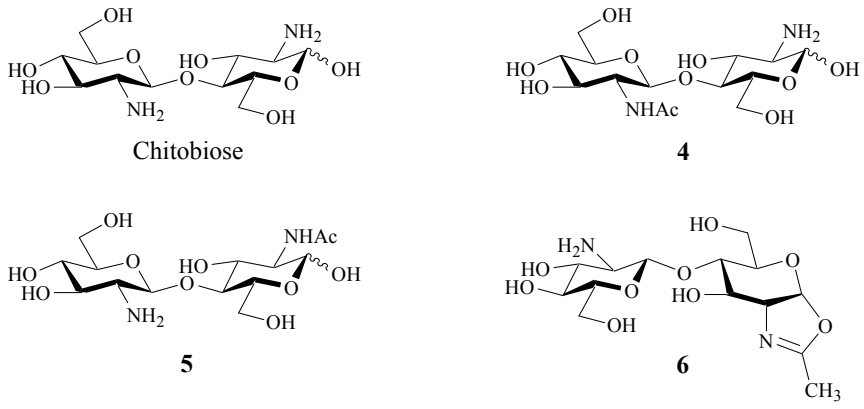
Kobayashi and co-workers described the synthesis of *N*,*N*'-diacetylchitobiose (**3**) via enzymatic glycosidation of an *N*-acetyl-D-glucosamine oxazoline derivative **1** as the glycosyl donor with GlcNAc (**2**) as the glycosyl acceptor (Scheme 1).[50]



1 **2** **3**

Scheme 1. Synthesis of *N*,*N*'-diacetylchitobiose via enzymatic glycosylation using an oxazoline derivative **1** as the glycosyl donor and GlcNAc (**2**) as the glycosyl acceptor.[50]

Tokuyasu et al. reported the preparation of monoacetylated chitobioses, i.e. GlcNAc-GlcN (**4**) and GlcN-GlcNAc (**5**) (Figure 2). GlcNAc-GlcN (**4**) was synthesized by enzymatic acetylation of chitobiose using chitin deacetylase from *Colletotrichum lindemuthianum*,[51] while its isomer **5** was prepared by enzymatic deacetylation of *N*,*N*'-diacetylchitobiose.[52] Formation of compound **5** was also observed during the slow chitinase-catalyzed hydrolysis of an *N*-acetylchitobiose oxazoline derivative (**6**).[53]



Chitobiose **4**

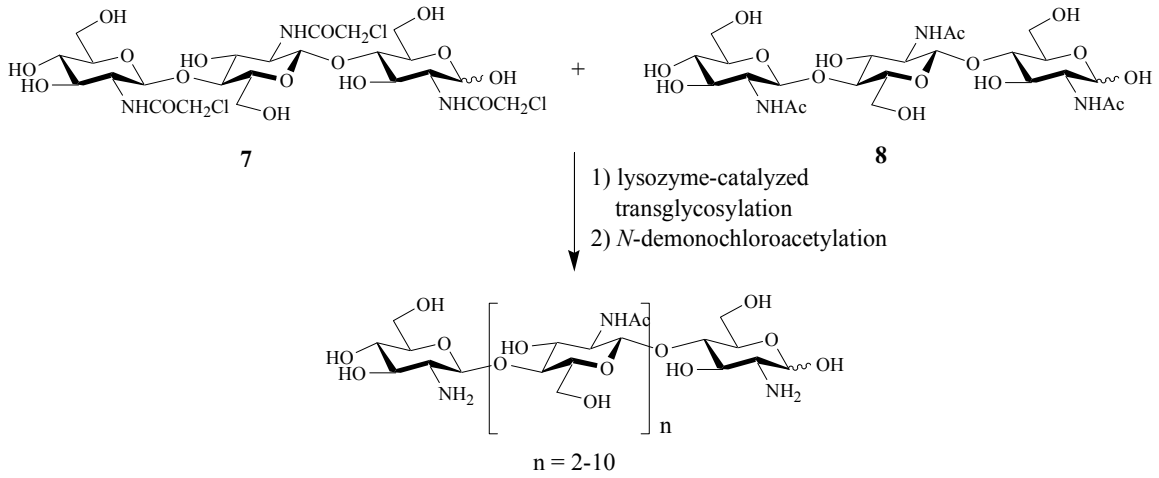
5 **6**

Figure 2. Structure of chitobiose, GlcNAc-GlcN (**4**), GlcN-GlcNAc (**5**) and oxazoline derivative (**6**).

For the preparation of chitooligosaccharides with higher degree of polymerization, *N*-hexa-acetylchitohexaose and *N*-hepta-acetylchitoheptaose were obtained by transglycosylation of the corresponding chitobiose and chitotetraose with lysozyme or chitinase, respectively.[47] Singh and co-workers reported the synthesis of homo chitooligosaccharides using glycosidase.[48] β-*N*-Acetylhexosaminidase of *Aspergillus oryzae* was used as a catalyst in the formation of *N*-penta-acetylchitopentaose and *N*-hexa-acetylchitohexaose from *N*-tri-acetylchitotriose and *N*-tetra-acetylchitotetraose.

The syntheses of partially *N*-acetylated oligosaccharides from chitosan utilizing various enzymes were also reported. Zhang and co-workers demonstrated the preparation of hetero-chitooligosaccharides, from the tetramer to octamer, by enzymatic depolymerization of chitosan using a mixture of cellulase, alpha amylase and proteinase.[54]

Akiyama et al. proposed an alternative chemo-enzymatic synthesis method using lysozyme and chitotriose derivative as depicted in Scheme 2.[43] *N*,*N*′,*N*″-Tri(monochloro)acetylchitotriose (**7**) and *N*,*N*′,*N*″-triacetylchitotriose (**8**) were successfully polymerized into higher-molecular-weight oligomers by a lysozyme-catalyzed transglycosylation reaction, and a following base-catalyzed *N*-demonochloroacetylation gave partially *N*-acetylated chitosan oligomers with DP 4-12 as a mixture of oligosaccharides.



Scheme 2. Chemo-enzymatic synthesis of partially *N*-acetylated chitosan oligomers utilizing a lysozyme-catalyzed transglycosylation reaction followed by *N*-demonochloroacetylation.[43]

Chemical and enzymatic methods feature exquisite stereo- and regioselectivity and catalyze the reaction under very mild conditions. Extensive protection-deprotection schemes are thus unnecessary, and the control of anomeric configuration is simple. However, some disadvantages still remain:

i) the cost associated with hydrolytic enzymes can be high;
ii) controlling of size of the oligomers obtained after depolymerization is sometimes difficult;
iii) the scope of substrates accepted by enzymes is narrow;
iv) for the preparation of partially *N*-acetylated chitosans with DP > 2, the products obtained are a mixture of randomly acetylated chitosan oligomers, which causes difficulty in identifying structural features of the components that possess the strongest biofunctions.

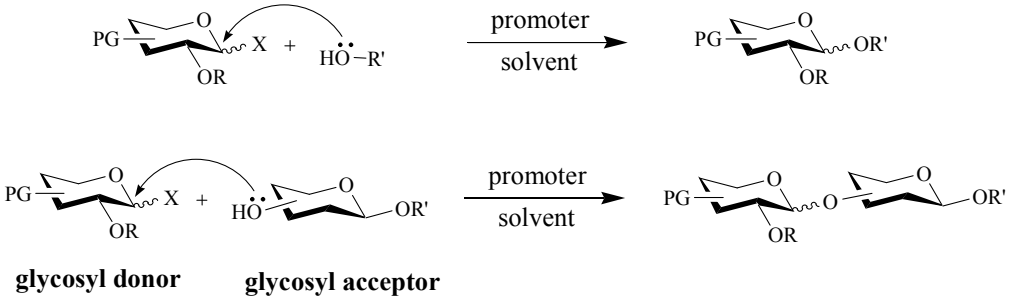
1.4 Chemical synthesis of oligosaccharides

The role of carbohydrates in many biological pathways has become more defined in recent years. The traditional view of carbohydrates as solely sources of energy has been enlarged by advances in glycobiology that establish oligosaccharides and glycoconjugates as essential components of information transfer in biological systems.[55]

Glycoconjugates are formed by a carbohydrate moiety joined to a protein (glycoproteins) or to a lipid moiety (glycolipids). These molecules together with proteins and nucleic acids are mainly responsible of information transfer between cells, which is a fundamental process of life and central to all cellular systems. It is well known that complex oligosaccharides in the form of glycolipids and glycoproteins are present in the membranes of cells and can mediate a large number of diverse and important biological functions.[56,57] Therefore, chemical syntheses of oligosaccharides are of interest in order to establish or confirm its structural assignment. In addition, defined oligosaccharide and their analogues are key tools for biochemical, biophysical and biological studies. The synthetic methods availible for glycosidic bond formation are becoming increasingly powerful and efficient which were evidenced by the ever increasing complexity of target oligosaccharides.

1.4.1 Formation of a glycosidic bond

The glycosylation reaction is the creation of a carbon-oxygen bond via a nucleophilic substitution of a leaving group (X) attached to the anomeric carbon of the carbohydrate by an alcohol ROH or by the OH group of partially protected mono or oligosaccharide (Scheme 3). The compound that provides the glycosyl moiety is called the glycosyl donor, and the alcohol that recives it is known as glycosyl acceptor. The reaction is generally performed in the presence of an activator called promoter. The role of the promoter is to assist the departure of the anomeric leaving group. Promoters are often used in catalytic amounts, although in some instances they are used stoichiometrically. In some cases, other additives such as molecular sieves or any base that may act as acid scavenger are also used.



Scheme 3. Formation of a glycosidic bond.

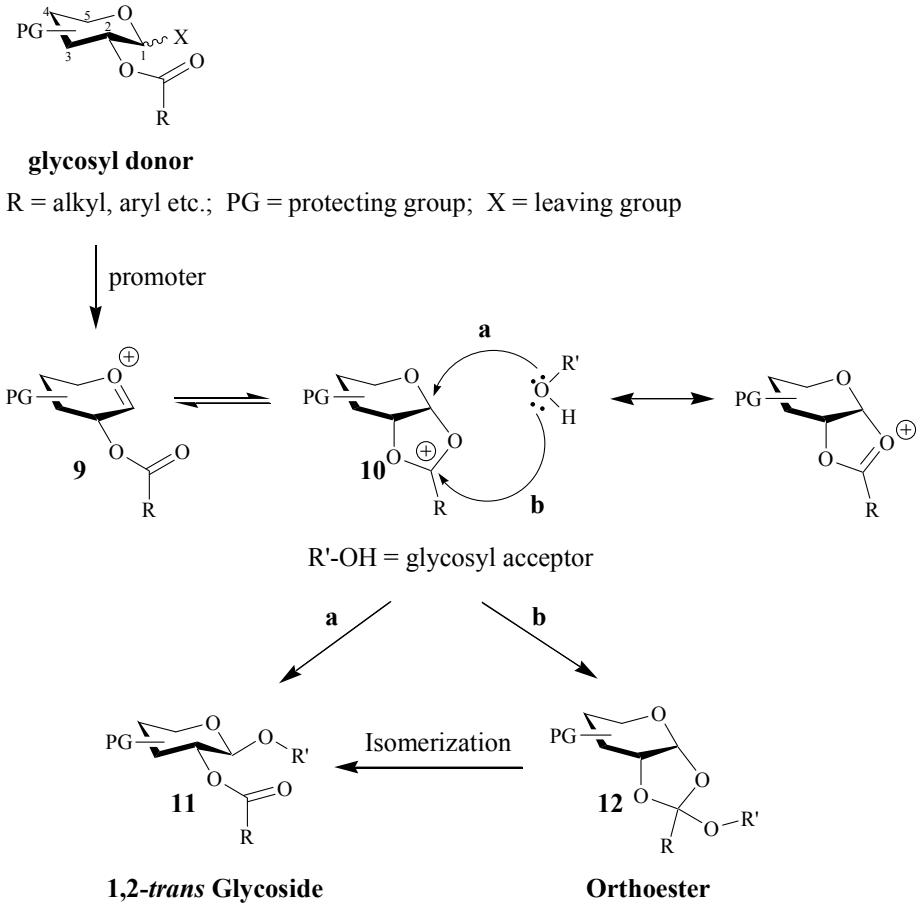
1.4.2 Stereoselectivity in glycosylation reaction

The stereoselective introduction of the glycosidic linkage is one of the most challenging aspects in chemical oligosaccharide synthesis. At present, various methods of controlling stereochemistry are well developed.

1.4.2.1 1,2-*trans* Glycosylation

The most widely used method involves using a glycosylation donor containing a participating neighbor group. The involvement of ester group at C-2 position in a glycosylation reaction leads to an oxocarbenium ion **9**, which then transforms to an acyloxonium ion intermediate **10** (Scheme 4). The acyloxonium ion can be opened stereoselectively to the 1,2-*trans* glycoside **11** by a reaction of the acceptor alcohol at the

anomeric center (route a). Nucleophilic attack of the alcohol component at the dioxolane ring leads to the formation of orthoester **12** (route b), which might be eventually isomerized to the respective glycoside. The formation of orthoester can become the main reaction when neutral or basic reaction conditions are being applied.

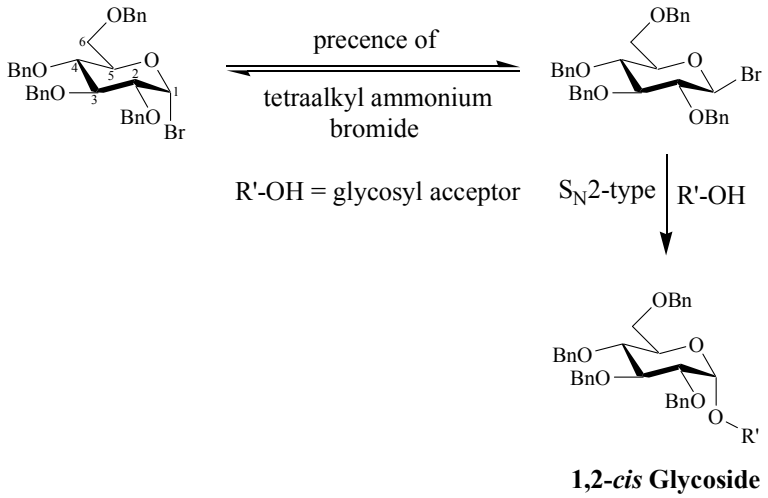


Scheme 4. Mechanism for 1,2-*trans* glycosylation.

1.4.2.2 1,2-*cis* Glycosylation

The synthesis of 1,2-*cis* glycosides should fit two main requirements, i.e. a non-participating group at C-2 position and a leaving group which is in a 1,2-*trans* orientation with respect to the C-2 substituent. An S_N2 reaction of a β-pyranosyl halide to give the α-glycoside would seem possible, but it is not practical, because β-pyranosyl halides are greatly destabilized by the anomeric effect. Lemieux et al.[58] solved this problem by reacting of α-pyranosyl bromide in the presence of tetraalkyl ammonium bromide with

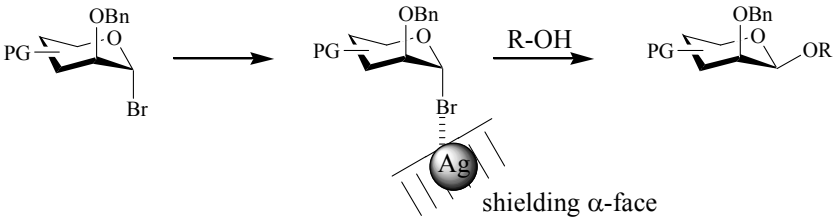
the bromide anion to produce the β-pyranosyl bromide *in situ* (Scheme 5). The highly reactive β-pyranosyl bromide reacts much faster than its α-analog affording the 1,2-*cis* glycoside in a large proportions in a kinetically-controlled reaction. This method has been called *in situ* anomerization. It works well with greater reactivity of the donor at the anomeric center. Thus, excellent results can be obtained when using galactose or fucose as donor and less good ones when using glucose.[59]



Scheme 5. 1,2-*cis* glycosylation using *in situ* anomerization method.[58]

1.4.2.3 Heterogeneous catalysis

Glycosylation of α-halides in the presence of an insoluble silver salt proceeds mainly with inversion of configuration and formation of β-glycoside. This probably functions by forming associations between the insoluble promoter and the glycosyl halides which are most likely to form on the least hindered α-face and therefore the nucleophile will preferentially attack from the β-face (Scheme 6). Silver silicate and silver silicate aluminate have often been used as the heterogeneous catalyst. These catalysts have proved to be valuable in the preparation of β-linked mannosides which can not be prepared by neighboring group participation or *in situ* anomerization.



PG = protecting group; R-OH = glycosyl acceptor

Scheme 6. Glycosylation using heterogeneous catalyst.

1.5 Glycosylation methods

Synthetic chemists have been addressing the challenges associated with the preparation of complex carbohydrates for over hundred years. During this time, a large number of glycosylation methods have been developed with efficient leaving groups, which lead to good yields and high stereoselectivities under mild conditions.[60-63]

A wide range of anomeric groups, including most notable glycosyl halide (Koenigs-Knorr method), thioglycoside, trichloacetimidate and *n*-pentenyl glycoside have been explored as glycosyl donors.

1.5.1 Koenigs-Knorr method

The use of glycosyl bromide and chloride as an effective glycosyl donor in the glycosylation reaction was first introduced by Koenigs and Knorr in 1901.[64] Insoluble catalysts such as Ag_2O and Ag_2CO_3, as well as soluble catalysts including $HgBr_2$, $Hg(CN)_2$ and AgOTf were employed as promoters. [59,65]

A strong advantage of the Koenigs-Knorr method is the easy availability of the donor molecules. Glycosyl bromides are obtained in one step from the reaction of the peracetylated sugars with HBr in acetic acid, for example.

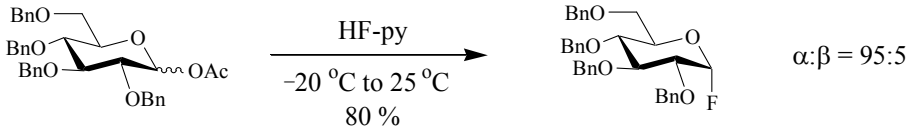
Despite the efficiency of several of the Koenigs-Knorr methods, partly inherent disadvantages of these methods for the synthesis of oligosaccharides could not be conquered. These disadvantages include the following:

i) the generation of the glycosyl halides require relatively harsh conditions;
ii) the glycosyl halides exhibit low thermal stability and can often be generated only *in situ* and at lower reaction temperatures;

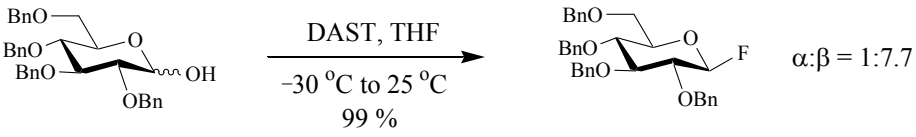
iii) the glycosyl halides are highly sensitive to hydrolysis;

iv) the heavy metal salts used as promoters in the reaction are expensive and toxic.

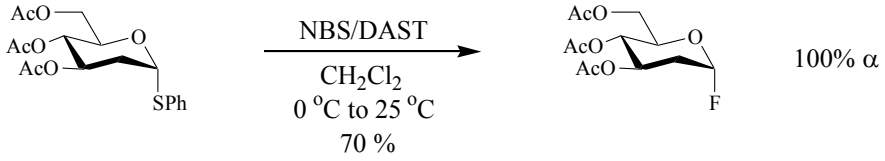
In 1981, Mukaiyama et al. demonstrated the use of anomeric fluorides for the preparation of *O*-glycosides.[66] The introduction of fluorine as a leaving group is a good alternative to the Koenigs-Knorr method due to the stability of the C-F bond. Glycosyl fluorides are easier to handle than glycosyl bromides or chlorides. They are typically prepared from the anomeric acetates by reaction with HF/pyridine[67] (Scheme 7), from hemiacetals by reaction with (diethylamino)sulfur trifluoride (DAST)[68] (Scheme 8) or from thioglycosides by reaction with NBS/DAST[69] (Scheme 9).



Scheme 7. Preparation of glycosyl fluoride from the anomeric acetates by reaction with HF/pyridine.[67]



Scheme 8. Preparation of glycosyl fluoride from hemiacetals by reaction with DAST.[68]

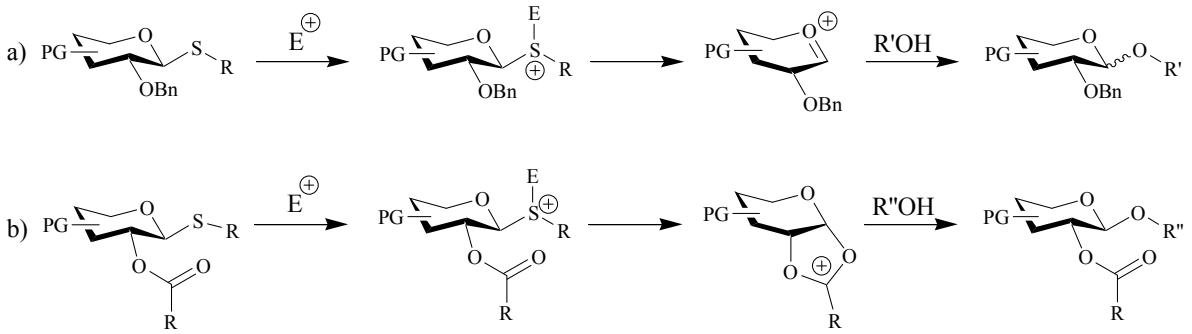


Scheme 9. Preparation of glycosyl fluoride from thioglycosides by reaction with NBS/DAST.[69]

The glycosylation reaction using glycosyl fluorides requires the use of other promoter systems besides silver salts. The first reaction using $SnCl_2$-$AgClO_4$ as a promoter was carried out by Mukaiyama and co-workers.[66] Apart from $SnCl_2$-$AgClO_4$, the following systems have been used: TMSOTf,[70] $BF_3{\cdot}Et_2O$,[71,72] Cp_2MCl_2-$AgClO_4$ (M = Zr, Hf),[73] Cp_2ZrCl_2-$AgBF_4$[74] and Cp_2HfCl_2-AgOTf.[74,75]

1.5.2 Thioglycoside method

The sulfur atom in a thioglycoside is a soft nucleophile and is able to react selectively with soft electrophiles such as heavy metal cations, halogens and alkylating or acylating reagents. This fact makes thioglycosides very versatile agents in carbohydrate chemistry. The mechanism of activation of this reaction is similar to the Koenigs-Knorr method. An electrophile activates the thioglycoside by producing intermediate sulfonium ions, which then give rise to glycosylating carbocationic intermediates that react with the alcohol affording the glycoside (Scheme 10).



PG = protecting group; R = alkyl, aryl; R'OH, R"OH = glycosyl acceptor

Scheme 10. Mechanism for thioglycoside method: a) glycosylation without neighboring group effect, b) glycosylation with neighboring group effect.

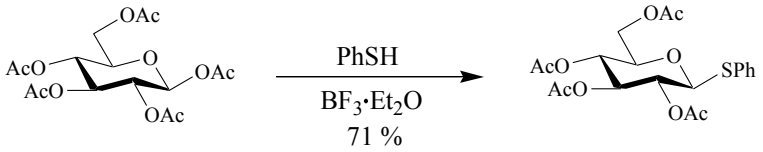
Thioglycoside can be activated by a variety of promoters, for instance MeOTf,[76] MeSOTf,[77] PhSeOTf,[78] NIS-TfOH,[79,80] $NOBF_4$,[81] dimethyl(methylthio)sulfonium triflate (DMTST),[82] iodonium dicollidine perchlorate (IDCP)[83] and tris(4-bromophenyl) ammoniumyl hexachloroantimonate (TBPA).[84]

With regard to the preparation of thioglycosides, they can be grouped into three categories:

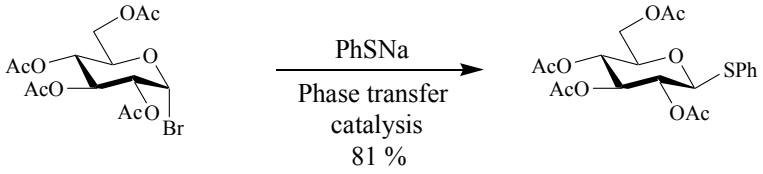
i) acid-promoted displacement at the anomeric center. This implies the synthesis from a sugar derivative with thiols such as thiophenol or thioethanol in the presence of a Lewis acid (Scheme 11).[85]

ii) base-promoted displacement at the anomeric center. This implies the synthesis by *S*-nucleophilic displacement at the anomeric center (Scheme 12).[86]

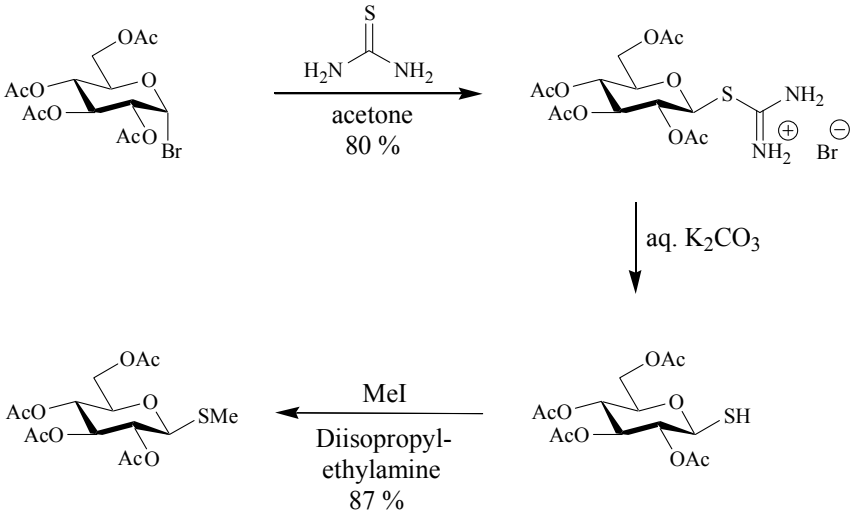
iii) synthesis by preparation of a 1-thioglycoside followed by *S*-alkylation (Scheme 13).[87]



Scheme 11. Synthesis of thioglycoside by acid-promoted displacement at the anomeric center.[85]



Scheme 12. Synthesis of thioglycoside by base-promoted displacement at the anomeric center.[86]



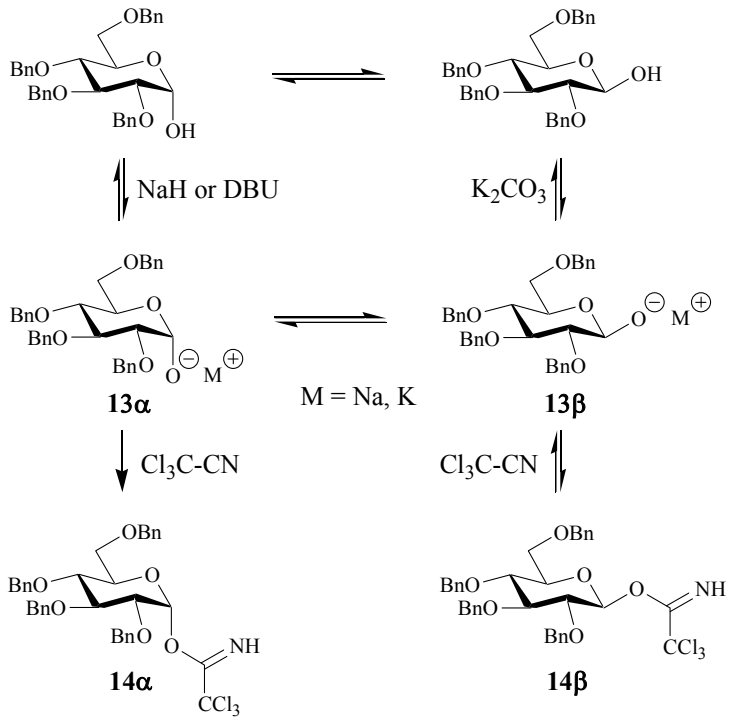
Scheme 13. Synthesis of thioglycoside by preparation of a 1-thioglycoside followed by *S*-alkylation.[87]

1.5.3 Trichloroacetimidate method

Trichloroimidate-mediated glycosylation was reported in 1980 by Schmidt et al.[88,89] as an alternative useful method to the classical Koenigs-Knor procedure. The glycosylation reaction is smoothly promoted by catalytic amount of $BF_3{\cdot}Et_2O$,[88] TMSOTf[90] or AgOTf [91,92] under mild conditions. Glycosyl trichloroacetimidates are more stable than the respective glycosyl bromides and can be stored at low temperature for many months.

The thermally and chemically stable trichloroacetimidate glycosyl donor is easily synthesized from the corresponding 1-hydroxyl sugar by treatment with trichloroacetonitrile in the presence of bases such as NaH or K_2CO_3. The stereochemistry of the obtained glycosyl trichloroacetimidate depends on the base used for deprotonation of the reducing sugar.[90] In the case of benzylated reducing glucose, for example, the β-trichloroacetimidate **14β** is formed under kinetic control in a rapid and reversible addition reaction (Scheme 14). However, this product anomerizes slowly in base-catalyzed reaction through retro-reaction and anomerization of the 1-oxide ion from **13β** to **13α** and renewed trichloroacetonitrile addition to form the thermodynamically more stable α-trichloroacetimidate **14α**. The equilibrium between the two trichloroacetimidates can be

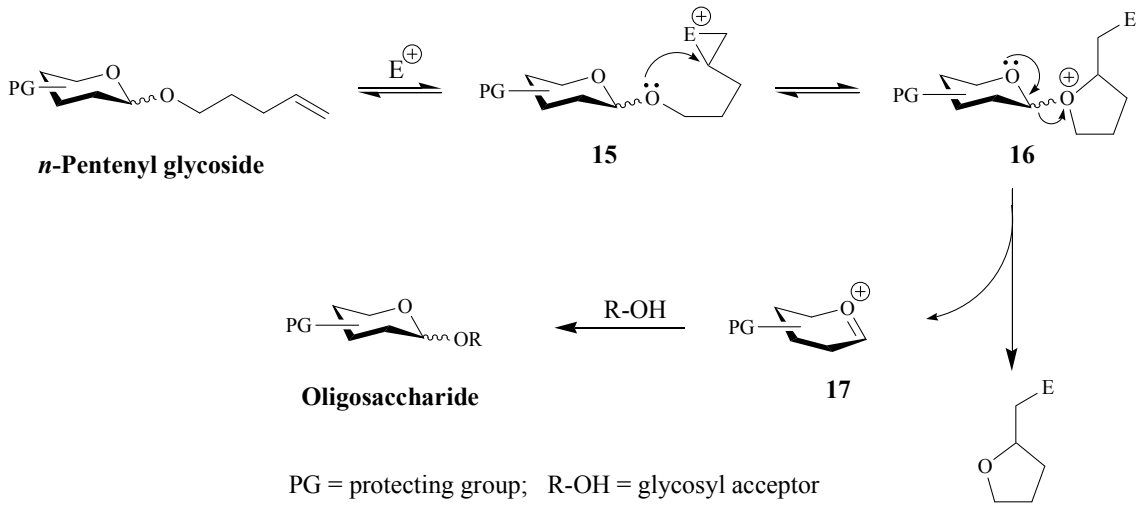
speeded up by stronger base. Thus, with different bases both anomers can be obtained in pure form. NaH and DBU are appropriate for axial trichloroacetimidates while weaker base, K_2CO_3, is appropriate for equatorial trichloroacetimidates.



Scheme 14. Synthesis of axial and equatorial trichloroacetimidates.[90]

1.5.4 *n*-Pentenyl glycoside method

Fraser-Reid et al. introduced a 4-pentenyl group as a new and effective leaving group at the anomeric center of the glycosyl donor in 1988.[93] The activation of the leaving group is based on an electrophilic addition to the double bond of the aglycone to form a cyclic halonium ion intermediate **15**. This intermediate then rearranges to a second intermediate containing the leaving group, 2-halomethyltetrahydrofuran **16**. Elimination of the leaving group results in an oxonium ion **17**, which then reacts with a glycosyl acceptor to provide the desired glycoside (Scheme 15).



Scheme 15. Mechanism for *n*-Pentenyl glycoside method.

4-Pentenyl glycosides can be prepared by Fischer glycosylation of the aldose of interest, by a Koenigs-Knorr procedure, or can be obtained from *n*-pentenyl 1,2-orthoesters.

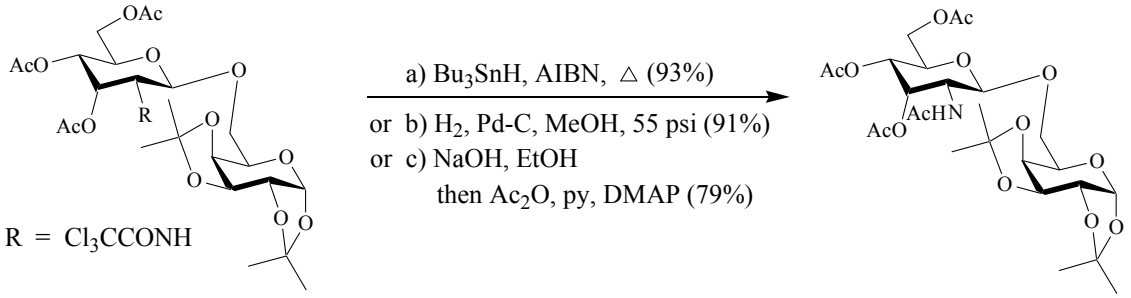
The most widely used electrophiles for the activation of *n*-pentenyl glycosides are *N*-bromosuccinimide (NBS) and *N*-iodosuccinimide (NIS) together with protic or Lewis acids such as triflic acid (TfOH)[94] or triethylsilyl triflate (TESOTf).[95] The iodonium dicollidine perchlorate (IDCP) has also often been used as an activator.[93,96]

1.6 Amino Protecting groups

An important constituent of glycoconjugates is D-glucosamine which is mainly found as an *N*-acetyl derivative in β-glycosidic linkages.[55,97] The synthesis of such glycosides requires a glycosyl donor where the amino group is protected. Glycoside bond formation with donors derived from *N*-acetyl-D-glucosamine generally occurs by neighboring group participation to give an oxazoline intermediate.[60,98] These oxazolines do not exert strong glycosyl donor properties because the methyl-substituted *N*-protonated oxazolinium system is rather stable. Consequently, the replacement of the *N*-acetyl group by strongly electron-withdrawing groups, for instance the trifluoroacetyl,[99,100] trichloroacetyl,[101,102] and trichloroethoxycarbonyl groups,[103-107] have

been investigated in order to avoid the formation of stable cyclic imidate intermediates which impede glycoside bond formation.

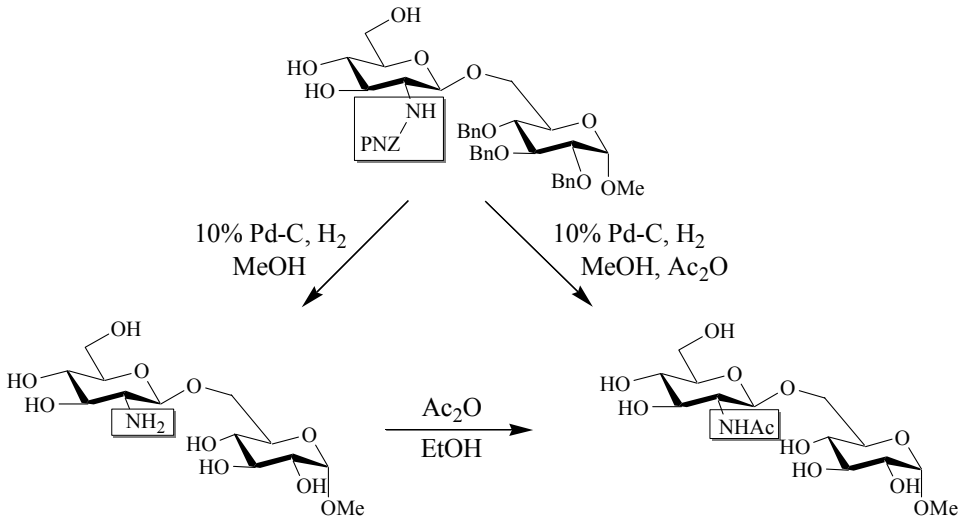
Blatter et al. have demonstrated the use of trichloroacetamido group as an amino-protecting group in trichloroacetimidate glycosylation with a high degree of 1,2-*trans* stereoselectivity.[101] The *N*-trichloroacetyl group are easily transformed into *N*-acetyl under neutral conditions by reaction with tributylstannane. Recently, Donohoe and co-workers have investigated a range of different methods for reducing the trichloroacetamido groups and found that, in addition to tin hydride, hydrogenolysis and cleavage with NaOH (followed by reacetylation) are also viable conditions (Scheme 16).[102]



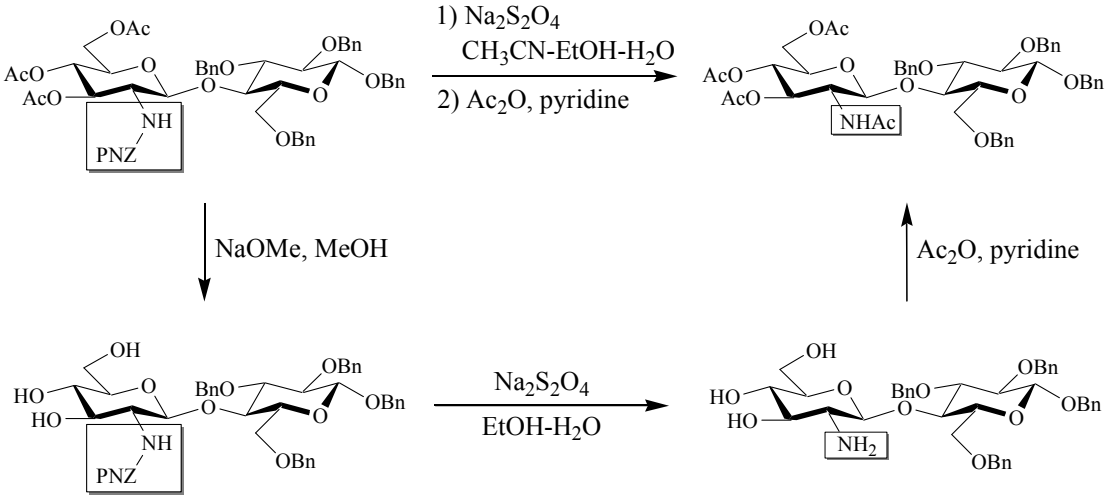
Scheme 16. Deprotection of *N*-trichloroacetyl group.[102]

The trichloroethoxycarbonyl (Troc) group has been evaluated as a protecting group for oligosaccharide syntheses and gave high yields and high β-selectivity in thioglycoside and trichloroacetimidate glycosylation.[107] The Troc group is readily established by reaction of the glucosamine hydrochloride with trichloroethoxycarbonyl chloride (Troc-Cl) and potassium carbonate in water. After the glycosylation step the *N*-Troc group can be converted to the *N*-acetyl group by using zinc in acetic anhydride.

The use of *p*-nitrobenzyloxycarbonyl (PNZ) group as a good participating substituent for 2-amino-β-glucoside formation has been described by Qian and Hindsgaul.[108] This *N*-protecting group can be conveniently removed either by hydrogenolysis along with *O*-benzyl ether (Scheme 17) or selectively by sodium dithionite under neutral conditions where carboxylate esters remain stable (Scheme 18).



Scheme 17. Deprotection of the *p*-nitrobenzyloxycarbonyl (PNZ) group by hydrogenolysis.[108]



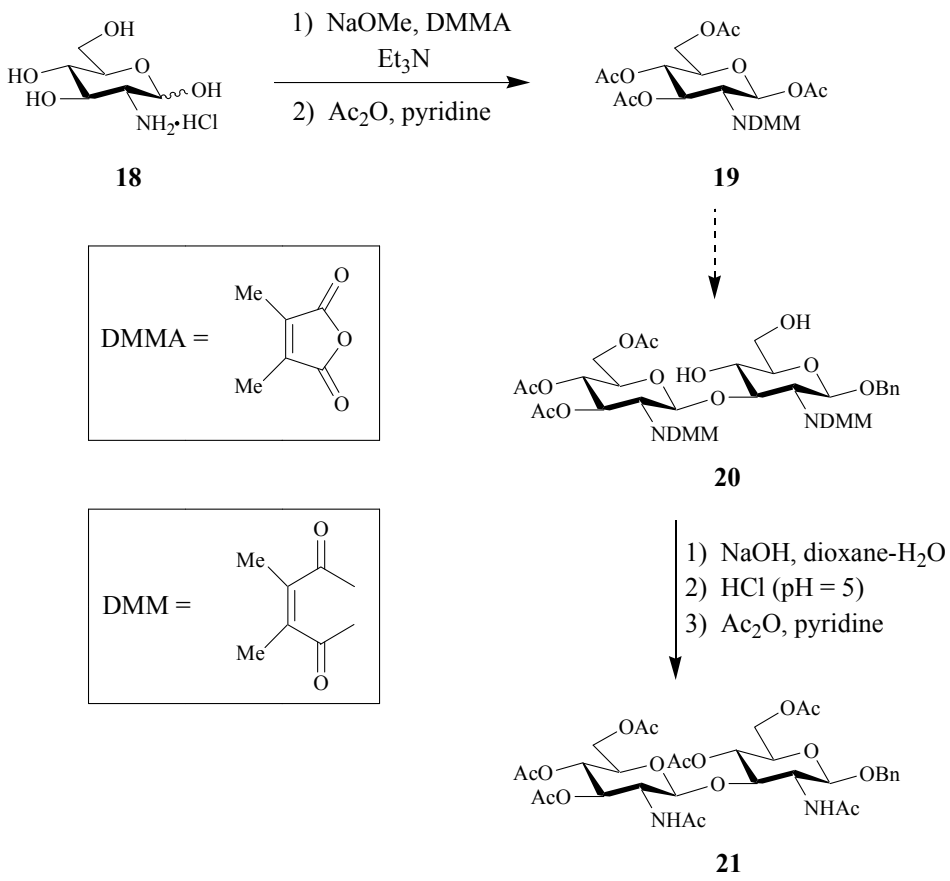
Scheme 18. Deprotection of the *p*-nitrobenzyloxycarbonyl (PNZ) group by sodium dithionite .[108]

Although the carbamate functionality serve as versatile amino-protecting groups to increase glycosylic donor property in glycoside bond formation, the structural assignment of these groups by NMR spectroscopy can be hampered when rotation around

the amidic CN bond is hindered.[108] This problem can be avoided by using symmetric *N*,*N*-diacyl compounds such as two noncyclic *N*-acyl groups or cyclic *N*,*N*-diacyl groups, for instance phthaloyl (Phth), trichlorophthaloyl (TCP), dimethylmaleoyl (DMM), dithiasuccinyl (Dts) and 2,5-dimethylpyrrole groups as an amino-protecting group.

The Phthalimido (Phth) protecting group[109,110] is widely used as *N*-protecting group in sugar chemistry. The *N*-Phth-protected sugars can be readily obtained from amino sugars by reacting with phthalic anhydride. In combination with trichloroacetimidate activation, good glycosyl donors are available for β-glycoside bond formation via reactive *N*-acylated oxazolinium intermediate. Removal of the Phth group can be achieved by using methylamine, ethylenediamine or hydrazine in refluxing methanol, ethanol or butanol.[111-114] It can also be cleaved with sodium borohydride.[115] The 4,4,5,5-tetrachlorophthaloyl (TCP) group[116-118] has been reported as an alternative to the *N*-Phth group which retains the advantageous β-directing influence but allow cleavage under milder conditions than the Phth group, either by a slight excess of ethylenediamine at 60 ^{o}C[117] or by sodium borohydride reduction.[118] Furthermore, it shows a useful level of compatibility with chloroacetyl, acetyl and benzoyl removal. The 4,5-dichlorophthaloyl (DCPhth) group has recently been evaluated for similar purposes.[119,120] It is also more labile than the Phth group but any advantages over the TCP group were not established.

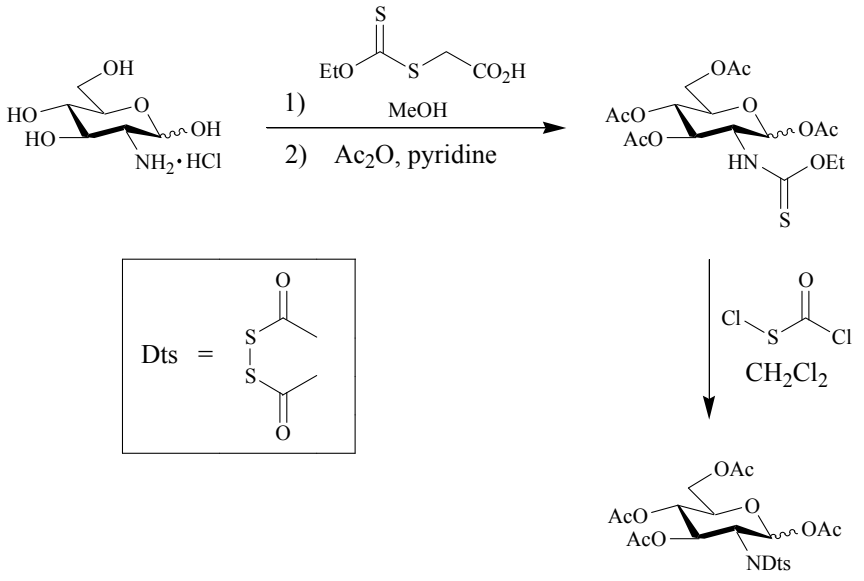
The dimethylmaleoyl (DMM) group has been investigated as an amino protecting group in oligosaccharide synthesis.[121] The protection was carried out by the reaction of glucosamine hydrochloride **18** (Scheme 19) with sodium methoxide in methanol followed by dimethylmaleic anhydride (DMMA) and triethylamine. Subsequent acetylation gave the protected amino sugar derivative **19** which was then transformed into the disaccharide **20**. The removal of the two DMM groups was accomplished under weakly basic aqueous and then acidic conditions to give **21** after acetylation. The results indicated that the DMM group exhibits neighboring group participation to enforce β linkage and is stable to acid and non-nucleophilic bases. It presents a useful alternative to phthalimido group.



Scheme 19. Synthesis of *N*-DMM-protected tetra-*O*-acetyl-β-D-glucopyranoside **19** and disaccharide **21**.[121]

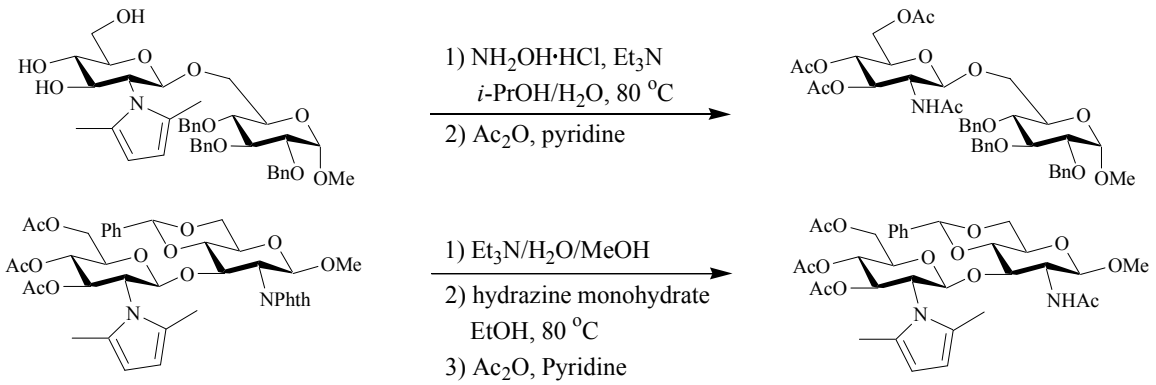
The dithiasuccinoyl (Dts) group[122,123] is another C_2-symmetric *N*,*N*-diacyl protecting group of glycosylamine which is introduced by treatment of the glycosylamine hydrochloride with *S*-carbomethyl *O*-ethyl dithiocarbonate in methanol and subsequently peracetylation with acetic anhydride in pyridine. The obtained ethoxythiocarbonyl intermediate is then treated with chlorocarbonylsulfenyl chloride, and in the accompanying cyclization reaction the dithiasuccinoyl group is formed with the extrusion of ethyl chloride and hydrogen chloride (Scheme 20). The *N*-Dts protecting group can be cleaved under thiolytic conditions using thiols such as dithiothreitol. It has been demonstrated that it is possible to reduce the Dts group in the presence of an azido group

selectively by sodium borohydride or the Dts and the azido group simultaneously by dithiothreitol, using *N*,*N*-diisopropylethylamine as a catalyst.[123]



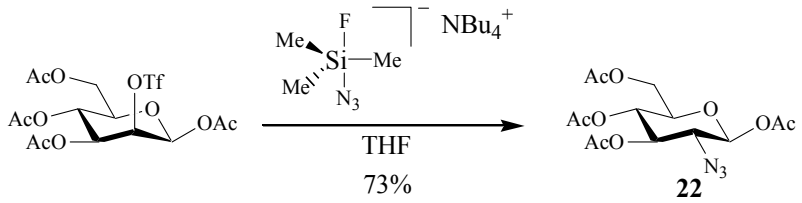
Scheme 20. Synthesis of *N*-Dts-protected tetra-*O*-acetyl-D-glucosamine.[123]

The 2,5-dimethylpyrrole functionality is a versatile amino protecting group that is compatible with many protecting-group manipulations commonly employed in oligosaccharide chemistry.[124-126] The dimethylpyrrole protecting group is readily installed by treatment of an amine with 2,5-hexanedione in the presence of triethylamine in methanol. Interestingly, it can be cleaved by treatment with hydroxylamine hydrochloride but is stable to conditions applied for cleavage of the *N*-Phth group (Scheme 21).[127] Furthermore, glycosyl trichloroacetimidate derived from 2-deoxy-2,5-dimethylpyrrole glycosides performed well in Lewis acid-mediated glycosylation leading selectively to 1,2-*trans*-glycosides.



Scheme 21. Deprotection of 2,5-dimethylpyrrole protecting group and selective deprotection of *N*-Phth group in the presence of the 2,5-dimethylpyrrole group.[127]

Azides can be considered as masked amino groups.[59,109,110] They serve as an excellent latent amino group, for instance in combination with trichloroacetimidate activation, reactive donors for the generation of α- and β-glycosidic linkages are available.[128] However, the preparation of the required azido sugars is still not very economical.[90,129] Recently, Soli et al. demonstrated the synthesis of glycosyl azide derivatives using azide displacement via hypervalent azidosilicate prepared in situ by the reaction of trimethylsilyl azide (TMS-N_3) with tetrabutylammonium fluoride (TBAF).[130] The 2-azidoglucopyranose **22** could be obtained in good yield starting from mannosyl triflate (Scheme 22). Azido groups are easily reduced to amino groups by catalytic hydrogenation. However, when C=C bonds or benzyl groups are present in the same molecule which would also be affected under these conditions, hydrogen sulfide in pyridine allows the chemoselective reduction of azide in the presence of these reducible groups.

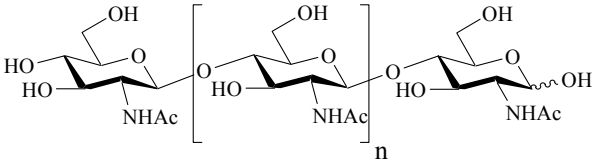


Scheme 22. Synthesis of glycosyl azide derivative using azide displacement via hypervalent azidosilicate.[130]

1.7 Syntheses of chitooligosaccharides and related compounds by chemical method

According to the biological significance of chitooligosaccharides, they have been required for investigating their functions in deep detail. A number of papers rely on the use of enzymatic methods, while there are few reports on the use of chemical methods, particularly for the preparation of hetero-chitooligosaccharides with defined structure.

Aly et al. demonstrated the synthesis of chitotetraose (**23**) and chitohexaose (**24**) (Figure 3) based on using DMM-protected glucosamine moieties as glycosyl donors and acceptors in combination with trichloroacetimidate as glycosylating agent.[131]

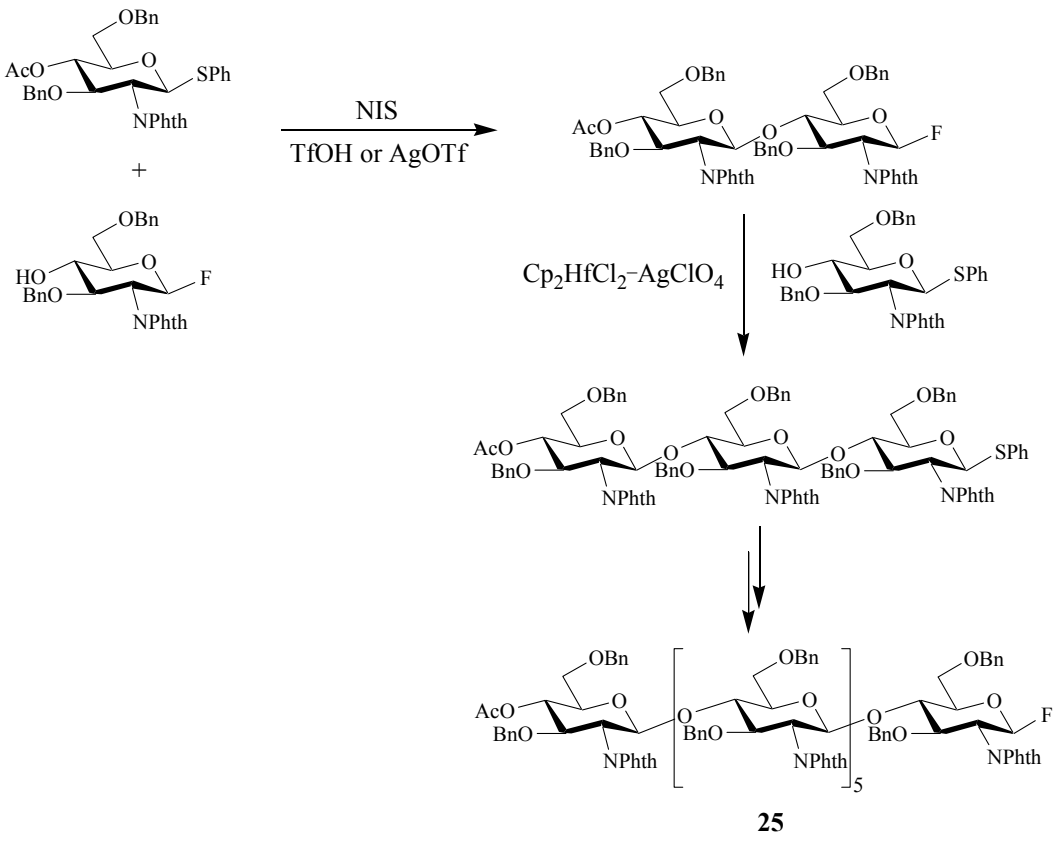


23: n = 2, **24**: n = 4

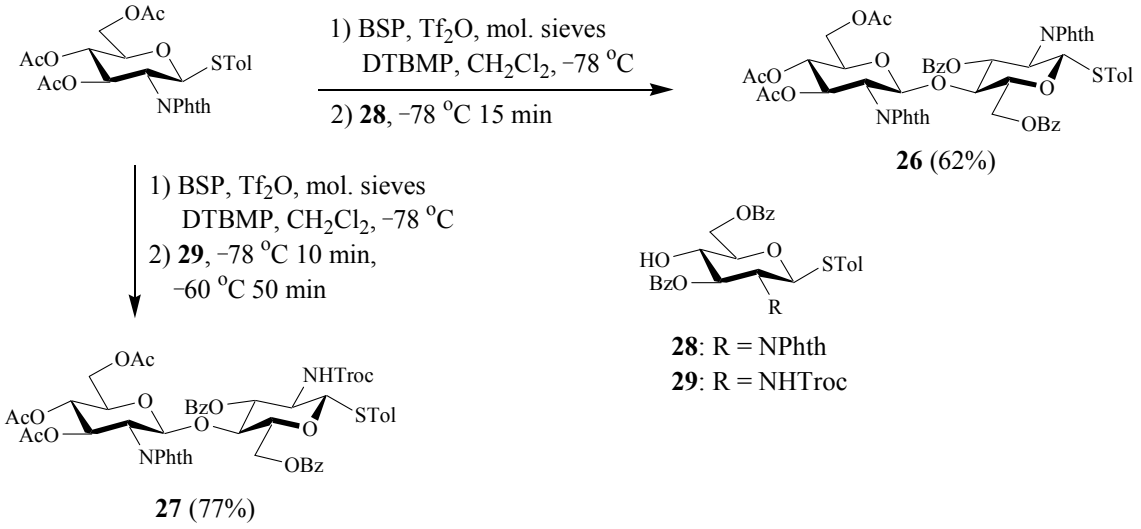
Figure 3. Structure of chitotetraose (**23**) and chitohexaose (**24**).

Ogawa and co-workers developed an orthogonal glycosylation strategy to synthesize heptasaccharide **25** by the combined used of phenylthioglycosides and glycosyl fluorides as both donors and acceptors (Scheme 23).[132] Extra steps, such as temporary protection of the anomeric position and subsequent conversion into donor, are thus eliminated. Thioglycosides are activated with NIS-TfOH or NIS-AgOTf, whereas glycosyl fluorides are activated with Cp_2HfCl_2–$AgClO_4$.

The synthesis of fully protected disaccharides **26** and **27** exploiting Crich's glycosylation conditions involving a glycosyl triflate intermediate was reported by Hansen and Skrydstrup (Scheme 24).[133]



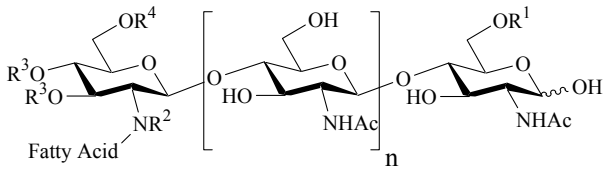
Scheme 23. Synthesis of heptasaccharide **25** by orthogonal glycosylation strategy.[132]



Scheme 24. Synthesis of disaccharides **25** and **26** employing Crich's glycosylation conditions.[133]

Recently, a preparative synthesis of chitobioses, i.e. $(GlcN)_2$, $(GlcNAc)_2$, GlcNAc-GlcN and GlcN-GlcNAc, using *N*-trichloroacetyl and *N*-benzyloxycarbonyl (*N*-Cbz) glucosamine building blocks as glycosyl donors and acceptors under catalysis of $BF_3{\cdot}Et_2O$, was described in a patent.[134]

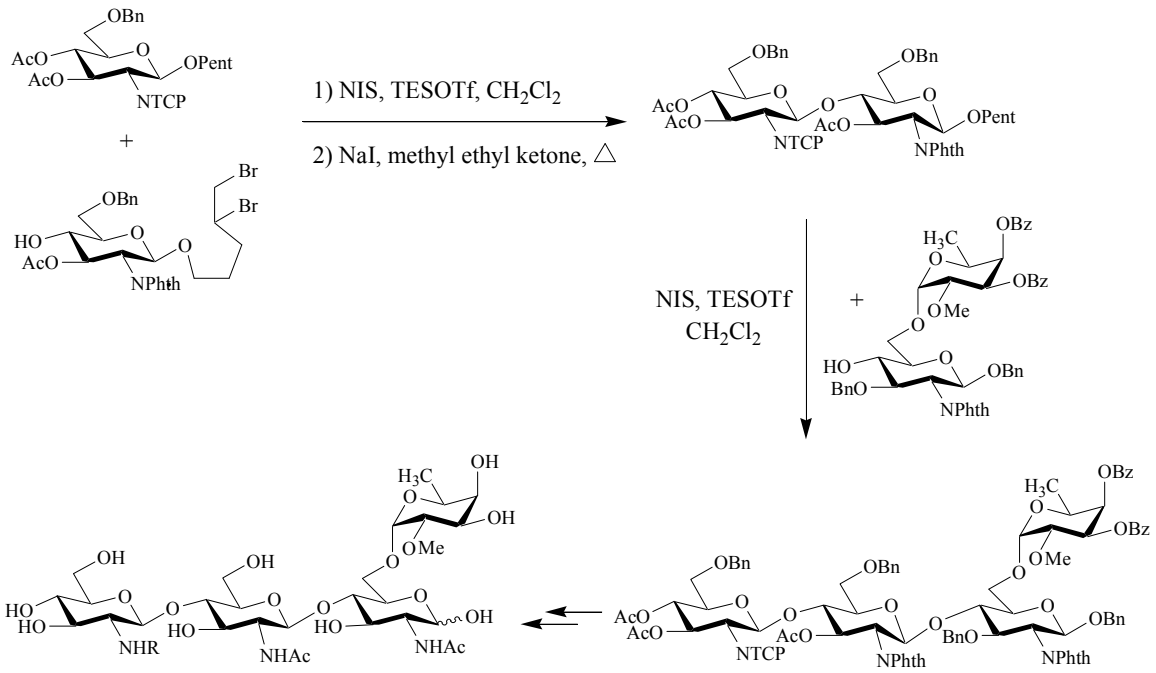
Lipochitooligosaccharides (LCOs), also called nodulation (Nod) factors, are important signal molecules secreted by rhizobia in the presence of leguminous plant inducers.[135,136] They are essential for the formation of symbiotic organs on the roots of host plants. The most common LCO structure is built up by a chitin backbone (Figure 4). The number of GlcNAc residues generally varies from three to five units. The non-reducing terminus of this backbone is always acylated with a fatty acid that can be mono- or polyunsaturated.[137] At both the reducing and non-reducing termini of the chitin chain, different substituents like acetyl, sulphatyl, carbamoyl and fucosyl groups may be present.



R^1 = H, SO_3^-, $CONH_2$, fucose or arabinose
R^2 = H or Me; R^3 = H, $CONH_2$ or Ac
R^4 = H, $CONH_2$ or Ac; n = 1 to 3

Figure 4. General structure of lipochitooligosaccharide (LOC).

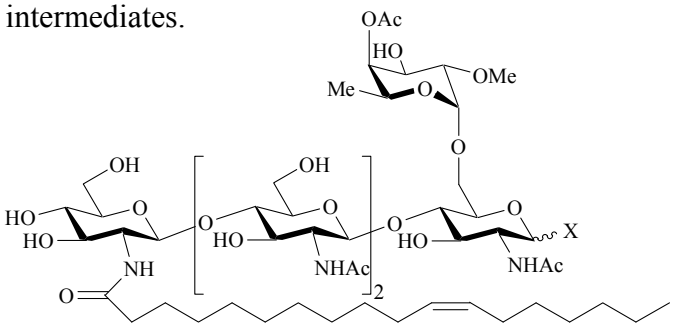
The important role of these molecules in nitrogen fixation, in the study of signal exchange process, host specificity and their natural scarcity, has encouraged several groups to perform their chemical synthesis.[138-143] Debenham and co-workers described a convergent stereocontrolled synthesis of the nodulation factor NodRf-III (C18:1, MeFuc) utilizing TCP- and Phth-protected *n*-pentenyl glucosamine as precursors.[140] Key to their synthetic approach was the use of the TCP group to provide for *N*-differentiation of the linear glucosamine backbone and the use of $FeCl_3$ for late-stage debenzylation of a complex tetrasaccharide (Scheme 25).



NodRf-III (C18:1, MeFuc)
R = 11-(*Z*)-octadecenoyl

Scheme 25. Synthesis of nodulation factor NodRf-III (C18:1, MeFuc).

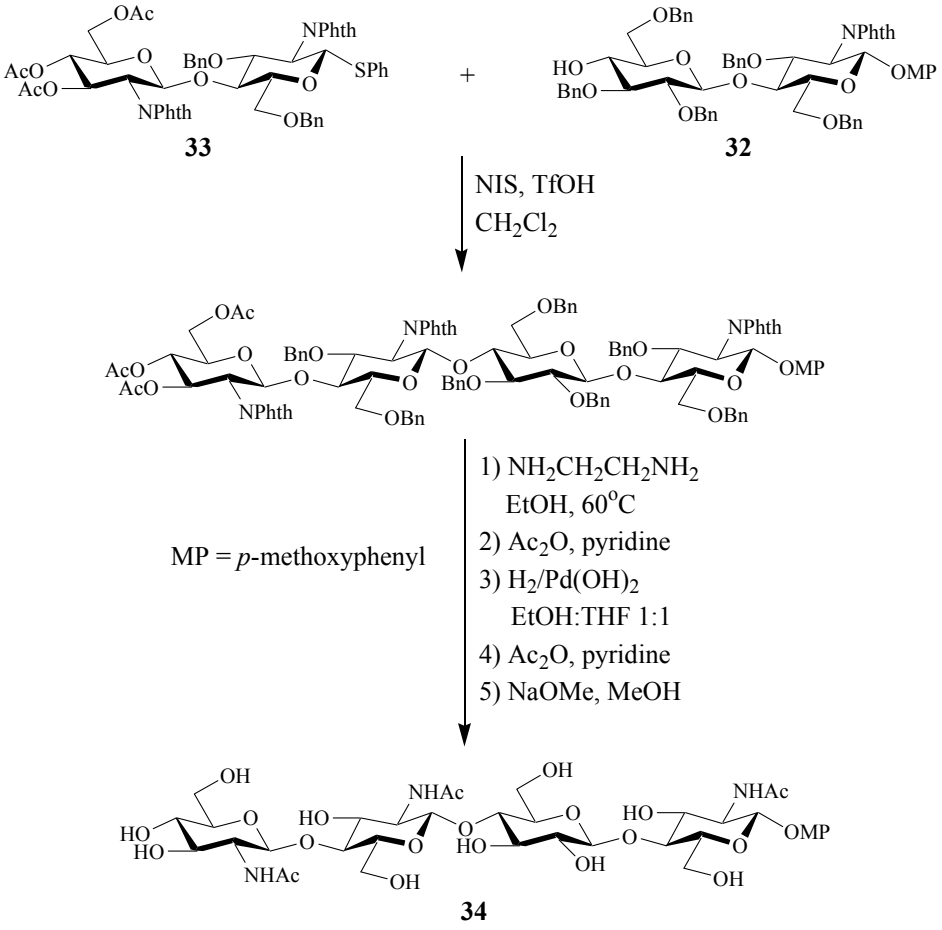
Two lipopentasaccharides related to the nodulation factors from *Rhizobium* sp. NGR 234, compounds **30** and **31** (Figure 5), were synthesized by Sedinkin et al. employing azide as masked amino group of the non-reducing glucosamine residue and Phth group as amino protecting group of three glucosamine residues.[141] Trichloroacetimidate and thioglycoside were used as glycosyl donors in the construction of oligosaccharide intermediates.



30: X = OH
31: X = β-OMe

Figure 5. Structure of *Rhizobium* sp. NGR 234 Nod factors analogues.

Robina et al. disclosed the synthesis of an analogue of the LOCs involved in the *Rhizobium*-legume symbiosis, which one central unit of GlcNAc has been substituted by Glc unit.[144] Compound **34**, containing GlcNAc and Glc units, was achieved by convergent synthesis where the tetrasaccharide backbone is formed by coupling the glycosyl acceptor **32** with thioglycoside **33** using NIS and TfOH as a promoter (Scheme 26).



Scheme 26. Synthesis of tetrasaccharide related to the chitinoligosaccharide involved in plant defence and *Rhizobium*-legume symbiosis.[144]

1.8 Objectives

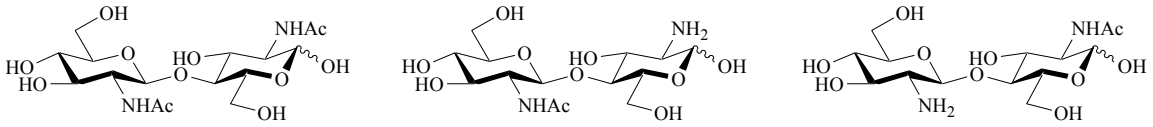
From what have been discussed in the previous sections, the biological properties of chitooligosaccharides are significantly dependent on their degree of polymerization (DP), degree of *N*-acetylation (DA), and GlcNAc/GlcN distribution pattern along the oligomeric chain. Therefore, structurally well-defined chitooligosaccharides would represent important tools for studying the relationship between structure and biological activity, as well as providing information concerning the preferred enzymatic cleavage site of different enzymes.

Even though enzymatic processes in principle present a very elegant access to chitooligosaccharides, the limited availability of suitable enzymes and rather small amounts of oligomers that can be prepared enzymatically render this approach presently below requirements of applications in preparative organic chemistry.

Aim of this project was therefore the development of synthetic strategies for the preparation of hetero-chitooligosaccharides, i.e. chitobioses and chitotetraoses. In order to achieve the goal, the objectives were: i) preparation of monosaccharides playing the role of donor and accepter of both GlcN and GlcNAc; ii) coupling reaction of GlcN/GlcNAc donor with GlcN/GlcNAc acceptor and iii) selective removal of protecting groups to generate the expected chitobioses. The strategy used for the synthesis of disaccharides was then applied to the preparation of chitotetraoses.

2. Results and Discussion

With the aim to develop strategies for the preparation of chitooligosaccharides, initially, we focused on the synthesis of the smallest unit of fully and partially *N*-acetylated chitosan oligomers, i.e. 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose [$(GlcNAc)_2$, **3**], 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranose (GlcNAc-GlcN, **4**) and 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (GlcN-GlcNAc, **5**) as shown in Figure 6.

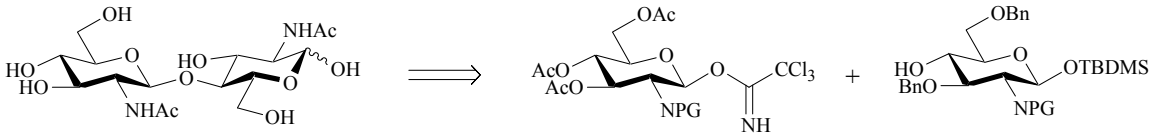


3 **4** **5**

Figure 6. Structure of $(GlcNAc)_2$ (**3**), GlcNAc-GlcN (**4**) and GlcN-GlcNAc (**5**).

2.1 Synthesis of $(GlcNAc)_2$ (3)

2.1.1 Retrosynthetic strategy



3 **35** **36**

PG = protecting group

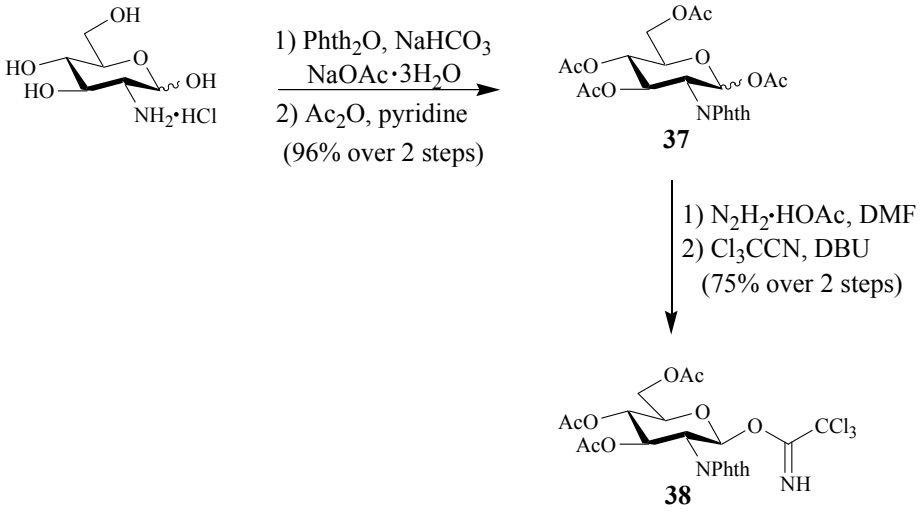
Scheme 27. Retrosynthetic analysis of $(GlcNAc)_2$ (**3**).

The synthesis of $(GlcNAc)_2$ (**3**) could be achieved by glycosylation reaction of glucosamine trichloroacetimidate **35** with glycosyl acceptor **36** as illustrated in Scheme 27. The participating amino protecting group is required to ensure the formation of β-(1→4) linkage. The phthaloyl (Phth) group[145] which is commonly used for the protection of glucosamines in the context of oligosaccharide syntheses was chosen for this purpose. Its electron-withdrawing property makes the D-glucosamine derivative a good glycosyl

donor and formation of a stable oxazoline during the following glycosylation step is avoided. Moreover, versatile glycosyl acceptors containing the phthalimido group could also be generated and successfully employed in glycosylation reactions.[146] The phth group could be readily converted into the *N*-acetyl group later by reacting with ethylenediamine[112] and subsequent acetylation. The method employing in our strategy was trimethylsilyl trifluoromethanesulfonate (TMSOTf) promoted glycosylation using trichloroacetimidate sugar[88] as glycosyl donor.

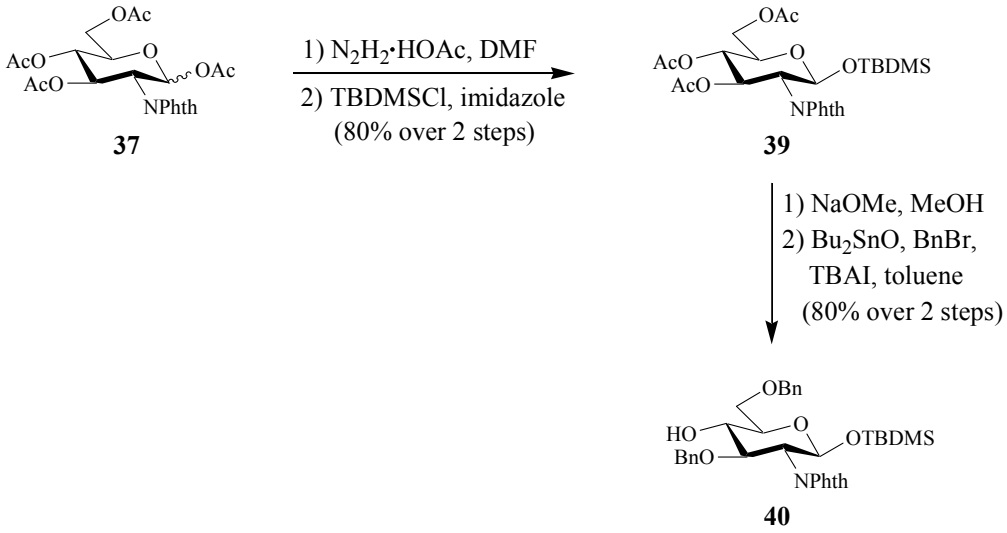
2.1.2 Preparation of *N*-phthaloyl-protected monosaccharide donor and acceptor

The *N*-phth glycosyl donor **38** was synthesized in four steps from commercial D-glucosamine hydrochloride (Scheme 28). The first step involved neutralization of the D-glucosamine hydrochloride with sodium acetate trihydrate in acetone-water, then *N*-acylation with phthalic anhydride in the presence of sodium hydrogencarbonate and *O*-acetylation using acetic anhydride in pyridine to provide *N*-phth-protected tetra-*O*-acetyl-D-glucosamine **37** as an anomeric mixture.[147] Thereafter, selective 1-*O*-deacetylation of **37** using hydrazine acetate in dimethylformamide (DMF) followed by imidation with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2[90] provided the β-trichloroacetimidate **38**[148] in 75% yield, as evidenced by ^{1}H NMR spectroscopy [δ 6.64 ppm, $J_{1,2}$ = 9.0 Hz, 1$_\beta$-H].



Scheme 28. Preparation of β-trichloroacetimidate **38**.

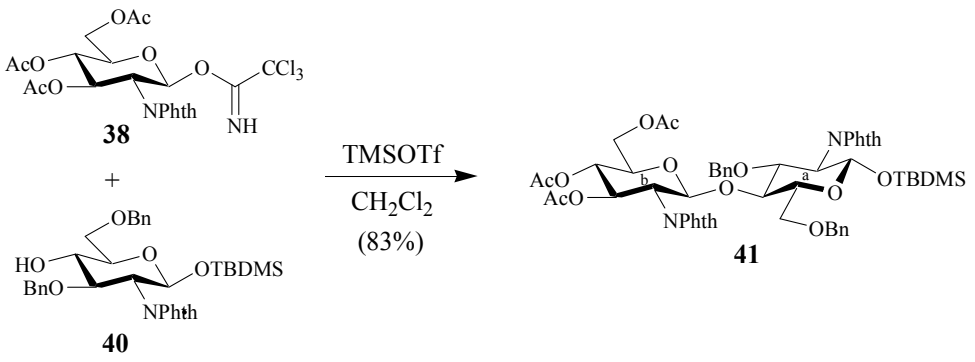
For the synthesis of the *N*-phth-protected glycosyl acceptor **40** (Scheme 29), anomeric deacetylation of the D-glucosamine derivative **37** by treatment with hydrazine acetate in DMF provided the corresponding 1-hydroxy compound. Reaction with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) in the presence of imidazole[149,150] afforded exclusively β-1-*O*-TBDMS protected **39** in 80% yield.[151] [^{1}H NMR: δ 5.52 ppm, $J_{1,2}$ = 7.8 Hz, 1_β-H]. Deacetylation of compound **39** with sodium methanolate in dry MeOH[152] furnished the triol intermediate, which was then converted into the dibutylstannyl acetal derivative by employing dibutyltin oxide (Bu_2SnO) in refluxing toluene.[153,154] Treatment of the obtained acetal with benzyl bromide and tetrabutylammonium iodide (TBAI) in the same solvent gave selectively 3,6-di-*O*-benzylated **40**.[155]



Scheme 29. Preparation of *N*-phthaloyl-protected glucosamine acceptor **40**.

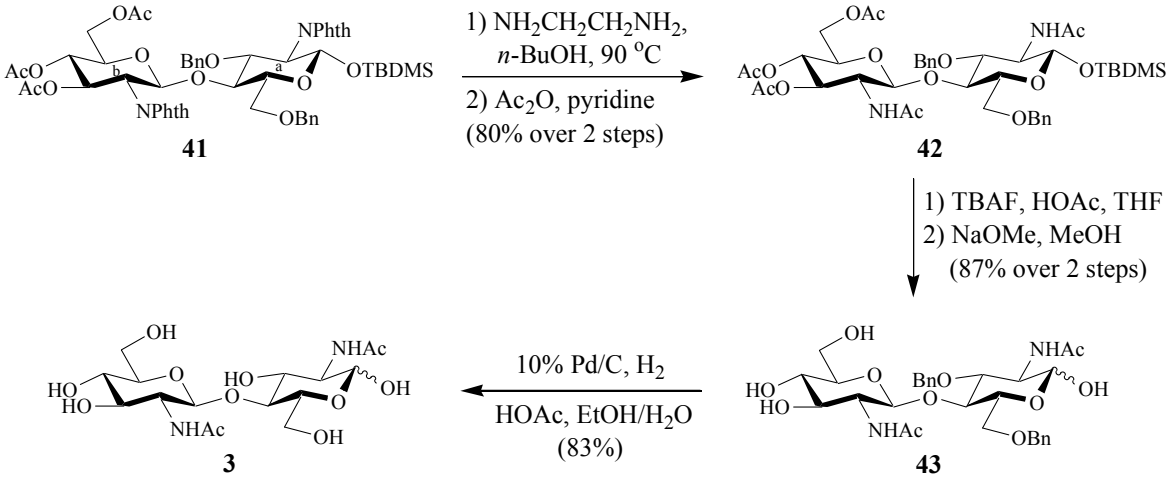
2.1.3 Glycosylation reaction and deprotection

Having the glycosyl donor and acceptor in hand, our attention was then focused on the preparation of the desired disaccharide **3**. Thus, glycosylation of acceptor **40** with donor **38** using 0.1 equiv. of TMSOTf as a catalyst in CH_2Cl_2 at $^{-}30$ ^{o}C provided the β-linked disaccharide **41** in 83% yield (Scheme 30). Its ^{1}H NMR spectrum supported the assignment; two doublets for 1a-H and 1b-H at δ 5.17 and 5.56 ppm with a *J* value of 8.0 and 8.5 Hz respectively indicated the axial nature of 1-H and 2-H in both rings.



Scheme 30. Glycosylation reaction of glycosyl acceptor **40** with donor **38**.

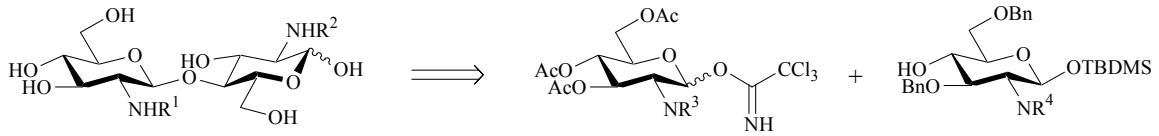
To reach the desired disaccharide **3**, selective deprotection was carried out as shown in Scheme 31. First, removal of *N*-phth groups in **41** by treatment with ethylenediamine in butanol at 90 ^{o}C[112] followed by *N*-acetylation gave *N*,*N*′-diacetyl derivative **42** in high yield. The disaccharide **42** was characterized by the upfield shift of the signal of 2-H proton from δ 4.07 (2a-H) and 4.34 (2b-H) ppm to 3.66 (2a-H) and 4.02 (2b-H) ppm respectively. The ^{13}C NMR spectrum exhibited the methyl signal of the NHAc groups at δ 23.2 ppm. Thereafter, anomeric *O*-desilylation[149,150] of **42** with tetrabutylammonium fluoride (TBAF) and acetic acid in dry tetrahydrofuran (THF) and subsequent *O*-deacetylation under Zemplén condition[152] afforded glycoside **43** (87%). Finally, hydrogenolytic *O*-debenzylation of **43** with 10% Pd/C catalyst furnished *N*,*N*'-diacetylchitobiose **3**[52] in 83% yield.



Scheme 31. Deprotection of disaccharide **41** to provide *N*,*N*'-diacetylchitobiose **3**.

2.2 Synthesis of partially *N*-acetylated chitobioses

2.2.1 Retrosynthetic aspect



4: R^1 = Ac, R^2 = H
5: R^1 = H, R^2 = Ac

38: R^3 = Phth
46: R^3 = Cbz

45: R^4 = Cbz
40: R^4 = Phth

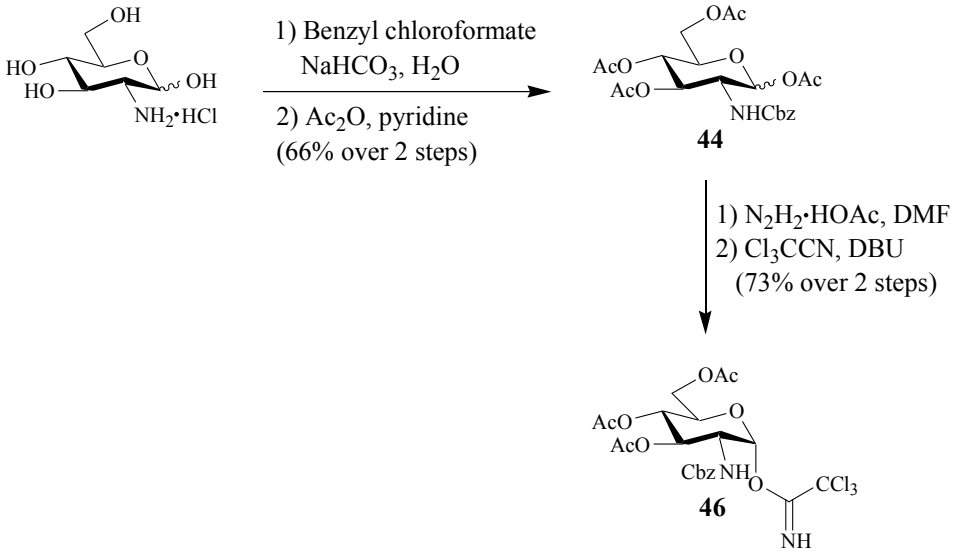
Scheme 32. Retrosynthetic analysis of partially *N*-acetyl chitobioses **4** and **5**.

For the synthesis of disaccharides **4** and **5** containing an *N*-acetate and a free amine, orthogonal nitrogen protections are required (Scheme 32). The phth group which has been proved to be useful for providing selective *trans* glycosylation and good yield in deprotection step for the synthesis of $(GlcNAc)_2$ was chosen as *N*-acetyl protecting group. The free amine was blocked as the benzyl carbamate, which can be removed by hydrogenolysis at the end of the synthesis along with the standard *O*-benzyl protecting group. The glycosyl donor was activated as trichloroacetimidate. The anomeric hydroxy group of the acceptor was masked as silyl ether in order to provide the disaccharide intermediate that can be easily transformed into the corresponding glycosyl donor in higher chitooligosaccharide synthesis.

2.2.2 Preparation of *N*-benzyloxycarbonyl-protected monosaccharide donor and its glycosylation reaction

In order to achieve the desired disaccharides **4** and **5**, the *N*-Cbz-protected glucosamine donor was prepared to investigate the coupling reaction with *N*-Phth-protected glucosamine acceptor. As depicted in Scheme 33, treatment of D-glucosamine hydrochloride with sodium hydrogencarbonate and benzyl chloroformate in water followed by *O*-acetylation provided acetate **44**[156] in 66 % yield as an anomeric mixture. Conversion of compound **44** to trichloroacetimidate building block **46** was achieved in 73% yield by 1-*O*-deacetylation of **44** using hydrazine acetate and subsequent imidation

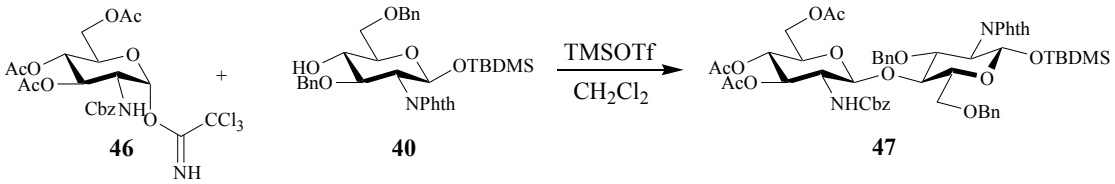
with trichloroacetonitrile in the presence of DBU. Only the α-anomer was observed, as evidenced by ^{1}H NMR spectroscopy [δ 6.41 ppm, $J_{1,2}$ = 3.6 Hz, 1_α-H].



Scheme 33. Preparation of α-trichloroacetimidate **46**.

With trichloroacetimidate **46** in hand, glycosylation reactions with glycosyl acceptor **40** employing TMSOTf as an activator at a range of temperatures and equivalents of promoter were investigated. Unfortunately, reaction at –30 ºC using 0.1 equiv. of TMSOTf did not give the desired disaccharide **47** (Table 1, entry 1). Increasing the temperature to 0 ºC or 0 ºC to room temperature resulted in decomposition of both glycosyl acceptor and donor (entry 2,3). The use of 0.01 equiv. of activator at 0 ºC to room temperature yielded no coupling product (entry 4). These results, along with complicacy in NMR signals assignment due to the rotation at the amide bond[108] rendered the Cbz group unattractive for amine protection in the context of our synthesis.

Table 1. Attempted glycosylation of glycosyl acceptor **40** with glycosyl donor **46**.



Entry	**46** (equiv.)	TMSOTf (equiv.)	Temperature	Time	Results[a]
1	1.2	0.1	–30 °C	2 h	No reaction
2	1.2	0.1	0 °C	6 h	Decomposition of **46**
3	1.3	0.1	0 °C → rt	6 h	Decomposition of **40** and **46**
4	1.3	0.01	0 °C → rt	15 h	Decomposition of **46**

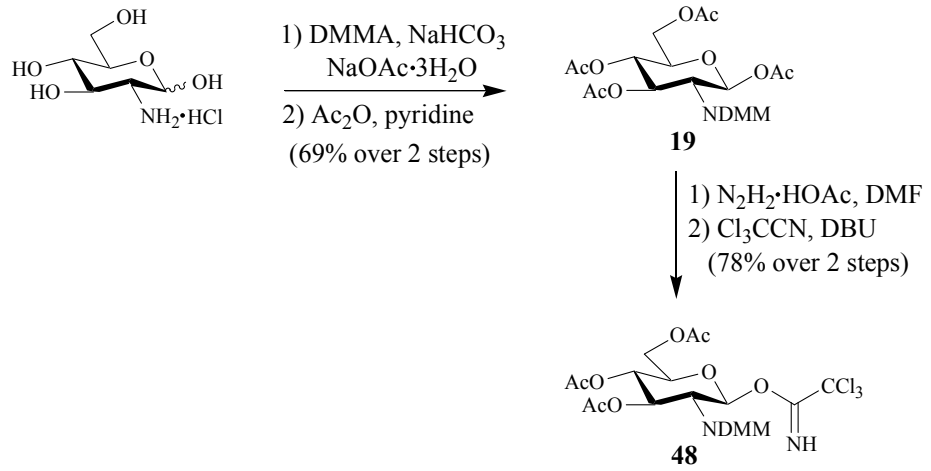
[a] Identified from TLC of crude product.

2.2.3 Preparation of *N*-dimethylmaleoyl-protected monosaccharide donor and acceptor

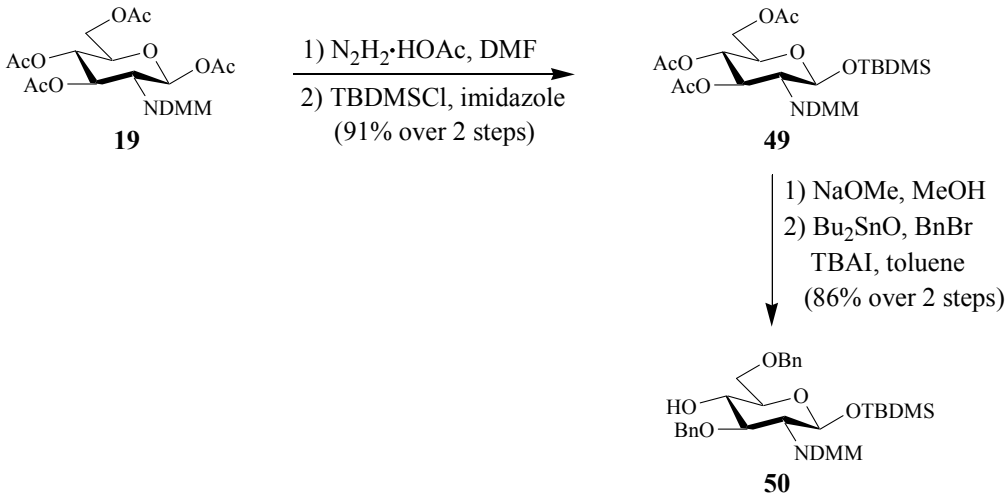
Having unsucceeded in glycosylation reaction using *N*-Cbz-protected donor, we next investigated dimethylmaleimido (DMM) glucosamine as glycosyl donor. Protection of the glucosamine nitrogen with DMM group was reported to effectively mask the glucosamine nitrogen while ensuring *trans* glycosylation products.[121,157] Moreover, glycosyl acceptors containing the DMM group could also be generated and successfully employed in glucosamine-containing oligosaccharide synthesis.[121,157,131] Removal of the DMM group could be readily accomplished under mild condition by treatment with aqueous NaOH and then with HCl (pH = 5).

Synthesis of glucosamine donor and acceptor containing the DMM group as amino protecting group was originally developed by Schmidt and co-workers.[121] We reproduced with minor changes in the first step to prepare the tetra-*O*-acetylated glucosamine derivative **19**. Thus, reaction of D-glucosamine hydrochloride with dimethylmaleic anhydride (DMMA) using sodium acetate trihydrate and sodium hydrogencarbonate as base and subsequent *O*-acetylation afforded acetate **19** in 69% yield (Lit.[121]: 57%), only the β anomer was found as reported in the literature[121] [^{1}H NMR: δ 6.34 ppm, $J_{1,2}$ = 9.0 Hz, 1_β-H]. Compound **19** could serve as a precursor for generation of either the glycosyl donor **48** or the glycosyl acceptor **50** (Scheme 34 and

35). Thus, anomeric *O*-deacetylation of **19** by treatment with hydrazine acetate in DMF**,** followed by imidation using trichloroacetonitrile and catalytic amounts of DBU furnished β-trichloroacetimidate **48**[121] (Scheme 34). [^{1}H NMR: δ 6.46 ppm, $J_{1,2}$ = 8.7 Hz, 1$_\beta$-H]. On the other hand, 1-*O*-deacetylation of **19** provided the corresponding hemiacetal, which was directly reacted with TBDMS-Cl in the presence of imidazole to yield β-1-*O*-TBDMS protected **49**[121] in 91% (Scheme 35). [^{1}H NMR: δ 5.38 ppm, $J_{1,2}$ = 8.1 Hz, 1$_\beta$-H]. Deacetylation of **49** under Zemplén condition[152] and subsequent regioselective 3,6-di-*O*-benzylation by treatment with Bu_2SnO and then benzyl bromide in the presence of TBAI gave glucosamine derivative **50,**[121] which was used as glycosyl acceptor.



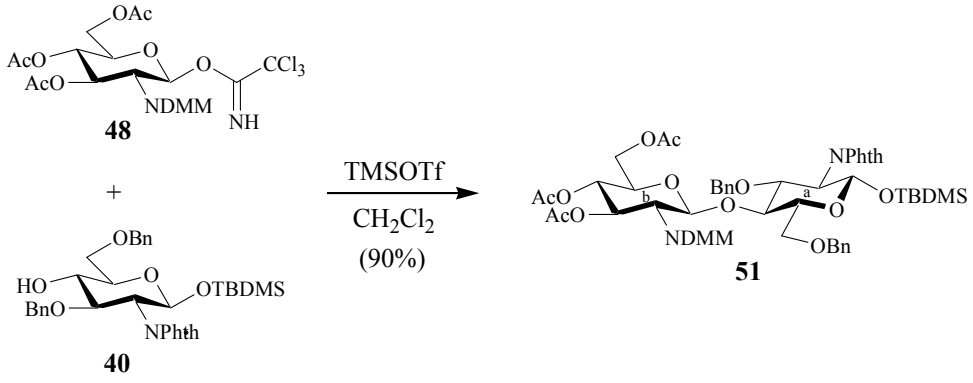
Scheme 34. Preparation of glycosyl donor **48**.



Scheme 35. Preparation of glycosyl acceptor **50**.

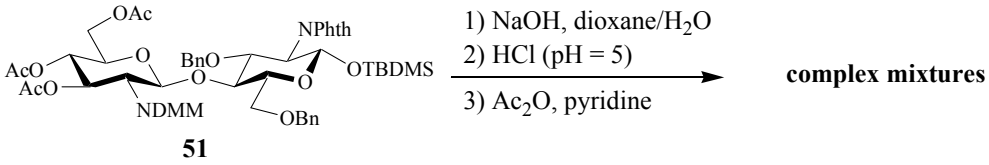
2.2.4 Synthesis of GlcNAc-GlcN (4)

The synthesis of GlcNAcGlcN (**4**) could be achieved by glycosylation of acceptor **40** with trichloroacetimidate **48** in the presence of TMSOTf (0.1 equiv.) as a catalyst in CH_2Cl_2 at –78 ^{o}C to provide the β-linked disaccharide **51** in 90% yield (Scheme 36). The ^{1}H NMR exhibited two doublets of 1a-H and 1b-H at δ 5.25 and 5.40 ppm respectively. The vicinal coupling constant of 8.4 Hz of the anomeric center of the glucosamine ring b supported the formation of the β-glycosidic linkage.



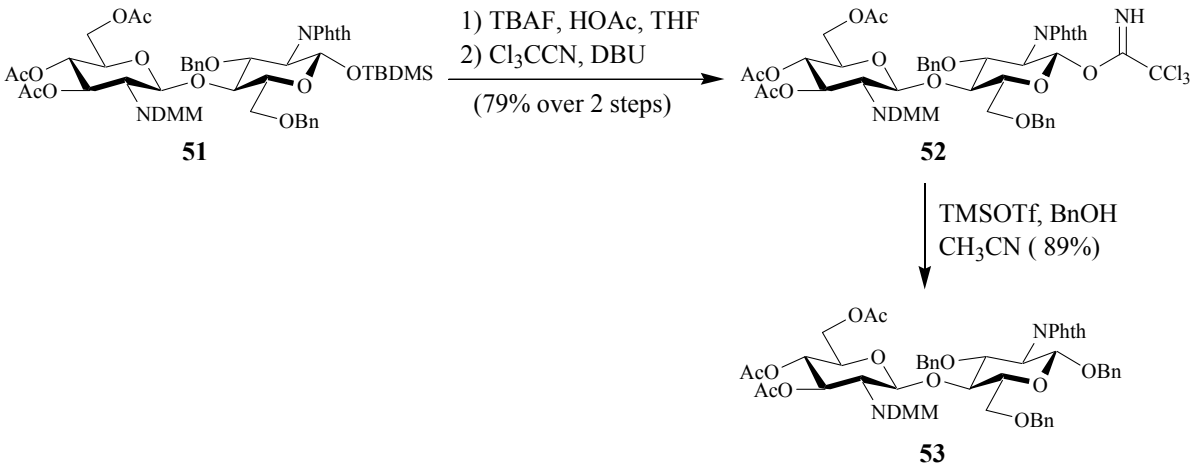
Scheme 36. Synthesis of fully protected disaccharide **51**.

For deprotection, we focused first on the removal of the *N*-DMM group to form an amine which will be transformed into the *N*-acetyl group in our final target. Unfortunately, removal of the DMM group in **51** by means NaOH in aqueous dioxane, followed by adjusting the pH = 5 with HCl and acetylation[121,131] resulted in complex mixtures, which were difficult to separate (Scheme 37).



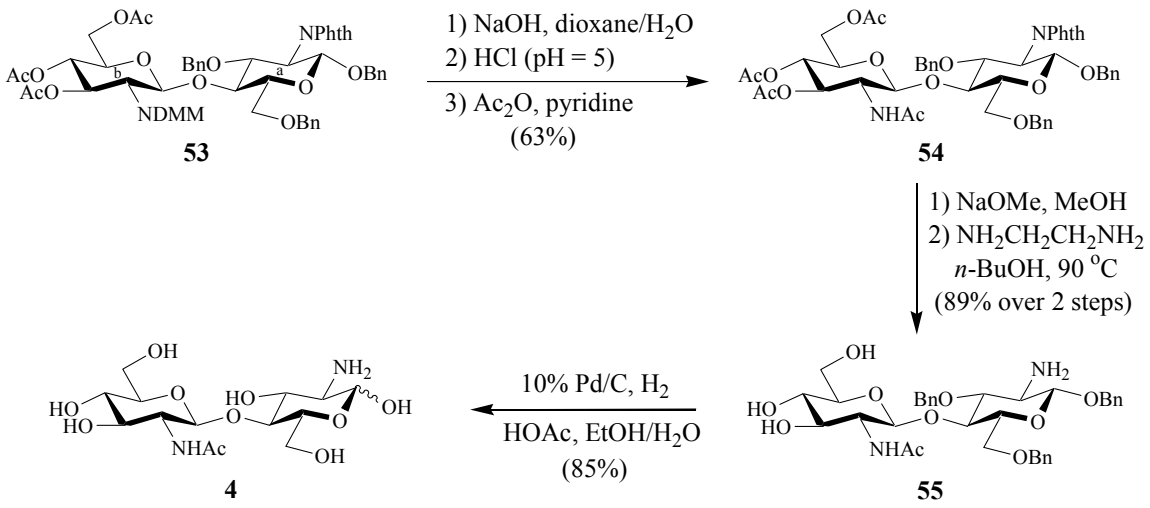
Scheme 37. Attempted deprtection of the DMM group of disaccharide **51**.

Assuming that the 1-*O*-silyl ether is unstable under our reaction conditions, thus the *O*-silyl ether was transformed into the corresponding benzyl ether. Selective *O*-desilylation of the disaccharide **51** with TBAF and acetic acid gave 1-hydroxy compound, which was directly treated with trichloroacetonitrile and DBU to afford trichloroacetimidate **52** (Scheme 38). Next, coupling of **52** as glycosyl donor with benzyl alcohol using 0.01 equiv. of TMSOTf as a catalyst in acetonitrile at room temperature led to benzyl glycoside **53** in 89% yield.



Scheme 38. Synthesis of benzyl glycoside **53**.

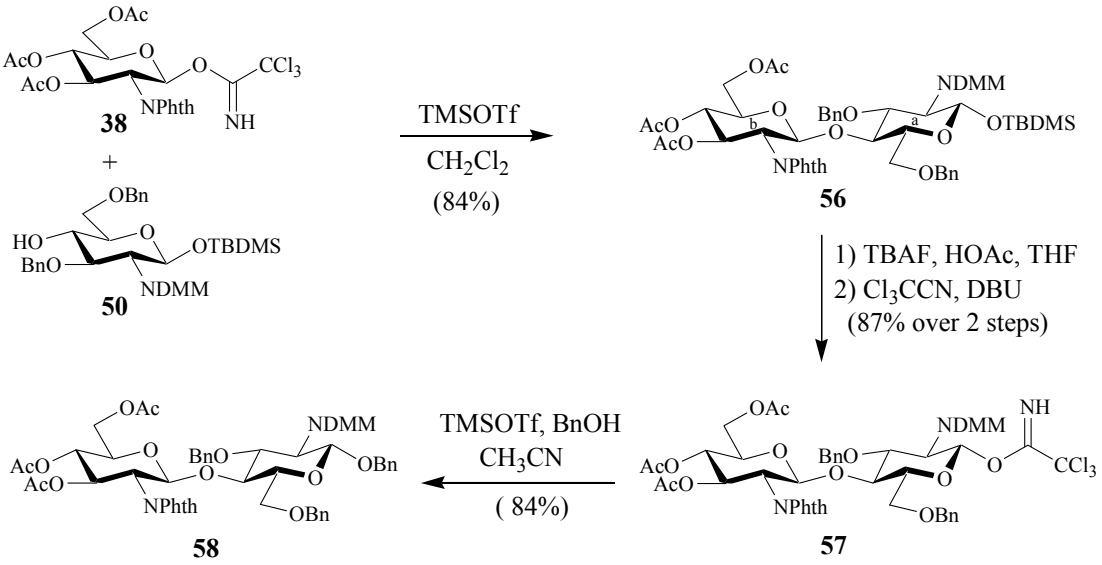
Deblocking of the DMM group of **53** by treatment with 0.5 M NaOH to open the DMM ring followed by addition of HCl in the presence of ethanolamine to pH 5 to cleave the presumably formed butenolide and then intermediate acetylation with acetic anhydride in pyridine[121,131] afforded *N*-acetyl-*N*′-phthaloyl derivative **54** in 63% yield (Scheme 39). The ^{1}H NMR spectrum confirmed the structure of **54**, as DMM methyl signals were absent and an acetamido methyl signal and an NH signal clearly appeared at δ 1.76 and 4.71 ppm respectively. Thereafter, *O*-deacetylation of compound **54** under Zemplén condition[152] and subsequent *N*-dephthaloylation using ethylenediamine in butanol at 90 ^{o}C[112] provided compound **55** in high yield. The success of this transformation was readily confirmed by the upfield shift of the 2a-H from δ 4.17 to 2.75 ppm. Finally, deprotection of benzyl groups using catalytic hydrogenation provided the mono-*N*-acetyl chitobiose **4**[51] in 85% yield.



Scheme 39. Deprotection of disaccharide **53** to provide mono-*N*-acetyl chitobiose **4**.

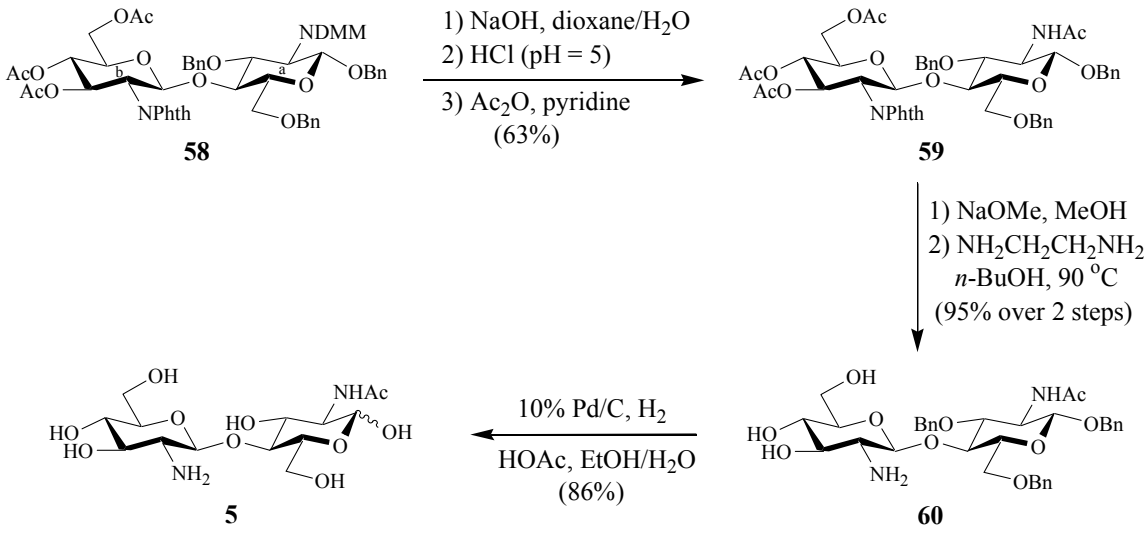
2.2.5 Synthesis of GlcN-GlcNAc (5)

The synthesis of disaccharide **5** was accomplished by TMSOTf (0.1 equiv.) catalyzed glycosylation of acceptor **50** with donor **38** at –30 °C in CH_2Cl_2 to furnish β-linked disaccharide **56** (84%) [^{1}H NMR: δ 5.55 ppm, $J_{1,2}$ = 8.4 Hz, 1b-H]. Anoneric *O*-desilylation of **56** followed by imidation provided imidate **57** in 87% yield. Coupling of **57** with benzyl alcohol in the presence of TMSOTf (0.01 equiv.) as a catalyst gave benzyl glycoside **58** in 84% yield (Scheme 40).



Scheme 40. Synthesis of disaccharide **56** and benzyl glycoside **58**.

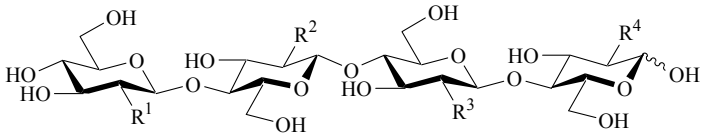
Deprotection steps were carried out starting from removal of the DMM group in **58** by treatment with 0.5 M NaOH in aqueous dioxane, followed by addition HCl in the presence of ethanolamine to pH 5,[121,131] then acetylation yielded *N*-phthaloyl-*N*′-acetyl derivative **59** in 63% (Scheme 41). The ^{1}H NMR spectroscopy exhibited the disappearance of the methyl signals of the DMM group and the appearance of an NH signal at δ 5.65 ppm and of a new methyl signal at δ 1.84 ppm. Next, de-*O*-acetylation of **59** and subsequent de-*N*-phthaloylation afforded disaccharide **60** (95%). Transformation of the *N*-phth group into the amino group was confirmed by the upfield shift of the signal of 2b-H from δ 4.31 to 2.60 ppm. Finally, catalytic hydrogenation of compound **60** using 10% Pd/C as a catalyst in EtOH/H_2O solution provided chitobiose **5**[52] in 86% yield.



Scheme 41. Deprotection of disaccharide **58** to provide mono-*N*-acetyl chitobiose **5**.

2.3 Synthesis of partially *N*-acetylated chitotetraoses

After we had succeeded in the synthesis of fully and partially *N*-acetylated chitobioses **3**-**5**, we then extended our attention to the synthesis of hetero chitotetrasaccharides, i.e. GlcN-GlcNAc-GlcN-GlcNAc (**61**), GlcNAc-$(GlcN)_2$-GlcNAc (**62**) and $(GlcNAc)_2$-$(GlcN)_2$ (**63**) (Figure 7), which can serve as useful substrates for studying the specificity and mechanism of chitinase and/or lysozymes, as well as providing an information concerning the relationship between structure and biological activity of chitooligosaccharide.



Compound	R^1	R^2	R^3	R^4
61	NH_2	NHAc	NH_2	NHAc
62	NHAc	NH_2	NH_2	NHAc
63	NHAc	NHAc	NH_2	NH_2

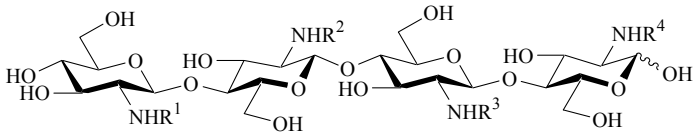
Figure 7. Structure of GlcN-GlcNAc-GlcN-GlcNAc (**61**), GlcNAc-$(GlcN)_2$-GlcNAc (**62**) and $(GlcNAc)_2$-$(GlcN)_2$ (**63**).

2.3.1 Retrosynthetic strategy

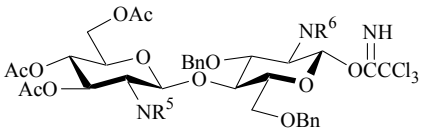
Based on a convergent synthesis strategy, the desired tetrasaccharide **61** was retrosynthesized into two disaccharide precursors (**57** and **68**) as illustrated in Scheme 42. Since disaccharide **57** had already been prepared in the previous section (2.2.5), another disaccharide **68** could be synthesized as the glycosyl acceptor via coupling monosacharide acceptor **50** with trichloroacetamidate **65**.

The construction of tetrasaccharide **62** required a disaccharide donor **52** which had already been prepared (Section 2.2.4) and disaccharide acceptor **68**. On the other hand, for the synthesis of tetrasaccharide **63**, two different building blocks should be synthesized, the disaccharide donor **67** and the disaccharide **69** as a key glycosyl acceptor. Intermediates **67** and **69** could be readily assembled from monosaccharides **40**, **48**, **50** and **65**. The levulinate ester[158,159] was chosen as the temporary protecting group in the

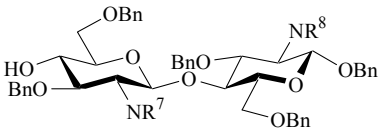
synthesis of the building block **65** which is easily removed with hydrazine[159] after coupling with an appropriate acceptor to provide the new disaccharide acceptor for further glycosylation.



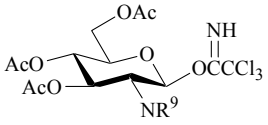
GlcN-GlcNAc-GlcN-GlcNAc (**61**): R^1 = H, R^2 = Ac, R^3 = H, R^4 = Ac
GlcNAc-$(GlcN)_2$-GlcNAc (**62**): R^1 = Ac, R^2, R^3 = H, R^4 = Ac
$(GlcNAc)_2$-$(GlcN)_2$ (**63**): R^1, R^2 = Ac, R^3, R^4 = H



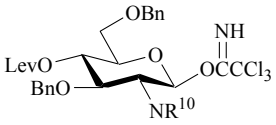
52: R^5 = DMM, R^6 = Phth
57: R^5 = Phth, R^6 = DMM
67: R^5 = DMM, R^6 = DMM



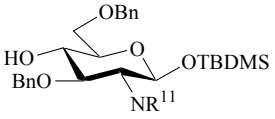
68: R^7 = Phth, R^8 = DMM
69: R^7 = Phth, R^8 = Phth



48: R^9 = DMM



65: R^{10} = Phth



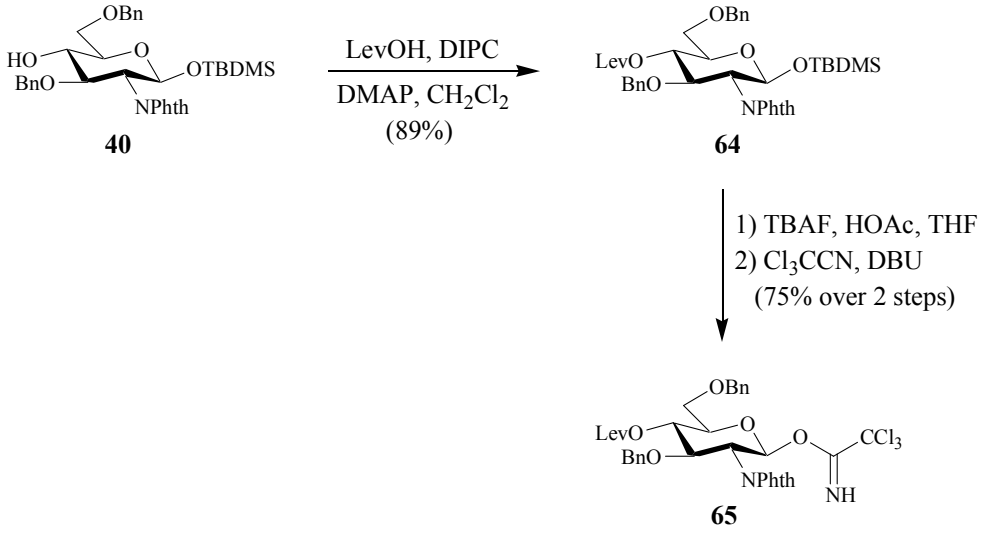
40: R^{11} = Phth
50: R^{11} = DMM

Scheme 42. Retrosynthetic analysis of GlcN-GlcNAc-GlcN-GlcNAc (**61**), GlcNAc-$(GlcN)_2$-GlcNAc (**62**) and $(GlcNAc)_2$-$(GlcN)_2$ (**63**).

2.3.2 Preparation of monosaccharide donor

The new *N*-Phth-protected donor **65** could be synthesized from monosaccharide **40** in a three steps sequence as depicted in Scheme 43. Levulinoylation[160] of the liberated hydroxyl group of **40** with levulinic acid, *N*,*N*'-diisopropylcarbodiimide (DIPC) and 4-(dimethylamino)pyridine (DMAP) afforded fully protected monosaccharide **64** (89%).

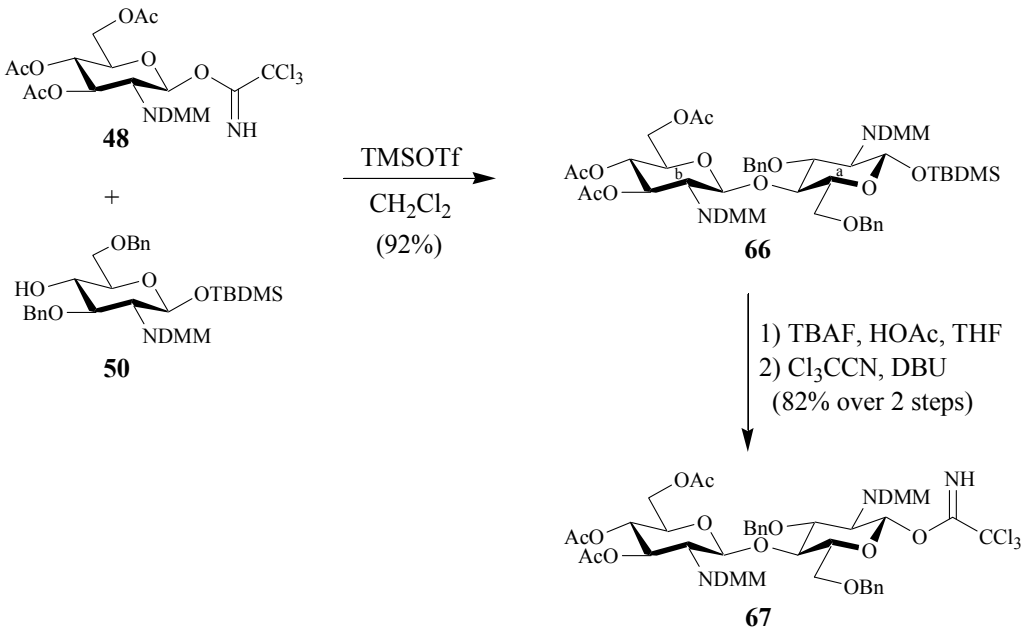
Conversion of **64** to the corresponding β-trichloroacetimidate **65** by cleavage of the anomeric silyl ether with TBAF and subsequent treatment of the hemiacetal intermediate with trichloroacetonitrile and catalytic amounts of DBU was readily achieved in 75% yield. [^{1}H NMR: δ 6.43 ppm, $J_{1,2}$ = 8.4 Hz, 1$_\beta$-H].



Scheme 43. Preparation of β-trichloroacetamidate **65**.

2.3.3 Preparation of disaccharide donor

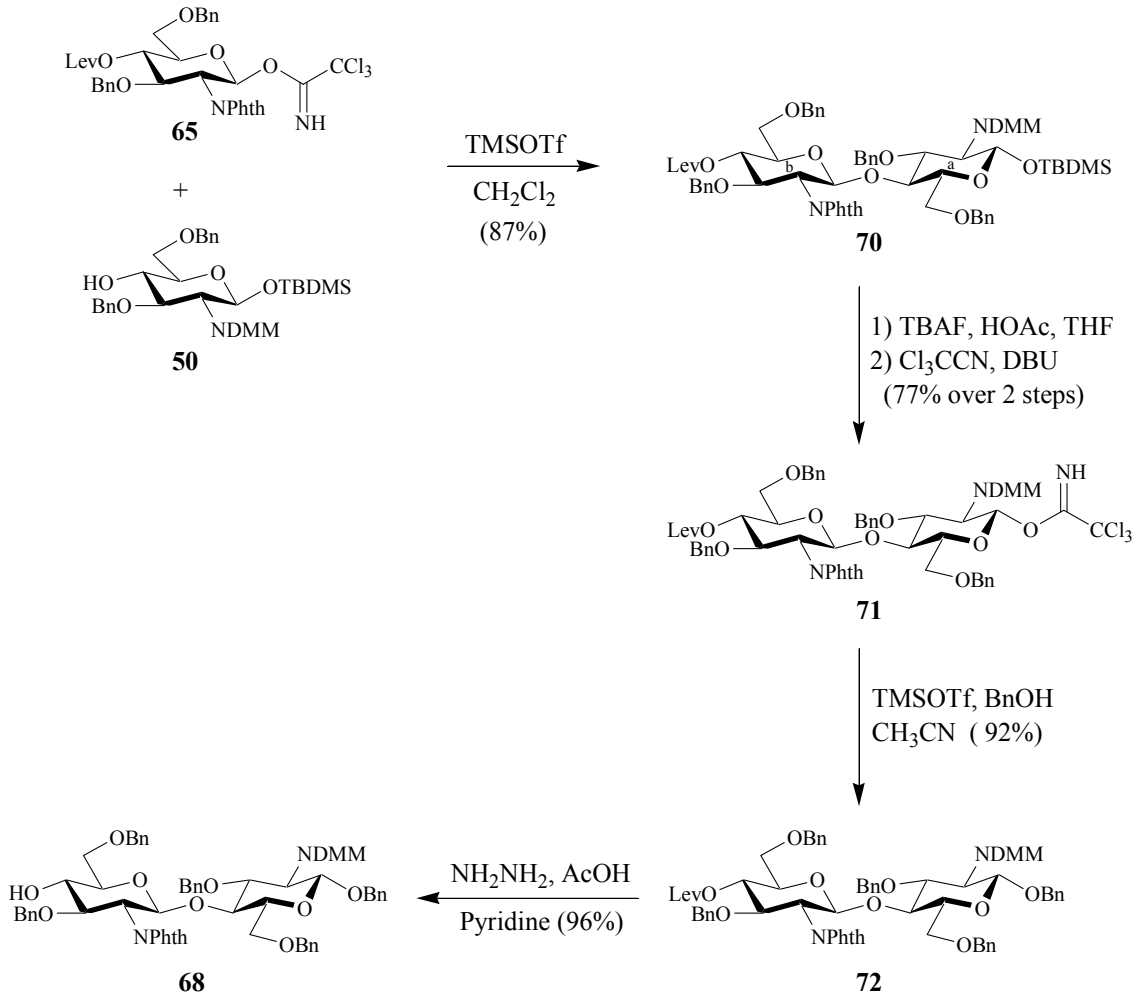
The required disaccharide donor **67** was synthesized from acceptor **50** by glycosylation with donor **48** under the catalysis of TMSOTf (0.1 equiv.) in CH_2Cl_2 at −78 ºC to yield the β-linked disaccharide **66**[131] in 92% (Scheme 44). [^{1}H NMR: δ 5.38 ppm, $J_{1,2}$ = 8.4 Hz, 1b-H]. Conversion of compound **66** to disaccharide trichloroacetimidate building block **67**[131] was achieved in 82% by cleavage of the TBDMS ether with TBAF and reaction with trichloroacetonitrile in the presence of DBU. Only the β-anomer was observed, as evidenced by ^{1}H NMR spectroscopy [δ 6.15 ppm, $J_{1,2}$ = 8.5 Hz, 1a$_\beta$-H].



Scheme 44. Preparation of disaccharide donor **67**.

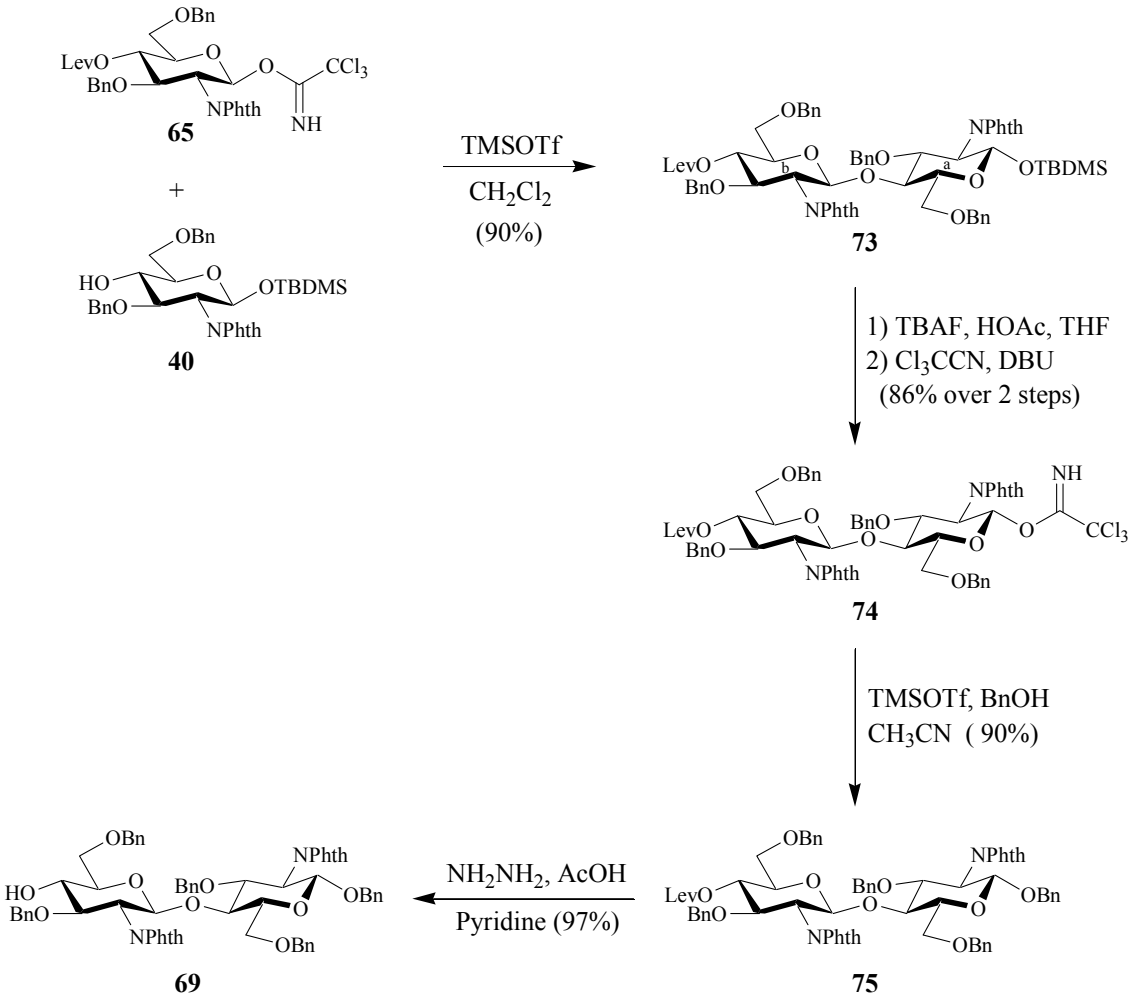
2.3.4 Preparation of disaccharide acceptors

The synthesis of disaccharide acceptor **68** was accomplished in a straightforward manner starting from acceptor **50** (Scheme 45). TMSOTf-catalysed glycosylation of **50** with donor **65** in CH_2Cl_2 at 0 ºC afforded the corresponding disaccharide **70** in 87% yield. The NMR data and coupling constant values indicated the presence of the 1,2-*trans* glycosidic linkage: ^{1}H NMR [δ = 4.99 ppm, $J_{1,2}$ = 8.5 Hz, 1a-H; δ = 5.35 ppm, $J_{1,2}$ = 8.5 Hz, 1b-H]. Anomeric *O*-desilylation of **70** with TBAF and subsequent imidation with trichloroacetonitrile and DBU yielded disaccharide **71** in 77%. Coupling of compound **71** as glycosyl donor with benzyl alcohol using 0.01 equiv. of TMSOTf as a catalyst in acetonitrile at room temperature led to benzyl glycoside **72** (92%). Removal of the levulinic (Lev) ester[160] of **72** with hydrazine monohydrate in a pyridine-AcOH solution proceeded in 96% yield to give acceptor **68**.



Scheme 45. Preparation of disaccharide acceptor **68**.

In an analogous manner, the reducing end disaccharide **69** was synthesized (Scheme 46). The glycosyl acceptor **40** was coupled with trichloroacetimidate **65** under catalysis by TMSOTf (0.05 equiv.) at 0 ºC providing disaccharide **73** in 90% yield. The ^{1}H NMR spectrum supported the formation of β linkage: δ = 5.15 ppm, $J_{1,2}$ = 8.0 Hz, 1a-H; δ = 5.35 ppm, $J_{1,2}$ = 8.0 Hz, 1b-H. Compound **73** was *O*-desilylated with TBAF and the resulting anomeric hydroxy group was transformed via the corresponding trichloroacetimidate **74** into the 1-*O*-benzyl ether **75**. Delevulinoylation of **75** with hydrazine monohydrate led to **69**,[161] a disaccharide glycosyl acceptor.



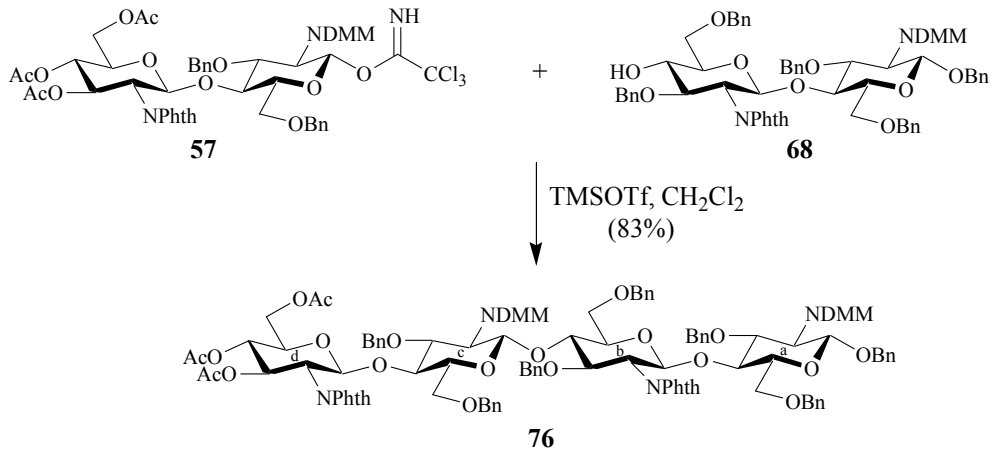
Scheme 46. Preparation of disaccharide acceptor **69**.

With the key intermediates disaccharide donors and acceptors in hand, the attention was directed to the assembly of the desired tetrasaccharides **61**-**63**.

2.3.5 Synthesis of GlcN-GlcNAc-GlcN-GlcNAc (61)

The reaction of donor **57** with disaccharide acceptor **68** proceeded smoothly in the presence of TMSOTf (0.1 equiv.) as a catalyst at –30 ºC affording the tetrasaccharide **76** in 83% yield (Scheme 47). Its ^{1}H NMR spectrum showed a doublet at δ 5.10 ppm for the newly formed glycosidic bond with a coupling constant of 7.5 Hz, thus confirming the β-

configuration. Moreover, its ^{13}C NMR spectrum showed signals at δ 96.7 (2C), 96.8 and 97.2 ppm, confirming the presence of the four anomeric centers.

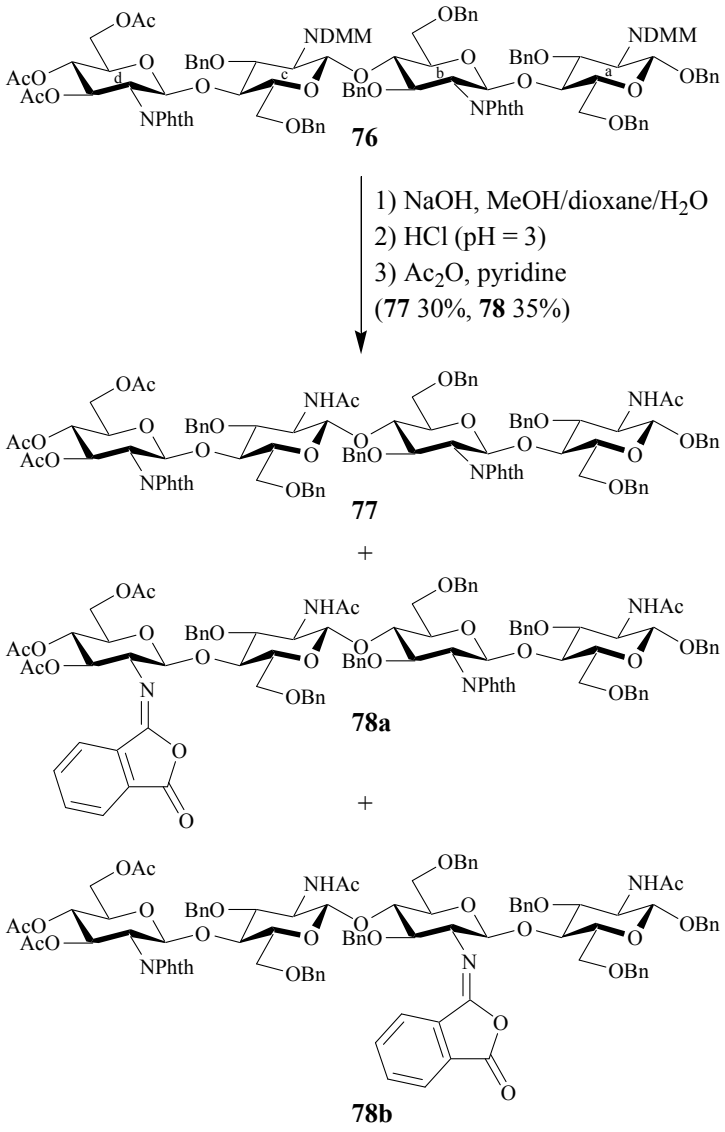


Scheme 47. Synthesis of tetrasaccharide **76**.

Conversion of the *N*-DMM groups of the fully protected tetrasaccharide **76** into the corresponding *N*-acetyl groups was performed by treatment of compound **76** with 1.5 M NaOH in a MeOH-dioxane-water mixture, followed by addition of HCl in the presence of ethanolamine to pH 3, and then acetylation (Scheme 48). The complete transformation of the starting material on the TLC confirmed the formation of new products. However, an unexpected result was obtained after chromatographic purification of the reaction mixture. Two fractions with same molecular mass were isolated almost in the same quantities, (MALDI-MS: *m/z* = 1785.92 $[M+Na]^+$). The analysis of the NMR spectra of the first fraction indicated the expected tetrasaccharide **77**. The ^{13}C NMR spectrum exhibited the disappearance of the methyl signals of the DMM groups and ^1H NMR spectrum showed the appearance of the NH signals at δ 4.92 (NHc) and 5.67 (NHa) ppm. The side-product obtained by the reaction was a mixture of isoimides **78a** and **78b** according to analysis by ^{13}C NMR spectroscopy: δ 164.1, 164.2 ppm (C=N).[162] The yield of compounds **77** and **78** were 30% and 35% respectively.

Varying the reaction conditions for removal of the DMM groups did not prevent the formation of the side-product. Variation of concentration of NaOH (0.5 and 1.0 M) affected the deblocking of the DMM groups. The reaction using 0.5 and 1.0 M NaOH

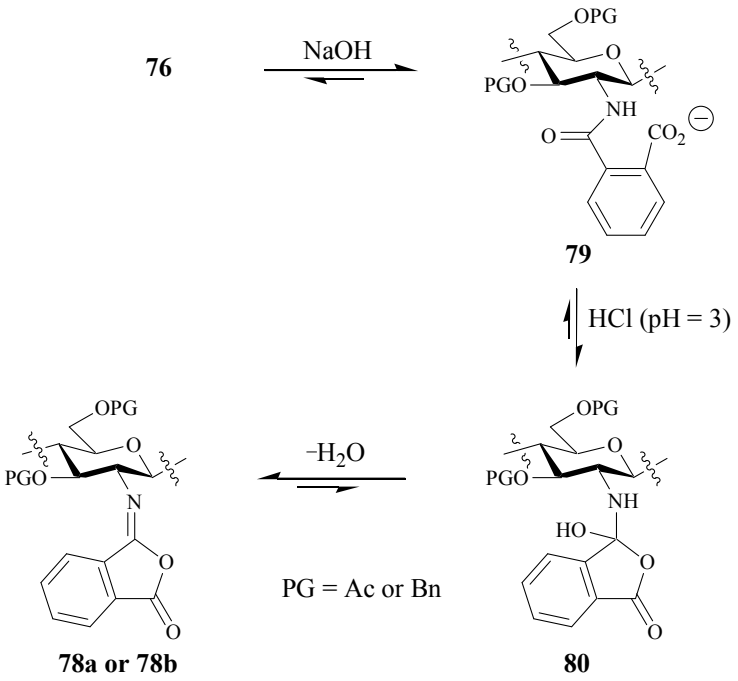
gave the partially DMM deprotected product together with the fully deprotected one as evidenced by MALDI-MS of the crude product. In addition, the undesired products **78a** and **78b** were always obtained after purification. Varying the pH of the reaction from pH 3 to 5 had no effect on the ratio of the desired product to the side-product.



Scheme 48. Deprotection of the DMM protecting groups of tetrasaccharide **76**.

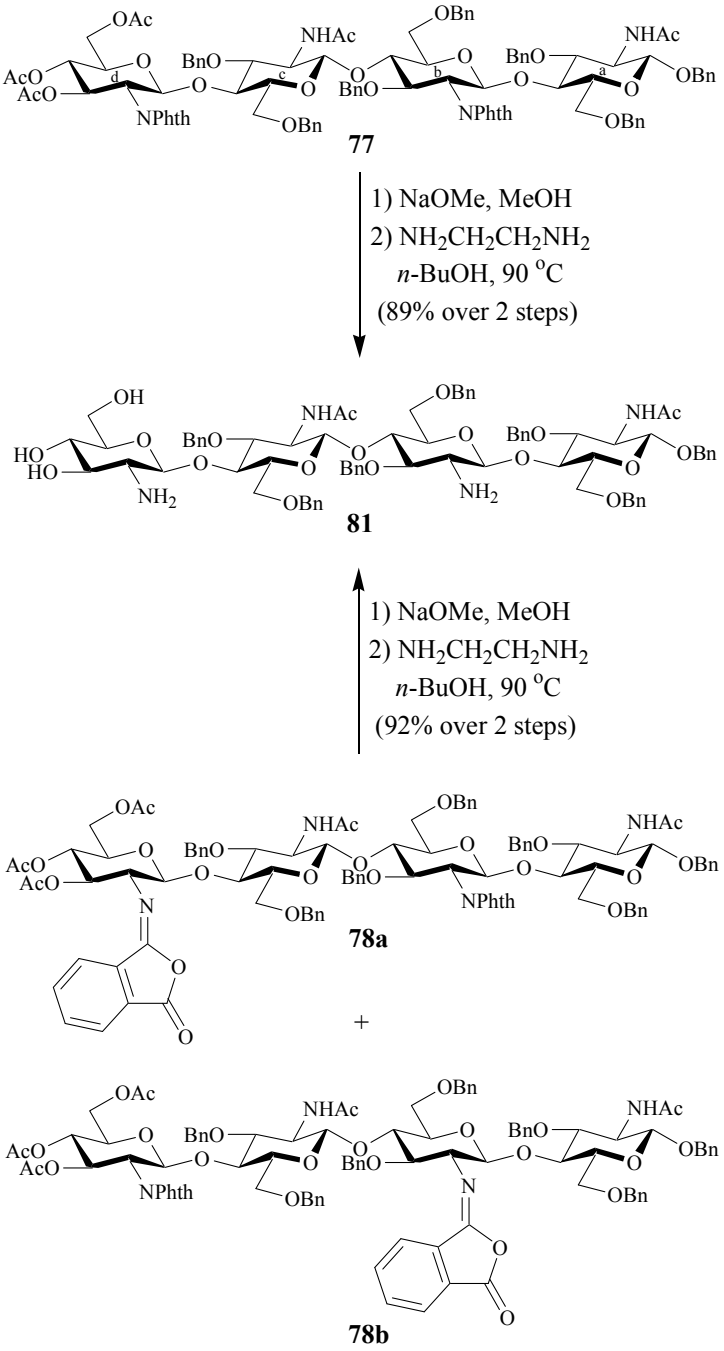
A proposed mechanism for the formation of the isoimides **78a** and **78b** is shown in Scheme 49. Under the reaction conditions, NaOH reacts with phthaloyl groups of tetrasaccharide **76** leading to an intermediate **79** which in the presence of acid is in

equilibrium with butenolide **80**. Elimination of water from **80** provides the isoimides **78a** and **78b**.



Scheme 49. Proposed mechanism for the formation of isoimides **78a** and **78b**.

Obviously, the Phth groups are not stable under the reaction conditions used to remove the DMM groups leading to the undesired products **78**. Fortunately, the further depotection step of both **77** and **78** furnished an identical product (Scheme 50). *O*-Deacetylation of tetrasaccharide **77** by treatment with sodium methanolate in MeOH provided triol intermediate which directly reacted with ethalenediamine in *n*-BuOH at 90 ºC overnight to provide the expected benzyl glycoside **81** in 89%. Reaction of isoimide derivatives **78**, under the same reaction conditions, gave **81** in 92% yield.[162]



Scheme 50. Removal of the phthalimide or the phthalisoimide group.

The structure of compound **81** was assessed by ^{1}H NMR spectroscopy. The proton at 2-position of **81** appeared upfield compared to the data of the fully protected compound **76**, as summarized in Table 2.

Table 2. Comparison of the ^{1}H NMR data of the tetrasaccharides **76** and **81**.

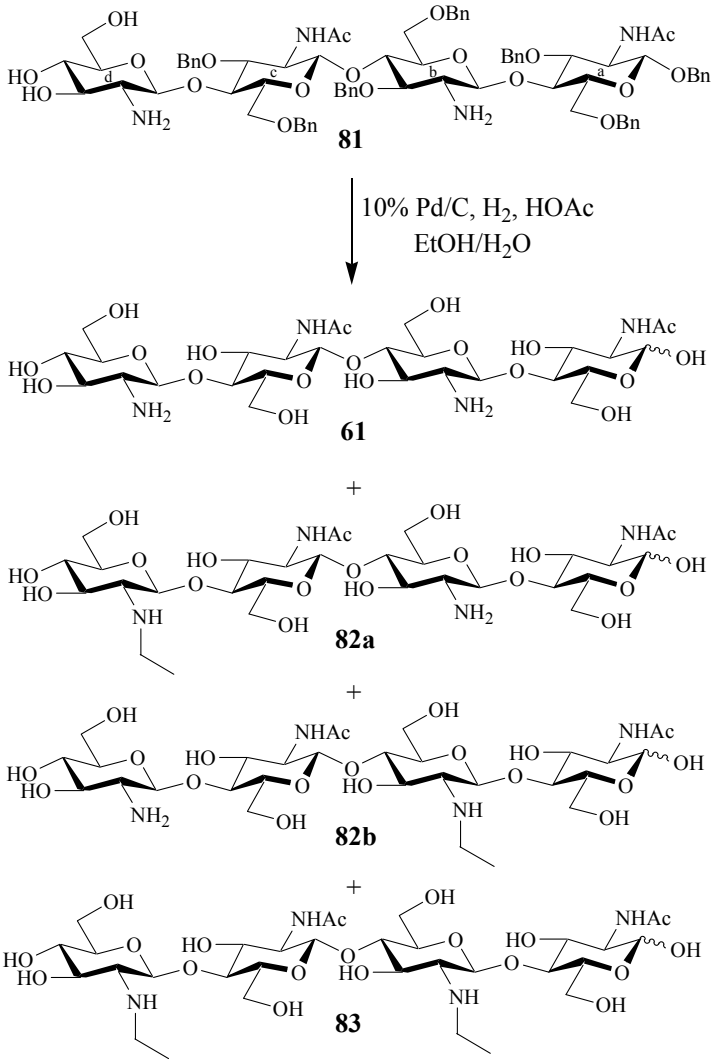
Compound		δ (ppm)	Multiplicity	Coupling constant (Hz)
76:	2a-H	3.87	m	-
	2b-H	3.88	m	-
	2c-H	4.07	m	-
	2d-H	4.30	dd	$J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5
81:	2a-H	3.60	m	-
	2b-H	2.76	dd	$J_{1,2}$ = 8.0, $J_{2,3}$ = 9.5
	2c-H	3.69	m	-
	2d-H	2.51	dd	$J_{1,2}$ = 8.0, $J_{2,3}$ = 9.0
	NHa	5.31	br s	-
	NHc	5.68	d	$J_{2,NH}$ = 8.0

br s = broad singlet, d = doublet, dd = double doublet, m = multiplet

To reach the target molecule **61**, the benzyl groups of the triol **81** were removed (Scheme 51). Thus, hydrogenation of **81** was performed by using 10% Pd/C (1:1 weight ratio) as a catalyst in 6:1 EtOH/H_2O as solvent at room temperature. The progression of the reaction was monitored by using MALDI-MS. After 6 days, the spectrum of crude product exhibited the absence of starting material and the appearance of a peak at *m/z* 769.73 $[M+Na]^+$ which belongs to the desired final product **61** and at *m/z* 797.71 and 825.74 of the side-products **82a**, **82b** and **83** (Figure 8). We assumed that the formation of undesired products **82** and **83** might result from *N*-alkylation of the free amine of benzyl glycoside **81** with EtOH[163-166] which was catalyzed by palladium used in the reaction. The NMR analysis of the crude product obtained after acetylation supported our hypothesis. Its ^{1}H NMR spectrum showed CH_3 signal of *N*-ethyl group at δ 1.1-1.2 ppm and CH_2 signal at δ 3.20-3.30 ppm.[167] In addition, its ^{13}C NMR spectrum showed CH_3 signal at δ 14.1 ppm and CH_2 signal at δ 37.5 ppm.

Changing the solvent used in the reaction from EtOH/H_2O into 2-PrOH/H_2O solely resulted in the expected tetrasaccharide **61**. We assumed that 2-PrOH is less reactive than EtOH to produce the *N*-alkylated product. Treatment of triol **81** with 10% Pd/C (1:1 weight ratio) in 4:1 2-PrOH/H_2O solution at room temperature for 7 days furnished partially *N*-acetylated chitotetraose **81** in 82% yield. *N*-Alkylated product was

not observed under this reaction conditions as evidenced by MALDI-MS and ^{1}H NMR analysis of the crude product. Increasing amount of the catalyst from 1:1 to 1:2.5 (**81**:Pd/C) reduced the reaction time to 2 days and slightly increased the yield to 86%.



Scheme 51. Debenzylation of tetrasaccharide **81** using 10% Pd/C as a catalyst in EtOH/H_2O solution. Note that *N*-alkylation is not observed when carrying out the hydrogenation in 2-PrOH/H_2O.

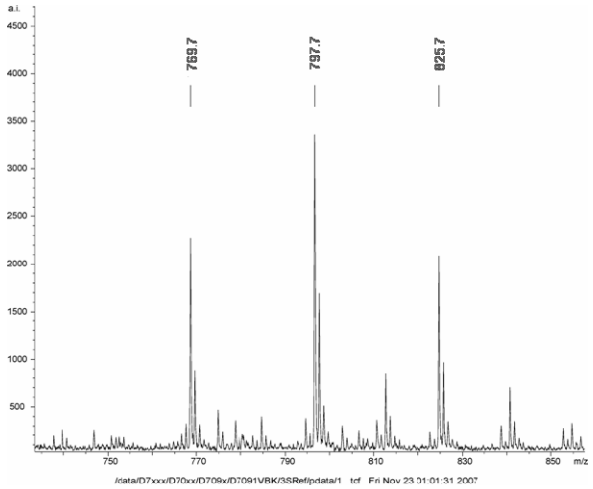
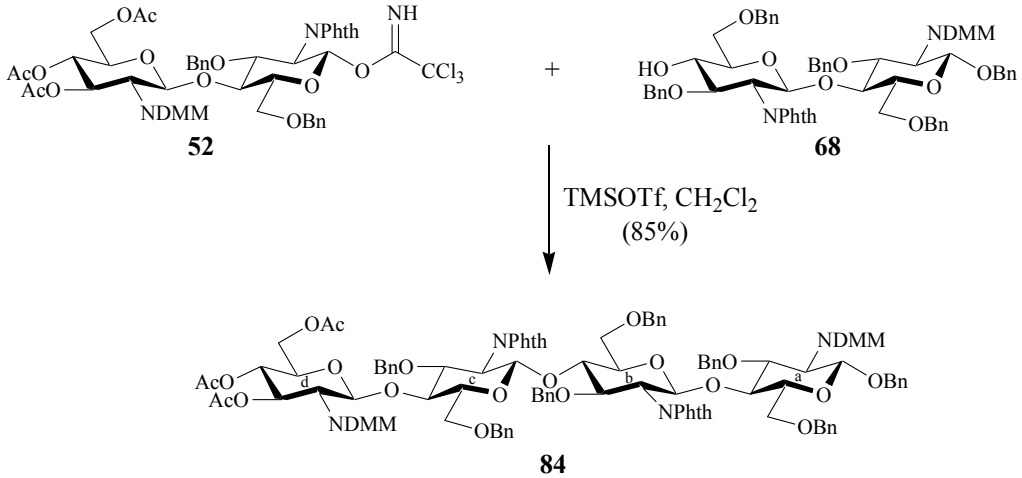


Figure 8. MALDI-MS of crude product obtained from hydrogenation of **81** with 10% Pd/C in EtOH/H_2O solution.

2.3.6 Synthesis of GlcNAc-(GlcN)$_2$-GlcNAc (62)

The synthesis of tetrasaccharide **62** was designed starting from the donor **52** and acceptor **68**. The fully protected tetrasaccharide **84** was obtained in 85% yield from glycosylation of **52** with **68** under standard conditions (CH_2Cl_2, –30 ºC) in the presence of catalytic amounts of TMSOTf (0.1 equiv.) (Scheme 52). The ^{1}H NMR spectrum showed a doublet of 1c-H proton at δ 5.18 ppm with a *J* value of 8.0 Hz, thus supporting the β-configuration. In addition, the ^{13}C-NMR spectrum showed signals at δ 96.6, 96.7, 96.8 and 97.2 ppm corresponding to the four β-linked anomeric carbons.



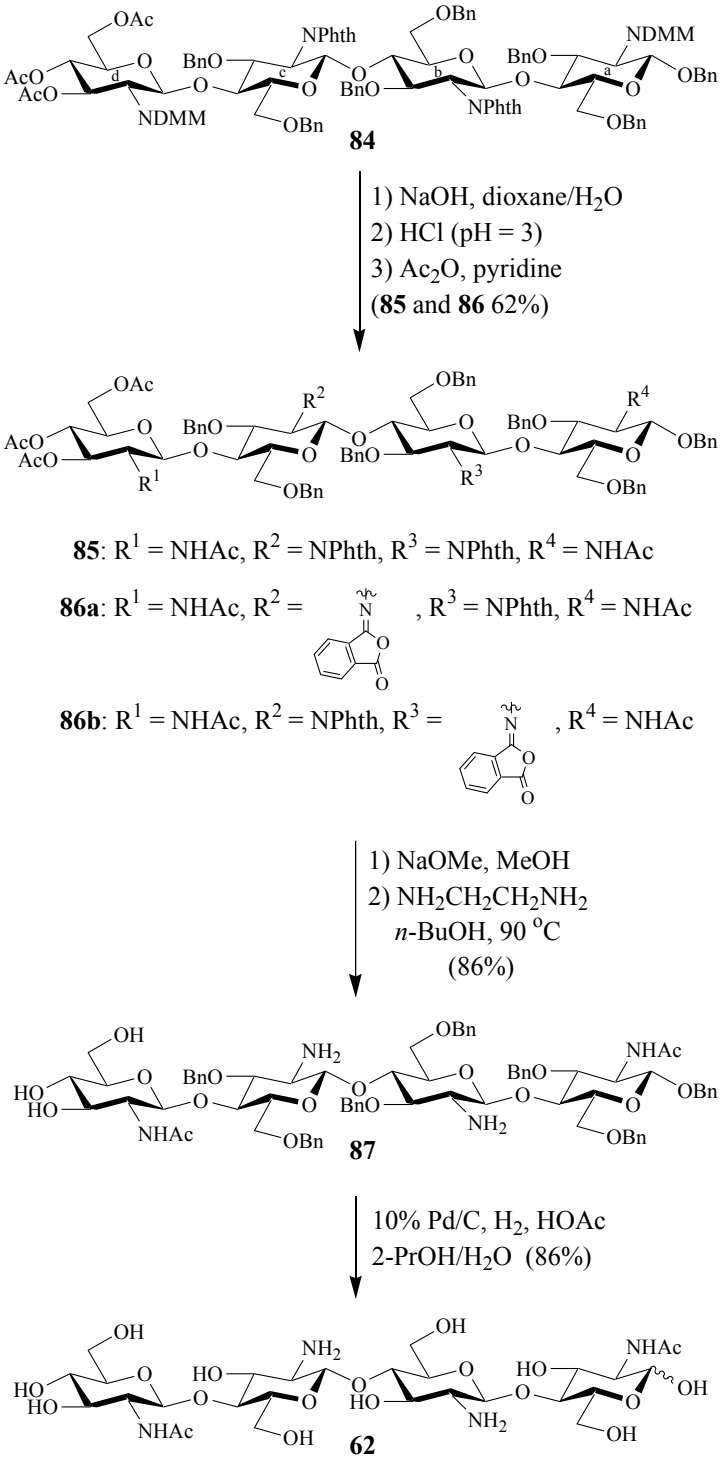
Scheme 52. Synthesis of fully protected tetrasaccharide **84**.

Sequential deblocking of tetrasaccharide **84** was achieved by first converting the *N*-DMM groups into *N*-acetyl groups by treatment with 1.5 M NaOH in aqueous dioxane, followed by addition of HCl in the presence of ethanolamine to pH 3, and then acetylation (Scheme 53).[121,131] Flash column chromatography on SiO_2 to remove the less polar impurity gave a mixture of tetrasaccharide derivative **85** and isoimides **86a** and **86b** in 62% yield. Removal of acetate groups from **85** and **86** under Zemplén condition[152] and subsequent *N*-dephthaloylation/*N*-dephthalisoimidation provided an intermediate **87** in 86% yield. The structural assignment of the dervatives **84** and **87** was performed by HMQC experiment. The data are summarized in Table 3. Finally, hydrogenolytic *O*-debenzylation of **87** with Pd/C catalyst furnished tetrasaccharide **62** in 86% yield.

Table 3. ^{1}H NMR data of the derivatives **84** and **87**.

Compound		δ (ppm)	Multiplicity	Coupling constant (Hz)
84:	2a-H	3.84	m	-
	2b-H	4.07	m	-
	2c-H	4.13	m	-
	2d-H	4.05	m	-
87:	2a-H	3.69	m	-
	2b-H	2.83	dd	$J_{1,2}$ = 8.0, $J_{2,3}$ = 9.5
	2c-H	2.77	dd	$J_{1,2}$ = 7.5, $J_{2,3}$ = 9.0
	2d-H	3.52	m	-
	NHa	5.69	d	$J_{2,NH}$ = 8.0
	NHd	6.37	d	$J_{2,NH}$ = 5.0

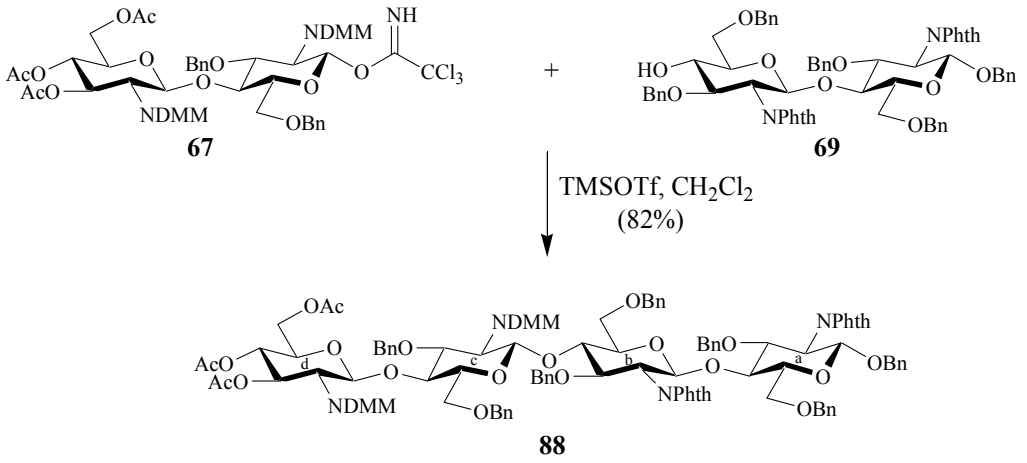
d = doublet, dd = double doublet, m = multiplet



Scheme 53. Deprotection of tetrasaccharide **84** to provide GlcNAc-$(GlcN)_2$-GlcNAc (**62**).

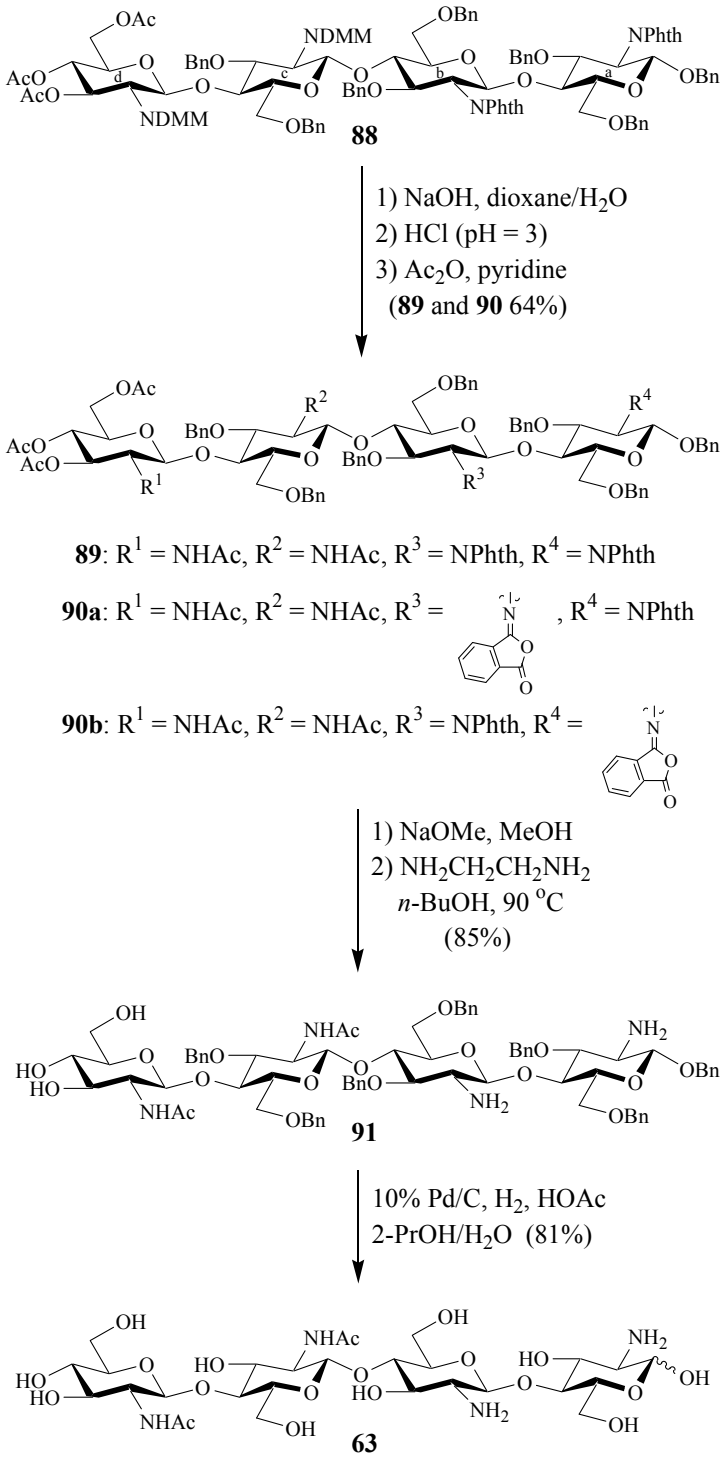
2.3.7 Synthesis of $(GlcNAc)_2$-$(GlcN)_2$ (63)

The synthesis of $(GlcNAc)_2$-$(GlcN)_2$ (**63**) started with the TMSOTf-promoted coupling of acceptor **69** with trichloroacetimidate **67** under standard conditions (CH_2Cl_2, −30 ºC) to afford the β-linked tetrasaccharide **88** in 82% yield (Scheme 54), as evidenced by ^1H and ^{13}C NMR spectroscopy [δ 5.14 ppm, $J_{1,2}$ = 7.5, 1c-H; δ 96.7, 96.8, 97.0, 97.1 ppm, 1-C].



Scheme 54. Synthesis of fully protected tetrasaccharide **88**.

The subsequent deprotection steps were carried out as depicted in Scheme 55. Firstly, removal of two DMM groups of the derivative **88** was effected in a one-pot procedure, as has been previously described for compound **84**. Flash column chromatography on SiO_2 provided a mixture of intermediate **89** and isoimides **90a** and **90b** in 64% yield. *O*-Deacetylation of **89** and **90** and then *N*-dephthaloylation/*N*-dephthalisoimidation led to tetrasaccharide **91** in 85% yield. The structural assignment of the derivatives **88** and **91** was performed by NMR spectroscopy, as summarized in Table 4. In the end, *O*-debenzylation of **91** proceeded smoothly in 81% yield, employing 10% Pd/C-catalyzed hydrogenation to provide tetrasaccharide **63**.



Scheme 55. Deprotection of tetrasaccharide **88** to provide $(GlcNAc)_2$-$(GlcN)_2$ (**63**).

Table 4. ^{1}H NMR data of the derivatives **88** and **91**.

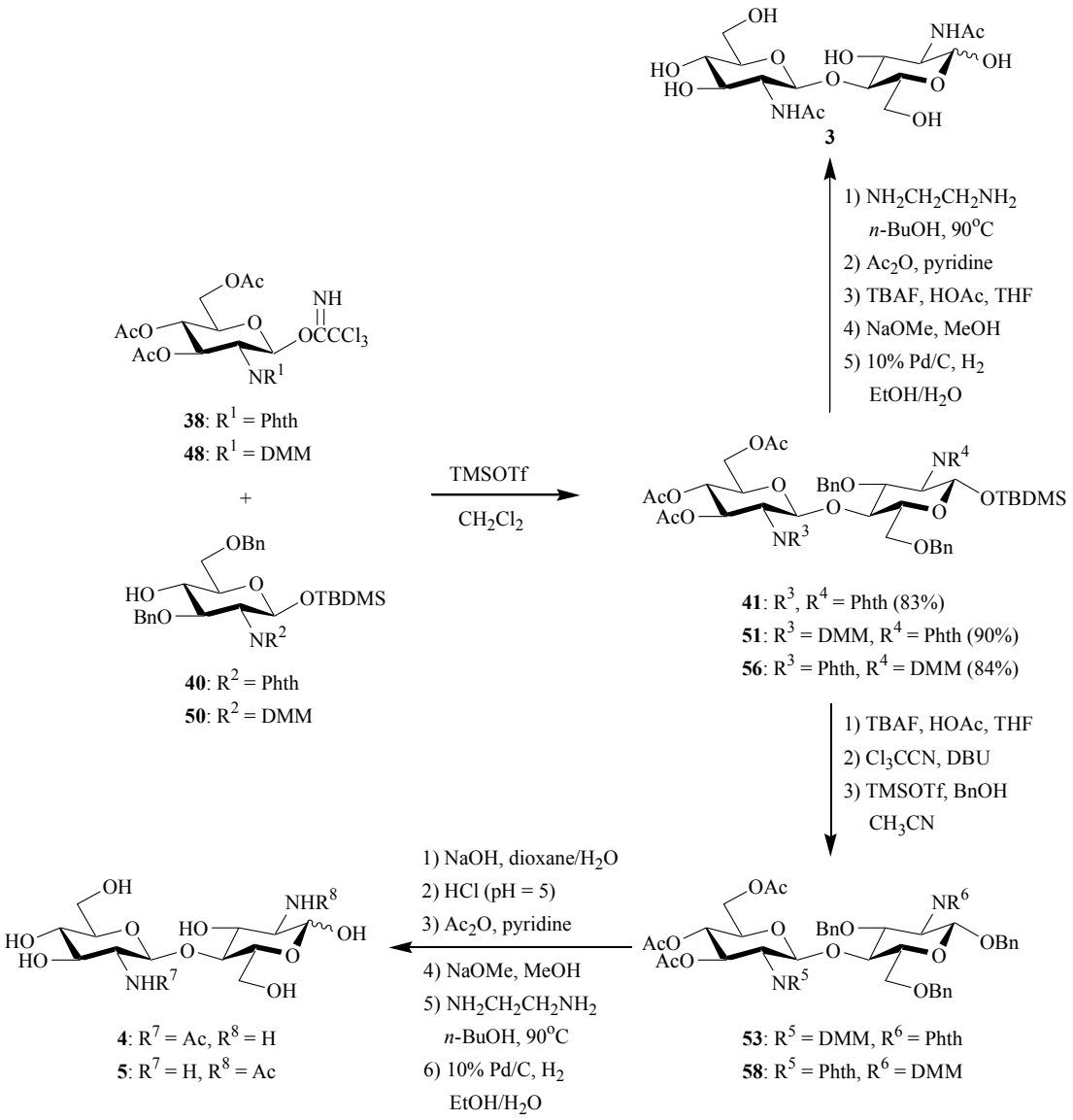
Compound		δ (ppm)	Multiplicity	Coupling constant (Hz)
88:	2a-H	4.13	m	-
	2b-H	3.90	dd	$J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5
	2c-H	4.12	m	-
	2d-H	4.04	m	-
91:	2a-H	2.80	dd	$J_{1,2}$ = 8.5, $J_{2,3}$ = 9.5
	2b-H	2.94	dd	$J_{1,2}$ = 8.5, $J_{2,3}$ = 9.0
	2c-H	3.63	dd	$J_{1,2}$ = 8.5, $J_{2,3}$ = 9.0
	2d-H	3.51	m	-
	NHc	5.22	d	$J_{2,NH}$ = 8.5
	NHd	6.18	d	$J_{2,NH}$ = 5.5

d = doublet, dd = double doublet, m = multiplet

3. Summary

We have demonstrated synthetic strategies for the preparation of hetero-chitooligosaccharides based on the use of dimethylmaleoyl and phthaloyl groups for protection of the amines. Formation of the β-glycosidic linkage was achieved using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoter of the glycosylation reaction with neighboring group participation. Trichloroacetimidate was used as glycosylating agent in the glycosylation step.

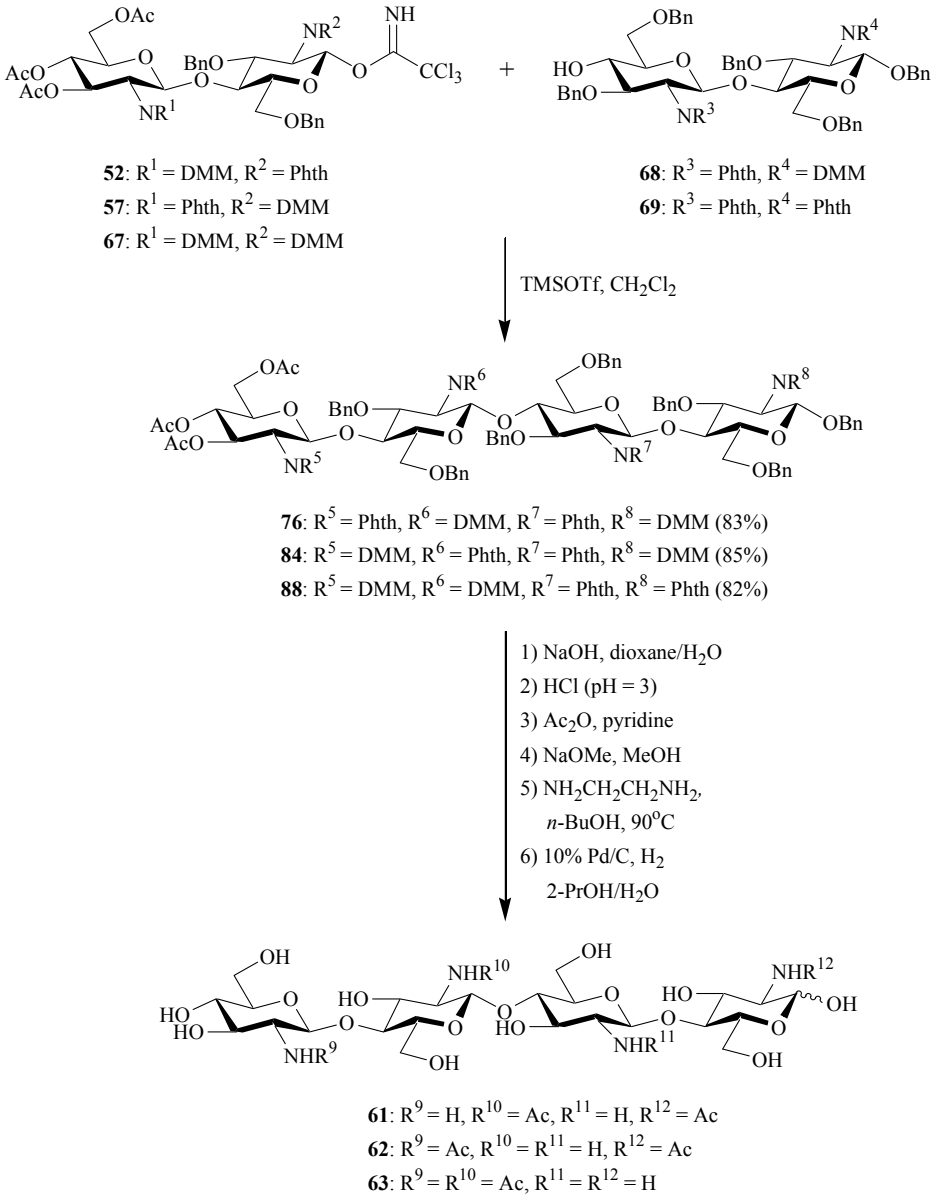
Initially, the synthesis of fully and partially *N*-acetylated chitobioses, i.e. $(GlcNAc)_2$ (**3**), GlcNAc-GlcN (**4**) and GlcN-GlcNAc (**5**), was reported. Dimethylmaleoyl- and phthaloyl-protected glucosamine donors and acceptors could be readily prepared in good yields from commercial D-glucosamine hydrochloride. Glycosylation in the presence of TMSOTf furnished β-linked disaccharide derivatives in excellent yields as illustrated in Scheme 56. Disaccharide **41** could be prepared from donor **38** and acceptor **40** in 83% yield, while disaccharide **51** resulted from coupling acceptor **40** with donor **48** (90%) and disaccharide **56** resulted from coupling acceptor **50** with donor **38** (84%). *N*- and *O*-deprotection of compound **41** via a five steps sequence including removal of *N*-Phth group, *N*-acetylation, subsequent deblocking of silyl ether and acetate groups and finally debenzylation afforded disaccharide **3** in 48% overall yield starting from glycosyl acceptor **40**. Due to an unstability of TBDMS group under the reaction conditions used for removal of the DMM group (addition of NaOH followed by HCl) disaccharides **51** and **56** were transformed via the corresponding trichloroacetimidate intermediates into benzyl glycosides **53** and **58** respectively. Sequential deblocking of **53** was accomplished by first converting the *N*-DMM group into the *N*-acetyl group, followed by removal of acetyl and phthaloyl groups and final hydrogenolysis of the intermediate afforded the desired disaccharide **4** in 30% overall yield starting from monosaccharide **40**. In an analogous manner, removal of the *N*- and *O*-protecting groups from **58** provided disaccharide **5** in 31% overall yield starting from monosaccharide **50**.



Scheme 56. Synthesis of fully and partially *N*-acetylated chitobioses.

Further, hetero-chitotetraoses, i.e. GlcN-GlcNAc-GlcN-GlcNAc (**61**), GlcNAc-$(GlcN)_2$-GlcNAc (**62**) and $(GlcNAc)_2$-$(GlcN)_2$ (**63**), were prepared based on the convergent synthesis strategy as shown in Scheme 57. Activation of **57** with TMSOTf in the presence of the acceptor **68** led to tetrasaccharide **76** in 83% yield. Removal of the *N*- and *O*-protecting groups from **76** by the similar sequence as described for disaccharide **53** afforded the desired tetrasaccharide **61** in 42% overall yield starting from disaccharide **68**. Tetrasaccharide **62** was synthesized in 39% overall yield through a coupling reaction of

disaccharide acceptor **68** with imidate **52** and subsequent *N*- and *O*-deprotection. Similary, condensation of acceptor **69** with donor **67** furnished compound **63** in 36% overall yield after deprotection steps.



Scheme 57. Synthesis of partially *N*-acetylated chitotetraoses.

The general strategy elaborated in this work can be applied to the synthesis of higher hetero-chitooligosaccharides as well as oligosaccharides containing GlcNAc and GlcN moieties.

4. Experimental Part

4.1 General remarks

Solvents

The solvents were dried according to standard methods by distillation over drying agents. DMF was heated under reflux for 3 h over calcium hydride and distilled. Methanol was treated with magnesium turnings, heated under reflux for 3 h and distilled. *n*-Butanol was purchased from Acros. Pyridine was purchased from ABCR, Karlsruhe. THF was freshly distilled from sodiumbenzophenone ketyl under nitrogen. Dichloromethane was freshly distilled from phosphorus pentoxide under nitrogen.

Chromatography

• **Thin layer chromatography (TLC)** was performed using aluminium 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 one of following reagents followed by gentle heating with a heat gun:

- Mostaine [ammonium molybdate (20 g) and cerium (IV) sulfate (0.4 g) in 10% aq. sulfuric acid (400 mL)]
- *m*-Methoxyphenol (0.45 mL) and conc. sulfuric acid (12 mL) in ethanol (450 mL).

• **Flash column chromatography** was performed using SiO_2 60 (Merck, 0.040-0.063 mm).

• **Size-exclusion chromatography** was carried out on Bio-Gel P-2 (extra fine).

Analytical data

• **Melting points** were recorded with an Electrothermal IA9100 and were uncorrected.

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

• **Infrared** spectra were determined with a Perkin Elmer FT-IR 16 PC. The absorption bands were reported in wave number (cm^{-1}). For the band characterization the following abbreviations were applied: br (broad), s (strong), m (medium), w (weak).

• **NMR** spectra were carried out with Bruker ARX-300, ARX-500 or DRX-600 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_3OD (δ_H: 4.84, δ_C: 49.0) or by reference to acetone (δ_H: 2.25, δ_C: 30.8) for solutions in D_2O. Coupling constants (*J*) are reported in Hertz.

All assignments were based on APT, COSY, HMQC and HMBC experiments. For the characterization of the observed signal multiplicities the following abbreviations were applied: br (broad), s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet). In the case of oligosaccharide the monosugar unit was indicated by a,b,c,…, beginning with the sugar at right end.

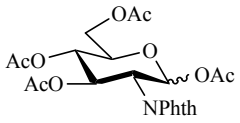
• **MALDI-MS** were determined with a Bruker Reflex II mass spectrometer using 2,4,6-trihydroxyacetophenone (THAP) or 2,5-dihydroxybenzoic acid (DHB) as a matrix.

• **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.

4.2 Experimental

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranoside (37)[147]



A solution of D-glucosamine hydrochloride (8.63 g, 40.0 mmol) in water (80 mL) was added dropwise to a mixture of phthalic anhydride (7.11 g, 48.0 mmol) and $NaOAc \cdot 3H_2O$ (6.53 g, 48.0 mmol) in 4:1 acetone-water (180 mL). After 15 min, $NaHCO_3$ (5.04 g, 60.0 mmol) was added in a small portions over 15 min. The reaction mixture was stirred at room temperature overnight, then dried well in vacuo. The residue was treated with 1:1 pyridine-acetic anhydride (120 mL), warmed to 60 ºC with stirring for 30 min and then continued stirring at room temperature overnight. The mixture was quenched with EtOH at 0 ºC for 15 min and then co-concentrated with toluene. A solution of the residue in

EtOAc (300 mL) was washed with aq. HCl (3%, 150 mL), water (200 mL), aq. saturated $NaHCO_3$ (150 mL), water (200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2) to yield **37** (18.4 g, 96%; Lit.[147]: 98%) as a white foam in the ratio of α:β = 1.5:1.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.41.

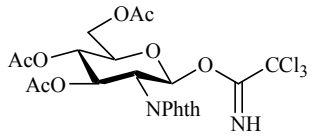
m.p. 89-91 °C. Lit.[168]: 91-94 °C.

IR (KBr): 2937 (w), 1756 (s), 1721 (s), 1613 (w), 1468 (w), 1432 (w), 1384 (s), 1221 (s), 1149 (m), 1079 (s), 1036 (s), 969 (w), 940 (w), 797 (w), 723 (s) cm^{-1}.

^{1}H and ^{13}C NMR: in accordance with literature data.[169]

ESI-MS calcd for $C_{22}H_{23}NO_{11}Na$ $[M+Na]^+$: 500.1169. Found: 500.1179.

_O_-[3,4,6-Tri-_O_-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl] trichloroacetimidate (38)[170]



A solution of **37** (15.3 g, 32.0 mmol) and hydrazine acetate (4.42 g, 48.0 mmol) in dry DMF (64 mL) was stirred at room temperature. After 1.5 h, the reaction mixture was diluted with EtOAc (250 mL) and washed with ice-cold aq. saturated $NaHCO_3$ (3 × 150 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. To a solution of the residue in dry CH_2Cl_2 (64 mL) was added trichloroacetonitrile (12.8 mL, 128 mmol) and DBU (0.9 mL, 6.40 mmol). The mixture was stirred at room temperature for 5 h, and then concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2 + 1% Et_3N) to yield **38** (13.6 g, 75%; Lit.[148]: 73%) as a pale yellow foam.

TLC (*n*-Hexane/EtOAc, 2:1): R_f = 0.38.

m.p. 73-75 °C. Lit.[148]: 146 °C.

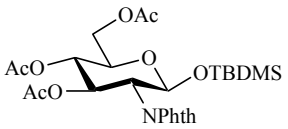
$[\alpha]^{33}_D = +72.7$ (c = 1.0, $CHCl_3$). Lit.[148]: $[\alpha]^{25}_D = +76.0$ (c = 1.0, $CHCl_3$).

IR (KBr): 3316 (w), 2954 (w), 1752 (s), 1721 (s), 1683 (m), 1468 (w), 1431 (w), 1386 (s), 1231 (s), 1077 (s), 1044 (s), 899 (w), 839 (m), 797 (m), 721 (m) cm^{-1}.

^{1}H NMR: in accordance with literature data.[148]

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 20.4, 20.6, 20.7 (3 CO*C*H_3), 53.6 (2-C), 61.6 (6-C), 68.5 (4-C), 70.4 (3-C), 72.8 (5-C), 93.6 (1-C), 123.7, 131.2, 134.4 (Phth-C), 160.6 (C=NH), 167.4, 169.4, 170.0, 170.6 (CO, 3 *C*OCH_3) ppm.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (39)**[121]



A solution of **37** (17.7 g, 37.0 mmol) and hydrazine acetate (5.11 g, 55.5 mmol) in dry DMF (75 mL) was stirred at room temperature. After 1.5 h, the reaction mixture was diluted with EtOAc (250 mL) and washed with ice-cold aq. saturated $NaHCO_3$ (3 × 150 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. To a solution of the residue and imidazole (5.04 g, 74.0 mmol) in dry CH_2Cl_2 (110 mL) was added TBDMSCl (6.69 g, 44.4 mmol). After stirring at room temperature overnight, the reaction mixture was diluted with water (250 mL), then extracted with CH_2Cl_2 (3 × 150 mL). The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 1:1) to yield **39** (16.3 g, 80%; Lit.[151]: 80%) as white crystals.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.62.

m.p. 138-139 °C. Lit.[151]: 139-140 °C.

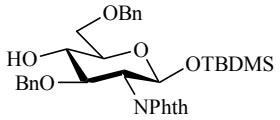
$[\alpha]^{26}_D = +25.7$ (c = 1.0, $CHCl_3$).

IR (KBr): 3474 (w), 2949 (m), 2930 (m), 2894 (m), 2859 (m), 1750 (s), 1715 (s), 1612 (w), 1469 (m), 1387 (s), 1336 (m), 1235 (s), 1171 (m), 1136 (m), 1079 (s), 1041 (s), 841 (s), 782 (m), 724 (s) cm^{-1}.

^{1}H and **^{13}C NMR**: in accordance with literature data.[151]

ESI-MS calcd for $C_{26}H_{35}NO_{10}NaSi$ $[M+Na]^+$: 572.1928. Found: 572.1936.

***tert*-Butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (40)**[121]



A mixture of **39** (12.9 g, 23.5 mmol), dry MeOH (230 mL) and NaOMe (0.65 g, 12.0 mmol) was stirred at room temperature. After 2 h, the reaction mixture was neutralized with Amberlite IR 120 (H^+) resin. The solution was filtered and concentrated in vacuo. A suspension of the crude product and dibutyltin oxide (12.9 g, 51.7 mmol) in toluene (220 mL) was heated under reflux (dean-Stark apparatus). After 15 h, TBAI (19.1 g, 51.7 mmol) and benzyl bromide (6.1 mL, 51.7 mmol) were added and the mixture was gently refluxed. After 3 h, the resulting mixture was cooled, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 4:1) to yield **40** (11.3 g, 80%; Lit.[155]: 71%) as a pale yellow solid.

TLC (*n*-Hexane/EtOAc, 4:1): $R_f = 0.38$.

m.p. 80-84 °C.

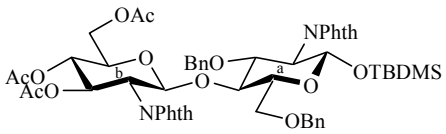
$[\alpha]^{25}_D = +17.7$ (c = 1.0, $CHCl_3$).

IR (KBr): 3473 (w), 3063 (w), 3028 (w), 2928 (m), 2886 (m), 2857 (m), 1773 (m), 1715 (s), 1614 (w), 1495 (w), 1470 (m), 1387 (s), 1256 (m), 1168 (m), 1128 (m), 1086 (s), 970 (w), 863 (s), 839 (s), 785 (m), 742 (s), 723 (s), 699 (m) cm^{-1}.

^{1}H and ^{13}C NMR: in accordance with literature data.[155]

MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 626.24 $[M+Na]^+$, 642.21 $[M+K]^+$.

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



Glycosyl donor **38** (1.87 g, 3.30 mmol) and glycosyl acceptor **40** (1.33 g, 2.20 mmol) were azeotroped with toluene (3 × 6 mL), dried under vacuo for 1 h, and dissolved in dry CH_2Cl_2 (9 mL). The solution was stirred under N_2 atmosphere at −30 °C for 10 min, and TMSOTf (0.1 M in CH_2Cl_2, 3.3 mL, 0.33 mmol) was added dropwise. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **41** (1.88 g, 83%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.69$.

m.p. 80-81 °C.

$[\alpha]^{27}_D = +10.1$ ($c = 0.6$, $CHCl_3$).

IR (neat): 1747 (s), 1713 (s), 1385 (s), 1223 (s), 1046 (s), 838 (s), 782 (m), 720 (s), 699 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = −0.22, −0.06 (2 s, 6 H, 2 $SiCH_3$), 0.60 [s, 9 H, $SiC(CH_3)_3$], 1.84, 1.98, 2.00 (3 s, 9 H, 3 $COCH_3$), 3.33-3.38 (m, 1 H, 5a-H), 3.44 (dd, $J_{gem} = 11.0$, $J_{5,6} = 3.5$ Hz, 1 H, 6a-H), 3.50 (br d, $J = 10.5$ Hz, 1 H, 6'a-H), 3.55-3.60 (m, 1 H, 5b-H), 3.96 (dd, $J_{gem} = 12.5$, $J_{5,6} = 2.0$ Hz, 1 H, 6b-H), 4.07 (dd, $J_{1,2} = 8.0$, $J_{2,3} = 10.0$ Hz, 1 H, 2a-H), 4.18-4.27 (m, 3 H, 3a-H, 4a-H, 6'b-H), 4.34 (dd, $J_{1,2} = 8.5$, $J_{2,3} = 10.5$ Hz, 1 H, 2b-H), 4.49, 4.82 (2 d, $J_{gem} = 12.5$ Hz, 2 H, CH_2Ph), 4.50, 4.59 (2 d, $J_{gem} = 12.0$ Hz, 2 H, CH_2Ph), 5.13 (dd, $J_{3,4} = J_{4,5} = 9.0$ Hz, 1 H, 4b-H), 5.17 (d, $J_{1,2} = 8.0$ Hz, 1 H, 1a-H), 5.56 (d, $J_{1,2} = 8.5$ Hz, 1 H, 1b-H), 5.82 (dd, $J_{2,3} = 10.5$, $J_{3,4} = 9.0$ Hz, 1 H, 3b-H), 6.85-7.94 (m, 18 H, 2 Ph, 2 Phth) ppm.

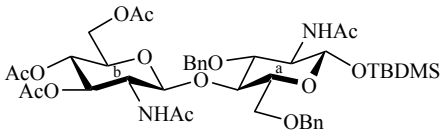
^{13}C NMR (125 MHz, $CDCl_3$): δ = −5.6, −4.4 (2 $SiCH_3$), 17.5 [Si*C*$(CH_3)_3$], 20.4, 20.6, 20.7 (3 CO*C*H_3), 25.2 [SiC(*C*$H_3)_3$], 55.3 (2b-C), 57.8 (2a-C), 61.6 (6b-C), 68.0 (6a-C), 68.9 (4b-C), 70.7 (3b-C), 71.5 (5b-C), 72.7, 73.9 (2 *C*H_2Ph), 74.4, 76.5 (3a-C, 4a-C), 93.2

(1a-C), 97.0 (1b-C), 126.9, 127.4, 127.9, 128.2, 131.4, 131.6, 133.6, 134.4, 138.3, 138.6 (2 Ph-C, 2 Phth-C), 169.5, 170.1, 170.7 (3 *C*OCH_3) ppm.

ESI-MS calcd for $C_{54}H_{60}N_2O_{16}NaSi$ $[M+Na]^+$: 1043.3610. Found: 1021.3574.

$C_{54}H_{60}N_2O_{16}Si$ (1021.14): calcd. C 63.51, H 5.92, N 2.74; found: C 63.44, H 5.95, N 2.74.

***tert*-Butyldimethylsilyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (42)**



A solution of **41** (0.79 g, 0.78 mmol) in *n*-BuOH (70 mL) was stirred at room temperature for 30 min, then ethylenediamine (5.2 mL, 78.0 mmol) was added. The mixture was stirred at 90 °C overnight, then co-concentrated with toluene. The crude product was treated with 2:1 pyridine-acetic anhydride (21 mL) and stirred at room temperature overnight, then co-concentrated with toluene. The residue was dissolved in EtOAc (50 mL) and washed successively with aq. HCl (3%, 50 mL), water (100 mL), aq. saturated $NaHCO_3$ (50 mL), water (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 1:7) to yield **42** (0.53 g, 80%) as a white foam.

TLC (EtOAc/cyclohexane, 7:1): $R_f = 0.49$.

m.p. 99-102 °C.

$[\alpha]^{29}_D = -31.3$ ($c = 0.6$, $CHCl_3$).

IR (neat): 1748 (s), 1655 (s), 1556 (m), 1367 (s), 1226 (s), 1040 (s), 837 (s), 781 (m), 736 (m), 697 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.06, 0.08 (2 s, 6 H, 2 $SiCH_3$), 0.85 [s, 9 H, $SiC(CH_3)_3$], 1.82, 1.86, 1.98, 2.01 (4 s, 15 H, 3 $COCH_3$, 2 $NCOCH_3$), 3.46-3.51 (m, 1 H, 5b-H), 3.53-3.59 (m, 1 H, 5a-H), 3.63-3.70 (m, 2 H, 2a-H, 6a-H), 3.76 (dd, J_{gem} = 10.5, $J_{5,6}$ = 5.0 Hz, 1 H, 6'a-H), 3.84 (dd, $J_{3,4} = J_{4,5}$ = 7.0 Hz, 1 H, 4a-H), 3.94-4.05 (m, 3 H, 3a-H, 2b-H, 6b-H), 4.19 (dd, J_{gem} = 12.5, $J_{5,6}$ = 5.0 Hz, 1 H, 6'b-H), 4.44, 4.63, 4.72, 4.79 (4 d, J_{gem} =

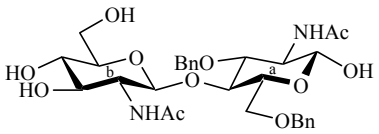
12.0 Hz, 4 H, 2 CH_2Ph), 4.46 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1b-H), 4.88 (d, $J_{1,2}$ = 6.0 Hz, 1 H, 1a-H), 4.96 (dd, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 1 H, 3b-H), 5.04 (dd, $J_{3,4}$ = $J_{4,5}$ = 10.0, 1 H, 4b-H), 5.33 (d, $J_{2,NH}$ = 9.0 Hz, 1 H, 2b-NH), 5.90 (d, $J_{2,NH}$ = 8.5 Hz, 1 H, 2a-NH), 7.23-7.46 (m, 10 H, 2 Ph) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = –5.3, –4.4 (2 $SiCH_3$), 17.9 [Si*C*$(CH_3)_3$], 20.5, 20.6 (3 CO*C*H_3), 23.2 (2 NCO*C*H_3), 25.6 [SiC(*C*$H_3)_3$], 54.4 (2b-C), 55.5 (2a-C), 61.9 (6b-C), 68.5 (4b-C), 69.5 (6a-C), 71.7 (3b-C), 72.7 (5b-C), 73.0, 73.7 (2 *C*H_2Ph), 74.3 (5a-C), 75.7 (3a-C), 77.7 (4a-C), 95.1 (1a-C), 100.3 (1b-C), 127.3, 127.7, 128.2, 128.3, 128.6, 138.0, 138.9 (2 Ph-C), 169.2, 170.0, 170.2, 170.5, 171.0 (3 *C*OCH_3, 2 N*C*OCH_3) ppm.

ESI-MS calcd for $C_{42}H_{61}N_2O_{14}Si$ $[M+H]^+$: 845.3892. Found: 845.3885.

$C_{42}H_{60}N_2O_{14}Si$ (845.02): calcd. C 59.70, H 7.16, N 3.32; found: C 59.30, H 7.37, N 3.26.

2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranose (43)



A solution of **42** (0.44 g, 0.52 mmol) in dry THF (10 mL) in an ice-salt bath was treated with glacial AcOH (35 µL, 0.59 mmol) and TBAF (1.0 M solution in THF, 1.6 mL, 1.60 mmol) with stirring. After 30 min, the ice bath was removed and the mixture was stirred at room temperature overnight. The resulted mixture was diluted with aq. saturated NaCl (30 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. A solution of the residue in dry MeOH (30 mL) was treated with NaOMe (42 mg, 1.04 mmol) and stirred at room temperature. After 2 h, the reaction mixture was neutralized with Amberlite IR 120 (H^+) resin, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, $CHCl_3$/EtOH, 4:1) to yield **43** (0.27 g, 87%) as a white powder.

TLC ($CHCl_3$/EtOH, 8:2): R_f = 0.33.

m.p. 206-207 °C.

IR (neat): 3270 (w), 2870 (w), 1654 (s), 1616 (s), 1537 (m), 1452 (w), 1372 (m), 1323 (w), 1110 (s), 1064 (s), 1052 (s), 1028 (s), 965 (w), 732 (s), 696 (s) cm^{-1}.

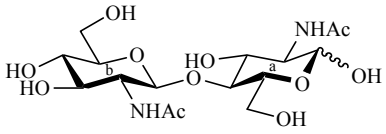
^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.86, 1.88 (2 s, 6 H, 2 $NCOCH_3$), 3.03-3.17 (m, 2 H, 4b-H, 5b-H), 3.31-3.41 (m, 2 H, 3b-H, 6b-H), 3.57-3.65 (m, 2 H, 2b-H, 6a-H), 3.66-3.75 (m, 3 H, 3a-H, 6'a-H, 6'b-H), 3.84-4.00 (m, 3 H, 2a-H, 4a-H, 5a-H), 4.46-4.62 (m, 4 H, 1b-H, C*H*HPh, CH_2Ph), 4.95 (d, J_{gem} = 11.1 Hz, 1 H, CH*H*Ph), 4.97 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1a-H), 7.14-7.32 (m, 10 H, 2 Ph) ppm.

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 22.7, 23.1 (2 $NCOCH_3$), 54.6 (2a-C), 58.4 (2b-C), 63.1 (6b-C), 69.8 (6a-C), 71.9 (5a-C), 72.7 (4b-C), 74.3 (CH_2Ph), 75.6 (3b-C), 76.7 (4a-C), 76.9 (CH_2Ph), 78.7 (5b-C), 80.2 (3a-C), 92.6 (1a-C), 101.5 (1b-C), 128.7, 128.8, 128.9, 129.3, 129.5, 129.6, 139.7, 139.8 (2 Ph-C), 173.2, 173.7 (2 $NCOCH_3$) ppm.

ESI-MS calcd for $C_{30}H_{41}N_2O_{11}$ $[M+H]^+$: 605.2710. Found: 605.2738.

$C_{30}H_{40}N_2O_{11}$ (604.64): calcd. C 59.59, H 6.67, N 4.63; found: C 59.73, H 6.91, N 4.52.

2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (3)



To a solution of **43** (50 mg, 83 μmol) in 2:1 EtOH-water (3 mL) was added 10% Pd/C (25 mg) and 3 drops of HOAc, and the mixture was stirred at room temperature under H_2 atmosphere. After 24 h, the reaction mixture was filtered through Celite, washed with 2:1 EtOH-water (3 × 2 mL), and concentrated in vacuo. Gel-filtration of the residue on a Bio-Gel P-2 column, eluted with water, and subsequent lyophilization yielded **3** (29 mg, 83%) as a white amorphous powder in the ratio of α:β = 3:2.

m.p. 163-165 °C.

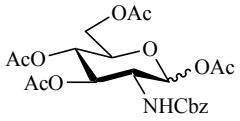
IR (neat): 3284 (br s), 1639 (s), 1550 (s), 1374 (s), 1311 (m), 1039 (s) cm^{-1}.

^{1}H NMR: in accordance with literature data.[52]

^{13}C NMR (75.5 MHz, D_2O): δ = 22.3, 22.5 (2 NCO*C*H_3), 54.0 (2a_α-C), 56.0 (2b-C), 56.5 (2a_β-C), 60.4, 60.6 (6a_α-C, 6a_β-C), 61.0 (6b-C), 69.7, 70.1, 70.4, 72.9, 73.9, 75.0, 76.3, (3a_α-C, 5a_α-C, 3a_β-C, 5a_β-C, 3b-C, 4b-C, 5b-C) 79.9, 80.3 (4a_α-C, 4a_β-C), 90.8 (1a_α-C), 95.2 (1a_β-C), 101.9 (1b-C), 174.9, 175.0, 175.1 (2 N*C*OCH$_3$) ppm.

ESI-MS calcd for $C_{16}H_{29}N_2O_{11}$ $[M+H]^+$: 425.1771. Found: 425.1778.

1,3,4,6-Tetra-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-D-glucopyranoside (44)[171]



To a solution of D-glucosamine hydrochloride (4.31 g, 20.0 mmol) and $NaHCO_3$ (4.20 g, 50.0 mmol) in water (40 mL) was added benzyl chloroformate (4.2 mL, 30.0 mmol). The mixture was stirred at room temperature overnight, then filtered and washed with diethylether. The crude product obtained was treated with 1:1 pyridine-acetic anhydride (60 mL) and stirred at room temperature for 20 h. Work-up and flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2) yielded **44** (6.36 g, 66%; Lit.[156]: 55%) as a white foam in the ratio of α:β = 4:1.

TLC (cyclohexane/EtOAc, 3:2): R_f = 0.53 (α-anomer), 0.64 (β-anomer).

m.p. 55-56 °C.

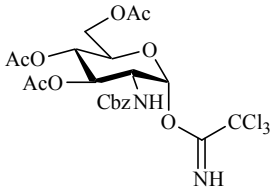
IR (neat): 1743 (s), 1531 (m), 1367 (m), 1213 (s), 1136 (m), 1012 (s), 921 (m), 742 (m), 698 (m), 599 (m) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.93, 2.02, 2.03, 2.08, 2.16 (5 s, 12 H, 4 COCH_3), 3.76-3.84 (m, 0.2 H, 5$_\beta$-H), 3.90-4.15 (m, 2 H, 5$_\alpha$-H, 6$_\alpha$-H, 2$_\beta$-H, 6$_\beta$-H), 4.16-4.33 (m, 1.8 H, 2$_\alpha$-H, 6'$_\alpha$-H, 6'$_\beta$-H), 4.92 (d, $J_{2,NH}$ = 9.6 Hz, 0.8 H, α-NH), 5.00-5.32 (m, 4.2 H, 3$_\alpha$-H, 4$_\alpha$-H, 3$_\beta$-H, 4$_\beta$-H, β-NH, CH_2Ph), 5.70 (d, $J_{1,2}$ = 8.7 Hz, 0.2 H, 1$_\beta$-H), 6.21 (d, $J_{1,2}$ = 3.6 Hz, 0.8 H, 1$_\alpha$-H), 7.28-7.43 (m, 5 H, Ph) ppm.

^{13}C NMR: in accordance with literature data.[156]

ESI-MS calcd for $C_{22}H_{27}NO_{11}Na$ $[M+Na]^+$: 504.1482. Found: 504.1481.

_O_-[3,4,6-Tri-_O_-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl] trichloroacetimidate (46)



According to the procedure as described for **38**, compound **44** (6.25 g, 13.0 mmol) was treated with hydrazine acetate (1.43 g, 15.6 mmol) in dry DMF (26 mL) at room temperature for 1.5 h. After work-up, the residue was dissolved in dry CH_2Cl_2 (26 mL) and trichloroacetonitrile (7.8 mL, 78.0 mmol) and DBU (0.4 mL, 2.80 mmol) were added. The mixture was stirred at room temperature for 5 h, and then concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2 + 1% Et_3N) to yield **46** (5.54 g, 73%) as a pale yellow foam.

TLC (cyclohexane/EtOAc, 3:2): $R_f = 0.69$.

m.p. 54-56 °C.

$[\alpha]^{28}_D = +63.7$ (c = 1.0, $CHCl_3$).

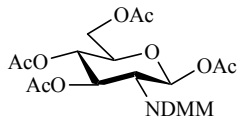
IR (neat): 3311 (w), 1722 (s), 1678 (m), 1520 (m), 1455 (w), 1366 (m), 1220 (s), 1137 (m), 1016 (s), 967 (s), 924 (m), 828 (m), 796 (m), 740 (m), 698 (m), 640 (m) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.93, 2.04, 2.06 (3 s, 9 H, 3 $COCH_3$), 4.08-4.18 (m, 2 H, 5-H, 6-H), 4.22-4.37 (m, 2 H, 2-H, 6'-H), 4.94 (d, $J_{2,NH}$ = 9.6 Hz, 1 H, NHCO), 5.02-5.36 (m, 4 H, 3-H, 4-H, CH_2Ph), 6.41 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 7.30-7.43 (m, 5 H, Ph), 8.78 (s, 1 H, NH) ppm.

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 20.5, 20.6 (3 COCH_3), 53.5 (2-C), 61.4 (6-C), 67.2 (CH_2Ph), 67.5 (4-C), 70.2, 70.6 (3-C, 5-C), 90.8 (CCl_3), 94.9 (1-C), 128.1, 128.2, 128.5, 136.0 (Ph-C), 155.6, (NHCOO), 160.3 (C=NH), 169.2, 170.5, 171.0 (3 $COCH_3$) ppm.

$C_{22}H_{25}Cl_3N_2O_{10}$ (583.80): calcd. C 45.26, H 4.32, N 4.80; found: C 45.49, H 4.12, N 5.09.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (19)[147]



According to the procedure as described for compound **37**, a solution of D-glucosamine hydrochloride (8.40 g, 38.9 mmol) in water (80 mL) was added dropwise to a mixture of dimethylmaleic anhydride (5.42 g, 43.0 mmol) and $NaOAc \cdot 3H_2O$ (6.39 g, 47.0 mmol) in 4:1 acetone-water (200 mL). After 15 min, $NaHCO_3$ (4.88 g, 58.0 mmol) was added in a small portions over 15 min. The reaction mixture was stirred at room temperature for 5 h, then dried well in vacuo. The residue was treated with 1:1 pyridine-acetic anhydride (120 mL), warmed to 60 °C with stirring for 30 min and then continued stirring at room temperature overnight. Work-up and flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2) yielded **19** (12.3 g, 69%; Lit.[121]: 57%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.41$. Lit.[121]: 0.39.

m.p. 109-110 °C. Lit.[121]: 109-110 °C.

$[\alpha]^{25}_D = +37.4$ ($c = 1.0$, $CHCl_3$). Lit.[121]: $[\alpha]_D = +40.0$ ($c = 1.0$, $CHCl_3$).

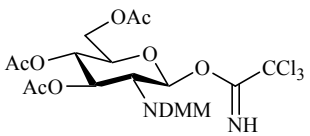
IR (KBr): 3644 (w), 3472 (w), 1756 (s), 1712 (s), 1405 (s), 1216 (s), 1081 (s), 1047 (s), 961 (w), 898 (m), 735 (s) cm^{-1}.

^{1}H NMR: in accordance with literature data.[121]

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 8.8 (2 CH_3), 20.4, 20.5, 20.7, 20.8 (4 $COCH_3$), 53.4 (2-C), 61.5 (6-C), 68.3, 70.6, 72.5 (3-C, 4-C, 5-C), 89.8 (1-C), 137.6 (C=C), 168.6, 169.4, 169.9, 170.6, 170.9 (CO, 4 $COCH_3$) ppm.

ESI-MS calcd for $C_{20}H_{25}NO_{11}Na$ $[M+Na]^+$: 478.1325. Found: 478.1331.

***O*-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl] trichloroacetimidate (48)**[121]



According to the procedure as described for **38**, a solution of **19** (12.7 g, 28.0 mmol) and hydrazine acetate (3.87 g, 42.0 mmol) in dry DMF (56 mL) was stirred at room temperature for 1.5 h. After work-up, the residue was dissolved in dry CH_2Cl_2 (56 mL) and trichloroacetonitrile (11.2 mL, 112 mmol) and DBU (0.8 mL, 5.6 mmol) were added. The mixture was stirred at room temperature for 5 h, and then concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2 + 1% Et_3N) to yield **48** (12.18 g, 78%; Lit.[121]: 75%) as a pale yellow foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.47. Lit.[121]: 0.4.

m.p. 60-62 °C.

$[\alpha]^{26}_D$ = +33.9 (*c* = 1.1, $CHCl_3$). Lit.[121]: $[\alpha]_D$ = +31.5 (*c* = 1.0, $CHCl_3$).

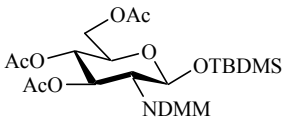
IR (KBr): 3316 (w), 2956 (w), 1752 (s), 1714 (s), 1403 (s), 1233 (s), 1078 (s), 1044 (s), 909 (w), 840 (m), 798 (m), 733 (m), 644 (m) cm^{-1}.

^{1}H NMR: in accordance with literature data.[121]

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 8.7 (2 CH_3), 20.5, 20.6, 20.7 (3 CO*C*H_3), 53.4 (2-C), 61.6 (6-C), 68.4, 70.6, 72.7 (3-C, 4-C, 5-C), 90.3 (CCl_3), 93.6 (1-C), 137.5 (C=C), 160.5 (C=NH), 169.4, 170.0, 170.6, 170.9 (CO, 3 *C*OCH_3) ppm.

ESI-MS calcd for $C_{20}H_{23}N_2O_{10}NaCl_3$ $[M+Na]^+$: 579.0316. Found: 579.0316.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (49)**[121]



According to the procedure as described for compound **39**, a solution of **19** (7.74 g, 17.0 mmol) and hydrazine acetate (1.88 g, 20.4 mmol) in dry DMF (34 mL) was stirred at room temperature for 1.5 h. After work-up, a solution of the residue and imidazole (2.31 g, 34.0 mmol) in dry CH_2Cl_2 (50 mL) was treated with TBDMSCl (3.01 g, 20.0 mmol) and stirred at room temperature overnight. Work-up and flash column chromatography (SiO_2, cyclohexane/EtOAc, 1:1) yielded **49** (8.19 g, 91%; Lit.[121]: 90%) as white crystals.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.75.

m.p. 129-130 °C. Lit.[121]: 126-127 °C.

$[\alpha]^{25}_D$ = +11.5 (*c* = 1.0, $CHCl_3$). Lit.[121]: $[\alpha]_D$ = +9.6 (*c* = 1.0, $CHCl_3$).

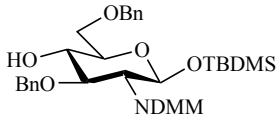
IR (KBr): 3462 (w), 2956 (m), 2932 (m), 2858 (m), 1751 (s), 1706 (s), 1406 (s), 1227 (s), 1176 (m), 1153 (m), 1080 (s), 1044 (s), 980 (w), 927 (w), 847 (s), 785 (m) cm^{-1}.

^{1}H NMR: in accordance with literature data.[121]

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = –5.6, –4.4 (2 $SiCH_3$), 8.6 (2 CH_3), 17.5 [Si*C*$(CH_3)_3$], 20.5, 20.6, 20.7 (3 CO*C*H_3), 25.3 [SiC(*C*$H_3)_3$], 56.5 (2-C), 62.4 (6-C), 69.5, 70.7, 71.7 (3-C, 4-C, 5-C), 93.2 (1-C), 137.3 (C=C), 169.5, 170.0, 170.5, 171.3 (CO, 3 *C*OCH_3) ppm.

ESI-MS calcd for $C_{24}H_{38}NO_{10}Si$ $[M+H]^+$: 528.2265. Found: 528.2294.

tert-Butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (50)[121]



According to the procedure as described for **40**, a mixture of **49** (10.5 g, 20.0 mmol), dry MeOH (200 mL) and NaOMe (0.54 g, 10.0 mmol) was stirred at room temperature for 2 h. After work-up, a suspension of the crude product and dibutyltin oxide (10.9 g, 44.0 mmol) in toluene (200 mL) was heated under reflux (dean-Stark apparatus). After 15 h, TBAI (16.2 g, 44.0 mmol) and benzyl bromide (5.3 mL, 44.0 mmol) were added and the mixture was gently refluxed for 3 h. After work-up, the residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 4:1) to yield **50** (10.1 g, 86%; Lit.[121]: 88%) as a pale yellow solid.

TLC (*n*-Hexane/EtOAc, 4:1): R_f = 0.48.

m.p. 67-69 °C. Lit.[121]: 66-69 °C.

$[\alpha]^{23}_D$ = +24.7 (*c* = 1.0, $CHCl_3$). Lit.[121]: $[\alpha]_D$ = +25.7 (*c* = 1.3, $CHCl_3$).

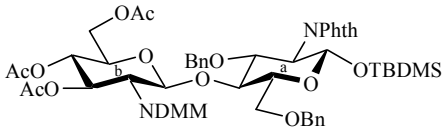
IR (KBr): 3462 (m), 3028 (w), 2927 (m), 2886 (m), 2587 (m), 1708 (s), 1495 (w), 1461 (w), 1408 (s), 1362 (w), 1318 (w), 1256 (m), 1207 (m), 1170 (m), 1077 (s), 978 (w), 938 (w), 861 (s), 838 (s), 785 (m), 747 (m), 733 (m), 701 (m) cm^{-1}.

^{1}H NMR: in accordance with literature data.[121]

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = –5.6, –4.2 (2 $SiCH_3$), 8.5 (2 CH_3), 17.6 [Si*C*$(CH_3)_3$], 25.3 [SiC(*C*$H_3)_3$], 57.3 (2-C), 70.9 (6-C), 73.6 (5-C), 73.7, 74.1 (2 CH_2Ph), 74.2 (4-C), 79.0 (3-C), 93.5 (1-C), 127.4, 127.7, 127.8, 127.9, 128.3, 128.5, 136.9, 137.7, 138.7 (C=C, 2 Ph-C) ppm.

ESI-MS calcd for $C_{32}H_{43}NO_7NaSi$ $[M+Na]^+$: 604.2707. Found: 604.2731.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (51)**



Glycosyl acceptor **40** (3.01 g, 5.00 mmol) and glycosyl donor **48** (4.18, 7.50 mmol) were azeotroped with toluene (3 × 8 mL), dried under vacuo for 1 h, and dissolved in dry CH_2Cl_2 (15 mL). The solution was stirred under N_2 atmosphere at –78 °C for 10 min, and TMSOTf (0.1 M in CH_2Cl_2, 7.5 mL, 0.75 mmol) was added dropwise. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **51** (4.49 g, 90%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.64.

m.p. 75-77 °C.

$[\alpha]^{25}_D$ = +17.3 (*c* = 1.2, $CHCl_3$).

IR (KBr): 3470 (w), 3031 (w), 2953 (m), 2859 (m), 1752 (s), 1714 (s), 1613 (w), 1497 (w), 1390 (s), 1227 (s), 1050 (s), 910 (w), 874 (w), 841 (m), 783 (m), 736 (m), 722 (m), 699 (m) cm^{-1}.

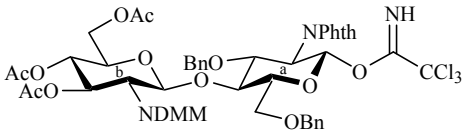
^{1}H NMR (300 MHz, $CDCl_3$): δ = −0.16, −0.01 (2 s, 6 H, 2 $SiCH_3$), 0.64 [s, 9 H, $SiC(CH_3)_3$], 1.91, 1.97, 1.98, 1,99 (4 s, 15 H, 3 $COCH_3$, 2 CH_3), 3.49-3.63 (m, 4 H, 5a-H, 6a-H, 6'a-H, 5b-H), 3.94 (dd, J_{gem} = 12.3, $J_{5,6}$ = 2.4 Hz, 1 H, 6b-H), 4.04-4.29 (m, 5 H, 2a-H, 3a-H, 4a-H, 2b-H, 6'b-H), 4.48, 4.80 (2 d, J_{gem} = 12.6 Hz, 2 H, CH_2Ph), 4.63, 4.68 (2 d, J_{gem} = 12.3 Hz, 2 H, CH_2Ph), 5.09 (dd, $J_{3,4}$ = 9.0, $J_{4,5}$ = 9.9 Hz, 1 H, 4b-H), 5.25 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1a-H), 5.40 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1b-H), 5.65 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 9.0 Hz, 1 H, 3b-H), 6.83-7.71 (m, 14 H, 2 Ph, Phth) ppm.

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = −5.6, −4.3 (2 $SiCH_3$), 8.8 (2 CH_3), 17.5 [Si*C*$(CH_3)_3$], 20.4, 20.5, 20.6 (3 CO*C*H_3), 25.3 [SiC(*C*$H_3)_3$], 55.1 (2b-C), 57.8 (2a-C), 61.6 (6b-C), 68.0 (6a-C), 68.9 (4b-C), 70.8 (3b-C), 71.4 (5b-C), 72.9, 73.8 (2 *C*H_2Ph), 74.5 (5a-C), 76.3 (4a-C), 76.4 (3a-C), 93.2 (1a-C), 96.9 (1b-C), 123.0, 126.9, 127.4, 127.8, 128.3, 131.6, 133.6, 137.4, 138.4, 138.6 (C=C, 2 Ph-C, Phth-C), 169.4, 170.0, 170.6 (3 *C*OCH_3) ppm.

ESI-MS calcd for $C_{52}H_{62}N_2O_{16}NaSi$ $[M+Na]^+$: 1021.3766. Found: 1021.3773.

$C_{52}H_{62}N_2O_{16}Si$ (999.14): calcd. C 62.51, H 6.25, N 2.80; found: C 62.88, H 5.97, N 2.82.

O-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl] trichloroacetimidate (52)



A solution of **51** (3.59 g, 3.60 mmol) in dry THF (10 mL) in an ice-salt bath was treated with glacial AcOH (0.3 mL, 5.00 mmol) and TBAF (1.0 M solution in THF, 4.3 mL, 4.30 mmol) with stirring. After 30 min, the ice bath was removed and the mixture was stirred at room temperature for 3 h. The resulting mixture was diluted with aq. saturated NaCl (60 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. A mixture of the crude product, trichloroacetonitrile (1.1 mL, 11.0 mmol), and DBU (0.1 mL, 0.72 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature overnight, and then concentrated in vacuo. The

residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2 + 1% Et_3N) to yield **52** (2.93 g, 79%) as a pale yellow foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.45.

m.p. 87-89 °C.

$[\alpha]^{32}_D$ = +32.1 (*c* = 0.8, $CHCl_3$).

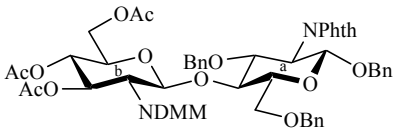
IR (KBr): 3316 (w), 3031 (w), 2946 (w), 1751 (s), 1715 (s), 1496 (w), 1388 (s), 1228 (s), 1052 (s), 912 (w), 876 (w), 837 (m), 797 (m), 736 (m), 721 (m), 700 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.91, 1.97, 2.00 (3 s, 15 H, 3 $COCH_3$, 2 CH_3), 3.37-3.42 (m, 1 H, 5a-H), 3.54 (dd, J_{gem} = 11.0, $J_{5,6}$ = 3.0 Hz, 1 H, 6a-H), 3.63-3.67 (m, 1 H, 5b-H), 3.74 (dd, J_{gem} = 11.0, $J_{5,6'}$ = 1.5 Hz, 1 H, 6'a-H), 3.93 (dd, J_{gem} = 12.5, $J_{5,6}$ = 2.0 Hz, 1 H, 6b-H), 4.09 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5 Hz, 1 H, 2b-H), 4.20 (dd, J_{gem} = 12.5, $J_{5,6'}$ = 4.5 Hz, 1 H, 6'b-H), 4.31 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 8.5 Hz, 1 H, 3a-H), 4.35 (dd, $J_{3,4}$ = $J_{4,5}$ = 8.5 Hz, 1 H, 4a-H), 4.41 (dd, $J_{1,2}$ = 9.0, $J_{2,3}$ = 10.5 Hz, 1 H, 2a-H), 4.49, 4.83 (2 d, J_{gem} = 12.5 Hz, 2 H, CH_2Ph), 4.63, 4.67 (2 d, J_{gem} = 12.0 Hz, 2 H, CH_2Ph), 5.06 (dd, $J_{3,4}$ = 9.0, $J_{4,5}$ = 10.0 Hz, 1 H, 4b-H), 5.38 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1b-H), 5.62 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 9.0 Hz, 1 H, 3b-H), 6.31 (d, $J_{1,2}$ = 9.0 Hz, 1 H, 1a-H), 6.83-7.70 (m, 14 H, 2 Ph, Phth), 8.5 (s, 1 H, NH) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.8 (2 CH_3), 20.4, 20.5, 20.6 (3 CO*C*H_3), 54.4 (2a-C), 55.0 (2b-C), 61.5 (6b-C), 67.7 (6a-C), 68.8 (4b-C), 70.7 (3b-C), 71.4 (5b-C), 72.8, 74.2 (2 *C*H_2Ph), 75.51, 75.52 (4a-C, 5a-C), 76.2 (3a-C), 90.4 (CCl_3), 93.9 (1a-C), 96.6 (1b-C), 123.2, 127.1, 127.6, 127.8, 127.9, 128.3, 131.4, 133.7, 138.2, 138.3 (C=C, 2 Ph-C, Phth-C), 160.8 (C=NH), 167.4, 169.4, 170.0, 170.6 (CO, 3 *C*OCH_3) ppm.

$C_{48}H_{48}Cl_3N_3O_{16}$ (1029.26): calcd. C 56.01, H 4.70, N 4.08; found: C 55.80, H 4.74, N 3.93.

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (53)



A mixture of **52** (1.75 g, 1.70 mmol) and benzyl alcohol (0.3 mL, 2.70 mmol) in dry CH_3CN (10 mL) was stirred under N_2 atmosphere at room temperature while TMSOTf (0.01 M in CH_3CN, 1.7 mL, 17 µmol) was added dropwise. After 5 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2) to yield **53** (1.48 g, 89%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.52.

m.p. 74-76 °C.

$[\alpha]^{25}_D$ = −7.5 (*c* = 0.5, $CHCl_3$).

IR (KBr): 3460 (br m), 3031 (w), 2922 (w), 1750 (s), 1713 (s), 1613 (w), 1497 (w), 1454 (w), 1389 (s), 1229 (s), 1049 (s), 912 (w), 875 (w), 826 (w), 736 (m), 723 (m), 670 (m) cm^{-1}.

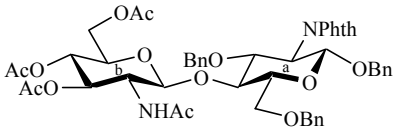
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.90, 1.95, 1.99 (3 s, 15 H, 3 $COCH_3$, 2 CH_3), 3.37-3.45 (m, 2 H, 5a-H, 5b-H), 3.52 (dd, J_{gem} = 11.0, $J_{5,6}$ = 3.5 Hz, 1 H, 6a-H), 3.68 (dd, J_{gem} = 11.0, $J_{5,6'}$ = 1.0 Hz, 1 H, 6'a-H), 3.92 (dd, J_{gem} = 12.5, $J_{5,6}$ = 2.0 Hz, 1 H, 6b-H), 4.08 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5 Hz, 1 H, 2b-H), 4.17-4.26 (m, 4 H, 2a-H, 3a-H, 4a-H, 6'b-H), 4.43, 4.44, 4.63, 4.67, 4.74, 4.78 (6 d, J_{gem} = 12.5 Hz, 6 H, 3 CH_2Ph), 5.01-5.08 (m, 2 H, 1a-H, 4b-H), 5.37 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1b-H), 5.61 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 9.0 Hz, 1 H, 3b-H), 6.81-7.66 (m, 19 H, 3 Ph, Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.8 (2 CH_3), 20.4, 20.5, 20.6 (3 CO*C*H_3), 55.1 (2b-C), 55.6 (2a-C), 61.5 (6b-C), 68.1 (6a-C), 68.8 (4b-C), 70.5 (*C*H_2Ph), 70.8 (3b-C), 71.4 (5b-C), 72.9, 74.0 (2 *C*H_2Ph), 74.5 (5a-C), 76.0, 76.5 (3a-C, 4a-C), 96.7 (1b-C), 97.1 (1a-C), 123.1, 126.9, 127.4, 127.5, 127.6, 127.8, 127.9, 128.0, 128.3, 131.6, 133.5, 137.1, 137.5, 138.4, 138.5 (C=C, 3 Ph-C, Phth-C), 163.4, 167.6, 169.4, 170.0, 170.6 (CO, 3 *C*OCH_3) ppm.

ESI-MS calcd for $C_{53}H_{54}N_2O_{16}Na$ $[M+Na]^+$: 997.3371. Found: 997.3348.

$C_{53}H_{54}N_2O_{16}$ (974.99): calcd. C 65.29, H 5.58, N 2.87; found: C 65.23, H 5.74, N 2.81.

Benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (54)



A mixture of **53** (0.61 g, 0.63 mmol) and NaOH (0.90 g, 22.5 mmol) in 4:1 dioxane-water (45 mL) was stirred at room temperature overnight. Then, the pH was adjusted to 5 using aq. 3 N HCl in the presence of ethanolamine. After 1 day, the solution was neutralized with ethanolamine and concentrated in vacuo. The residue was treated with 2:1 pyridine-acetic anhydride (30 mL) and stirred at room temperature overnight, then co-concentrated with toluene. After work-up, the residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 1:7) to yield **54** (0.36 g, 63%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:3): $R_f = 0.40$.

m.p. 80-82 °C.

$[\alpha]^{23}_D = -15.4$ ($c = 0.5$, $CHCl_3$).

IR (KBr): 3305 (w), 3063 (w), 3030 (w), 2945 (w), 2873 (w), 1750 (s), 1715 (s), 1537 (m), 1497 (w), 1454 (m), 1388 (s), 1232 (s), 1048 (s), 910 (w), 875 (w), 742 (m), 723 (m), 670 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.76 (s, 3 H, $NCOCH_3$), 1.95, 1.99, 2.01 (3 s, 9 H, 3 $COCH_3$), 3.49-3.58 (m, 2 H, 5a-H, 5b-H), 3.65 (dd, $J_{gem} = 10.5$, $J_{5,6} = 2.0$ Hz, 1 H, 6a-H), 3.72 (dd, $J_{gem} = 10.5$, $J_{5,6'} = 2.5$ Hz, 1 H, 6'a-H), 3.97 (dd, $J_{gem} = 12.0$, $J_{5,6} = 2.5$ Hz, 1 H, 6b-H), 3.98-4.06 (m, 2 H, 4a-H, 2b-H), 4.14-4.21 (m, 2 H, 2a-H, 3a-H), 4.27 (dd, J_{gem} = 12.0, $J_{5,6'} = 4.5$ Hz, 1 H, 6'b-H), 4.35 (d, $J_{gem} = 12.5$ Hz, 1 H, C*H*HPh), 4.41-4.50 (m, 3 H, 1b-H, CH_2Ph), 4.71 (d, $J_{2,NH} = 9.5$ Hz, 1 H, NH), 4.76, 4.78 (2 d, $J_{gem} = 12.5$ Hz, 2 H, CH_2Ph), 4.88 (dd, $J_{2,3} = 10.5$, $J_{3,4} = 9.5$, 1 H, 3b-H), 4.94 (d, $J_{gem} = 12.0$ Hz, 1 H, C*H*HPh), 4.98 (dd, $J_{3,4} = J_{4,5} = 10.0$ Hz, 1 H, 4b-H), 5.08 (d, $J_{1,2} = 8.0$ Hz, 1 H, 1a-H), 6.71-7.67 (m, 19 H, 3 Ph, Phth) ppm.

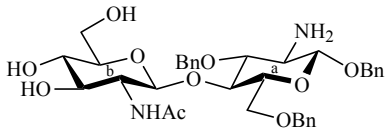
^{13}C NMR (125 MHz, $CDCl_3$): δ = 20.6 (3 CO*C*H_3), 23.1 (NCO*C*H_3), 54.2 (2b-C), 55.7 (2a-C), 61.8 (6b-C), 67.6 (6a-C), 68.5 (4b-C), 70.8 (*C*H_2Ph), 71.3 (5b-C), 73.1 (5a-C), 74.0 (*C*H_2Ph), 74.1 (3b-C), 74.6 (*C*H_2Ph), 76.8 (3a-C), 78.8 (4a-C), 97.4 (1a-C), 100.8

(1b-C), 123.1, 126.8, 127.5, 127.8, 127.8, 128.1, 128.9, 129.0, 129.2, 131.6, 133.4, 137.1, 137.7, 138.7 (Ph-C), 167.6, 169.3, 169.7, 170.7, 170.8 (CO, 3 *C*OCH$_3$, N*C*OCH$_3$) ppm.

ESI-MS calcd for $C_{49}H_{53}N_2O_{15}$ $[M+H]^+$: 909.3446. Found: 909.3425.

$C_{49}H_{52}N_2O_{15}$ (908.94): calcd. C 64.75, H 5.77, N 3.08; found: C 64.43, H 5.45, N 3.10.

Benzyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl -2-deoxy-β-D-glucopyranoside (55)



A solution of **54** (0.10 g, 0.11 mmol) in dry MeOH (2 mL) was treated with NaOMe (3 mg, 55 μmol) and stirred at room temperature. After 1 h, the reaction mixture was neutralized with Amberlite IR 120 (H^+) resin, filtered and concentrated in vacuo. The residue was dissolved in *n*-BuOH (25 mL), then ethylenediamine (0.7 mL, 11.0 mmol) was added. The mixture was stirred at 90 °C overnight, then co-concentrated with toluene. The residue was purified by flash column chromatography (SiO_2, $CHCl_3$/EtOH, 4:1) to yield **55** (66 mg, 89%) as a white powder.

TLC ($CHCl_3$/EtOH, 4:1): R_f = 0.47.

m.p. 205-206 °C.

$[\alpha]^{27}_D$ = −39.8 (*c* = 0.5, $CHCl_3$).

IR (KBr): 3361 (br s), 3288 (br s), 2916 (s), 2867 (s), 1954 (br m), 1660 (s), 1548 (s), 1497 (m), 1454 (s), 1377 (s), 1315 (s), 1214 (m), 1057 (br s), 892 (m), 827 (w), 753 (s), 698 (s) cm^{-1}.

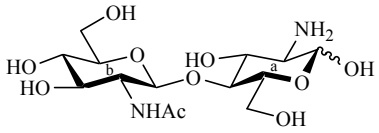
^{1}H NMR (500 MHz, CD_3OD): δ = 1.97 (s, 3 H, NCOCH$_3$), 2.75 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.0 Hz, 1 H, 2a-H), 3.15-3.20 (m, 1 H, 5b-H), 3.24 (dd, $J_{3,4}$ = 8.5, $J_{4,5}$ = 10.0 Hz, 1 H, 4b-H), 3.41-3.52 (m, 4 H, 3a-H, 5a-H, 3b-H, 6b-H), 3.67 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5 Hz, 1 H, 2b-H), 3.75-3.82 (m, 3 H, 6a-H, 6'a-H, 6'b-H), 4.08 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1 H, 4a-H), 4.33 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1a-H), 4.58-4.70 (m, 6 H, C*H*HPh, 2 CH$_2$Ph, 1b-H), 5.13 (d, J_{gem} = 10.5 Hz, 1H, CH*H*Ph), 7.25-7.41 (m, 15 H, 3 Ph) ppm.

^{13}C NMR (125 MHz, CD_3OD): δ = 23.1 ($NCOCH_3$), 57.7 (2a-C), 58.3 (2b-C), 63.1 (6b-C), 69.5 (6a-C), 72.1 (CH_2Ph), 72.7 (4b-C), 74.3 (CH_2Ph), 75.4 (3b-C), 76.1 (4a-C), 76.6 (5a-C), 76.8 (CH_2Ph), 78.5 (5b-C), 84.0 (3a-C), 101.4 (1b-C), 103.7 (1a-C), 128.7, 128.8, 128.9, 129.1, 129.3, 129.4, 129.5, 129.9, 138.9, 139.5, 139.7 (3 Ph-C), 173.6 ($NCOCH_3$) ppm.

ESI-MS calcd for $C_{35}H_{45}N_2O_{10}$ $[M+H]^+$: 653.3074. Found: 653.3066.

$C_{35}H_{44}N_2O_{10}$ (652.73): calcd. C 64.40, H 6.79, N 4.29; found: C 64.05, H 7.03, N 4.21.

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



To a solution of **55** (50 mg, 76 μmol) in 2:1 EtOH-water (3 mL) was added 10% Pd/C (25 mg) and 3 drops of HOAc, and then stirred at room temperature under H_2 atmosphere. After 24 h, the reaction mixture was filtered through Celite, washed with 2:1 EtOH-water (3 × 2 mL), and concentrated in vacuo. Gel-filtration of the residue on a Bio-Gel P-2 column, eluted with water, and subsequent lyophilization yielded **4** (25 mg, 85%) as a white amorphous powder in the ratio of α:β = 2:3.

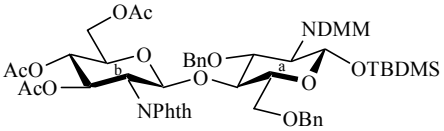
m.p. 114-116 °C.

IR (neat): 3285 (br s), 1644 (s), 1557 (s), 1375 (s), 1317 (s), 1158 (s), 1028 (br s), 894 (s) cm^{-1}.

^{1}H and **^{13}C NMR**: in accordance with literature data.[51]

ESI-MS calcd for $C_{14}H_{27}N_2O_{10}$ $[M+H]^+$: 383.1666. Found: 383.1652.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (56)**



Glycosyl donor **38** (4.20, 7.40 mmol) and glycosyl acceptor **50** (2.90 g, 4.98 mmol) were azeotroped with toluene (3 × 8 mL), dried under vacuo for 1 h, and dissolved in dry CH_2Cl_2 (15 mL). The solution was stirred under N_2 atmosphere at –30 °C for 10 min, and TMSOTf (0.1 M in CH_2Cl_2, 7.5 mL, 0.75 mmol) was added dropwise. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **56** (4.18 g, 84%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.75.

m.p. 51-53 °C.

$[\alpha]^{33}_D$ = +22.1 (*c* = 0.6, $CHCl_3$).

IR (KBr): 3303 (w), 3031 (w), 5953 (w), 2859 (w), 1751 (s), 1717 (s), 1601 (w), 1497 (w), 1387 (s), 1228 (s), 1075 (s), 839 (s), 783 (m), 723 (m), 698 (m) cm^{-1}.

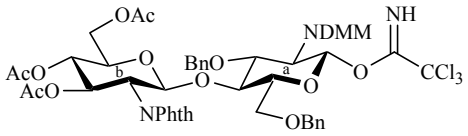
^{1}H NMR (300 MHz, $CDCl_3$): δ = –0.16, –0.04 (2 s, 6 H, 2 $SiCH_3$), 0.69 [s, 9 H, $SiC(CH_3)_3$], 1.76 (br s, 6 H, 2 CH_3), 1.84, 1.98, 2.00 (3 s, 9 H, $COCH_3$), 3.27-3.33 (m, 1 H, 5a-H), 3.41 (dd, J_{gem} = 11.4, $J_{5,6}$ = 3.6 Hz, 1 H, 6a-H), 3.48 (dd, J_{gem} = 11.4, $J_{5,6'}$ = 1.5 Hz, 1 H, 6'a-H), 3.54-3.61 (m, 1 H, 5b-H), 3.83 (dd, $J_{1,2}$ = 8.1, $J_{2,3}$ = 10.5 Hz, 1 H, 2a-H), 3.97 (dd, J_{gem} = 12.3, $J_{5,6}$ = 2.4 Hz, 1 H, 6b-H), 4.14-4.27 (m, 2 H, 3a-H, 4a-H), 4.23 (dd, J_{gem} = 12.3, $J_{5,6'}$ = 4.5 Hz, 1 H, 6'b-H), 4.33 (dd, $J_{1,2}$ = 8.4, $J_{2,3}$ = 10.5 Hz, 1 H, 2b-H), 4.43-4.58 (m, 3 H, C*H*HPh, CH_2Ph), 4.87 (d, J_{gem} = 12.6 Hz, 1 H, CH*H*Ph), 5.01 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1a-H), 5.13 (dd, $J_{3,4}$ = 9.0, $J_{4,5}$ = 10.2 Hz, 1 H, 4b-H), 5.55 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1b-H), 5.81 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 9.0 Hz, 1 H, 3b-H), 7.13-7.95 (m, 14 H, 2 Ph, Phth) ppm.

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = –5.7, –4.3 (2 $SiCH_3$), 8.4 (2 CH_3), 17.5 [Si*C*$(CH_3)_3$], 20.4, 20.5, 20.6 (3 CO*C*H_3), 25.3 [SiC(*C*$H_3)_3$], 55.3 (2b-C), 57.6 (2a-C), 61.6 (6b-C), 67.9 (6a-C), 68.9 (4b-C), 70.7 (3b-C), 71.5 (5b-C), 72.7, 73.9 (2 *C*H_2Ph), 74.3 (5a-C), 76.4, 77.2 (3a-C, 4a-C), 93.2 (1a-C), 97.0 (1b-C), 123.6, 127.0, 127.4, 128.0, 128.1, 128.2, 131.3, 134.4, 136.7, 138.3, 139.1 (C=C, 2 Ph-C, Phth-C), 169.5, 170.1, 170.7 (3 *C*OCH_3) ppm.

ESI-MS calcd for $C_{52}H_{62}N_2O_{16}NaSi$ $[M+Na]^+$: 1021.3766. Found: 1021.3780.

$C_{52}H_{62}N_2O_{16}Si$ (999.14): calcd. C 62.51, H 6.25, N 2.80; found: C 62.92, H 6.19, N 3.03.

_O_-[3,4,6-Tri-_O_-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-_O_-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl] trichloroacetimidate (57)



According to the procedure as described for **52**, a solution of **56** (4.05 g, 4.05 mmol) in dry THF (12 mL) in an ice-salt bath was treated with glacial AcOH (0.3 mL, 5.0 mmol) and TBAF (1.0 M solution in THF, 4.9 mL, 4.90 mmol). After work-up, a mixture of the crude product, trichloroacetonitrile (1.2 mL, 12.0 mmol), and DBU (120 µL, 0.80 mmol) in dry CH_2Cl_2 (12 mL) was stirred at room temperature overnight, and then concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2 + 1% Et_3N) to yield **57** (3.62 g, 87%) as a pale yellow foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.53$.

m.p. 95-97 °C.

$[\alpha]^{35}_D = +31.2$ (c = 1.1, $CHCl_3$).

IR (KBr): 3470 (w), 3318 (w), 3031 (w), 2928 (m), 1752 (s), 1715 (s), 1611 (w), 1497 (w), 1453 (m), 1387 (s), 1228 (s), 1049 (s), 899 (w), 837 (w), 797 (m), 723 (s), 699 (m), 643 (w) cm^{-1}.

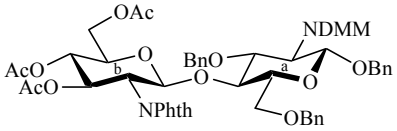
^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.73, 1.85, 1.97, 2.00 (4 s, 15 H, 3 $COCH_3$, 2 CH_3), 3.41-3.53 (m, 3 H, 5a-H, 6a-H, 5b-H), 3.62 (dd, J_{gem} = 12.6, $J_{5,6'}$ = 3.0 Hz, 1 H, 6'a-H), 3.94 (dd, J_{gem} = 12.3, $J_{5,6}$ = 2.1 Hz, 1 H, 6b-H), 4.08-4.38 (m, 5 H, 2a-H, 3a-H, 4a-H, 2b-H, 6'b-H), 4.48, 4.90 (2 d, J_{gem} = 12.6 Hz, 2 H, CH_2Ph), 4.53, 4.60 (2 d, J_{gem} = 12.0 Hz, 2 H, CH_2Ph), 5.12 (dd, $J_{3,4}$ = 9.0, $J_{4,5}$ = 9.9 Hz, 1 H, 4b-H), 5.53 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1b-H), 5.80 (dd, $J_{2,3}$ = 10.8, $J_{3,4}$ = 9.0 Hz, 1 H, 3b-H), 6.09 (d, $J_{1,2}$ = 8.7 Hz, 1 H, 1a-H), 7.13-7.95 (m, 14 H, 2 Ph, Phth) 8.45 (s, 1 H, NH) ppm.

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 8.5 (2 CH_3), 20.4, 20.5, 20.6 (3 COCH_3), 54.2 (2a-C), 55.2 (2b-C), 61.5 (6b-C), 67.7 (6a-C), 68.9 (4b-C), 70.6 (3b-C), 72.7, 74.2 (2 CH_2Ph),

75.3 (5a-C), 75.5, 76.9 (3a-C, 4a-C), 90.5 (CCl_3), 94.0 (1a-C), 96.6 (1b-C), 123.7, 127.1, 127.5, 127.6, 128.0, 128.1, 128.3, 131.3, 134.4, 136.8, 138.2, 138.8 (C=C, 2 Ph-C, Phth-C), 160.8 (C=NH), 169.4, 170.0, 170.6, 171.1 (CO, 3 *C*OCH_3) ppm.

$C_{48}H_{48}Cl_3N_3O_{16}$ (1029.26): calcd. C 56.01, H 4.70, N 4.08; found: C 56.22, H 4.51, N 3.98.

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (58)



A mixture of **57** (0.62 g, 0.60 mmol) and benzyl alcohol (0.1 mL, 0.90 mmol) in dry CH_3CN (4 mL) was stirred under N_2 atmosphere at room temperature while TMSOTf (0.01 M in CH_3CN, 0.6 mL, 6 μmol) was added dropwise. After 5 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2) to yield **58** (0.49 g, 84%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.66$.

m.p. 72-73 °C.

$[\alpha]^{25}_D = -13.4$ ($c = 0.3$, $CHCl_3$).

IR (neat): 1746 (s), 1704 (s), 1385 (s), 1222 (s), 1027 (s), 899 (w), 722 (s), 698 (m) cm^{-1}.

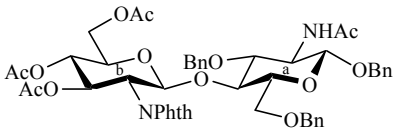
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.69 (br s, 6 H, 2 CH_3), 1.84, 1.96, 2.00 (3 s, 9 H, 3 $COCH_3$), 3.28-3.32 (m, 1 H, 5a-H), 3.43-3.50 (m, 2 H, 6a-H, 5b-H), 3.58 (d, J_{gem} = 11.0 Hz, 1 H, 6'a-H), 3.91-3.97 (m, 2 H, 2a-H, 6b-H), 4.04 (dd, $J_{3,4} = J_{4,5}$ = 9.5 Hz, 1 H, 4a-H), 4.15-4.23 (m, 2 H, 3a-H, 6'b-H), 4.32 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5 Hz, 1 H, 2b-H), 4.37, 4.43 (2 d, J_{gem} = 12.5 Hz, 2 H, CH_2Ph), 4.52, 4.56 (2 d, J_{gem} = 12.0 Hz, 2 H, CH_2Ph), 4.70, 4.86 (2 d, J_{gem} = 12.5 Hz, 2 H, CH_2Ph), 4.81 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1a-H), 5.12 (dd, $J_{3,4} = J_{4,5}$ = 9.5 Hz, 1 H, 4b-H), 5.52 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1b-H), 5.79 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 9.5 Hz, 1 H, 3b-H), 7.03-7.93 (m, 19 H, 3 Ph, Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.5 (2 CH_3), 20.4, 20.6 (3 $COCH_3$), 55.3 (2b-C), 55.4 (2a-C), 61.5 (6b-C), 68.0 (6a-C), 68.8 (4b-C), 70.4 (CH_2Ph), 70.6 (5b-C), 71.5 (5a-C), 72.7, 74.1 (2 CH_2Ph), 74.3 (3a-C), 76.0 (4a-C), 96.8 (1b-C), 97.3 (1a-C), 127.0, 127.4, 127.5, 127.6, 128.0, 128.1, 128.2, 128.3, 131.4, 134.4, 136.6, 137.3, 138.3, 139.0 (C=C, 3 Ph-C, Phth-C), 169.4, 170.0, 170.6, 171.3 (CO, 3 $COCH_3$) ppm.

ESI-MS calcd for $C_{53}H_{54}N_2O_{16}Na$ $[M+Na]^+$: 997.3371. Found: 997.3372.

$C_{53}H_{54}N_2O_{16}$ (974.99): calcd. C 65.29, H 5.58, N 2.87; found: C 64.89, H 5.64, N 2.80.

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (59)



A mixture of **58** (0.30 g, 0.31 mmol) and NaOH (0.44 g, 11.0 mmol) in 4:1 dioxane-water (22 mL) was stirred at room temperature overnight. Then, the pH was adjusted to 5 using aq. 3 N HCl in the presence of ethanolamine. After 1 day, the solution was neutralized with ethanolamine and concentrated in vacuo. The residue was treated with 2:1 pyridine-acetic anhydride (21 mL) and stirred at room temperature overnight. After work-up, the residue was purified by flash column chromatography (SiO_2, $CHCl_3$/EtOH, 98:2) to yield **59** (0.18 g, 63%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:3): R_f = 0.62.

m.p. 78-79 °C.

$[\alpha]^{27}_D$ = -21.2 (*c* = 0.5, $CHCl_3$).

IR (neat): 2871 (w), 1745 (s), 1715 (s), 1656 (m), 1549 (w), 1454 (w), 1384 (s), 1223 (s), 1029 (s), 901 (m), 738 (m), 722 (s), 697 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.84 (s, 3 H, $NCOCH_3$), 1.99, 2.00 (2 s, 9 H, 3 $COCH_3$), 3.37-3.42 (m, 1 H, 5a-H), 3.45-3.53 (m, 2 H, 6a-H, 5b-H), 3.61-3.68 (m, 2 H, 2a-H, 6'a-H), 3.89-3.96 (m, 2 H, 3a-H, 6b-H), 4.12 (dd, $J_{3,4}$ = $J_{4,5}$ = 7.5 Hz, 1 H, 4a-H), 4.20 (dd, J_{gem} = 12.5, $J_{5,6'}$ = 4.5 Hz, 1 H, 6'b-H), 4.31 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 11.0 Hz, 1 H, 2b-H), 4.36, 4.42, 4.47 (3 d, J_{gem} = 12.0 Hz, 3 H, C*H*HPh, CH_2Ph), 4.63-4.68 (m, 2 H, 1a-H,

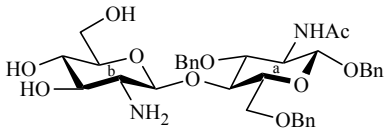
CH*H*Ph), 4.72, 4.82 (2 d, J_{gem} = 12.0 Hz, 2 H, CH_2Ph), 5.13 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, 1 H, 4b-H), 5.47 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1b-H), 5.65 (br s, 1 H, NH), 5.81 (dd, $J_{2,3}$ = 11.0, $J_{3,4}$ = 9.0 Hz, 1 H, 3b-H), 7.18-7.89 (m, 19 H, 3 Ph, Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 21.4, 21.6, 21.7 (3 CO*C*H_3), 24.4 (NCO*C*H_3), 55.4 (2a-C), 56.1 (2b-C), 62.6 (6b-C), 69.6 (6a-C), 69.7 (4b-C), 71.4 (3b-C), 71.5 (*C*H_2Ph), 72.7 (5b-C), 73.8, 74.2 (2 *C*H_2Ph), 75.2 (5a-C), 75.9 (4a-C), 78.6 (3a-C), 97.8 (1b-C), 100.1 (1a-C), 124.7, 128.5, 128.7, 128.8, 129.3, 129.4, 132.3, 132.4, 135.4, 138.4, 139.3, 139.6 (3 Ph-C, Phth-C), 170.5, 171.1, 171.2, 171.6 (3 *C*OCH_3, N*C*OCH_3) ppm.

ESI-MS calcd for $C_{49}H_{53}N_2O_{15}$ $[M+H]^+$: 909.3446. Found: 909.3446.

$C_{49}H_{52}N_2O_{15}$ (908.94): calcd. C 64.75, H 5.77, N 3.08; found: C 64.49, H 5.62, N 3.03.

Benzyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl -2-deoxy-β-D-glucopyranoside (60)



According to the procedure as described for **55**, a solution of **59** (0.18 g, 0.20 mmol) in dry MeOH (4 mL) was treated with NaOMe (6 mg, 0.11 mmol) and stirred at room temperature for 1 h. After work-up, the residue was dissolved in *n*-BuOH (40 mL), then ethylenediamine (1.3 mL, 20.0 mmol) was added. The mixture was stirred at 90 ^{o}C overnight, then co-concentrated with toluene. The residue was purified by flash column chromatography (SiO_2, $CHCl_3$/EtOH, 4:1) to yield **60** (0.12 g, 95%) as a white powder.

TLC ($CHCl_3$/EtOH, 4:1): R_f = 0.15.

m.p. 198-200 ^{o}C.

$[\alpha]^{26}_{D}$ = −27.6 (*c* = 0.5, MeOH).

IR (neat): 3305 (w), 2869 (w), 1651 (s), 1548 (s), 1497 (m), 1453 (m), 1371 (s), 1311 (s), 1159 (s), 1063 (s), 734 (s), 695 (s) cm^{-1}.

^{1}H NMR (500 MHz, CD_3OD): δ = 1.87 (s, 3 H, NCOCH_3), 2.55-2.65 (m, 1 H, 2b-H), 3.12-3.20 (m, 2 H, 3b-H, 5b-H), 3.46 (dd, J_{gem} = 12.5, $J_{5,6}$ = 4.5 Hz, 1 H, 6b-H), 3.50-

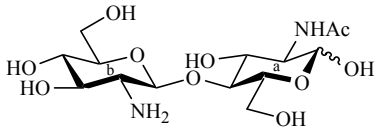
3.54 (m, 1 H, 5a-H), 3.60-3.68 (m, 2 H, 3a-H, 4b-H), 3.75 (br d, J = 12.0 Hz, 1 H, 6'b-H), 3.77-3.88 (m, 2 H, 2a-H, 6a-H), 3.90 (dd, J_{gem} = 11.0, $J_{5,6'}$ = 3.5 Hz, 1 H, 6'a-H), 4.12 (dd, $J_{3,4} = J_{4,5}$ = 9.5 Hz, 1 H, 4a-H), 4.40 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1b-H), 4.50 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1a-H), 4.52-4.60 (m, 3 H, 3 C*H*HPh), 4.66 (d, J_{gem} = 12.0 Hz, 1 H, CH*H*Ph), 4.82 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 4.94 (d, J_{gem} = 11.0 Hz, 1 H, CH*H*Ph), 7.22-7.40 (m, 15 H, 3 Ph) ppm.

^{13}C NMR (125 MHz, CD_3OD): δ = 23.0 (NCO*C*H$_3$), 56.4 (2a-C), 59.4 (2b-C), 63.1 (6b-C), 69.5 (6a-C), 71.5 (4b-C), 71.7 (*C*H$_2$Ph), 72.3 (3b-C), 74.2 (*C*H$_2$Ph), 75.9 (4a-C), 76.2 (5a-C), 76.7 (*C*H$_2$Ph), 78.9 (5b-C), 82.5 (3a-C), 101.6 (1a-C), 102.9 (1b-C), 128.7, 128.8, 128.9, 129.0, 129.3, 129.4, 129.5, 129.6, 139.1, 139.5, 139.6 (3 Ph), 173.2 (N*C*OCH$_3$) ppm.

ESI-MS calcd for $C_{35}H_{45}N_2O_{10}$ $[M+H]^+$: 653.3074. Found: 653.3049.

$C_{35}H_{44}N_2O_{10}$ (652.73): calcd. C 64.40, H 6.79, N 4.29; found: C 63.82, H 7.27, N 3.94.

2-Amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (5)



To a solution of **60** (50 mg, 76 μmol) in 2:1 EtOH-water (3 mL) was added 10% Pd/C (25 mg) and 3 drops of HOAc, and then stirred at room temperature under H_2 atmosphere. After 24 h, the reaction mixture was filtered through Celite, washed with 2:1 EtOH-water (3 × 2 mL), and concentrated in vacuo. Gel-filtration of the residue on a Bio-Gel P-2 column, eluted with water, and subsequent lyophilization yielded **5** (25 mg, 86%) as a white amorphous powder in the ratio of α:β = 3:2.

m.p. 146-148 ^{o}C.

IR (neat): 3285 (br s), 2874 (m), 1634 (s), 1556 (s), 1375 (s), 1032 (s) cm^{-1}.

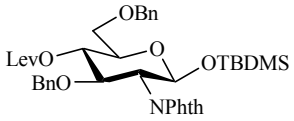
^{1}H NMR: in accordance with literature data.[52]

^{13}C NMR (125 MHz, D_2O): δ = 22.9 (NCO*C*H$_3$), 54.9 (2a$_\alpha$-C), 56.9 (2b-C), 57.6 (2a$_\beta$-C), 61.3, 61.4 (6a$_\alpha$-C, 6a$_\beta$-C, 6b-C), 69.8, 70.5, 70.9, 72.9, 73.4, 75.4 (3a$_\alpha$-C, 5a$_\alpha$-C, 3a$_\beta$-C,

5a$_\beta$-C, 3b-C, 4b-C), 77.3 (5b-C), 78.0, 78.3 (4a$_\alpha$-C, 4a$_\beta$-C), 91.5 (1a$_\alpha$-C), 95.8 (1a$_\beta$-C), 99.3 (1b-C), 175.5 (N*C*OCH$_3$) ppm.

ESI-MS calcd for $C_{14}H_{27}N_2O_{10}$ $[M+H]^+$: 383.1666. Found: 383.1664.

***tert*-Butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-2-phthalimido-β-D-glucopyranoside (64)**[160]



A solution of **40** (4.98 g, 8.20 mmol) in dry CH_2Cl_2 (80 mL) was cooled to 0 °C. DIPC (1.9 mL, 12.3 mmol) and DMAP (1.02 g, 8.20 mmol) were added followed by addition of levulinic acid (1.3 mL, 12.6 mmol). The reaction mixture was shielded from light and allowed to stir and warm for 15 h. CH_2Cl_2 (100 mL) was added and the organic layer was washed with aq. saturated $NaHCO_3$ (150 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 80 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **64** (5.15 g, 89%) as viscous pale yellow liquid.

TLC (*n*-Hexane/EtOAc, 4:1): R_f = 0.25.

$[\alpha]^{32}_D$ = +42.7 (*c* = 0.56, $CHCl_3$).

IR (KBr): 3478 (w), 3064 (m), 3031 (m), 2930 (s), 2858 (s), 1777 (s), 1715 (s), 1612 (m), 1497 (m), 1470 (s), 1388 (s), 1255 (s), 1148 (s), 1064 (s), 1027 (s), 963 (m), 912 (m), 840 (s), 783 (s), 740 (s), 721 (s), 698 (s) cm^{-1}.

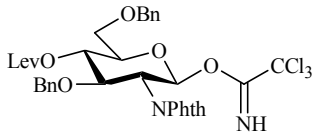
^{1}H NMR (300 MHz, $CDCl_3$): δ = −0.10, −0.04 (2 s, 6 H, 2 SiCH$_3$), 0.65 [s, 9 H, SiC(CH$_3$)$_3$], 2.13 (s, 3 H, COCH$_3$), 2.42-2.68 (m, 4 H, C*H*$_2$CO, C*H*$_2$COO), 3.57-3.65 (m, 2 H, 6-H, 6'-H), 3.74-3.83 (m, 1 H, 5-H), 4.20 (dd, $J_{1,2}$ = 8.1, $J_{2,3}$ = 11.1 Hz, 1 H, 2-H), 4.32-4.68 (m, 5 H, 3-H, 2 C*H*$_2$Ph), 5.15 (dd, $J_{3,4}$ = 9.0, $J_{4,5}$ = 9.9 Hz, 1 H, 4-H), 5.37 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1-H), 6.85-7.80 (m, 14 H, 2 Ph, Phth) ppm.

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = −5.6, −4.2 (2 SiCH$_3$), 17.5 [Si*C*(CH$_3$)$_3$], 25.3 [SiC(*C*H$_3$)$_3$], 27.9 (*C*H$_2$COO), 29.7 (*C*H$_3$CO), 37.7 (*C*H$_2$CO), 57.6 (2-C), 69.9 (6-C), 72.8 (5-C), 73.4 (2 *C*H$_2$Ph), 73.6 (4-C), 76.7 (3-C), 93.4 (1-C), 123.1, 127.3, 127.5, 127.6,

127.8, 128.0, 128.2, 131.6, 133.8, 138.0, 138.2 (2 Ph-C, Phth-C), 171.6 ($CH_2\mathit{C}OO$), 206.1 ($CH_2\mathit{C}OCH_3$) ppm.

ESI-MS calcd for $C_{39}H_{47}NO_9NaSi$ $[M+Na]^+$: 724.2918. Found: 724.2933.

_O_-[3,6-Di-_O_-benzyl-2-deoxy-4-_O_-levulinoyl-2-phthalimido-β-D-glucopyranosyl] trichloroacetimidate (65)[160]



A solution of **64** (4.91 g, 7.00 mmol) in dry THF (21 mL) in an ice-salt bath was treated with glacial AcOH (0.5 mL, 8.40 mmol) and TBAF (1.0 M solution in THF, 8.4 mL, 8.40 mmol) with stirring. After 30 min, the ice bath was removed and the mixture was stirred at room temperature for 3 h. The resulting mixture was diluted with aq. saturated NaCl (100 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. A mixture of the crude product, trichloroacetonitrile (4.2 mL, 42.0 mmol), and DBU (0.2 mL, 1.40 mmol) in dry CH_2Cl_2 (21 mL) was stirred at room temperature overnight, and then concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2 + 1% Et_3N) to yield **65** (3.83 g, 75%) as a pale yellow foam.

TLC (*n*-Hexane/EtOAc, 2:1): $R_f = 0.44$.

m.p. 136-138 °C.

$[\alpha]^{33}_D = +36.9$ (c = 1.0, $CHCl_3$).

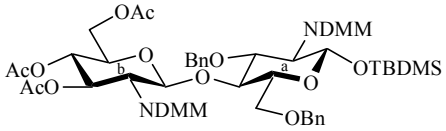
IR (KBr): 3305 (w), 3030 (w), 2914 (w), 1777 (m), 1748 (m), 1717 (s), 1681 (m), 1612 (w), 1496 (w), 1454 (w), 1388 (s), 1297 (m), 1205 (m), 1154 (s), 1068 (s), 910 (w), 874 (w), 839 (m), 796 (m), 721 (m), 700 (m) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$)[172]: δ = 2.13 (s, 3 H, $COCH_3$), 2.40-2.68 (m, 4 H, CH_2CO, CH_2COO), 3.61-3.74 (m, 2 H, 6-H, 6'-H), 3.92-3.99 (m, 1 H, 5-H), 4.32-4.72 (m, 6 H, 2-H, 3-H, 2 CH_2Ph), 5.28 (dd, $J_{3,4}$ = 8.7, $J_{4,5}$ = 9.9 Hz, 1 H, 4-H), 6.43 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1-H), 6.82-7.75 (m, 14 H, 2 Ph, Phth), 8.56 (s, 1 H, NH) ppm.

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 27.9 (*C*H_2COO), 29.7 (*C*H_3CO), 37.7 (*C*H_2CO), 54.5 (2-C), 69.0, (6-C), 72.2 (4-C), 73.5, 74.1 (2 *C*H_2Ph), 74.5 (5-C), 76.7 (3-C), 90.3 (CCl_3), 93.9 (1-C), 123.4, 127.4, 127.6, 127.8, 127.9, 128.0, 128.3, 131.4, 133.9, 137.7, 138.0 (2 Ph-C, Phth-C), 160.8 (C=NH), 167.6 (CO), 171.4 (CH_2*C*OO), 206.0 (CH_2*C*OCH_3) ppm.

MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 570.12 $[M-OC(=NHCCl_3)]^+$.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (66)**



Glycosyl donor **48** (5.57 g, 10.0 mmol) and glycosyl acceptor **50** (3.84 g, 6.60 mmol) were azeotroped with toluene (3 × 8 mL), dried under vacuo for 1 h, and dissolved in dry CH_2Cl_2 (25 mL). The solution was stirred under N_2 atmosphere at –78 °C for 10 min, and TMSOTf (0.1 M in CH_2Cl_2, 10 mL, 1.0 mmol) was added dropwise. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **66** (5.93 g, 92%; Lit.[131]: 91%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.64.

m.p. 59-60 °C.

$[\alpha]^{26}_D$ = +12.8 (*c* = 0.99, $CHCl_3$). Lit.[131]: $[\alpha]_D$ = +14.4 (*c* = 0.53, $CHCl_3$).

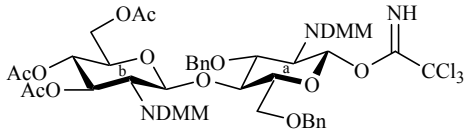
IR (KBr): 3465 (w), 2953 (m), 2858 (m), 1752 (s), 1709 (s), 1497 (w), 1403 (s), 1227 (s), 1076 (s), 912 w), 842 (s), 783 (m), 735 (s), 699 (m), 599 (w), 521 (m), 480 (w) cm^{-1}.

^{1}H and **^{13}C NMR**: in accordance with literature data.[131]

ESI-MS calcd for $C_{50}H_{64}N_2O_{16}NaSi$ $[M+Na]^+$: 999.3923. Found: 999.3922.

$C_{50}H_{64}N_2O_{16}Si$ (977.13): calcd. C 61.46, H 6.60, N 2.87; found: C 61.78, H 6.62, N 2.94.

_O_-[3,4,6-Tri-_O_-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-_O_-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl] trichloroacetimidate (67)



According to the procedure as described for **52**, a solution of **66** (4.40 g, 4.50 mmol) in dry THF (14 mL) in an ice-salt bath was treated with glacial AcOH (0.4 mL, 6.70 mmol) and TBAF (1.0 M solution in THF, 5.4 mL, 5.40 mmol). After work-up, a mixture of the crude product, trichloroacetonitrile (2.7 mL, 27.0 mmol), and DBU (0.2 mL, 1.30 mmol) in dry CH_2Cl_2 (14 mL) was stirred at room temperature overnight, and then concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2 + 1% Et_3N) to yield **67** (3.72 g, 82%; Lit.[131]: 82%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.49.

m.p. 83-85 °C.

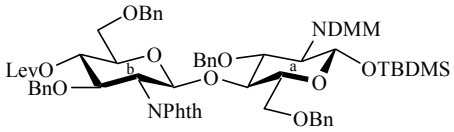
IR (KBr): 3309 (w), 3031 (w), 2951 (w), 1751 (s), 1709 (s), 1599 (w), 1497 (w), 1404 (s), 1228 (s), 1150 (m), 1049 (s), 916 (w), 834 (m), 798 (m), 734 (m), 700 (m), 643 (w), 596 (w), 520 (w), 483 (w) cm^{-1}.

^{1}H NMR: in accordance with literature data.[131]

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.5 (4 CH_3), 20.5, 20.6 (3 CO*C*H_3), 54.2 (2a-C), 55.0 (2b-C), 61.5 (6b-C), 67.7 (6a-C), 68.8 (4b-C), 70.7 (3b-C), 71.4 (5b-C), 72.8 (*C*H_2Ph), 74.2 (*C*H_2Ph), 75.5 (4a-C, 5a-C), 76.8 (3a-C), 90.5 (CCl_3), 94.0 (1a-C), 96.6 (1b-C), 127.1, 127.5, 127.6, 128.0, 128.1, 128.3, 136.9, 138.8 (C=C, 2 Ph-C), 160.9 (C=NH), 169.4, 170.0, 170.6, 171.1 (CO, 3 *C*OCH_3) ppm.

$C_{46}H_{50}Cl_3N_3O_{16}$ (1007.26): calcd. C 54.85, H 5.00, N 4.17; found: C 54.73, H 5.18, N 4.13.

***tert*-Butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (70)**



Glycosyl donor **65** (3.51 g, 4.80 mmol) and glycosyl acceptor **50** (1.86 g, 3.20 mmol) were azeotroped with toluene (3 × 6 mL), dried under vacuo for 1 h, and dissolved in dry CH_2Cl_2 (13 mL). The solution was stirred under N_2 atmosphere at 0 °C for 10 min, and TMSOTf (0.1 M in CH_2Cl_2, 2.4 mL, 0.24 mmol) was added dropwise. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **70** (3.20 g, 87%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.73$.

m.p. 48-50 °C.

$[\alpha]^{27}_D = +34.9$ ($c = 0.9$, $CHCl_3$).

IR (KBr): 3372 (m), 3245 (m), 3063 (w), 3031 (w), 2929 (m), 2858 (m), 1775 (m), 1711 (s), 1613 (m), 1497 (m), 1454 (m), 1388 (s), 1254 (m), 1206 (m), 1070 (s), 1027 (m), 928 (w), 836 (s), 783 (w), 723 (s), 698 (s), 649 (w), 619 (m), 520 (w), 464 (w), 437 (w) cm^{-1}.

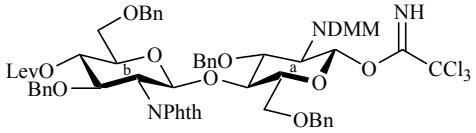
^{1}H NMR (500 MHz, $CDCl_3$): δ = −0.18, −0.06 (2 s, 6 H, 2 $SiCH_3$), 0.68 [s, 9 H, $SiC(CH_3)_3$], 1.75 (br s, 6 H, 2 CH_3), 2.10 (s, 3 H, $COCH_3$), 2.36-2.68 (m, 4 H, CH_2CO, CH_2COO), 3.24-3.28 (m, 1 H, 5a-H), 3.33 (dd, $J_{gem} = 11.0$, $J_{5,6} = 4.0$ Hz, 1 H, 6a-H), 3.40 (dd, $J_{gem} = 11.0$, $J_{5,6'} = 1.0$ Hz, 1 H, 6'a-H), 3.48 (dd, $J_{gem} = 11.0$, $J_{5,6} = 5.0$ Hz, 1 H, 6b-H), 3.57 (dd, $J_{gem} = 11.0$, $J_{5,6'} = 4.0$ Hz, 1 H, 6'b-H), 3.58-3.64 (m, 1 H, 5b-H), 3.83 (dd, $J_{1,2} = 8.5$, $J_{2,3} = 10.5$ Hz, 1 H, 2a-H), 4.02-4.08 (m, 2 H, 3a-H, 4a-H), 4.27 (dd, $J_{1,2} = 8.5$, $J_{2,3} = 11.0$ Hz, 1 H, 2b-H), 4.33, 4.66 (2 d, $J_{gem} = 12.0$ Hz, 2 H, CH_2Ph), 4.37-4.54 (m, 6 H, 3b-H, C*H*HPh, 2 CH_2Ph), 4.88 (d, $J_{gem} = 13.0$ Hz, 1 H, CH*H*Ph), 4.99 (d, $J_{1,2} = 8.5$ Hz, 1 H, 1a-H), 5.18 (dd, $J_{3,4} = J_{4,5} = 9.5$ Hz, 1 H, 4b-H), 5.35 (d, $J_{1,2} = 8.5$ Hz, 1 H, 1b-H), 6.85-7.85 (m, 24 H, 4 Ph, Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = –5.7, –4.4 (2 $SiCH_3$), 8.4 (2 CH_3), 17.5 [$SiC(CH_3)_3$], 25.2 [$SiC(CH_3)_3$], 27.9 (CH_2COO), 29.7 (CH_3CO), 37.6 (CH_2CO), 56.2 (2b-C), 57.6 (2a-C), 67.9 (6a-C), 69.3 (6b-C), 72.5 (CH_2Ph), 72.9 (4b-C), 73.2 (5b-C), 73.4, 73.9, 74.2 (3 CH_2Ph), 74.4 (5a-C), 76.3, 76.8, 77.2 (3a-C, 4a-C, 3b-C), 93.2 (1a-C), 97.2 (1b-C), 126.8, 127.2, 127.3, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2, 133.8, 136.5, 137.8, 138.2, 138.3, 139.2 (C=C, 4 Ph-C, Phth-C), 171.4 (CH_2COO), 206.1 (CH_2COCH_3) ppm.

ESI-MS calcd for $C_{65}H_{74}N_2O_{15}NaSi$ $[M+Na]^+$: 1173.4756. Found: 1173.4731.

$C_{65}H_{74}N_2O_{15}Si$ (1151.37): calcd. C 67.81, H 6.48, N 2.43; found: C 67.37, H 6.80, N 2.12.

_O_-[3,6-Di-_O_-benzyl-2-deoxy-4-_O_-levulinoyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-_O_-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl] trichloroacetimidate (71)



According to the procedure as described for **52**, a solution of **70** (2.65 g, 2.30 mmol) in dry THF (7 mL) in an ice-salt bath was treated with glacial AcOH (0.2 mL, 3.40 mmol) and TBAF (1.0 M solution in THF, 2.8 mL, 2.80 mmol). with stirring. After work-up, a mixture of the crude product, trichloroacetonitrile (1.4 mL, 14.0 mmol), and DBU (70 µL, 0.46 mmol) in dry CH_2Cl_2 (7 mL) was stirred at room temperature overnight, and then concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2 + 1% Et_3N) to yield **71** (2.09 g, 77%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.59.

m.p. 70-72 °C.

$[\alpha]^{33}_D$ = +57.1 (*c* = 0.5, $CHCl_3$).

IR (KBr): 3317 (w), 3026 (w), 3030 (w), 2872 (m), 1775 (m), 1748 (m), 1713 (s), 1497 (w), 1454 (m), 1389 (s), 1297 (m), 1207 (m), 1149 (m), 1061 (s), 911 (w), 873 (w), 836 (m), 796 (m), 723 (s), 699 (s), 643 (w), 571 (w), 521 (w), 465 (w) cm^{-1}.

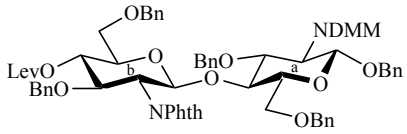
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.73 (s, 6 H, 2 CH_3), 2.11 (s, 3 H, $COCH_3$), 2.34-2.69 (m, 4 H, CH_2CO, CH_2COO), 3.37-3.50 (m, 3 H, 5a-H, 6a-H, 6b-H), 3.51-3.60 (m, 3 H,

6'a-H, 5b-H, 6'b-H), 4.09-4.18 (m, 2 H, 2a-H, 3a-H), 4.23 (dd, $J_{3,4} = J_{4,5} = 9.0$ Hz, 1 H, 4a-H), 4.26 (dd, $J_{1,2} = 8.5$, $J_{2,3} = 11.0$ Hz, 1 H, 2b-H), 4.32, 4.66 (2 d, $J_{gem} = 12.0$ Hz, 2 H, C*H*$_2$Ph), 4.42-4.55 (m, 6 H, 3b-H, C*H*HPh, 2 C*H*$_2$Ph), 4.90 (d, $J_{gem} = 12.5$ Hz, 1 H, CH*H*Ph), 5.18 (dd, $J_{3,4} = J_{4,5} = 9.5$ Hz, 1 H, 4b-H), 5.34 (d, $J_{1,2} = 8.5$ Hz, 1 H, 1b-H), 6.06 (d, $J_{1,2} = 8.5$ Hz, 1 H, 1a-H), 6.85-7.89 (m, 24 H, 4 Ph, Phth), 8.43 (s, 1 H, NH) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.4 (2 CH_3), 27.9 (*C*H_2COO), 29.7 (*C*H_3CO), 37.6 (*C*H_2CO), 54.3 (2a-C), 56.1 (2b-C), 67.5 (6a-C), 69.2 (6b-C), 72.6 (*C*H_2Ph), 72.9 (4b-C), 73.3 (5b-C), 73.4, 73.9, 74.5 (3 *C*H_2Ph), 75.4 (4a-C, 5a-C), 76.8 (3a-C, 3b-C), 90.5 (CCl_3), 94.0 (1a-C), 96.9 (1b-C), 127.0, 127.3, 127.4, 127.5, 127.6, 127.9, 128.0, 128.2, 128.3, 133.9, 136.8, 137.8, 138.2, 1383, 138.9 (C=C, 4 Ph-C, Phth-C), 160.7 (C=NH), 171.4 (CH_2*C*OO), 206.1 (CH_2*C*OCH_3) ppm.

$C_{61}H_{60}Cl_3N_3O_{15}$ (1181.49): calcd. C 62.01, H 5.12, N 3.56; found: C 62.38, H 5.22, N 3.56.

Benzyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (72)



A mixture of **71** (2.24 g, 1.90 mmol) and benzyl alcohol (0.3 mL, 2.70 mmol) in dry CH_3CN (8 mL) was stirred under N_2 atmosphere at room temperature while TMSOTf (0.01 M in CH_3CN, 1.9 mL, 19 μmol) was added dropwise. After 5 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **72** (1.97 g, 92%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.62$.

m.p. 55-56 °C.

$[\alpha]^{25}_D = +25.1$ ($c = 1.0$, $CHCl_3$).

IR (KBr): 3062 (w), 3030 (w), 2872 (m), 1775 (m), 1748 (m), 1712 (s), 1609 (w), 1496 (m), 1454 (m), 1389 (s), 1362 (s), 1207 (m), 1072 (s), 1027 (s), 910 (w), 874 (w), 821 (w), 724 (s), 699 (s), 522 (w), 464 (w) cm^{-1}.

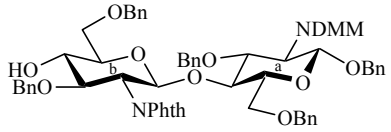
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.70 (br s, 6 H, 2 CH_3), 2.10 (s, 3 H, $COCH_3$), 2.35-2.69 (m, 4 H, CH_2CO, CH_2COO), 3.22-3.28 (m, 1 H, 5a-H), 3.39 (dd, J_{gem} = 11.0, $J_{5,6}$ = 4.0 Hz, 1 H, 6a-H), 3.42-3.52 (m, 2 H, 6'a-H, 6b-H), 3.52-3.59 (m, 2 H, 5b-H, 6'b-H), 3.93 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5 Hz, 1 H, 2a-H), 4.01 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 8.5 Hz, 1 H, 3a-H), 4.10 (dd, $J_{3,4}$ = 8.5, $J_{4,5}$ = 10.0 Hz, 1 H, 4a-H), 4.25 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 11.0 Hz, 1 H, 2b-H), 4.31, 4.65 (2 d, J_{gem} = 12.0 Hz, 2 H, CH_2Ph), 4.35, 4.68 (2 d, J_{gem} = 12.5 Hz, 2 H, CH_2Ph), 4.39-4.52 (m, 6 H, 3b-H, C*H*HPh, 2 CH_2Ph), 4.80 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1a-H), 4.86 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 5.17 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1 H, 4b-H), 5.32 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1b-H), 6.85-7.88 (m, 29 H, 5 Ph, Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.5 (2 CH_3), 27.9 (*C*H_2COO), 29.7 (*C*H_3CO), 37.6 (*C*H_2CO), 55.4 (2a-C), 56.2 (2b-C), 68.0 (6a-C), 69.3 (6b-C), 70.3, 72.6 (2 *C*H_2Ph), 72.9 (4b-C), 73.2 (5b-C), 73.4, 73.9, 74.4 (3 *C*H_2Ph), 74.4 (5a-C), 76.0 (4a-C), 76.8, 77.2 (3a-C, 3b-C), 97.1 (1b-C), 97.2 (1a-C), 126.8, 127.2, 127.3, 127.4, 127.5, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 133.9, 136.5, 137.4, 137.9, 138.2, 138.3, 139.0 (C=C, 5 Ph-C, Phth-C), 171.4 (CH_2*C*OO), 206.1 (CH_2*C*OCH_3) ppm.

ESI-MS calcd for $C_{66}H_{66}N_2O_{15}Na$ $[M+Na]^+$: 1149.4361. Found: 1149.4332.

$C_{66}H_{66}N_2O_{15}$ (1127.23): calcd. C 70.32, H 5.90, N 2.49; found: C 70.52, H 6.18, N 2.58.

Benzyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (68)[160]



Disaccharide **72** (1.35 g, 1.20 mmol) was dissolved in pyridine (6 mL) and AcOH (4 mL) at 0 °C. Hydrazine monohydrate (110 μL, 3.60 mmol) was added, and the reaction mixture was stirred for 1 h, then diluted with EtOAc (30 mL), washed with aq. HCl (10%, 3 × 15 mL), water, brine and dried over Na_2SO_4. After filtration and concentration in

vacuo, the crude product was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **68** (1.19 g, 96%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.74$.

m.p. 56-58 °C.

$[\alpha]^{34}_D = +1.5$ ($c = 0.6$, $CHCl_3$).

IR (KBr): 3461 (m), 3062 (w), 3030 (w), 2871 (m), 1775 (m), 1711 (s), 1496 (m), 1453 (m), 1389 (s), 1208 (m), 1072 (s), 1027 (s), 874 (w), 819 (w), 735 (s), 698 (s), 658 (w), 598 (w), 521 (w), 462 (w) cm^{-1}.

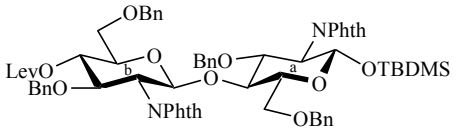
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.70 (br s, 6 H, 2 CH_3), 3.22-3.27 (m, 1 H, 5a-H), 3.34-3.44 (m, 2 H, 6a-H, 5b-H), 3.49-3.57 (m, 2 H, 6'a-H, 6b-H), 3.69 (dd, J_{gem} = 10.0, $J_{5,6'}$ = 4.5 Hz, 1 H, 6'b-H), 3.81 (dd, $J_{3,4}$ = 8.0, $J_{4,5}$ = 9.0 Hz, 1 H, 4b-H), 3.93 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.5 Hz, 1 H, 2a-H), 3.98 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 8.5 Hz, 1 H, 3a-H), 4.10 (dd, $J_{3,4}$ = 8.5, $J_{4,5}$ = 9.0 Hz, 1 H, 4a-H), 4.14 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5 Hz, 1 H, 2b-H), 4.24 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 8.0 Hz, 1 H, 3b-H), 4.35, 4.69 (2 d, J_{gem} = 12.5 Hz, 2 H, CH_2Ph), 4.40-4.54 (m, 6 H, 2 C*H*HPh, 2 CH_2Ph), 4.75-4.83 (m, 3 H, 1a-H, 2 CH*H*Ph), 5.29 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1b-H), 6.91-7.89 (m, 29 H, 5 Ph, Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.5 (2 CH_3), 55.4 (2a-C), 56.0 (2b-C), 68.1 (6a-C), 70.4 (*C*H_2Ph), 70.9 (6b-C), 72.6 (*C*H_2Ph), 72.8 (5b-C), 73.7, 74.1, 74.3 (3 *C*H_2Ph), 74.5 (5a-C), 75.3 (4b-C), 75.6 (4a-C), 77.1 (3a-C), 78.3 (3b-C), 96.9 (1b-C), 97.2 (1a-C), 123.1, 123.6, 126.8, 127.3, 127.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.5, 131.4, 131.7, 133.8, 133.9, 136.5, 137.4, 138.3, 138.4, 139.0 (C=C, 5 Ph-C, Phth-C), 167.6, 168.4 (CO) ppm.

ESI-MS calcd for $C_{61}H_{61}N_2O_{13}$ $[M+H]^+$: 1029.4174. Found: 1029.4126.

$C_{61}H_{60}N_2O_{13}$ (1029.13): calcd. C 71.19, H 5.88, N 2.72; found: C 70.85, H 6.01, N 2.75.

***tert*-Butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (73)**



Glycosyl donor **65** (2.78 g, 3.80 mmol) and glycosyl acceptor **40** (1.51 g, 2.50 mmol) were azeotroped with toluene (3 × 6 mL), dried under vacuo for 1 h, and dissolved in dry CH_2Cl_2 (10 mL). The solution was stirred under N_2 atmosphere at 0 °C for 10 min, and TMSOTf (0.1 M in CH_2Cl_2, 1.9 mL, 0.19 mmol) was added dropwise. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **73** (2.64 g, 90%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.75$.

m.p. 48-49 °C.

$[\alpha]^{28}_D = +36.8$ (c = 1.2, $CHCl_3$).

IR (KBr): 3030 (w), 2930 (w), 2858 (w), 1776 (m), 1748 (m), 1715 (s), 1612 (w), 1496 (w), 1469 (w), 1454 (w), 1388 (s), 1254 (w), 1204 (m), 1072 (s), 1027 (m), 874 (w), 841 (m), 783 (w), 722 (m), 699 (m), 530 (w), 460 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = −0.22, −0.07 (2 s, 6 H, 2 $SiCH_3$), 0.60 [s, 9 H, $SiC(CH_3)_3$], 2.11 (s, 3 H, $COCH_3$), 2.35-2.67 (m, 4 H, CH_2CO, CH_2COO), 3.28-3.32 (m, 1 H, 5a-H), 3.35 (dd, J_{gem} = 11.0, $J_{5,6}$ = 4.0 Hz, 1 H, 6a-H), 3.42 (dd, J_{gem} = 11.0, $J_{5,6'}$ = 1.5 Hz, 1 H, 6'a-H), 3.47 (dd, J_{gem} = 10.5, $J_{5,6}$ = 4.5 Hz, 1 H, 6b-H), 3.56 (dd, J_{gem} = 10.5, $J_{5,6'}$ = 3.5 Hz, 1 H, 6'b-H), 3.58-3.63 (m, 1 H, 5b-H), 4.06 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.5 Hz, 1 H, 2a-H), 4.13 (dd, $J_{3,4} = J_{4,5}$ = 9.0 Hz, 1 H, 4a-H), 4.18 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 8.5 Hz, 1 H, 3a-H), 4.27 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.5 Hz, 1 H, 2b-H), 4.32 (d, J_{gem} = 12.5 Hz, 1 H, C*H*HPh), 4.41 (d, J_{gem} = 12.0 Hz, 1 H, C*H*HPh), 4.43 (d, J_{gem} = 12.5 Hz, 1 H, C*H*HPh), 4.47 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 9.0 Hz, 1 H, 3b-H), 4.49, 4.51 (2 d, J_{gem} = 12.0 Hz, 2 H, CH_2Ph), 4.53 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 4.65 (d, J_{gem} = 12.0 Hz, 1 H, CH*H*Ph), 4.82 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 5.15 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1a-H), 5.17 (dd, $J_{3,4}$ = 9.0, $J_{4,5}$ = 10.0 Hz, 1 H, 4b-H), 5.35 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1b-H), 6.80-7.86 (m, 28 H, 4 Ph, 2 Phth) ppm.

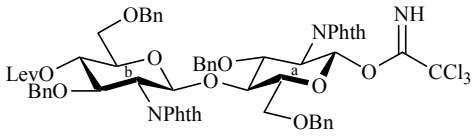
^{13}C NMR (125 MHz, $CDCl_3$): δ = −5.6, −4.4 (2 $SiCH_3$), 17.4 [Si*C*$(CH_3)_3$], 25.2 [SiC(*C*$H_3)_3$], 27.9 (*C*H_2COO), 29.7 (*C*H_3CO), 37.6 (*C*H_2CO), 56.2 (2b-C), 57.9 (2a-C), 68.0 (6a-C), 69.3 (6b-C), 72.6 (*C*H_2Ph), 73.0 (4b-C), 73.3 (5b-C), 73.4, 73.9, 74.2 (3 *C*H_2Ph), 74.5 (5a-C), 76.4, 76.5 (3a-C, 4a-C), 76.8 (3b-C), 93.1 (1a-C), 97.2 (1b-C),

126.8, 127.2, 127.3, 127.6, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 131.6, 133.5, 137.9, 138.2, 138.3, 138.7 (4 Ph-C, 2 Phth-C), 171.4 ($CH_2\mathit{C}OO$), 206.1 ($CH_2\mathit{C}OCH_3$) ppm.

MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 1195.51 $[M+Na]^+$, 1121.48 $[M+K]^+$.

$C_{67}H_{72}N_2O_{15}Si$ (1173.38): calcd. C 68.58, H 6.18, N 2.39; found: C 68.20, H 6.36, N 2.42.

_O_-[3,6-Di-_O_-benzyl-2-deoxy-4-_O_-levulinoyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-_O_-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl] trichloroacetimidate (74)



According to the procedure as described for **52**, a solution of **73** (2.11 g, 1.80 mmol) in dry THF (5 mL) in an ice-salt bath was treated with glacial AcOH (0.2 mL, 3.40 mmol) and TBAF (1.0 M solution in THF, 2.2 mL, 2.20 mmol). After work-up, a mixture of the crude product, trichloroacetonitrile (1.1 mL, 11.0 mmol), and DBU (60 µL, 0.40 mmol) in dry CH_2Cl_2 (5 mL) was stirred at room temperature overnight, and then concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 3:2 + 1% Et_3N) to yield **74** (1.86 g, 86%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.52.

m.p. 66-68 °C.

$[\alpha]^{26}_D$ = +48.2 (*c* = 0.5, $CHCl_3$).

IR (KBr): 3312 (w), 3030 (w), 2872 (w), 1777 (m), 1716 (s), 1611 (w), 1496 (w), 1468 (w), 1454 (w), 1388 (s), 1298 (m), 1206 (m), 1148 (m), 1061 (s), 874 (w), 836 (w), 796 (m), 721 (s), 699 (m), 644 (w), 572 (w), 530 (w) cm^{-1}.

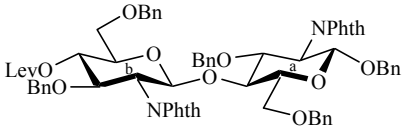
^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.11 (s, 3 H, $COCH_3$), 2.37-2.68 (m, 4 H, CH_2CO, CH_2COO), 3.43 (dd, J_{gem} = 11.5, $J_{5,6}$ = 3.5 Hz, 1 H, 6a-H), 3.45-3.52 (m, 2 H, 5a-H, 6b-H), 3.53-3.59 (m, 3 H, 6'a-H, 5b-H, 6'b-H), 4.24-4.35 (m, 4 H, 3a-H, 4a-H, 2b-H, C*H*HPh), 4.39 (dd, $J_{1,2}$ = 9.0, $J_{2,3}$ = 10.0 Hz, 1 H, 2a-H), 4.43-4.59 (m, 6 H, 3b-H, C*H*HPh, 2 CH_2Ph), 4.66 (d, J_{gem} = 12.0 Hz, 1 H, C*H*HPh), 4.85 (d, J_{gem} = 13.0 Hz, 1 H,

C*H*HPh), 5.18 (dd, $J_{3,4} = J_{4,5} = 9.5$ Hz, 1 H, 4b-H), 5.35 (d, $J_{1,2} = 8.5$ Hz, 1 H, 1b-H), 6.24 (d, $J_{1,2} = 9.0$ Hz, 1 H, 1a-H), 6.80-7.90 (m, 28 H, 4 Ph, 2 Phth), 8.40 (s, 1 H, NH) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 27.9 (*C*H_2COO), 29.7 (*C*H_3CO), 37.7 (*C*H_2CO), 54.5 (2a-C), 56.2 (2b-C), 67.6 (6a-C), 69.3 (6b-C), 72.7 (*C*H_2Ph), 72.9, 73.3 (5a-C, 4b-C), 73.4, 74.0, 74.5 (3 *C*H_2Ph), 75.5, 76.3 (3a-C, 4a-C, 5b-C), 76.9 (3b-C), 90.4 (CCl_3), 94.0 (1a-C), 96.9 (1b-C), 123.2, 127.0, 127.3, 127.4, 127.5, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 131.5, 133.7, 137.9, 138.2, 138.3, 138.4 (4 Ph-C, 2 Phth-C), 160.8 (C=NH), 171.5 (CH_2*C*OO), 206.1 (CH_2*C*OCH_3) ppm.

$C_{63}H_{58}Cl_3N_3O_{15}$ (1203.50): calcd. C 62.87, H 4.86, N 3.49; found: C 62.62, H 4.78, N 3.59.

Benzyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (75)



A mixture of **74** (1.80 g, 1.50 mmol) and benzyl alcohol (0.3 mL, 2.70 mmol) in dry CH_3CN (6 mL) was stirred under N_2 atmosphere at room temperature while TMSOTf (0.01 M in CH_3CN, 1.5 mL, 15 μmol) was added dropwise. After stirring overnight, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **75** (1.55 g, 90%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.64$.

m.p. 62-63 °C.

$[\alpha]^{29}_D = +18.6$ (*c* = 1.0, $CHCl_3$).

IR (KBr): 3062 (w), 3030 (w), 2872 (w), 1776 (m), 1747 (m), 1715 (s), 1611 (w), 1496 (w), 1454 (m), 1388 (s), 1206 (m), 1073 (s), 1027 (m), 911 (w), 874 (w), 741 (m), 722 (s), 699 (s), 530 (w), 467 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.10 (s, 3 H, $COCH_3$), 2.35-2.67 (m, 4 H, C*H*$_2$CO, C*H*$_2$COO), 3.28-3.33 (m, 1 H, 5a-H), 3.41 (dd, $J_{gem} = 11.0$, $J_{5,6} = 3.5$ Hz, 1 H, 6a-H), 3.46

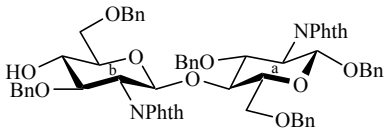
(dd, J_{gem} = 11.5, $J_{5,6}$ = 5.5 Hz, 1 H, 6b-H), 3.50-3.59 (m, 3 H, 6'a-H, 5b-H, 6'b-H), 4.10-4.20 (m, 3 H, 2a-H, 3a-H, 4a-H), 4.26 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5 Hz, 1 H, 2b-H), 4.32 (d, J_{gem} = 12.5 Hz, 1 H, C*H*HPh), 4.36 (d, J_{gem} = 12.5 Hz, 1 H, C*H*HPh), 4.40-4.55 (m, 6 H, 3b-H, C*H*HPh, 2 CH_2Ph), 4.65 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 4.68 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 4.81 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 4.95 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1a-H), 5.17 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1 H, 4b-H), 5.33 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1b-H), 6.79-7.88 (m, 33 H, 5 Ph, 2 Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 27.9 (*C*H_2COO), 29.7 (*C*H_3CO), 37.6 (*C*H_2CO), 55.6 (2a-C), 56.2 (2b-C), 68.0 (6a-C), 69.3 (6b-C), 70.4, 72.7 (2 *C*H_2Ph), 72.9 (4b-C), 73.2 (5b-C), 73.4, 74.0, 74.3 (3 *C*H_2Ph), 74.4 (5a-C), 76.1, 76.5, 76.7 (3a-C, 4a-C, 3b-C), 97.0 (1a-C, 1b-C), 123.1, 126.8, 127.2, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 131.6, 133.4, 137.1, 137.9, 138.3, 138.4, 138.5 (5 Ph-C, 2 Phth-C), 167.6 (CO), 171.5 (CH_2*C*OO), 206.2 (CH_2*C*OCH_3) ppm.

MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 1171.47 $[M+Na]^+$, 1187.44 $[M+K]^+$.

$C_{68}H_{64}N_2O_{15}$ (1149.24): calcd. C 71.07, H 5.61, N 2.44; found: C 70.87, H 5.67, N 2.44.

Benzyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (69)[160]



According to the procedure as described for **68**, disaccharide **75** (1.26 g, 1.10 mmol) was dissolved in pyridine (6 mL) and AcOH (4 mL) at 0 °C. Hydrazine monohydrate (0.1 mL, 3.30 mmol) was added, and the reaction mixture was stirred for 1 h. After work-up, the crude product was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 7:3) to yield **69** (1.12 g, 97%) as a white foam.

TLC (Toluene/EtOAc, 3:1): R_f = 0.49. Lit.[161]: 0.50.

m.p. 64-65 °C.

$[\alpha]^{30}_D$ = −7.8 (*c* = 0.7, $CHCl_3$). Lit.[161]: $[\alpha]_D$ = −7.8 (*c* = 1.1, $CHCl_3$).

IR (KBr): 3477 (w), 3062 (w), 3030 (w), 2872 (m), 1775 (m), 1714 (s), 1611 (w), 1496 (w), 1454 (m), 1388 (s), 1206 (m), 1073 (s), 1027 (m), 874 (w), 740 (m), 722 (s), 699 (s), 658 (w), 531 (w), 462 (w) cm^{-1}.

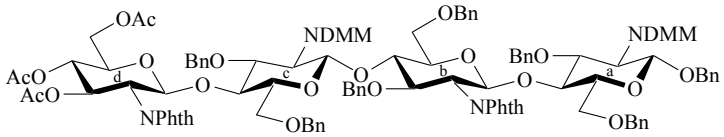
^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.09 (br s, 1 H, OH), 3.26-3.33 (m, 1 H, 5a-H), 3.33-3.40 (m, 1 H, 5b-H), 3.44 (dd, J_{gem} = 11.1, $J_{5,6}$ = 3.9 Hz, 1 H, 6a-H), 3.51-3.59 (m, 2 H, 6'a-H, 6b-H), 3.70 (dd, J_{gem} = 9.9, $J_{5,6'}$ = 4.2 Hz, 1 H, 6'b-H), 3.82 (dd, $J_{3,4} = J_{4,5}$ = 8.9 Hz, 1 H, 4b-H), 4.07-4.09 (m, 5 H, 2a-H, 3a-H, 4a-H, 2b-H, 3b-H), 4.36, 4.69 (2 d, J_{gem} = 12.3 Hz, 2 H, C*H*$_2$Ph), 4.42-4.57 (m, 6 H, 2 C*H*HPh, 2 C*H*$_2$Ph), 4.77 (d, J_{gem} = 12.3 Hz, 1 H, CH*H*Ph), 4.79 (d, J_{gem} = 12.0 Hz, 1 H, CH*H*Ph), 4.94 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1a-H), 5.31 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1b-H), 6.80-7.90 (m, 33 H, 5 Ph, 2 Phth) ppm.

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 55.7 (2b-C), 56.1 (2a-C), 68.1 (6a-C), 70.5 (6b-C), 71.0, 72.6 (2 *C*H$_2$Ph), 72.7 (5b-C), 73.7, 74.1, 74.3 (3 *C*H$_2$Ph), 74.5 (5a-C), 75.5, 75.6, 76.4 (3a-C, 4a-C, 4b-C), 78.3 (3b-C), 96.9 (1b-C), 97.1 (1a-C), 123.1, 126.9, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.2, 128.5, 131.6, 133.5, 133.9, 134.0, 137.2, 137.5, 138.3, 138.4, 138.6 (5 Ph-C, 2 Phth-C), 167.2 (CO) ppm.

ESI-MS calcd for $C_{63}H_{59}N_2O_{13}$ $[M+H]^+$: 1051.4017. Found: 1051.4004.

$C_{63}H_{58}N_2O_{13}$ (1051.14): calcd. C 71.99, H 5.56, N 2.67; found: C 71.71, H 5.63, N 2.64.

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (76)



Glycosyl donor **57** (0.43 g, 0.42 mmol) and glycosyl acceptor **68** (0.29 g, 0.28 mmol) were azeotroped with toluene (3 × 3 mL), dried under vacuo for 1h, and dissolved in dry CH_2Cl_2 (2 mL). The solution was stirred under N_2 atmosphere at –30 °C for 10 min, and TMSOTf (0.1 M in CH_2Cl_2, 0.42 mL, 42 µmol) was added dropwise. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was

purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 6:4) to yield **76** (0.44 g, 83%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): $R_f = 0.54$.

m.p. 106-108 ^{o}C.

$[\alpha]^{23}_{D} = +11.9$ ($c = 0.5$, $CHCl_3$).

IR (KBr): 3062 (w), 3030 (w), 2872 (m), 1752 (s), 1713 (s), 1610 (w), 1497 (m), 1453 (m), 1388 (s), 1227 (s), 1076 (s), 900 (w), 873 (w), 795 (w), 723 (s),699 (s), 598 (w), 522 (w), 479 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.67, 1.71, 1.74, 1.83, 1.86, 1.87, 1.97 (7 s, 21 H, 4 CH_3, 3 $COCH_3$), 2.85-2.90 (m, 1 H, 5c-H), 2.99-3.04 (m, 1 H, 5a-H), 3.10 (dd, J_{gem} = 11.0, $J_{5,6}$ = 3.0 Hz, 1 H, 6a-H), 3.16-3.21 (m, 2 H, 5b-H, 6c-H), 3.30 (dd, J_{gem} = 11.0, $J_{5,6}$ = 4.0 Hz, 1 H, 6b-H), 3.37-3.47 (m, 4 H, 6'a-H, 6'b-H, 6'c-H, 5d-H), 3.83-3.94 (m, 4 H, 2a-H, 2b-H, 3b-H, 6d-H), 3.95-4.10 (m, 5 H, 3a-H, 4a-H, 4b-H, 2c-H, 3c-H), 4.15-4.23 (m, 2 H, 4c-H, 6'd-H), 4.30 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5 Hz, 1 H, 2d-H), 4.31-4.45 (m, 9 H, 5 C*H*HPh, 2 CH_2Ph), 4.50 (d, J_{gem} = 12.0 Hz, 1 H, CH*H*Ph), 4.65 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 4.74 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1a-H), 4.79, 4.92 (2 d, J_{gem} = 13.0 Hz, 3 H, 3 CH*H*Ph), 4.94 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1b-H), 5.10 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1c-H), 5.11 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1 H, 4d-H), 5.47 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1d-H), 5.77 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 9.5 Hz, 1 H, 3d-H), 6.66-7.95 (m, 43 H, 7 Ph, 2 Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.4 (4 CH_3), 20.4, 20.5, 20.6 (3 CO*C*H_3), 55.2, 55.4 (2a-C, 2d-C), 56.3, 56.6 (2b-C, 2c-C), 61.3 (6d-C), 67.1 (6a-C, 6c-C), 68.0 (6b-C), 68.7 (4d-C), 70.3 (*C*H_2Ph), 70.7 (3d-C), 71.3 (5d-C), 72.3, 72.5 (3 *C*H_2Ph), 73.9 (5a-C, 5b-C, 5c-C), 74.3, 74.4 (3 *C*H_2Ph), 75.3, 75.7, 75.8, 77.6 (3a-C, 4a-C, 3b-C, 4b-C, 3c-C, 4c-C), 96.7 (1b-C, 1d-C), 96.8 (1c-C), 97.2 (1a-C), 123.0, 123.4, 123.6, 126.6, 126.7, 126.9, 127.0, 127.2, 127.3, 137.4, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 131.4, 131.8, 133.5, 133.7, 134.4, 136.4, 137.3, 138.3, 138.4, 138.7, 139.1, 139.2 (C=C, 7 Ph-C, 2 Phth-C), 167.5, 168.1, 169.4, 170.0, 170.6, 171.2 (CO, 3 *C*OCH_3) ppm.

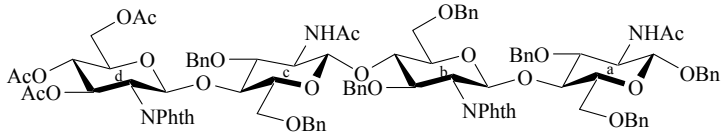
MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 1917.69 $[M+Na]^+$, 1933.66 $[M+K]^+$.

$C_{107}H_{106}N_4O_{28}$ (1895.99): calcd. C 67.78, H 5.64, N 2.96; found: C 67.48, H 5.48, N 2.92.

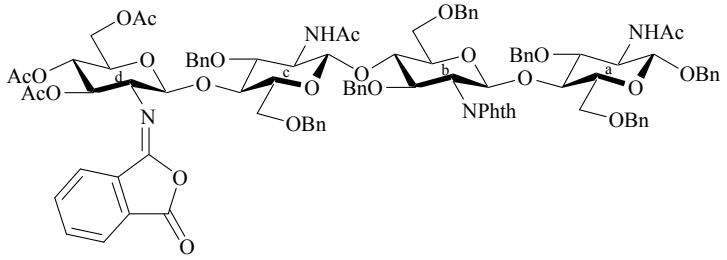
Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (77)

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalisoimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (78a)

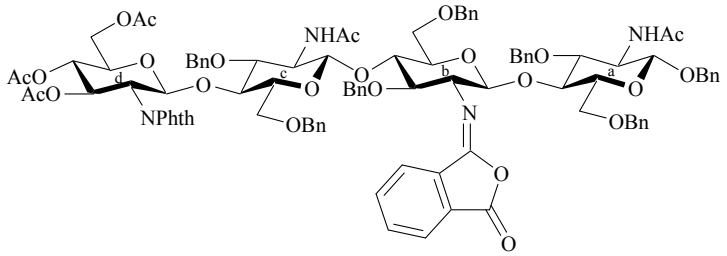
Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalisoimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (78b)



77



78a



78b

A mixture of **76** (0.19 g, 0.10 mmol) and NaOH (0.56 g, 14.0 mmol) in 5:2:1 MeOH-dioxane-water (14 mL) was stirred at room temperature overnight. Then, the pH was adjusted to 3 using aq. 3 N HCl in the presence of ethanolamine (20 µL, 0.33 mmol).

After 1 day, the solution was neutralized with ethanolamine and concentrated in vacuo. The residue was treated with 2:1 pyridine-acetic anhydride (18 mL) and stirred at room temperature overnight, then co-concentrated with toluene. Work-up and flash column chromatography (SiO_2, $CHCl_3$/EtOH, 97:3) yielded **77** (53 mg, 30%) as a white foam and a mixture of **78a** and **78b** (62 mg, 35%) as a white foam.

Compound **77**:

TLC ($CHCl_3$/EtOH, 96:4): R_f = 0.29.

m.p. 109-111 ^{o}C.

$[\alpha]^{25}_{D}$ = –9.4 (*c* = 1.0, $CHCl_3$).

IR (neat): 2867 (w), 1748 (m), 1713 (s), 1664 (m), 1529 (w), 1452 (w), 1388 (s), 1224 (s), 1046 (s), 723 (s), 697 (s), 599 (m), 558 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.72, 1.84, 1.86, 1.91, 1.98 (5 s, 15 H, 3 $COCH_3$, 2 $NCOCH_3$), 2.94-2.99 (m, 1 H, 5c-H), 3.24-3.29 (m, 2 H, 5b-H, 6c-H), 3.31-3.38 (m, 2 H, 5a-H, 5d-H), 3.41-3.61 (m, 7 H, 6a-H, 6'a-H, 6b-C, 6'b-H, 2c-H, 3c-H, 6'c-H), 3.74 (dd, $J_{2,3} = J_{3,4}$ = 6.0 Hz, 1 H, 3a-H), 3.81-3.89 (m, 2 H, 2a-H, 6d-H), 3.93 (dd, $J_{3,4} = J_{4,5}$ = 9.0 Hz, 1 H, 4b-H), 4.00 (dd, $J_{3,4} = J_{4,5}$ = 6.0 Hz, 1 H, 4a-H), 4.07 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 11.0 Hz, 1 H, 2b-H), 4.08-4.15 (m, 2 H, 4c-H, 6'd-H), 4.19-4.25 (m, 2 H, 3b-H, C*H*HPh), 4.27-4.39 (m, 6 H, 2d-H, 4 C*H*HPh, CH*H*Ph), 4.41-4.47 (m, 2 H, 1a-H, CH*H*Ph), 4.50 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1c-H), 4.54-4.72 (m, 5 H, C*H*HPh, 2 CH*H*Ph, CH_2Ph), 4.77 (d, J_{gem} = 13.0 Hz, 1 H, CH*H*Ph), 4.89-4.96 (m, 2 H, NH-2c, CH*H*Ph), 5.08-5.15 (m, 2 H, 1b-H, 4d-H), 5.43 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1d-H), 5.67 (br s, 1 H, NH-2a), 5.78 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 9.0 Hz, 1 H, 3d-H), 6.67-7.93 (m, 43 H, 7 Ph, 2 Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 20.4, 20.6 (3 CO*C*H_3), 23.3, 23.4 (2 NCO*C*H_3), 52.5 (2a-C), 55.2 (2d-C), 56.3 (2b-C, 2c-C), 61.4 (6d-C), 67.5 (6a-C), 67.8 (6c-C), 68.6 (4d-C), 68.9 (6b-C), 70.2 (*C*H_2Ph), 70.5 (3d-C), 71.4 (5d-C), 72.4, 72.6, 73.1, 73.6 (5 *C*H_2Ph), 74.1, 74.2, 74.3 (4a-C, 5a-C, 5b-C, 5c-C), 74.7 (*C*H_2Ph), 74.8 (4c-C), 77.1, 77.2, 77.3 (3a-C, 3b-C, 4b-C), 79.3 (3c-C), 96.7 (1d-C), 96.9 (1b-C), 99.4 (1a-C), 99.7 (1c-C), 123.2, 123.6, 126.7, 127.2, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 128.1, 128.2, 128.3, 128.4, 131.3, 133.7, 134.5, 137.5, 138.0, 138.2, 138.6, 138.7, 139.0 (7 Ph-C, 2 Phth-C), 167.9, 169.4, 169.8, 169.9, 170.0, 170.6 (CO, 3 *C*OCH_3, 2 N*C*OCH_3) ppm.

MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 1785.92 $[M+Na]^+$, 1801.86 $[M+K]^+$.

$C_{99}H_{102}N_4O_{26}$ (1763.88): calcd. C 67.41, H 5.83, N 3.18; found: C 66.95, H 5.84, N 3.12.

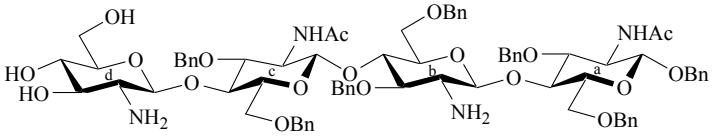
Compounds **78a** and **78b**:

IR (neat): 2917 (w), 2849 (w), 1800 (m), 1748 (m), 1716 (s), 1667 (m), 1538 (w), 1496 (w), 1453 (w), 1366 (s), 1227 (s), 1048 (s), 920 (s), 736 (s), 697 (s), 599 (m) cm^1.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 20.4, 20.5, 20.6 ($COCH_3$), 23.3, 23.4 ($NCOCH_3$), 54.5, 55.2, 55.9, 56.0, 62.8, 64.7 (2-C), 61.3, 61.8, 68.0, 68.1, 68.2, 68.4, 68.7, 70.4, 70.5, 71.4, 71.7, 72.3, 73.1, 73.2, 73.3, 73.4, 73.6, 73.9, 74.3, 74.6, 74.7, 74.9, 76.4, 76.6, 77.8, 79.5, 82.3, 82.4 (3-C, 4-C, 5-C, 6-C, CH_2Ph), 96.5, 99.3, 100.2, 101.5, 101.6 (1-C), 123.0, 123.7, 125.0, 126.5, 127.0, 127.1, 127.2, 127.4, 127.5, 127.6, 127.7, 127.9, 128.1, 128.2, 128.3, 128.4, 132.7, 133.4, 134.9, 136.2, 137.5, 138.0, 138.2, 138.3, 138.9, 139.2, 139.4 (Ph-C, Phth-C), 164.1, 164.2 (C=N), 169.4, 169.8, 169.9, 170.0, 170.5, 170.6 (CO, $COCH_3$, $NCOCH_3$) ppm.

MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 1785.92 $[M+Na]^+$, 1801.86 $[M+K]^+$.

Benzyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (81)



A solution of **77** (0.14 g, 0.08 mmol) in dry MeOH (3 mL) was treated with NaOMe (10 mg, 0.18 mmol) and stirred at room temperature. After 1 h, the reaction mixture was neutralized with Amberlite IR 120 (H^+) resin, filtered and concentrated in vacuo. The residue was dissolved in *n*-BuOH (14 mL), then ethylenediamine (0.3 mL, 4.50 mmol) was added. The mixture was stirred at 90 ^oC overnight, then co-concentrated with toluene.

The residue was purified by flash column chromatography (SiO_2, $CHCl_3$/EtOH, 9:1) to yield **81** (98 mg, 89%) as a white powder.

The reaction of a mixture of **78a** and **78b** (35 mg, 0.02 mmol) under the same conditions yielded **81** (25 mg, 92%) as a white powder after flash column chromatography.

TLC (EtOAc/MeOH, 4:1): R_f = 0.78.

m.p. 98-100 °C.

$[\alpha]^{28}_D$ = −9.3 (c = 0.5, $CHCl_3$).

IR (neat): 2866 (m), 1652 (m), 1453 (m), 1367 (m), 1052 (s), 735 (s), 696 (s), 597 (m) cm^{-1}.

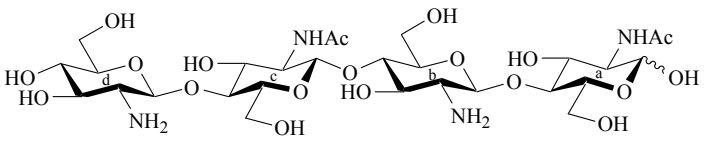
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.71, 1.78 (2 s, 6 H, 2 $NCOCH_3$), 2.51 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.0 Hz, 1 H, 2d-H), 2.76 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.5 Hz, 1 H, 2b-H), 2.99-3.06 (m, 1 H, 5b-H), 3.12-3.33 (m, 5 H, 5a-H, 3b-H, 5c-H, 3d-H, 4d-H), 3.41 (dd, J_{gem} = 11.5, $J_{5,6}$ = 4.5 Hz, 1 H, 6d-H), 3.47-3.73 (m, 10 H, 2a-H, 3a-H, 6a-H, 6'a-H, 2c-H, 3c-H, 6c-H, 6'c-H, 5d-H, 6'd-H), 3.82 (dd, J_{gem} = 10.0, $J_{5,6}$ = 2.0 Hz, 1 H, 6b-H), 3.88-4.06 (m, 4 H, 4a-H, 4b-H, 6'b-H, 4c-H), 4.22 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1d-H), 4.28 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1b-H), 4.32-4.45 (m, 3 H, 3 C*H*HPh), 4.46-4.65 (m, 8 H, 1a-H, 4 C*H*HPh, 3 CH*H*Ph), 4.77-4.89 (m, 4 H, 1c-H, 3 CH*H*Ph), 5.12 (d, J_{gem} = 11.5 Hz, 1 H, CH*H*Ph), 5.31 (br s, 1 H, NH-2a), 5.68 (d, $J_{2,NH}$ = 8.0 Hz, 1 H, NH-2c), 7.12-7.38 (m, 35 H, 7 Ph) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 23.3, 23.4 (2 $NCOCH_3$), 54.5 (2c-C), 56.2 (2a-C), 57.1 (2b-C), 57.6 (2d-C), 62.0 (6d-C), 68.4, 68.5, 68.7 (6a-C, 6b-C, 6c-C), 70.5 (CH_2Ph), 70.9 (4d-C, 5d-C), 73.2, 73.3, 74.1 (6 CH_2Ph), 74.5, 74.8, 75.7, 76.0, 76.1, 77.6 (3a-C, 4a-C, 5a-C, 4b-C, 5b-C, 3c-C, 4c-C, 5c-C, 3d-C), 83.0 (3b-C), 99.1 (1c-C), 99.6 (1a-C), 102.1 (1d-C), 103.1 (1b-C), 127.3, 127.4, 127.6, 127.7, 127.8, 127.9, 128.2, 128.3, 128.4, 128.5, 128.6, 137.5, 137.9, 138.0, 138.1, 138.5, 138.9, 139.0 (7 Ph-C), 170.1, 170.2 (2 $NCOCH_3$) ppm.

ESI-MS calcd for $C_{77}H_{93}N_4O_{19}$ $[M+H]^+$: 1377.6434. Found: 1377.6400.

$C_{77}H_{92}N_4O_{19}$ (1377.57): calcd. C 67.13, H 6.73, N 4.07; found: C 66.70, H 7.14, N 3.86.

2-Amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (61)



To a solution of **81** (40 mg, 29 μmol) in 4:1 2-PrOH-water (4 mL) was added 10% Pd/C (100 mg) and 4 drops of HOAc, and then stirred at room temperature under H_2 atmosphere. After 2 days, the reaction mixture was filtered through Celite and concentrated. The residue was dissolved in water (1 mL) and applied on an ISOLUTE SPE C_{18} column which was eluted with water, concentrated and lyophilized to yield **61** (14 mg, 86%) as a white amorphous powder in the ratio of α:β = 1:1.

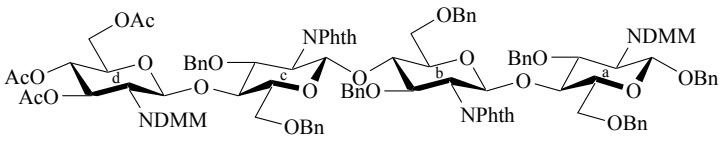
IR (neat): 3278 (br s), 1637 (s), 1557 (s), 1376 (s), 1316 (m), 1028 (s) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 2.06, 2.08 (2 s, 6 H, 2 $NCOCH_3$), 2.83-2.92 (m, 2 H, 2b-H, 2d-H), 3.43-4.03 (m, 22 H, 2a-H, 2c-H, 3a-H, 3b-H, 3c-H, 3d-H, 4a-H, 4b-H, 4c-H, 4d-H, 5a-H, 5b-H, 5c-H, 5d-H, 6a-H, 6b-H, 6c-H, 6d-H, 6'a-H, 6'b-H, 6'c-H, 6'd-H), 4.61 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1c-H), 4.63 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1d-H), 4.66 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1b-H), 4.74 (d, $J_{1,2}$ = 8.4 Hz, 0.5 H, $1a_\beta$-H), 5.22 (d, $J_{1,2}$ = 2.4 Hz, 0.5 H, $1a_\alpha$-H) ppm.

^{13}C NMR (150 MHz, D_2O): δ = 23.2, 23.4, 23.5 ($NCOCH_3$), 55.2 ($2a_\alpha$-C), 56.8 (2c-C), 57.0 (2b-C), 57.2 (2d-C), 57.9 ($2a_\beta$-C), 61.0 (6b-C), 61.5 ($6a_\alpha$-C, 6c-C), 61.6 ($6a_\beta$-C, 6d-C), 70.1 ($3a_\alpha$-C), 70.8 (4d-C), 71.2 ($5a_\alpha$-C), 72.6 (3c-C), 72.7 ($3a_\beta$-C), 73.2 (3b-C), 73.8 (3d-C), 75.6 (5c-C), 75.7 ($5a_\beta$-C), 76.3 (5b-C), 77.6 (5d-C), 78.0, 78.1 ($4a_\beta$-C, 4c-C), 78.4 ($4a_\alpha$-C), 79.7 (4b-C), 91.8 ($1a_\alpha$-C), 96.0 ($1a_\beta$-C), 99.5 (1b-C), 99.8 (1d-C), 102.6 (1c-C), 175.8, 175.9, 176.0 ($COCH_3$) ppm.

ESI-MS calcd for $C_{28}H_{51}N_4O_{19}$ $[M+H]^+$: 747.3148. Found: 747.3168.

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (84)



Glycosyl donor **52** (1.65 g, 1.60 mmol) and glycosyl acceptor **68** (1.13 g, 1.10 mmol) were azeotroped with toluene (3 × 5 mL), dried under vacuo for 1 h, and dissolved in dry CH_2Cl_2 (6 mL). The solution was stirred under N_2 atmosphere at −30 °C for 10 min, and TMSOTf (0.1 M in CH_2Cl_2, 1.6 mL, 0.16 mmol) was added dropwise. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 6:4) to yield **84** (1.77 g, 85%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.43.

m.p. 105-107 °C.

$[\alpha]^{33}_D$ = +1.9 (*c* = 0.6, $CHCl_3$).

IR (KBr): 3469 (w), 3062 (w), 3030 (w), 2872 (m), 1752 (s), 1713 (s), 1611 (w), 1497 (m), 1454 (m), 1389 (s), 1227 (s), 1077 (s), 910 (w), 874 (w), 795 (w), 723 (s), 699 (s), 598 (w), 521 (w), 478 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.65 (br s, 6 H, 2 CH_3), 1.87, 1.89, 1.96, 1.98 (4 s, 15 H, 2 CH_3, 3 $COCH_3$), 2.80-2.84 (m, 1 H, 5b-H), 3.09 (dd, J_{gem} = 11.0, $J_{5,6}$ = 2.5 Hz, 1 H, 6b-H), 3.10-3.19 (m, 2 H, 5a-H, 5c-H), 3.22-3.34 (m, 3 H, 6a-H, 6'b-H, 6c-H), 3.34-3.39 (m, 1 H, 5d-H), 3.44 (br d, J_{gem} = 10.0 Hz, 1 H, 6'a-H), 3.57 (br d, J_{gem} = 11.0 Hz, 1 H, 6'c-H), 3.82-3.92 (m, 3 H, 2a-H, 3a-H, 6d-H), 3.98 (dd, $J_{3,4}$ = 9.5, $J_{4,5}$ = 8.5 Hz, 1 H, 4a-H), 4.03-4.27 (m, 8 H, 2b-H, 3b-H, 4b-H, 2c-H, 3c-H, 4c-H, 2d-H, 6'd-H), 4.28-4.50 (m, 9 H, 5 C*H*HPh, 2 CH_2Ph), 4.58 (d, J_{gem} = 12.0 Hz, 1 H, CH*H*Ph), 4.64 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 4.73 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1a-H), 4.76 (d, J_{gem} = 13.0 Hz, 1 H, CH*H*Ph), 4.83 (d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 4.88 (d, J_{gem} = 13.0 Hz, 1 H, CH*H*Ph), 5.02-5.08 (m, 2

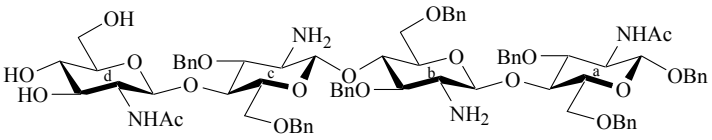
H, 1b-H, 4d-H), 5.18 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1c-H), 5.33 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1d-H), 5.59 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 9.0 Hz, 1 H, 3d-H), 6.70-7.88 (m, 43 H, 7 Ph, 2 Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.4, 8.8 (4 CH_3), 20.4, 20.5 (3 CO*C*H_3), 55.1, 55.4 (2a-C, 2d-C), 56.5, 56.6 (2b-C, 2c-C), 61.4 (6d-C), 67.0, 67.2 (6b-C, 6c-C), 68.1 (6a-C), 68.6 (4d-C), 70.3 (*C*H_2Ph), 70.8 (3d-C), 71.2 (5d-C), 72.2, 72.4, 72.5 (3 *C*H_2Ph), 74.1, 74.4 (5a-C, 5b-C, 5c-C), 74.2, 74.3, 74.6 (3 *C*H_2Ph), 75.3, 75.5, 75.9, 76.9, 77.1 (3a-C, 4a-C, 3b-C, 4b-C, 3c-C, 4c-C), 96.6, 96.7, 96.8 (1b-C, 1c-C, 1d-C), 97.2 (1a-C), 123.0, 123.1, 123.3, 123.4, 126.6, 126.7, 126.9, 127.0, 127.2, 127.3, 127.4, 127.5, 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 131.5, 131.8, 133.5, 133.7, 133.9, 136.4, 137.3, 138.2, 138.4, 138.5, 138.6, 139.1 (C=C, 7 Ph-C, 2 Phth-C), 167.5, 168.1, 169.4, 170.0, 170.5, 171.2 (CO, 3 *C*OCH_3) ppm.

MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 1917.48 $[M+Na]^+$, 1933.47 $[M+K]^+$.

$C_{107}H_{106}N_4O_{28}$ (1895.99): calcd. C 67.78, H 5.64, N 2.96; found: C 67.68, H 5.89, N 2.90.

Benzyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (87)



A mixture of **84** (0.47 g, 0.25 mmol) and NaOH (2.40 g, 60.0 mmol) in 4:1 dioxane-water (40 mL) was stirred at room temperature overnight. Then, the pH was adjusted to 3 using aq. 3 N HCl in the presence of ethanolamine (40 µL, 0.66 mmol). After 1 day, the solution was neutralized with ethanolamine and concentrated in vacuo. The residue was treated with 2:1 pyridine-acetic anhydride (30 mL) and stirred at room temperature overnight, then co-concentrated with toluene. Work-up and flash column chromatography (SiO_2, $CHCl_3$/EtOH, 97:3) yielded a mixture of **85**, **86a** and **86b** (0.27 g, 62%) as a white foam.

A solution of the product obtained in dry MeOH (6 mL) was treated with NaOMe (18 mg, 0.33 mmol) and stirred at room temperature. After 1 h, the reaction mixture was neutralized with Amberlite IR 120 (H^+) resin, filtered and concentrated in vacuo. The residue was dissolved in *n*-BuOH (30 mL), then ethylenediamine (0.7 mL, 10.5 mmol) was added. The mixture was stirred at 90 ^{o}C overnight, then co-concentrated with toluene. The residue was purified by flash column chromatography (SiO_2, $CHCl_3$/EtOH, 9:1) to yield **87** (0.18 g, 86%) as a white powder.

TLC (EtOAc/MeOH, 4:1): R_f = 0.60.

m.p. 102-103 ^{o}C.

$[\alpha]^{25}_{D}$ = –27.2 (*c* = 0.5, $CHCl_3$).

IR (neat): 3392 (br m), 3087 (w), 3062 (w), 3030 (w), 2869 (m), 1956 (w), 1879 (w), 1813 (w), 1714 (w), 1659 (s), 1556 (m), 1496 (m), 1454 (s), 1369 (s), 1311 (m), 1208 (m), 1058 (s), 1028 (s), 913 (w), 821 (w), 738 (s), 698 (s), 601 (w), 562 (w), 462 (w), 413 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.70, 1.78 (2 s, 6 H, 2 $NCOCH_3$), 2.77 (dd, $J_{1,2}$ = 7.5, $J_{2,3}$ = 9.0 Hz, 1 H, 2c-H), 2.83 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.5 Hz, 1 H, 2b-H), 3.13-3.20 (m, 3 H, 5b-H, 3c-H, 5d-H), 3.22-3.42 (m, 5 H, 3b-H, 5c-H, 3d-H, 4d-H, 6d-H), 3.41-3.53 (m, 2 H, 6a-H, 2d-H), 3.55 (dd, J_{gem} = 10.5, $J_{5,6}$ = 3.0 Hz, 1 H, 6'a-H), 3.62-3.76 (m, 5 H, 2a-H, 5a-H, 6b-H, 4c-H, 6'd-H), 3.83-3.90 (m, 2 H, 6'b-H, 6c-H), 3.92-4.01 (m, 2 H, 3a-H, 6'c-H), 4.03-4.10 (m, 2 H, 4a-H, 4b-H), 4.29 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1c-H), 4.33 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1b-H), 4.33-4.48 (m, 4 H, 1d-H, C*H*HPh, CH_2Ph), 4.50-4.66 (m, 7 H, 4 C*H*HPh, CH*H*Ph, CH_2Ph), 4.82-4.89 (m, 3 H, 1a-H, 2 CH*H*Ph), 5.00 (d, J_{gem} = 11.0 Hz, 1 H, CH*H*Ph), 5.09 (d, J_{gem} = 11.5 Hz, 1 H, CH*H*Ph), 5.69 (d, $J_{2,NH}$ = 8.0 Hz, 1 H, NH-2a), 6.37 (d, $J_{2,NH}$ = 5.0 Hz, 1 H, NH-2d), 7.18-7.40 (m, 35 H, 7 Ph) ppm.

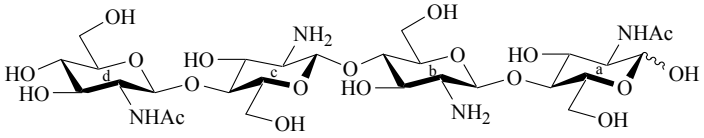
^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.7, 23.4 (2 NCO*C*H_3), 54.7 (2a-C), 57.0, 57.2 (2b-C, 2c-C), 57.9 (2d-C), 62.5 (6d-C), 68.1 (6b-C), 68.9 (6c-C), 70.5 (6a-C), 72.0 (*C*H_2Ph), 72.3 (4d-C), 72.9, 73.1 (2 *C*H_2Ph), 73.2 (3d-C), 73.4 (*C*H_2Ph), 74.4, 74.6, 74.9 (3 *C*H_2Ph), 74.8, 75.0, 75.3, 75.8, 76.1, 77.1 (4a-C, 5a-C, 4b-C, 5b-C, 5c-C, 5d-C), 77.6 (3a-C), 80.1 (4c-C), 82.6, 83.0 (3b-C, 3c-C), 99.1, 99.4 (1a-C, 1d-C), 102.6, 103.0 (1b-C, 1c-C), 127.2,

127.3, 127.5, 127.6, 127.7, 127.8, 127.9, 128.2, 128.3, 128.5, 128.8, 128.9, 129.1, 136.2, 137.5, 138.2, 138.5, 138.9, 139.0 (7 Ph-C), 170.0, 173.1 (2 N*C*OCH$_3$) ppm.

ESI-MS calcd for $C_{77}H_{93}N_4O_{19}$ $[M+H]^+$: 1377.6434. Found: 1377.6420.

$C_{77}H_{92}N_4O_{19}$ (1377.57): calcd. C 67.13, H 6.73, N 4.07; found: C 67.63, H 6.77, N 3.92.

2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (62)



To a solution of **87** (45 mg, 33 μmol) in 4:1 2-PrOH-water (5 mL) was added 10% Pd/C (112 mg) and 5 drops of HOAc, and then stirred at room temperature under H_2 atmosphere. After 2 days, the reaction mixture was filtered through Celite and concentrated. The residue was dissolved in water (1 mL) and applied on an ISOLUTE SPE C_{18} column which was eluted with water, concentrated and lyophilized to yield **62** (21 mg, 86%) as a white amorphous powder in the ratio of α:β = 1:1.

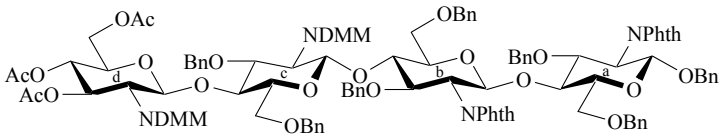
IR (neat): 3257 (br s), 1636 (s), 1551 (s), 1376 (s), 1025 (s) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 2.06, 2.08 (2 s, 6 H, 2 NCOCH$_3$), 2.93-3.00 (m, 2 H, 2b-H, 2c-H), 3.46-3.54 (m, 2 H, 4d-H, 5d-H), 3.57-3.62 (m, 2 H, 5c-H, 3d-H), 3.62-3.98 (m, 17.5 H, 2a-H, 2d-H, 3a-H, 3b-H, 3c-H, 4a-H, 4b-H, 4c-H, 5a$_\beta$-H, 5b-H, 6a-H, 6b-H, 6c-H, 6d-H, 6'a-H, 6'b-H, 6'c-H, 6'd-H), 4.00-4.04 (m, 0.5 H, 5a$_\alpha$-H), 4.59 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1d-H), 4.70 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1c-H), 4.72 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1b-H), 4.75 (d, $J_{1,2}$ = 7.8 Hz, 0.5 H, 1a$_\beta$-H), 5.22 (d, $J_{1,2}$ = 2.4 Hz, 0.5 H, 1a$_\alpha$-H) ppm.

^{13}C NMR (150 MHz, D_2O): δ = 23.2, 23.4, 23.5 (NCO*C*H$_3$), 55.2 (2a$_\alpha$-C), 56.9 (2d-C), 57.0 (2c-C), 57.2 (2b-C), 57.9 (2a$_\beta$-C), 61.1, 61.3, 61.6, 61.7 (6a$_\alpha$-C, 6a$_\beta$-C, 6b-C, 6c-C), 61.9 (6d-C), 70.1 (3a$_\alpha$-C), 71.1 (4d-C), 71.2 (5a$_\alpha$-C), 72.1 (3a$_\beta$-C), 72.4 (3b-C), 73.2 (3c-C), 74.6 (3d-C), 75.7 (5a$_\beta$-C), 76.1 (5b-C), 76.3 (5c-C), 77.2 (5d-C), 77.8 (4b-C), 78.2 (4a$_\beta$-C), 78.5 (4a$_\alpha$-C), 80.0 (4c-C), 91.9 (1a$_\alpha$-C), 96.1 (1a$_\beta$-C), 99.4 (1c-C), 99.5 (1b-C), 102.8 (1d-C), 175.8, 175.9, 176.0 (*C*OCH$_3$) ppm.

ESI-MS calcd for $C_{28}H_{51}N_4O_{19}$ $[M+H]^+$: 747.3148. Found: 747.3156.

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (88)



Glycosyl donor **67** (0.76 g, 0.75 mmol) and glycosyl acceptor **69** (0.53 g, 0.50 mmol) were azeotroped with toluene (3 × 3 mL), dried under vacuo for 1 h, and dissolved in dry CH_2Cl_2 (3 mL). The solution was stirred under N_2 atmosphere at −30 °C for 10 min, and TMSOTf (0.1 M in CH_2Cl_2, 0.75 mL, 75 µmol) was added dropwise. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, cyclohexane/EtOAc, 6:4) to yield **88** (0.78 g, 82%) as a white foam.

TLC (*n*-Hexane/EtOAc, 1:1): R_f = 0.41.

m.p. 106-108 °C.

$[\alpha]^{33}_D$ = +5.9 (*c* = 0.6, $CHCl_3$).

IR (KBr): 3062 (w), 3030 (m), 2872 (m), 1752 (s), 1713 (s), 1611 (w), 1497 (w), 1453 (m), 1389 (s), 1227 (s), 1077 (s), 1049 (s), 911 (w), 874 (w), 736 (s), 722 (s), 699 (s), 599 (w), 521 (w), 480 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.75 (br s, 3 H, CH_3), 1.86, 1.88, 1.95 (3 s, 18 H, 3 CH_3, 3 $COCH_3$), 3.02-3.06 (m, 1 H, 5c-H), 3.06-3.10 (m, 1 H, 5b-C), 3.13 (dd, J_{gem} = 11.5, $J_{5,6}$ = 3.0 Hz, 1 H, 6c-H), 3.20-3.27 (m, 2 H, 5a-H, 6b-H), 3.33-3.38 (m, 2 H, 6a-H, 5d-H), 3.44-3.58 (m, 3 H, 6'a-H, 6'b-H, 6'c-H), 3.87 (dd, J_{gem} = 11.5, $J_{5,6}$ = 1.5 Hz, 1 H, 6d-H), 3.90 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.5 Hz, 1 H, 2b-H), 4.01-4.19 (m, 10 H, 2a-H, 3a-H, 4a-H, 3b-H, 4b-H, 2c-H, 3c-H, 4c-H, 2d-H, 6'd-H), 4.33, 4.65 (2 d, J_{gem} = 12.5 Hz, 2 H, CH_2Ph), 4.36, 4.88 (2 d, J_{gem} = 13.0 Hz, 2 H, CH_2Ph), 4.39-4.47 (m, 6 H, 4 C*H*HPh, CH_2Ph), 4.53

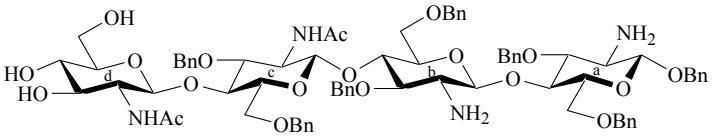
(d, J_{gem} = 12.5 Hz, 1 H, CH*H*Ph), 4.55 (d, J_{gem} = 12.0 Hz, 1 H, CH*H*Ph), 4.75, 4.84 (2 d, J_{gem} = 13.0 Hz, 2 H, 2 CH*H*Ph), 4.90 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1a-H), 5.02 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1b-H), 5.05 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1 H, 4d-H), 5.14 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1c-H), 5.3 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1d-H), 5.58 (dd, $J_{2,3}$ = 10.0, $J_{3,4}$ = 9.5 Hz, 1 H, 3d-H), 6.65-7.83 (m, 43 H, 7 Ph, 2 Phth) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.8 (4 CH_3), 20.4, 20.5, 20.6 (3 CO*C*H_3), 55.1, 55.7 (2c-C, 2d-C), 56.3 (2b-C), 56.6 (2a-C), 61.4 (6d-C), 67.0, 67.3 (6b-C, 6c-C), 68.1 (6a-C), 68.7 (4d-C), 70.4 (*C*H_2Ph), 70.8 (3d-C), 71.2 (5d-C), 72.4, 72.5, 72.6, 74.3, 74.4 (6 *C*H_2Ph), 74.2, 74.5, 74.6 (5a-C, 5b-C, 5c-C), 75.6, 75.7, 75.8, 77.2, 77.6 (3a-C, 4a-C, 3b-C, 4b-C, 3c-C, 4c-C), 96.7, 96.8 (1c-C, 1d-C), 97.0, 97.1 (1a-C, 1b-C), 123.0, 126.6, 126.7, 127.0, 127.1, 127.2, 127.3, 127.4, 127.5, 127.6, 127.7, 127.9, 128.0, 128.2, 128.3, 131.7, 133.3, 133.5, 133.7, 137.2, 138.4, 138.5, 138.7, 138.8, 139.2 (C=C, 7 Ph-C, 2 Phth-C), 167.5, 169.4, 170.0, 170.6 (CO, 3 *C*OCH$_3$) ppm.

MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 1917.28 $[M+Na]^+$, 1933.24 $[M+K]^+$.

$C_{107}H_{106}N_4O_{28}$ (1895.99): calcd. C 67.78, H 5.64, N 2.96; found: C 67.89, H 5.21, N 2.85.

Benzyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (91)



A mixture of **88** (0.25 g, 0.13 mmol) and NaOH (1.26 g, 31.5 mmol) in 4:1 dioxane-water (21 mL) was stirred at room temperature overnight. Then, the pH was adjusted to 3 using aq. 3 N HCl in the presence of ethanolamine (20 µL, 0.33 mmol). After 1 day, the solution was neutralized with ethanolamine and concentrated in vacuo. The residue was treated with 2:1 pyridine-acetic anhydride (30 mL) and stirred at room temperature overnight, then co-concentrated with toluene. Work-up and flash column chromatography (SiO_2, $CHCl_3$/EtOH, 97:3) yielded a mixture of **89**, **90a** and **90b** (0.15 g, 64%) as a white foam.

A solution of the product obtained in dry MeOH (3 mL) was treated with NaOMe (10 mg, 0.18 mmol) and stirred at room temperature. After 1 h, the reaction mixture was neutralized with Amberlite IR 120 (H^+) resin, filtered and concentrated in vacuo. The residue was dissolved in *n*-BuOH (14 mL), then ethylenediamine (0.4 mL, 6.00 mmol) was added. The mixture was stirred at 90 °C overnight, then co-concentrated with toluene. The residue was purified by flash column chromatography (SiO_2, $CHCl_3$/EtOH, 9:1) to yield **91** (0.10 g, 85%) as a white powder.

TLC (EtOAc/MeOH, 4:1): R_f = 0.78.

m.p. 87-89 °C.

$[\alpha]^{26}_D$ = −32.0 (*c* = 0.5, $CHCl_3$).

IR (KBr): 3393 (s), 3062 (m), 3030 (m), 2868 (s), 1956 (w), 1665 (s), 1549 (m), 1497 (m), 1453 (s), 1370 (s), 1311 (m), 1209 (m), 1059 (s), 738 (s), 699 (s), 601 (m), 562 (m), 465 (w), 433 (w), 407 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.70, 1.71 (2 s, 6 H, 2 $NCOCH_3$), 2.80 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 9.5 Hz, 1 H, 2a-H), 2.94 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 9.0 Hz, 1 H, 2b-H), 3.10-3.23 (m, 3 H, 3a-H, 5a-H, 5d-H), 3.25-3.35 (m, 3 H, 5c-H, 6c-H, 3d-H), 3.35-3.45 (m, 4 H, 3b-H, 5b-H, 4d-H, 6d-H), 3.46-3.59 (m, 5 H, 6a-H, 6'a-H, 3c-H, 6'c-H, 2d-H), 3.63 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 9.0 Hz, 1 H, 2c-H), 3.68 (dd, J_{gem} = 12.0, $J_{5,6'}$ = 2.5 Hz, 1 H, 6'd-H), 3.75 (dd, $J_{3,4}$ = $J_{4,5}$ = 8.5 Hz, 1 H, 4c-H), 3.82 (br d, *J* = 10.0 Hz, 1 H, 6b-H), 3.92 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1 H, 4a-H), 3.98 (dd, J_{gem} = 11.0, $J_{5,6'}$ = 3.0 Hz, 1 H, 6'b-H), 4.11 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1 H, 4b-H), 4.25-4.40 (m, 6 H, 1a-H, 1b-H, 1d-H, 3 C*H*HPh), 4.45-4.52 (m, 7 H, 1c-H, 3 C*H*HPh, 3 CH*H*Ph), 4.71 (d, J_{gem} = 12.0 Hz, 1 H, CH*H*Ph), 4.79 (d, J_{gem} = 11.5 Hz, 1 H, CH*H*Ph), 4.91 (d, J_{gem} = 11.5 Hz, 1 H, CH*H*Ph), 5.08, 5.16 (2 d, J_{gem} = 11.5 Hz, 2 H, CH_2Ph), 5.22 (d, $J_{2,NH}$ = 8.5 Hz, 1 H, NH-2c), 6.18 (d, $J_{2,NH}$ = 5.5 Hz, 1 H, NH-2d), 7.15-7.44 (m, 35 H, 7 Ph) ppm.

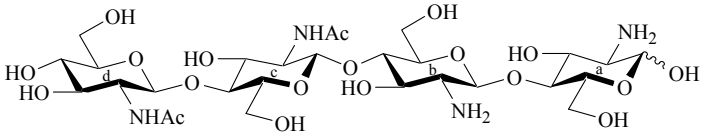
^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.8, 23.3 (2 $NCOCH_3$), 55.7 (2c-C), 56.4 (2b-C), 57.4 (2d-C), 57.6 (2a-C), 62.3 (6d-C), 68.2, 68.6 (6a-C, 6b-C), 70.9 (*C*H_2Ph), 71.3 (6c-C), 72.0 (4d-C), 73.2, 74.2, 74.3, 74.6, 74.7 (6 *C*H_2Ph), 73.3, 74.5, 75.3, 75.6, 75.8, 76.6 (4a-C, 5a-C, 4b-C, 5b-C, 5c-C, 3d-C, 5d-C), 79.0 (4c-C), 79.6 (3c-C), 80.0 (3b-C), 83.3 (3a-C), 99.4 (1d-C), 99.8 (1c-C), 102.8, 102.9 (1a-C, 1b-C), 127.2, 127.3, 127.4, 127.5, 127.6,

127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6, 128.8, 128.9, 136.5, 137.3, 138.1, 138.2, 138.4, 139.1, 139.2 (7 Ph-C), 170.1, 172.8 (2 N*C*OCH$_3$) ppm.

MALDI-MS (positive mode, THAP/MeOH matrix); *m/z*: 1399.58 $[M+Na]^+$, 1415.55 $[M+K]^+$.

$C_{77}H_{92}N_4O_{19}$ (1377.57): calcd. C 67.13, H 6.73, N 4.07; found: C 66.71, H 6.69, N 3.92.

2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranose (63)



To a solution of **91** (30 mg, 22 μmol) in 4:1 2-PrOH-water (3 mL) was added 10% Pd/C (75 mg) and 3 drops of HOAc, and then stirred at room temperature under H_2 atmosphere. After 2 days, the reaction mixture was filtered through Celite and concentrated. The residue was dissolved in water (1 mL) and applied on an ISOLUTE SPE C_{18} column which was eluted with water, concentrated and lyophilized to yield **63** (13 mg, 81%) as a white amorphous powder in the ratio of α:β = 1:1.

IR (neat): 3277 (br s), 1637 (s), 1557 (s), 1376 (s), 1027 (s) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 2.07, 2.08 (2 s, 6 H, 2 NCOCH$_3$), 2.97 (dd, $J_{1,2}$ = 8.4, $J_{2,3}$ = 9.6 Hz, 1 H, 2b-H), 3.02 (dd, $J_{1,2}$ = 8.4, $J_{2,3}$ = 9.6 Hz, 0.5 H, 2a$_\beta$-H), 3.32 (dd, $J_{1,2}$ = 3.0, $J_{2,3}$ = 10.2 Hz, 0.5 H, 2a$_\alpha$-H), 3.46-4.07 (m, 22 H, 2c-H, 2d-H, 3a-H, 3b-H, 3c-H, 3d-H, 4a-H, 4b-H, 4c-H, 4d-H, 5a-H, 5b-H, 5c-H, 5d-H, 6a-H, 6'a-H, 6b-H, 6'b-H, 6c-H, 6'c-H, 6d-H, 6'd-H), 4.58 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1c-H), 4.61 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1d-H), 4.71 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1b-H), 4.91 (d, $J_{1,2}$ = 8.4 Hz, 0.5 H, 1a$_\beta$-H), 5.45 (d, $J_{1,2}$ = 3.0 Hz, 0.5 H, 1a$_\alpha$-H) ppm.

^{13}C NMR (150 MHz, D_2O): δ = 23.4 (2 NCO*C*H$_3$), 55.6 (2a$_\alpha$-C), 56.3 (2c-C), 56.9 (2d-C), 57.2 (2b-C), 58.1 (2a$_\beta$-C), 60.1 (6a$_\alpha$-C), 61.2 (6a$_\beta$-C, 6b-C), 61.4 (6c-C), 61.8 (6d-C), 69.6 (3a$_\alpha$-C), 71.0 (4d-C), 71.4 (5a$_\alpha$-C), 71.7 (3a$_\beta$-C), 73.4 (3b-C, 3c-C), 74.7 (3d-C), 75.8 (5c-C), 76.0 (5a$_\beta$-C), 76.2 (5b-C),77.2 (5d-C), 78.2 (4a$_\alpha$-C, 4a$_\beta$-C), 80.1 (4b-C), 80.7

(4c-C), 90.4 (1a$_\alpha$-C), 94.6 (1a$_\beta$-C), 100.8 (1b-C), 102.6 (1d-C), 102.8 (1c-C), 175.8 (2 *C*OCH$_3$) ppm.

ESI-MS calcd for $C_{28}H_{51}N_4O_{19}$ $[M+H]^+$: 747.3148. Found: 747.3189.

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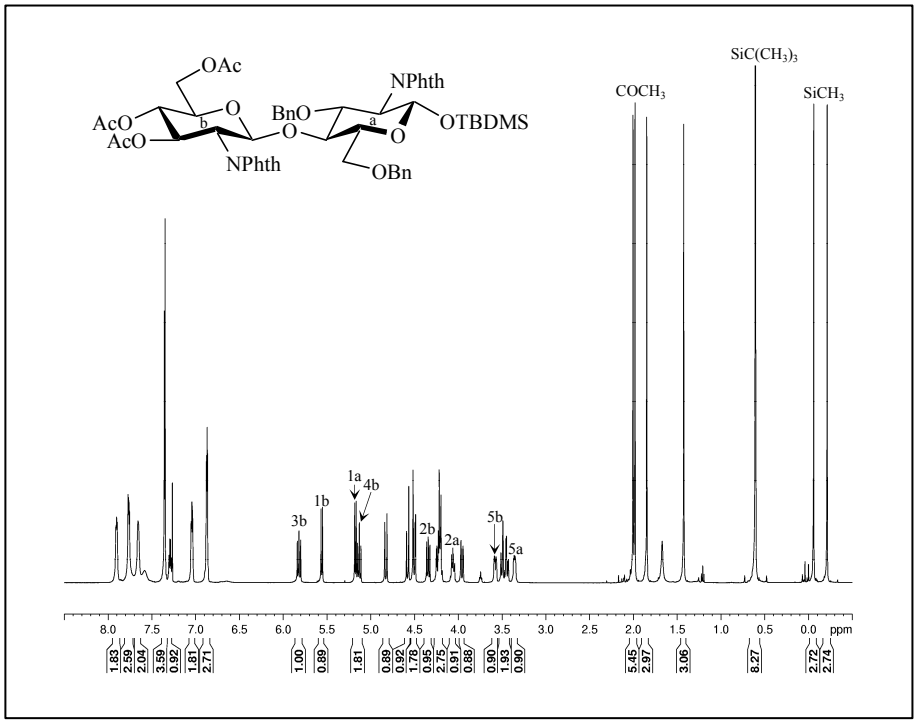
6. Appendix

6.1 Abbreviations

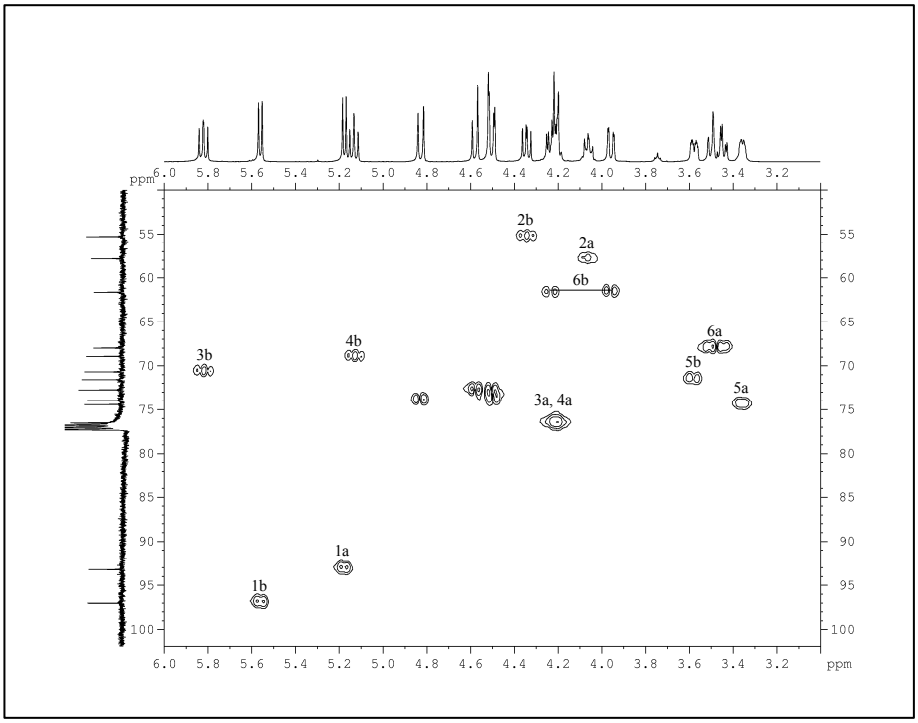
Ac	acetyl
APT	attached proton test
aq.	aqueous
Bn	benzyl
br	broad
BSP	1-benzenesulfinylpiperidine
°C	degree celcius
Cbz	benzyloxycarbonyl
COSY	correlation spectroscopy
d	doublet
DA	degree of acetylation
DAST	(diethylamino)sulfur trifluoride
DBU	1,8-diaza[5.4.0]bicycloundec-7-ene
DD	degree of deacetylation
dd	double doublet
DHB	2,5-dihydroxybenzoic acid
DIPC	*N*,*N*'-diisopropylcarbodiimide
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMM	dimethylmaleoyl
DMMA	dimethylmaleic anhydride
DMTST	dimethyl(methylthio)sulfonium triflate
DP	degree of polymerization
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
Dts	dithiasuccinoyl
equiv.	equivalent
ESI	electronspray ionization
EtOAc	ethyl acetate
Glc	glucose
GlcN	2-amino-2-deoxy-β-D-glucopyranose
GlcNAc	2-acetamido-2-deoxy-β-D-glucopyranose
h	hour
HMBC	heteronuclear multiple bond coherence
HMQC	heteronuclear multiple quantum coherence
Hz	hertz

IDCP	iodonium dicollidine perchlorate
i-Pr	isopropyl
IR	infrared spectroscopy
J	coupling constant
LCOs	lipochitooligosaccharides
Lev	levulinoyl
M	molar
m	multiplet
m.p.	melting point
MALDI	matrix-assisted laser desorption ionization
Me	methyl
min	minute
mmol	milimole
MP	*p*-methoxyphenyl
MS	mass spectroscopy
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
pent	pentenyl
Phth	phthaloyl
PNZ	*p*-nitrobenzyloxycarbonyl
ppm	parts per million
py	pyridine
rt	room temperature
s	singlet
t	triplet
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBDMS	*tert*-butyldimethylsilyl
TBPA	tris(4-bromophenyl) ammoniumyl hexachloroantimonate
TCP	tetrachlorophthaloyl
TESOTf	triethylsilyl trifluoromethanesulfonate
TfOH	triflic acid
THAP	2,4,6-trihydroxyacetophenone
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Troc	trichloroethoxycarbonyl
δ	chemical shift

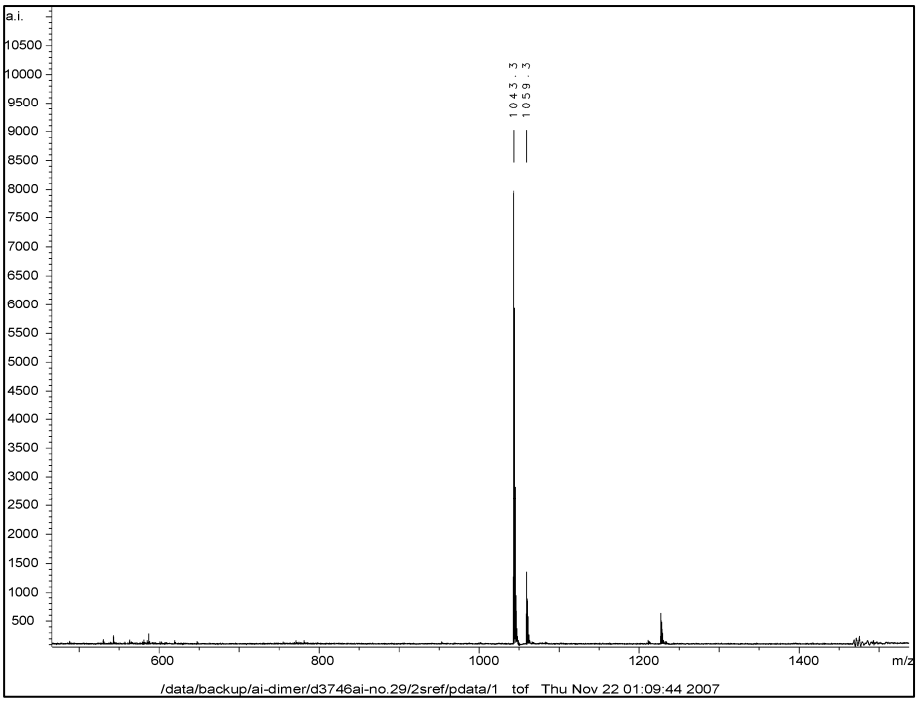
6.2 ^{1}H-NMR, HMQC and MALDI spectra



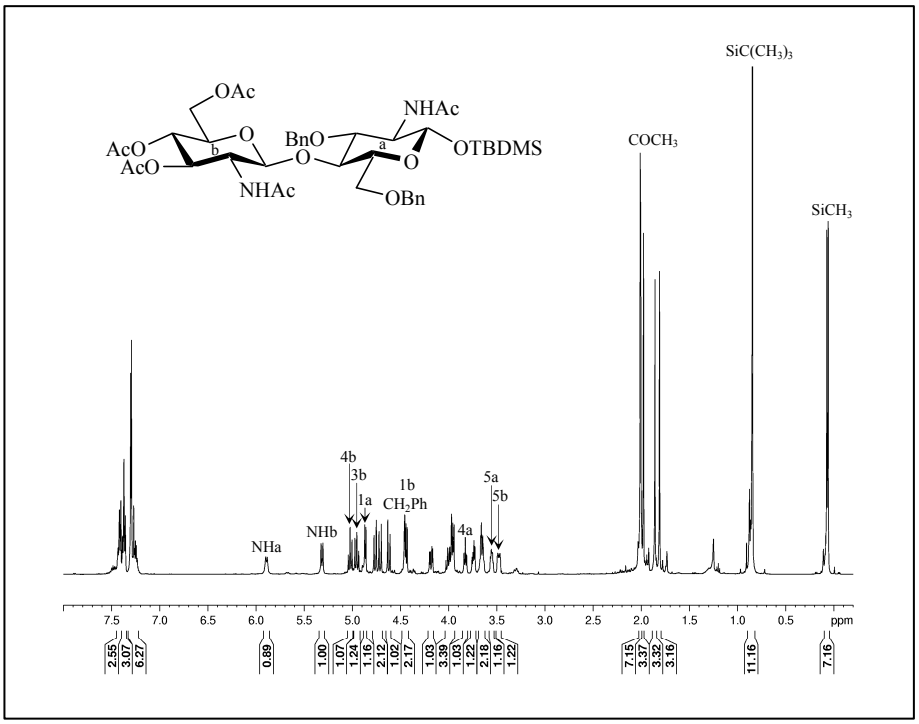
^{1}H-NMR spectrum of compound **41** (500 MHz, $CDCl_3$)



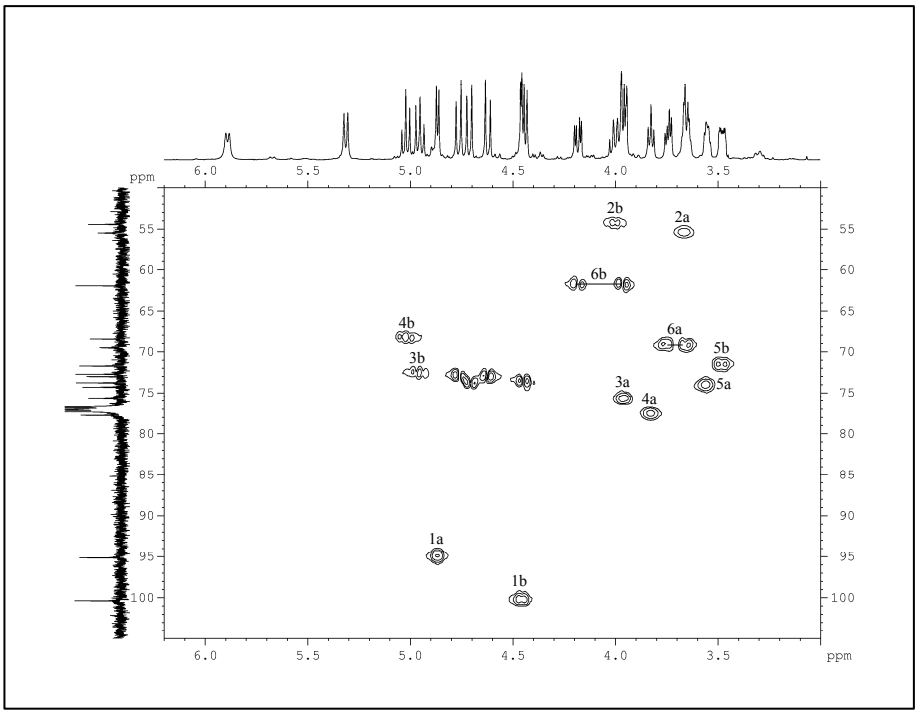
HMQC spectrum of compound **41**



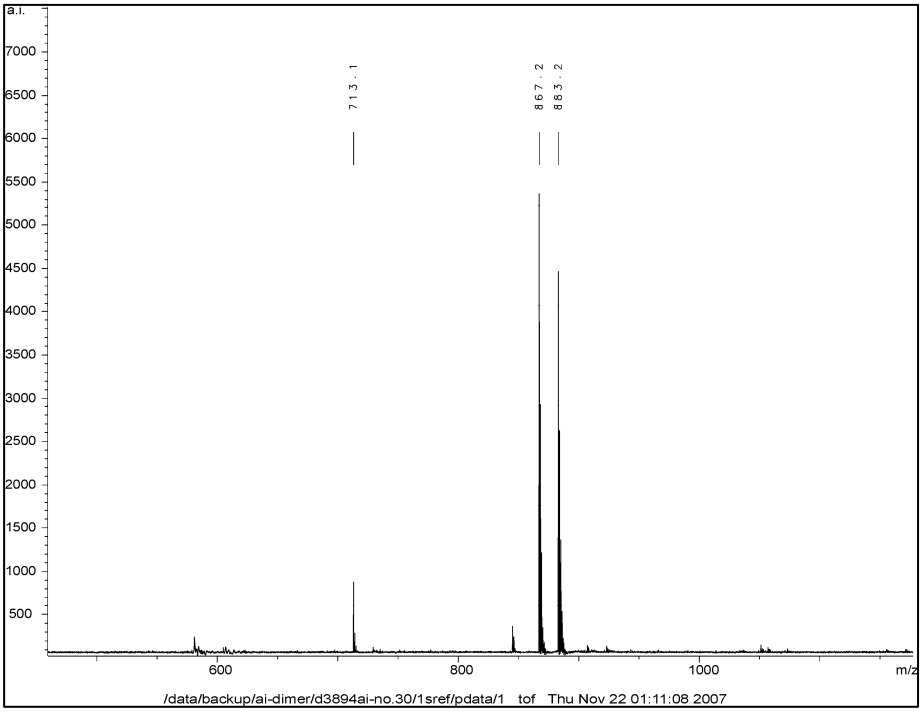
MALDI spectrum of compound **41**



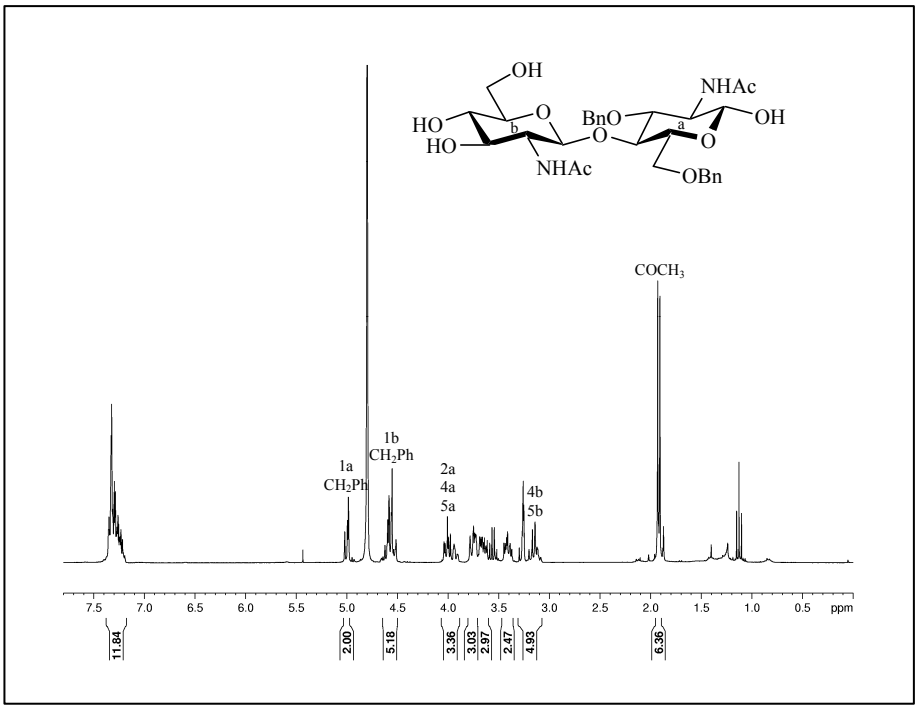
^{1}H-NMR spectrum of compound **42** (500 MHz, $CDCl_3$)



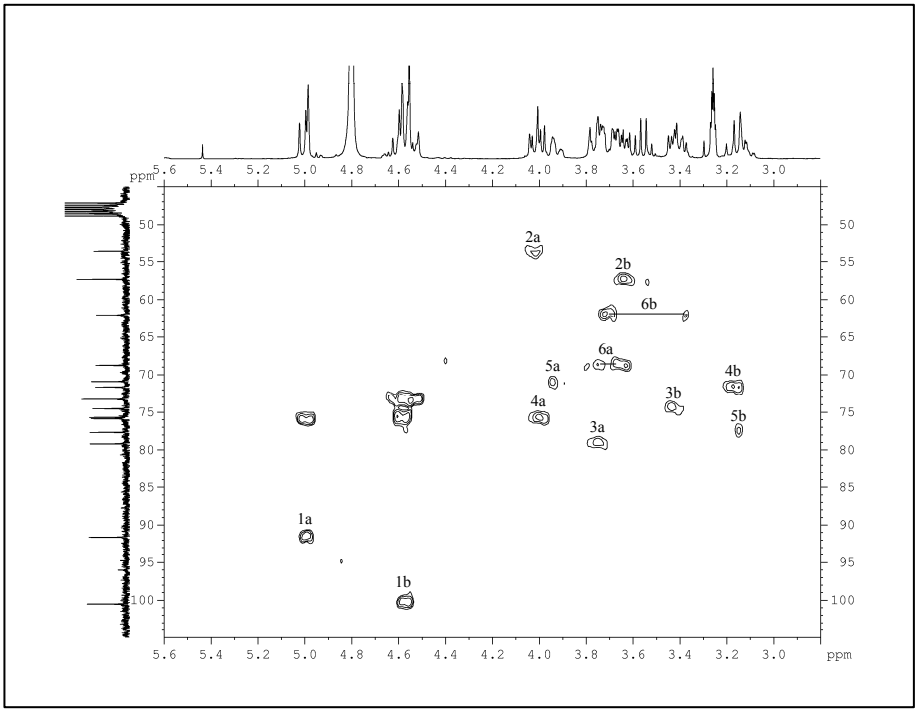
HMQC spectrum of compound **42**



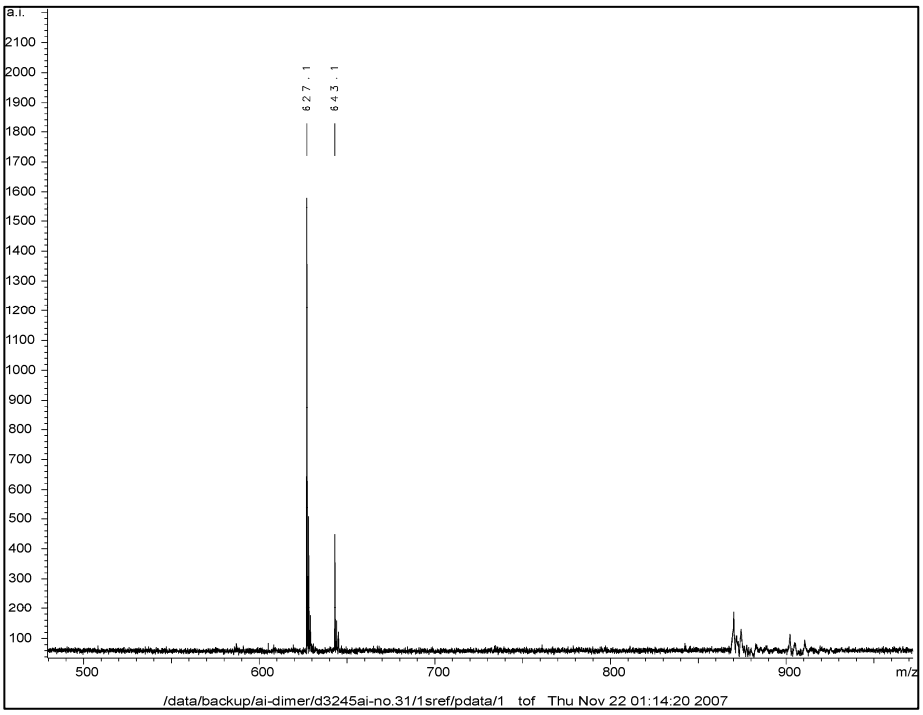
MALDI spectrum of compound **42**



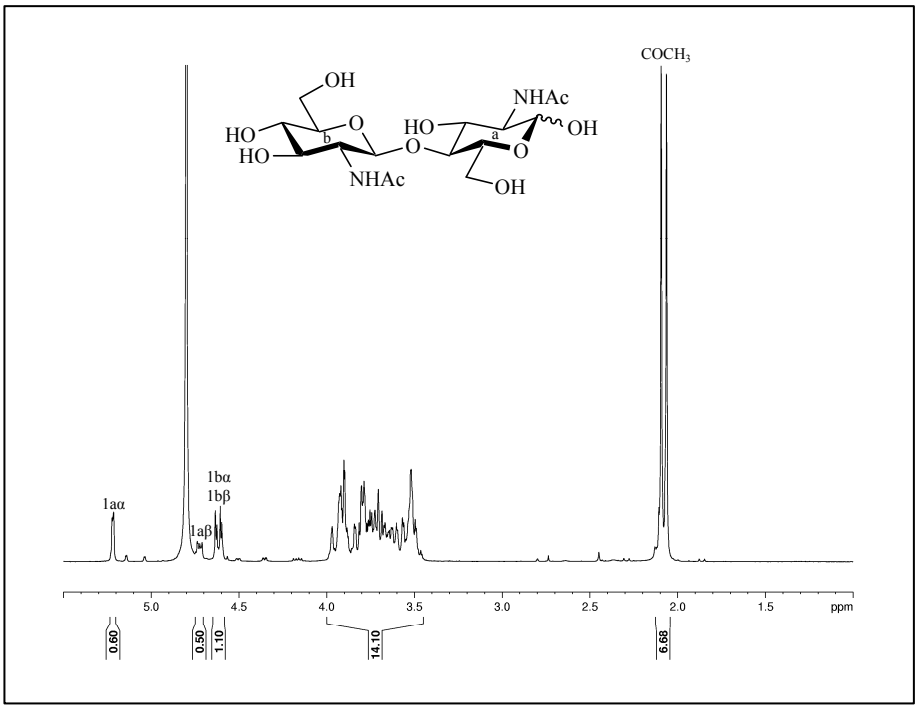
^{1}H-NMR spectrum of compound **43** (300 MHz, CD_3OD)



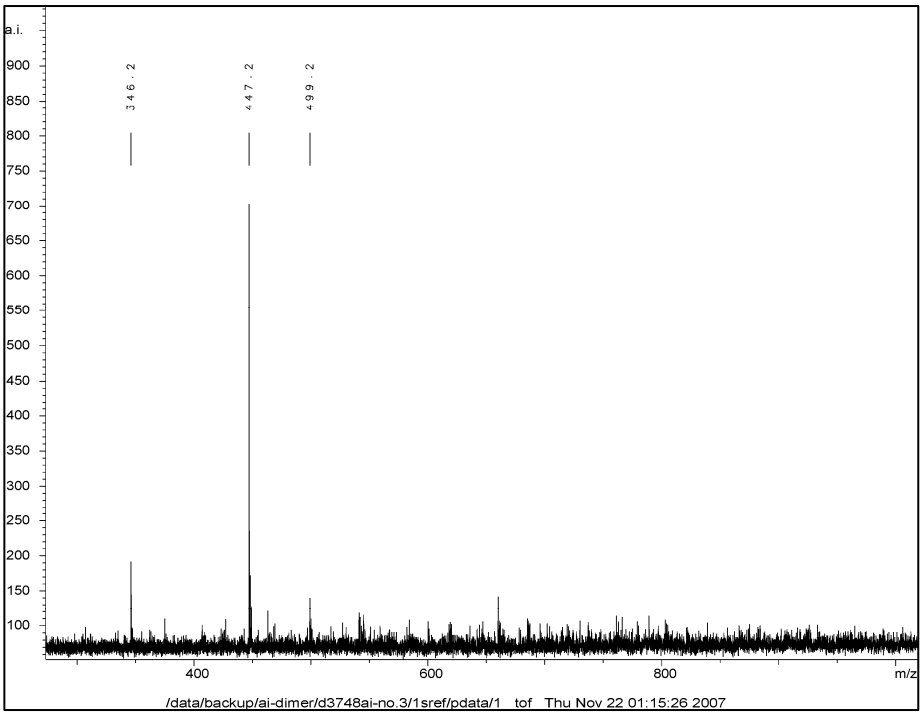
HMQC spectrum of compound **43**



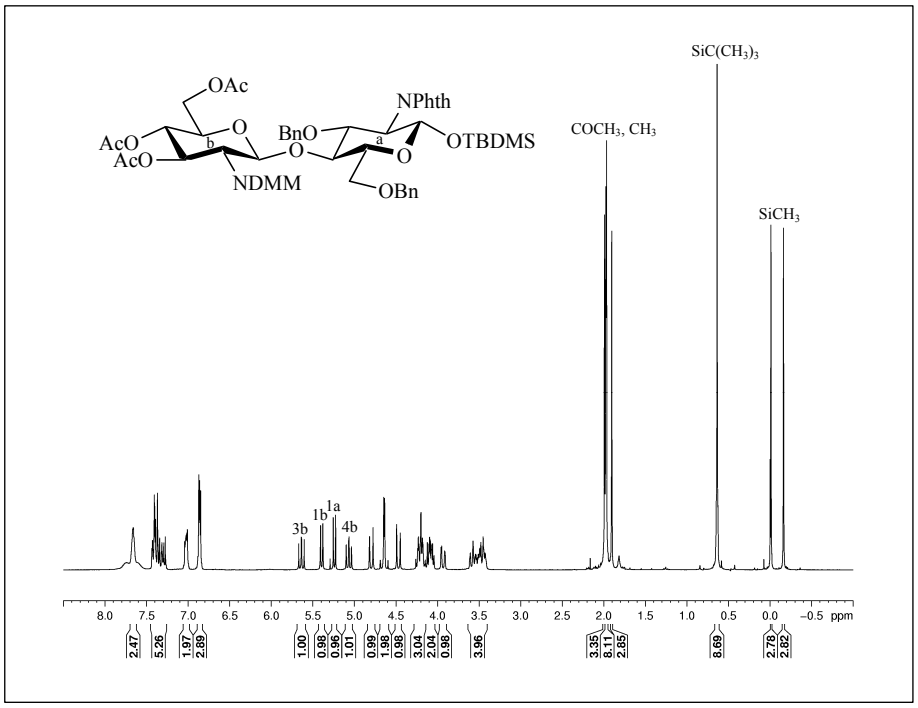
MALDI spectrum of compound **43**



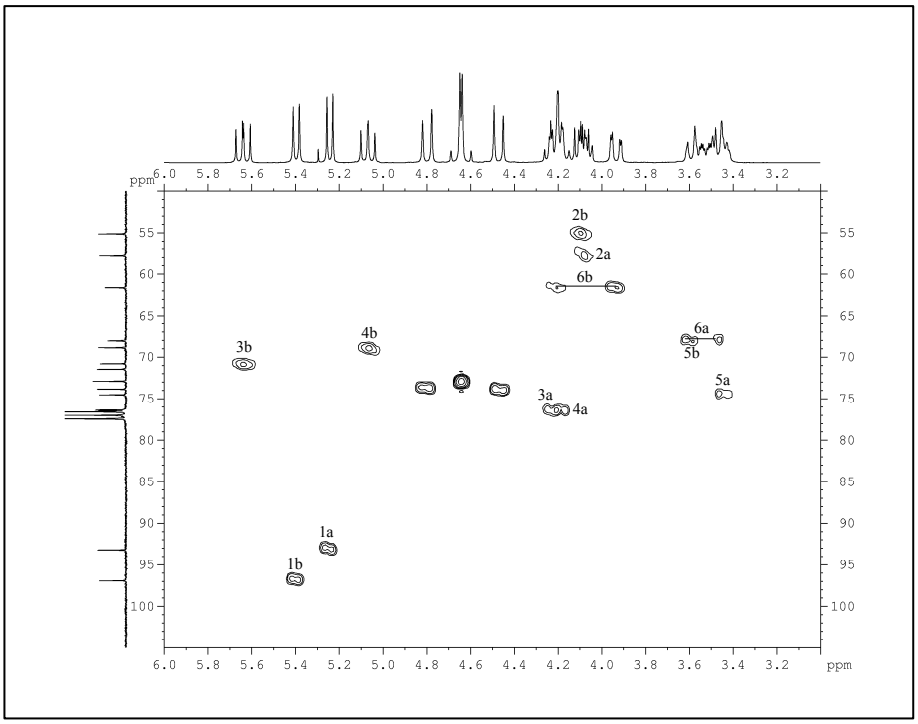
^{1}H-NMR spectrum of compound **3** (300 MHz, D_2O)



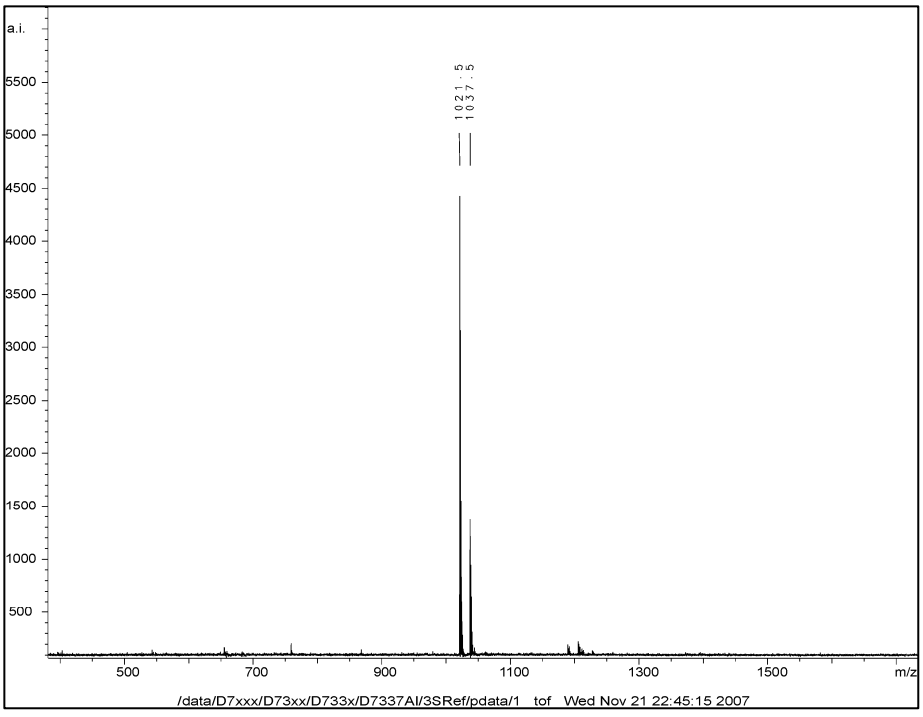
MALDI spectrum of compound **3**



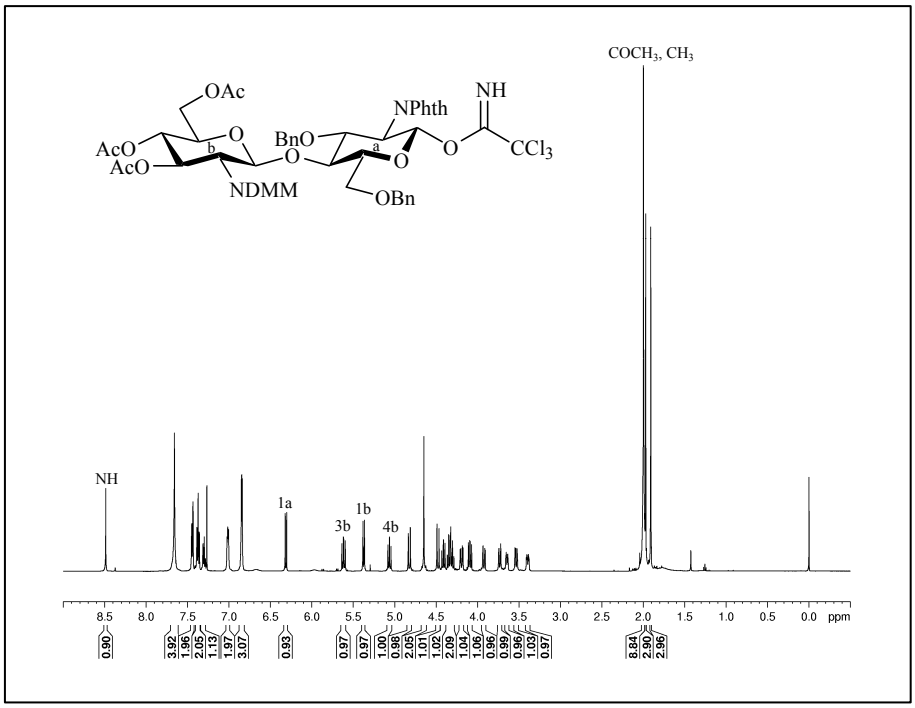
^{1}H-NMR spectrum of compound **51** (300 MHz, $CDCl_3$)



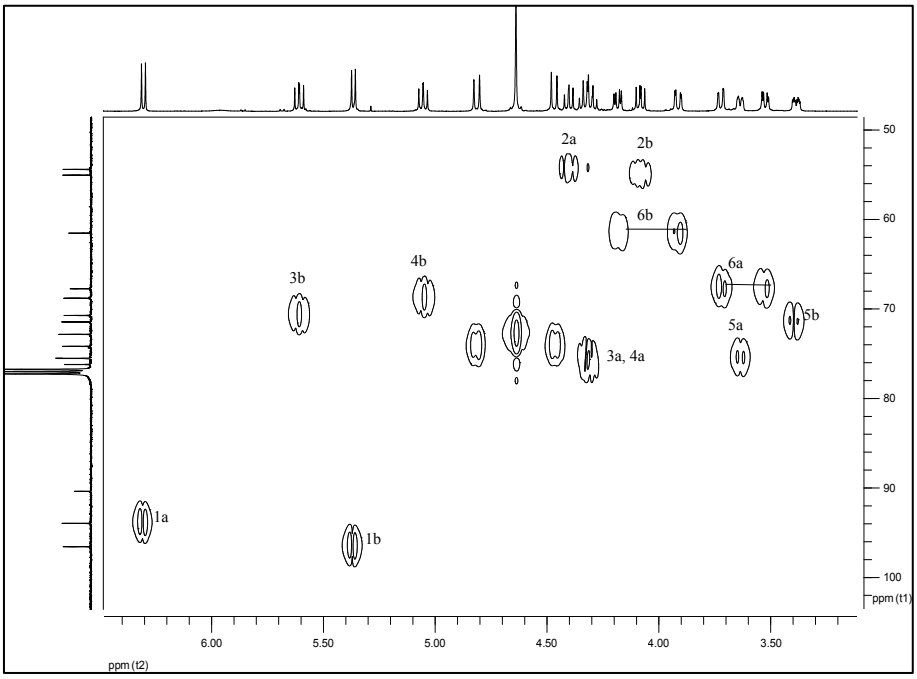
HMQC spectrum of compound **51**



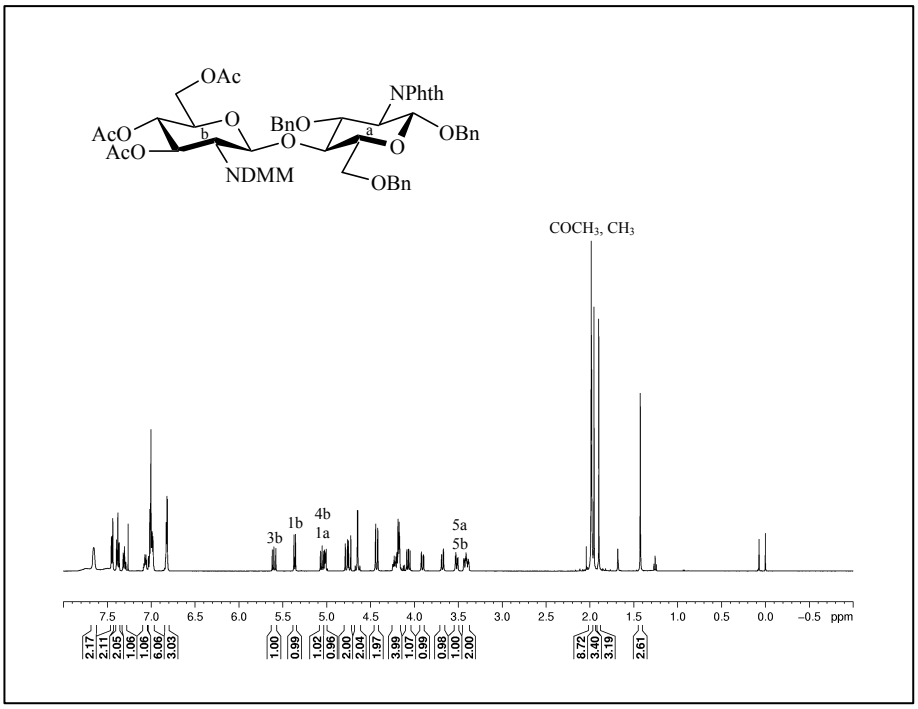
MALDI spectrum of compound **51**



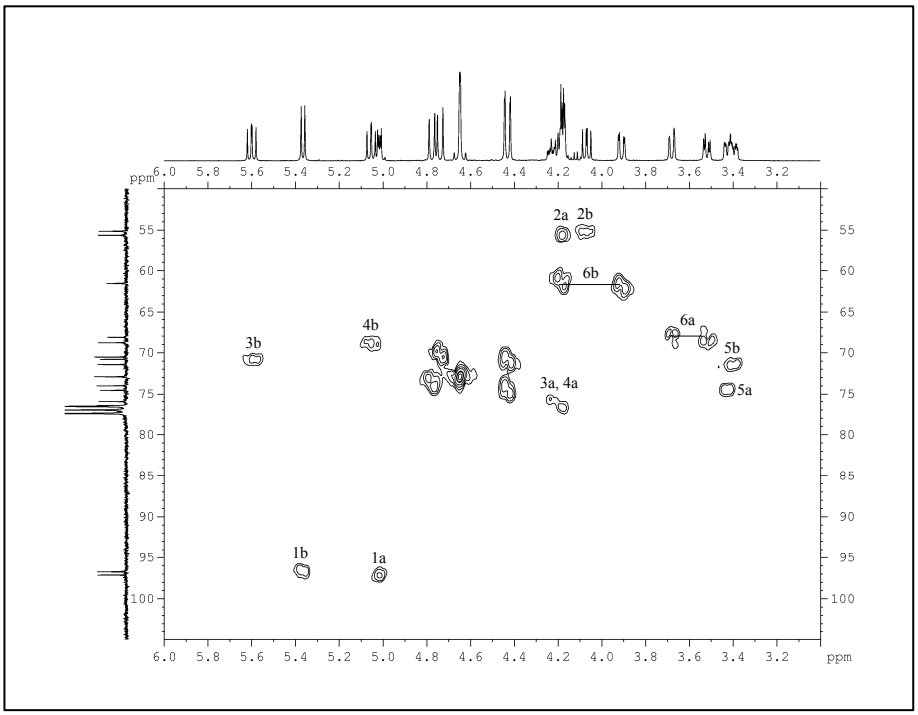
^{1}H-NMR spectrum of compound **52** (500 MHz, $CDCl_3$)



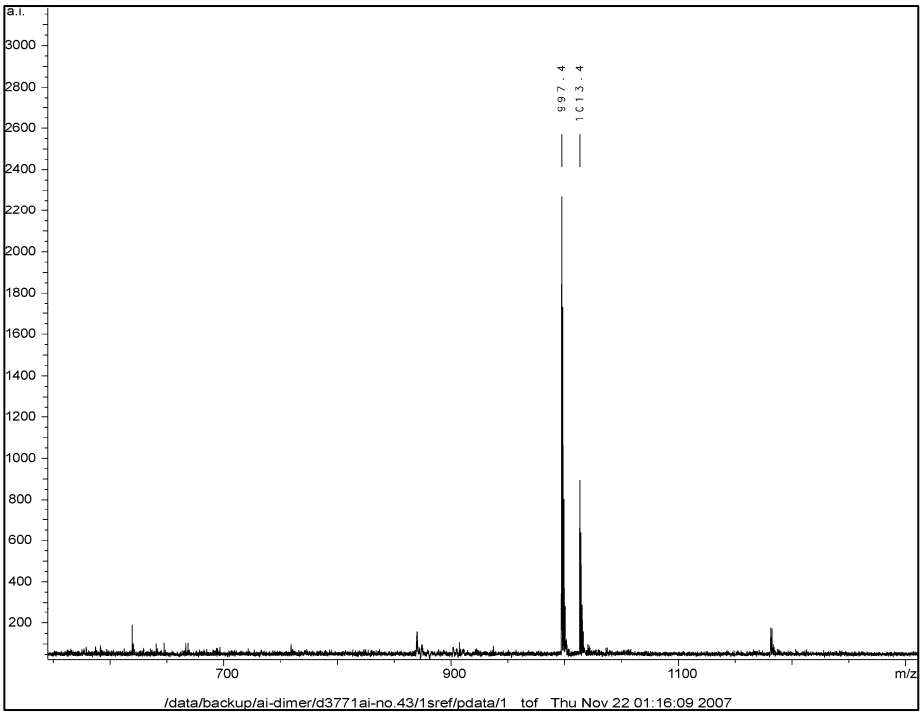
HMQC spectrum of compound **52**



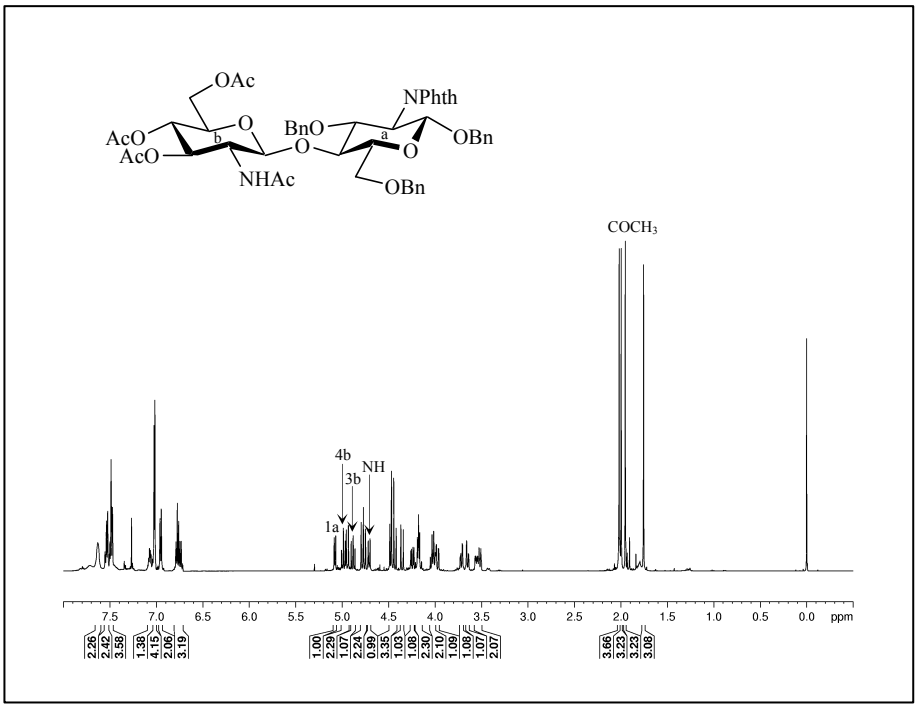
^{1}H-NMR spectrum of compound **53** (500 MHz, $CDCl_3$)



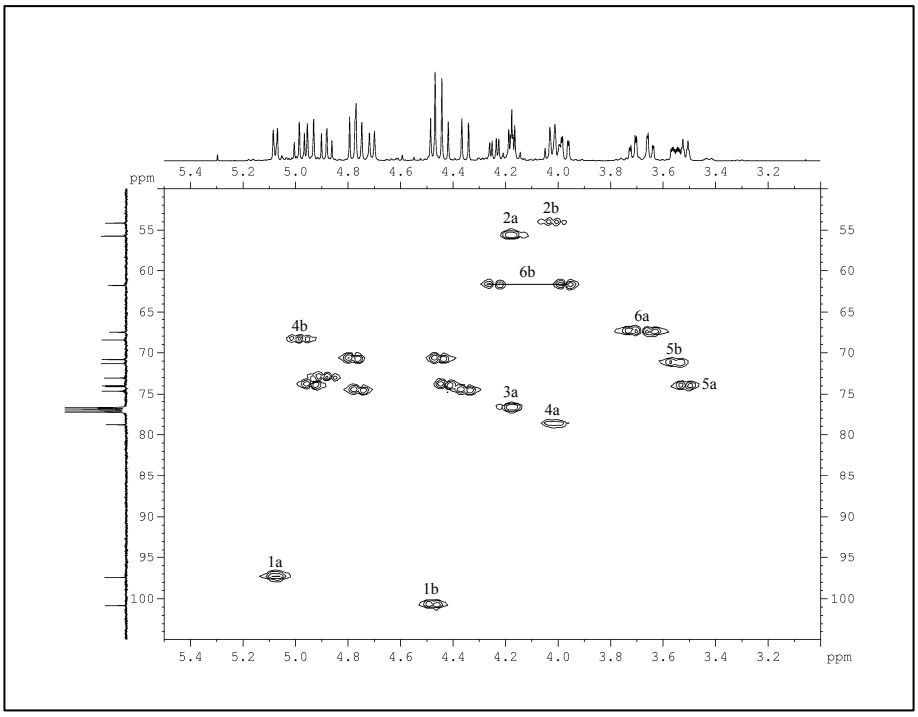
HMQC spectrum of compound **53**



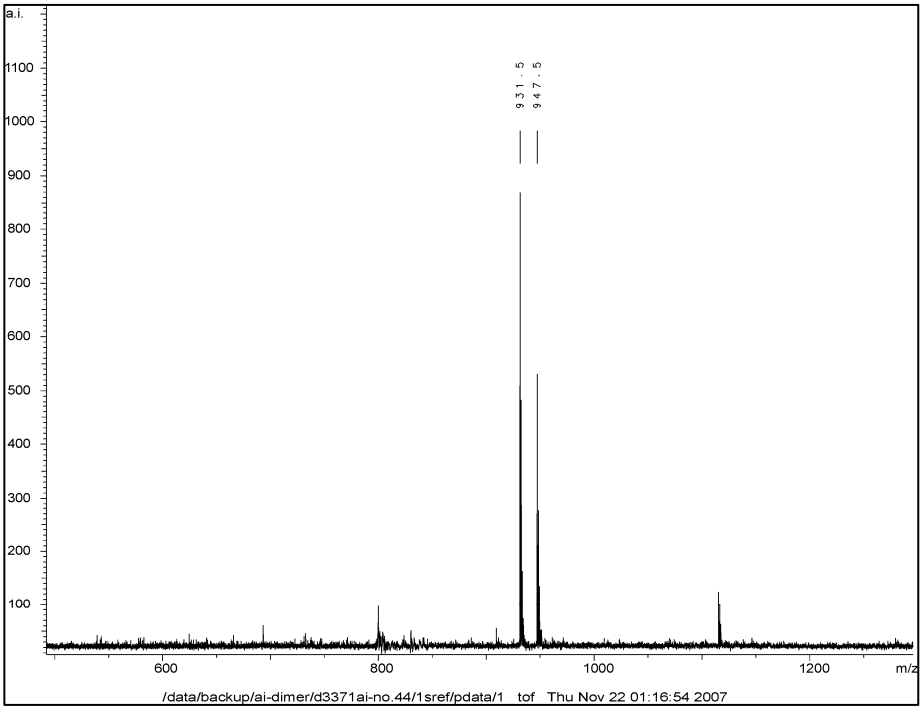
MALDI spectrum of compound **53**



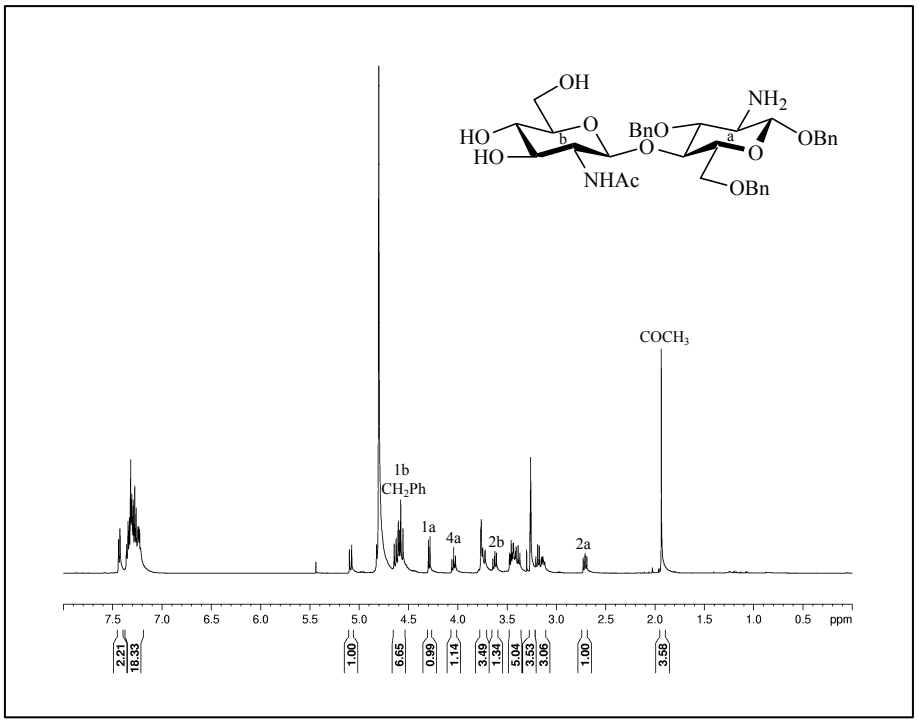
^{1}H-NMR spectrum of compound **54** (500 MHz, $CDCl_3$)



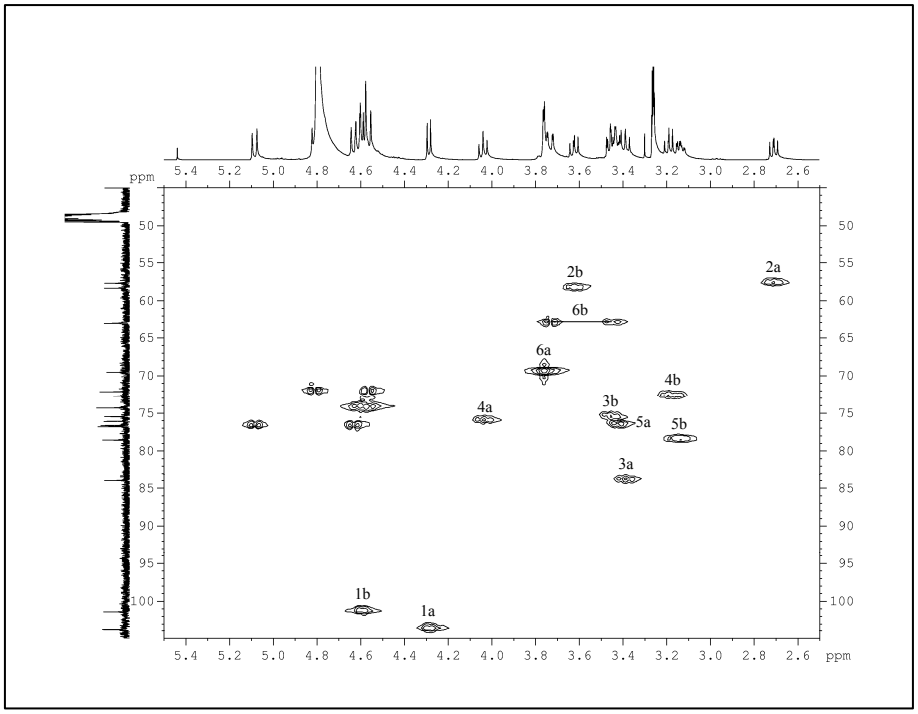
HMQC spectrum of compound **54**



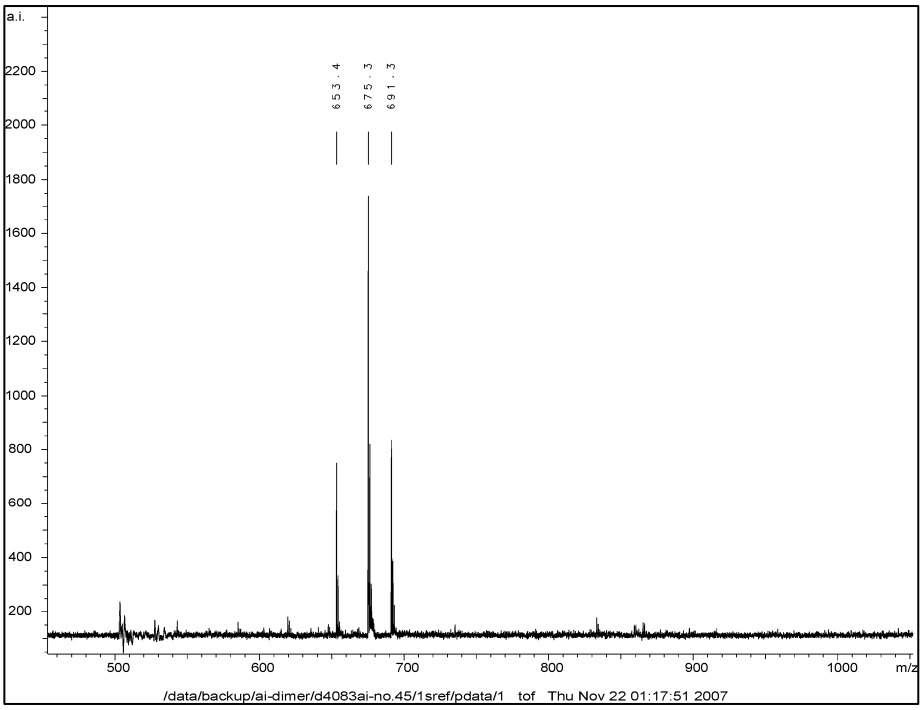
MALDI spectrum of compound **54**



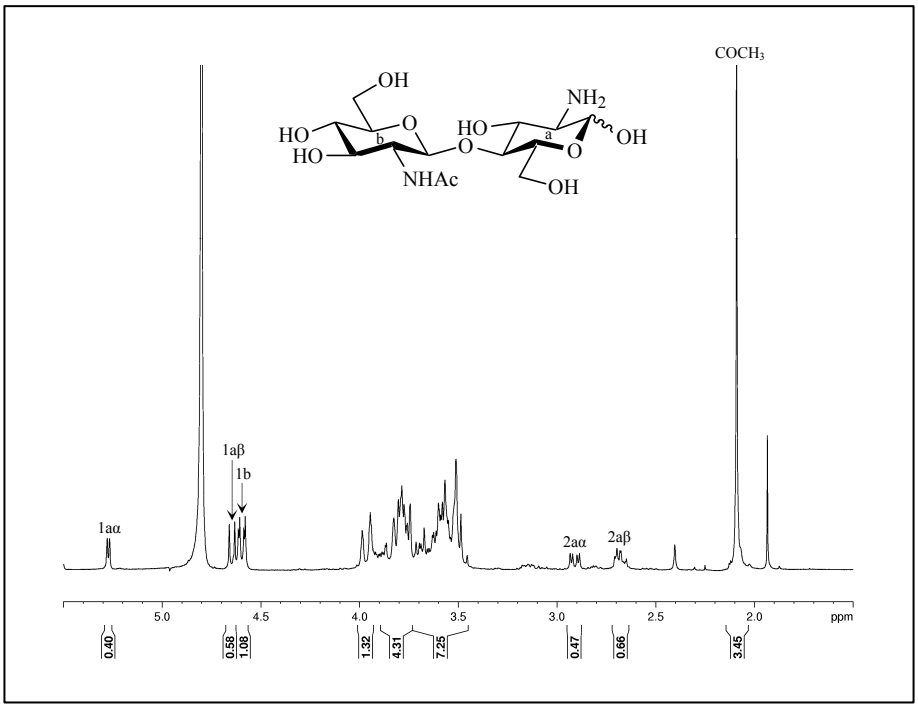
^{1}H-NMR spectrum of compound **55** (500 MHz, CD_3OD)



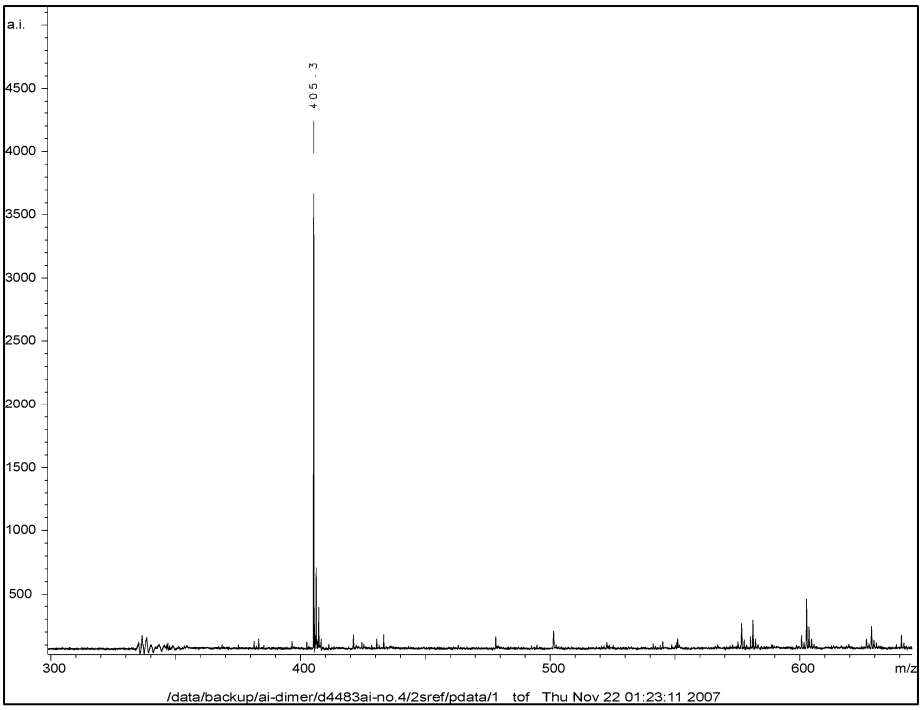
HMQC spectrum of compound **55**



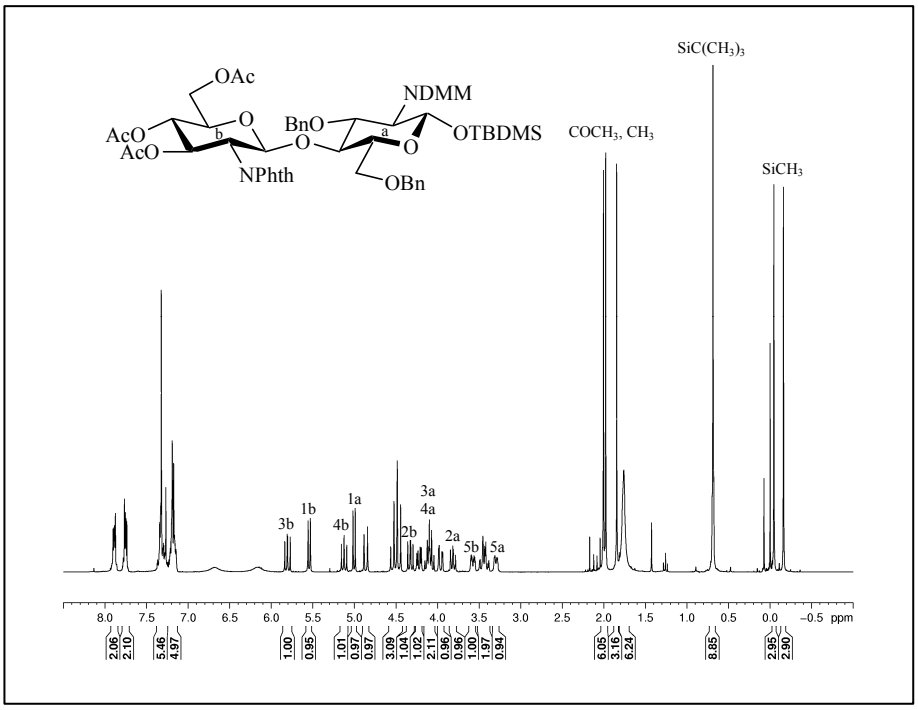
MALDI spectrum of compound **55**



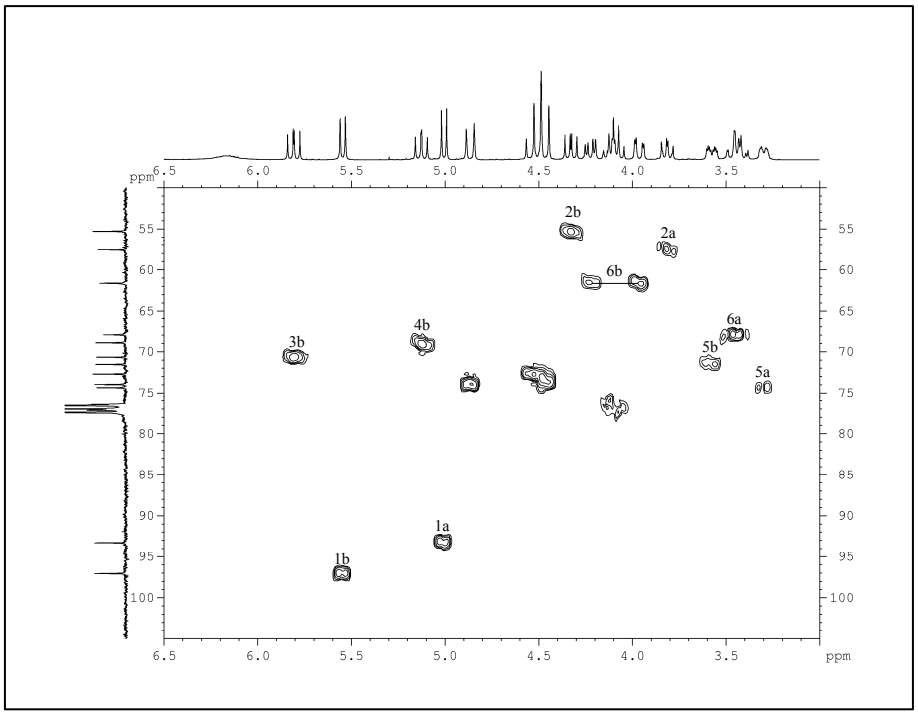
^{1}H-NMR spectrum of compound **4** (300 MHz, D_2O)



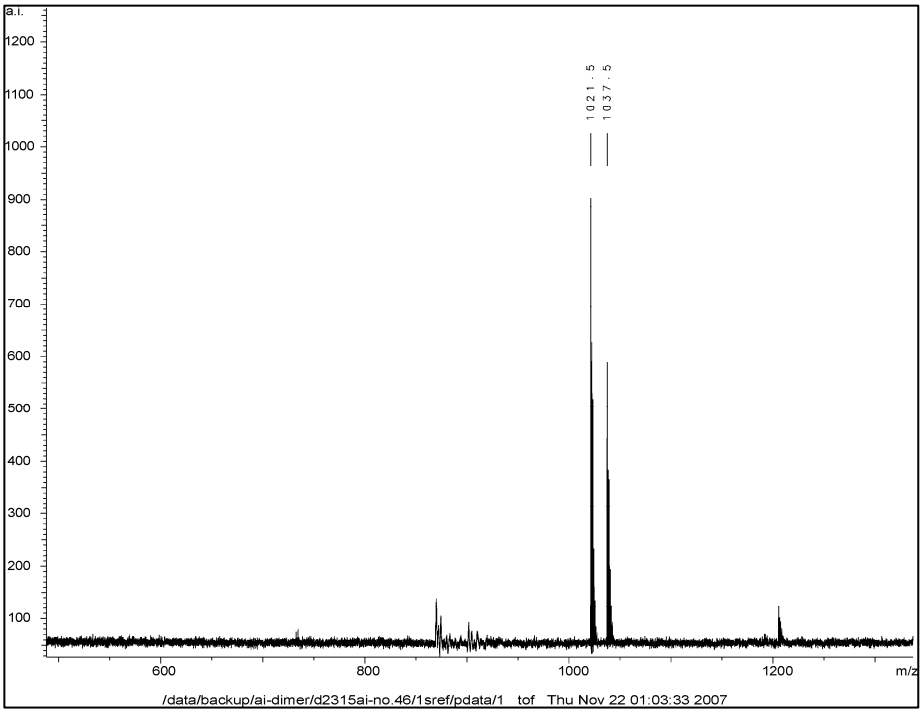
MALDI spectrum of compound **4**



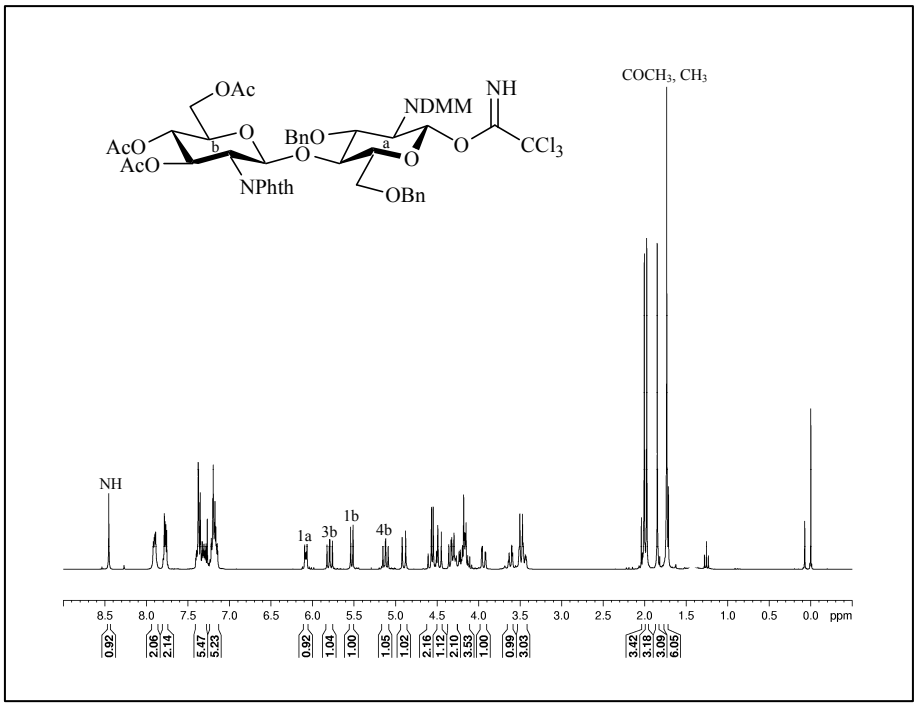
^{1}H-NMR spectrum of compound **56** (300 MHz, $CDCl_3$)



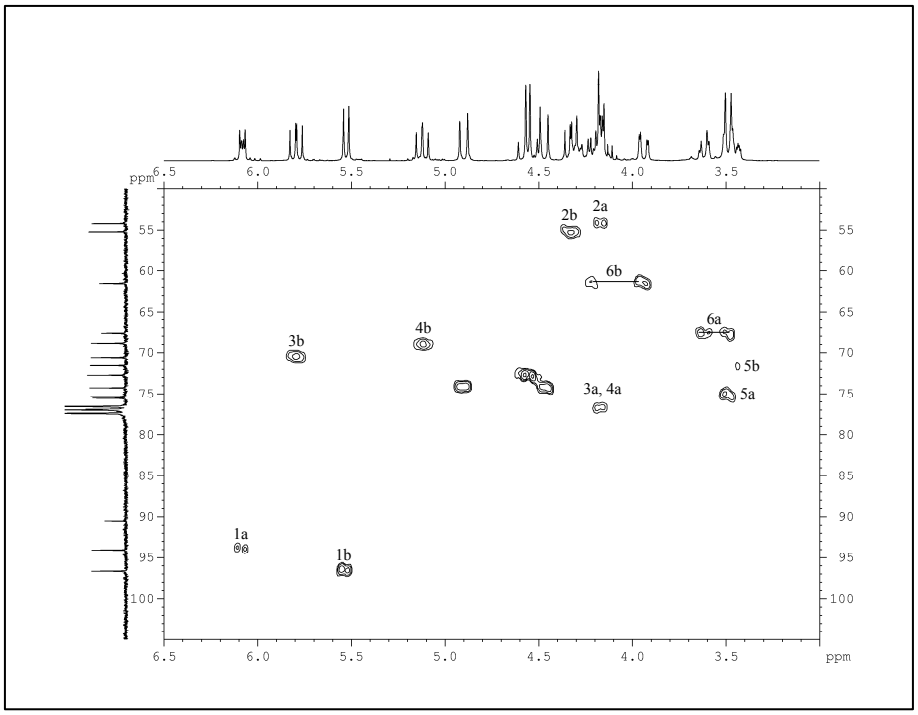
HMQC spectrum of compound **56**



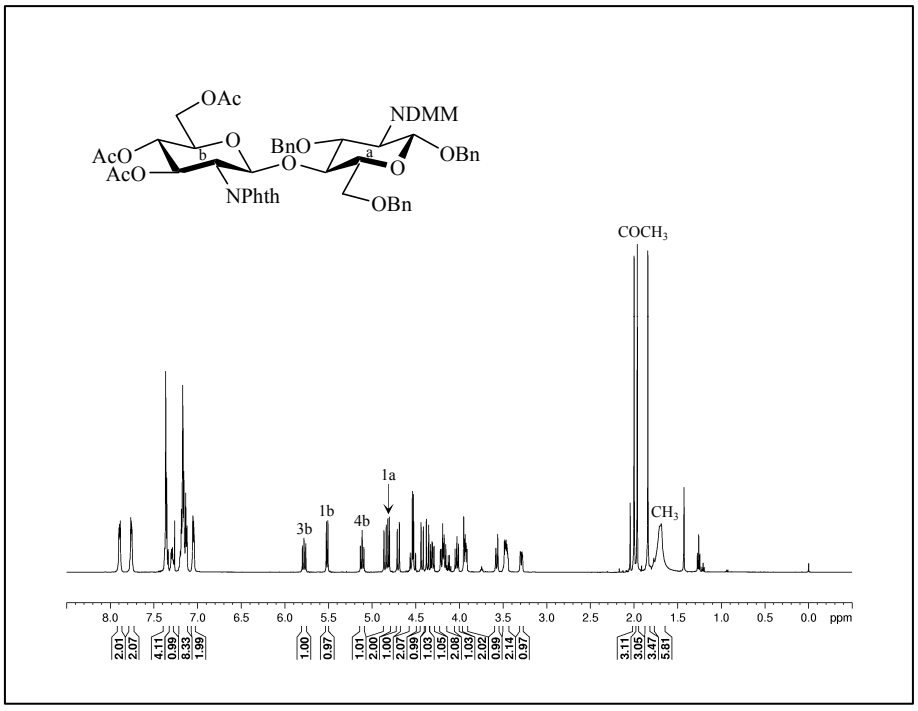
MALDI spectrum of compound **56**



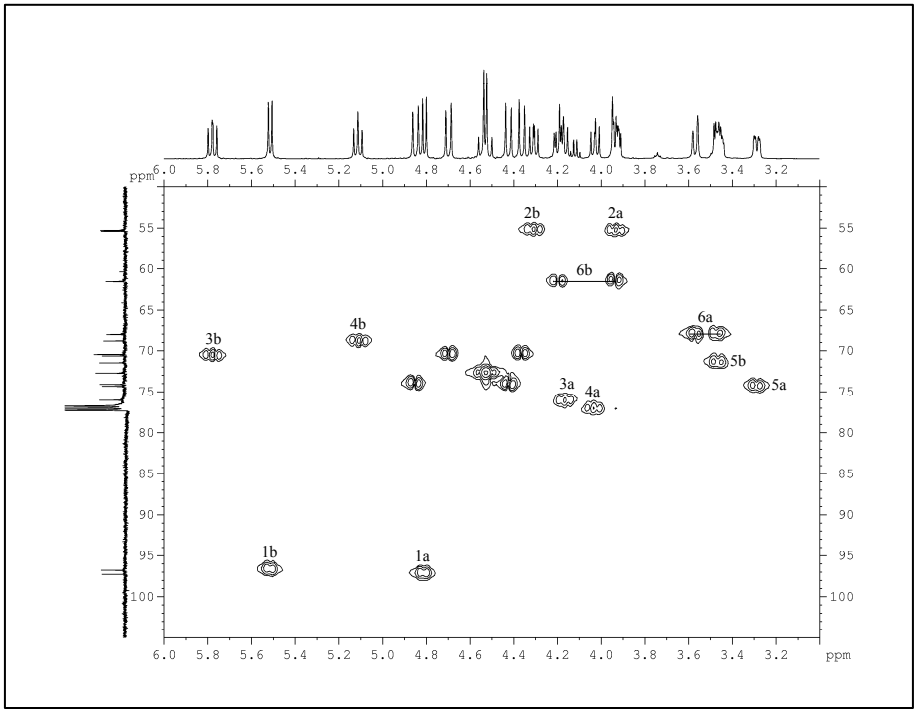
^{1}H-NMR spectrum of compound **57** (300 MHz, $CDCl_3$)



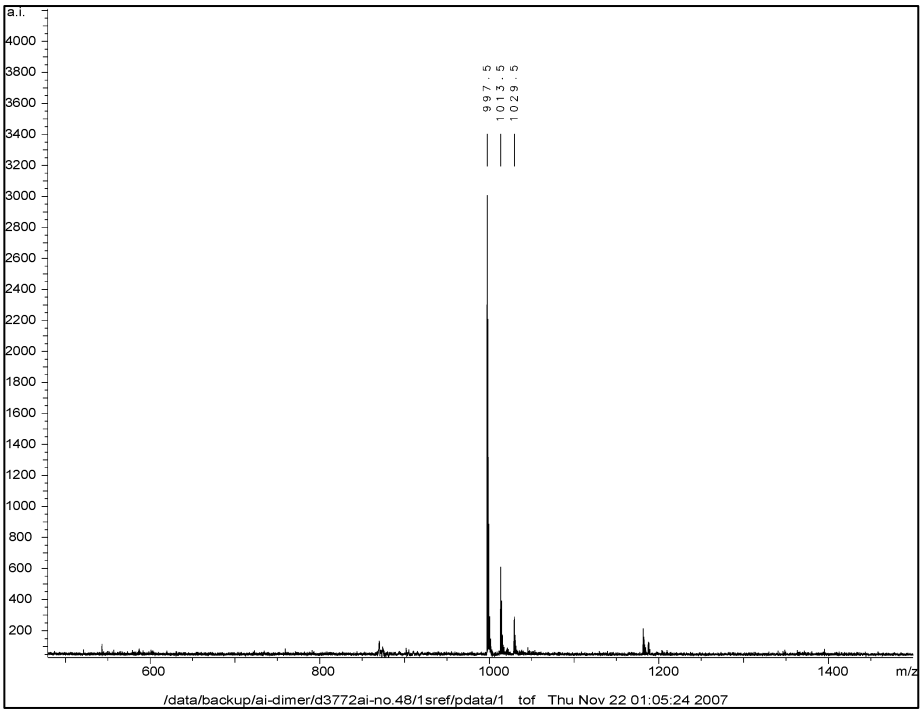
HMQC spectrum of compound **57**



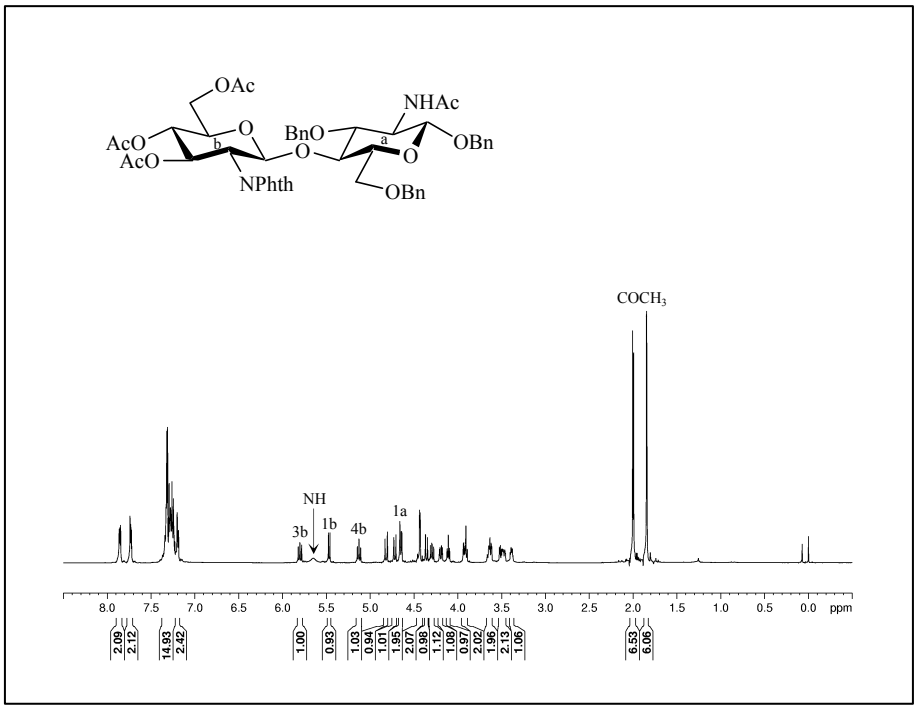
^{1}H-NMR spectrum of compound **58** (500 MHz, $CDCl_3$)



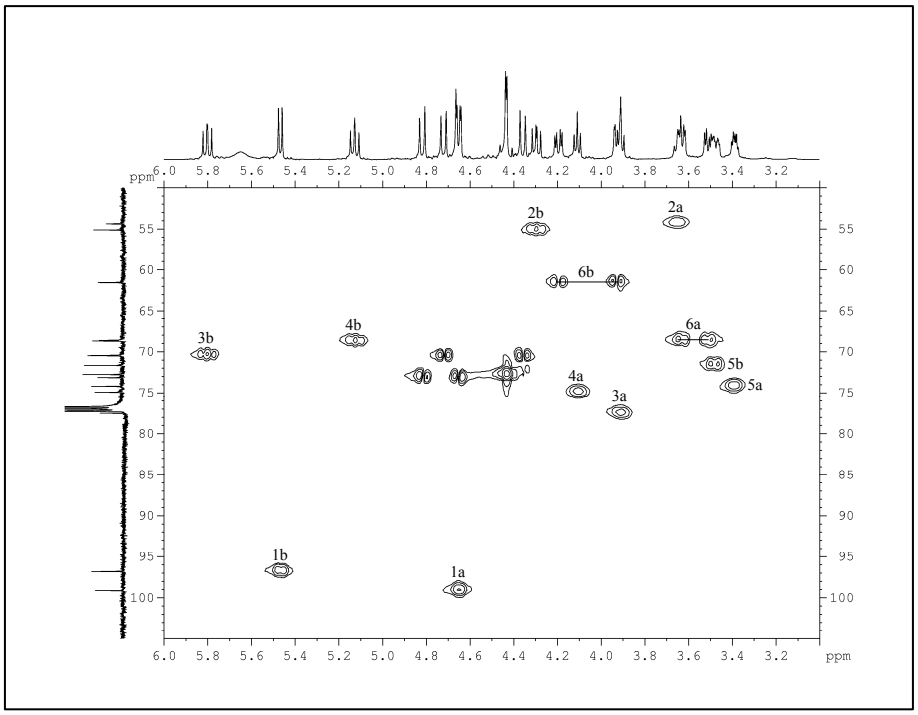
HMQC spectrum of compound **58**



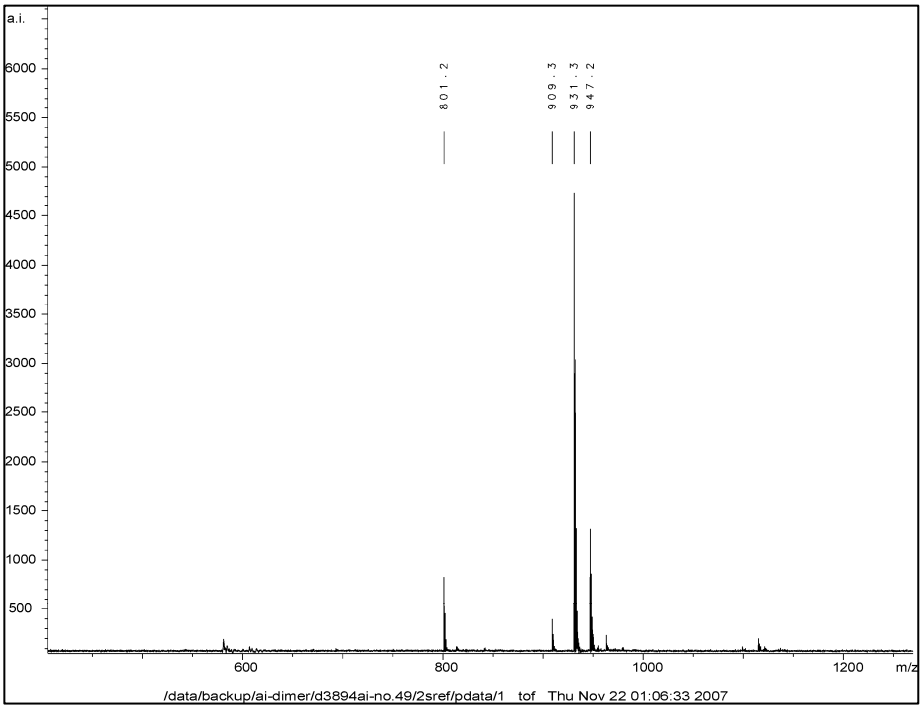
MALDI spectrum of compound **58**



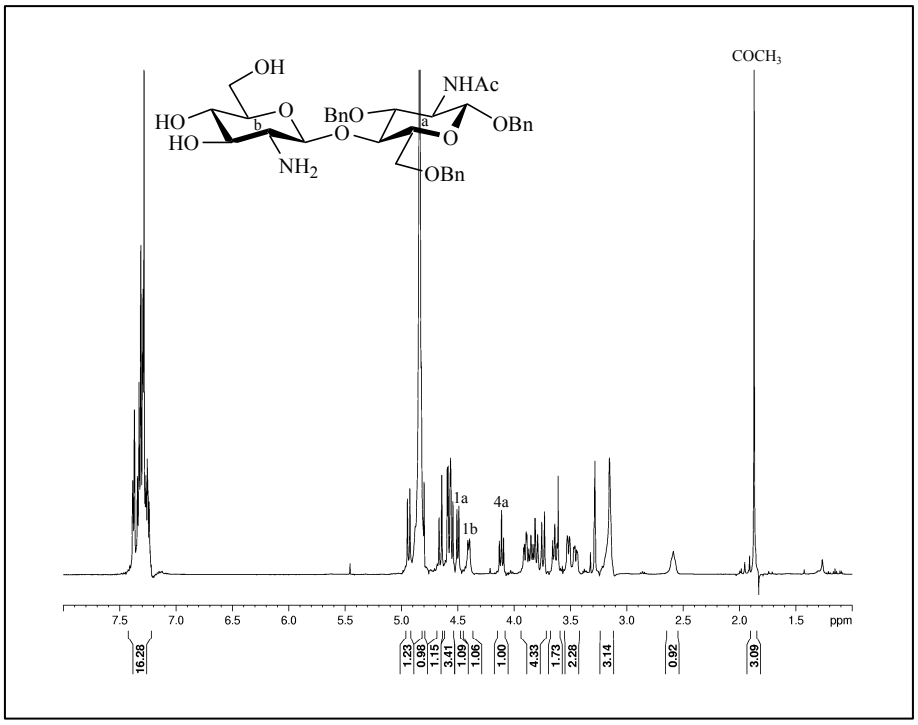
^{1}H-NMR spectrum of compound **59** (500 MHz, $CDCl_3$)



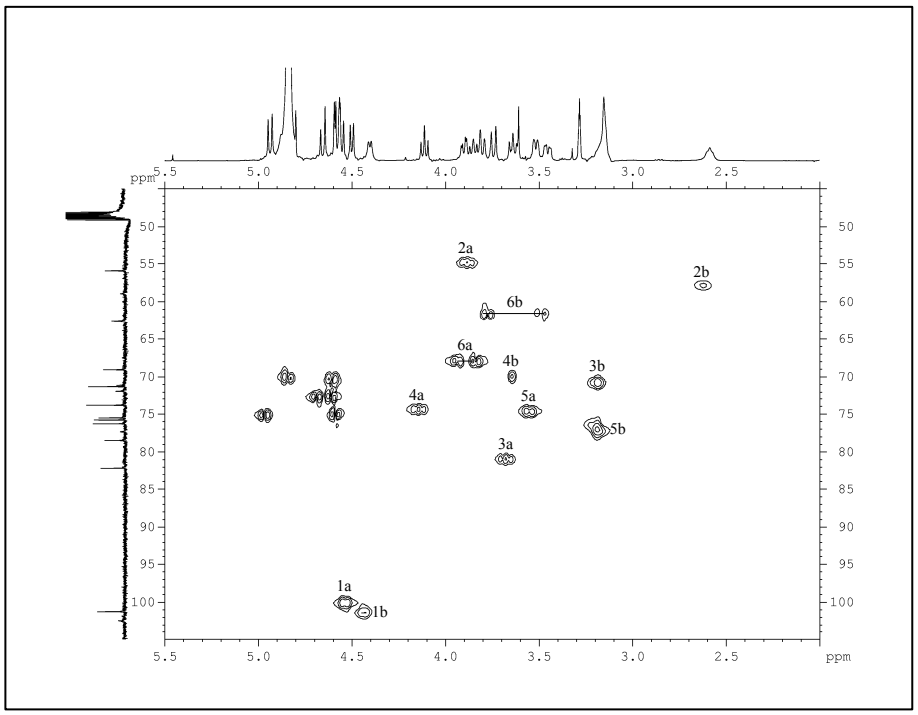
HMQC spectrum of compound **59**



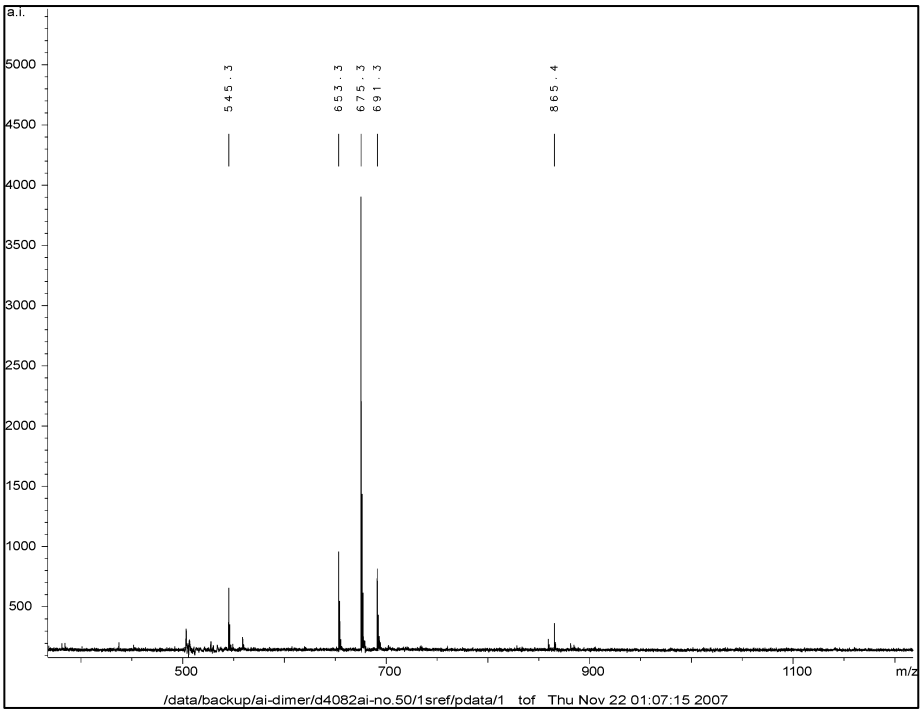
MALDI spectrum of compound **59**



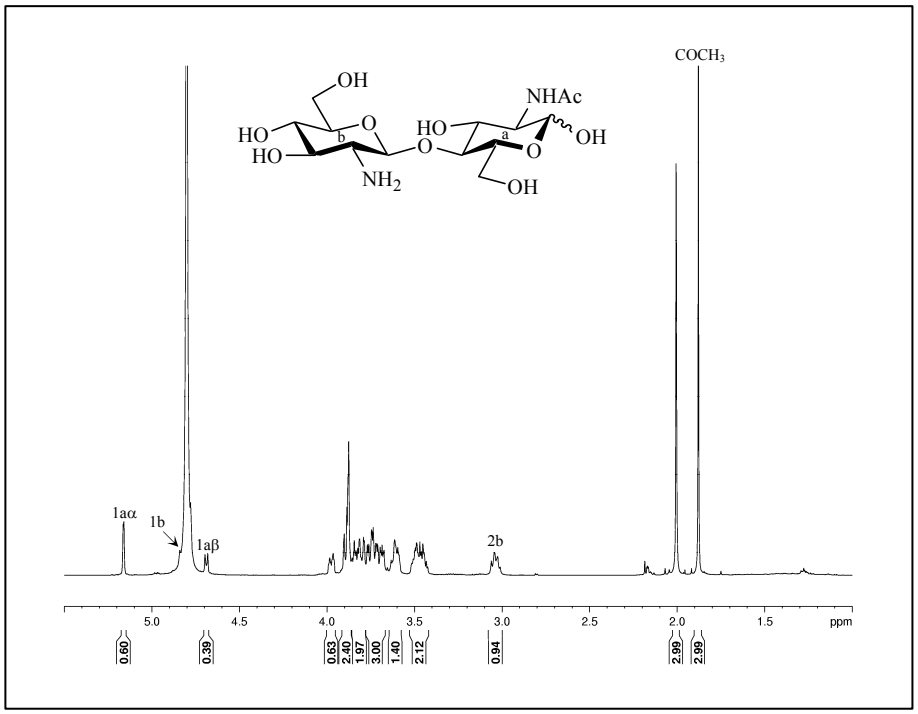
^{1}H-NMR spectrum of compound **60** (500 MHz, CD_3OD)



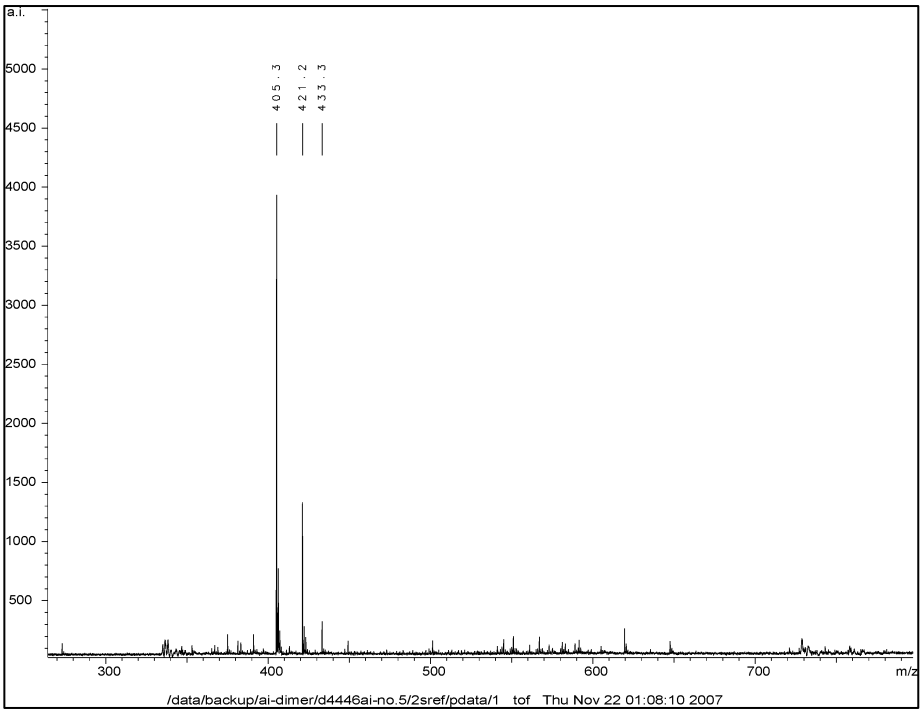
HMQC spectrum of compound **60**



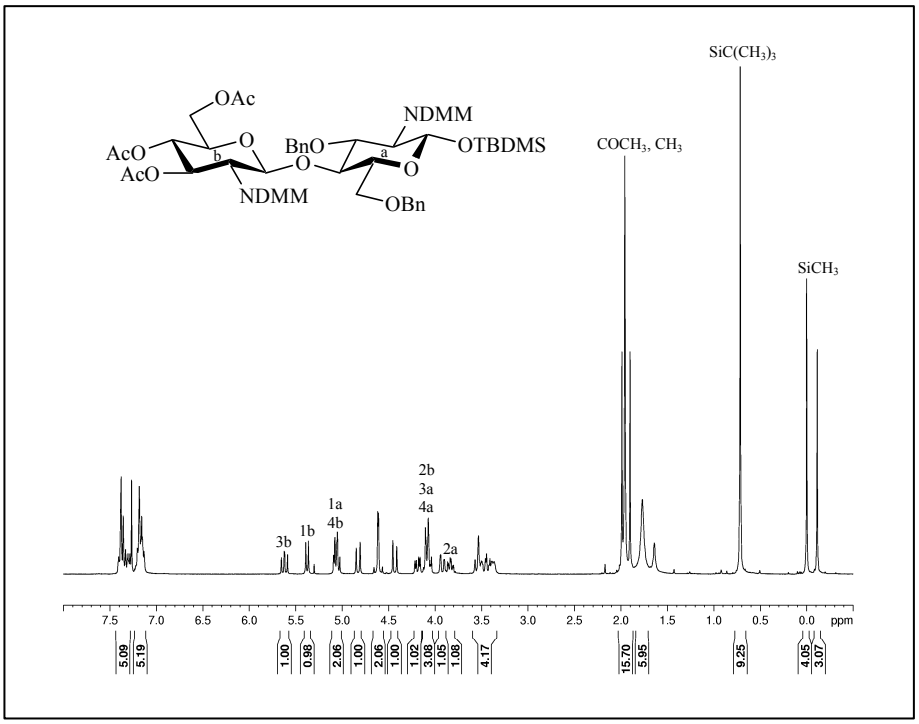
MALDI spectrum of compound **60**



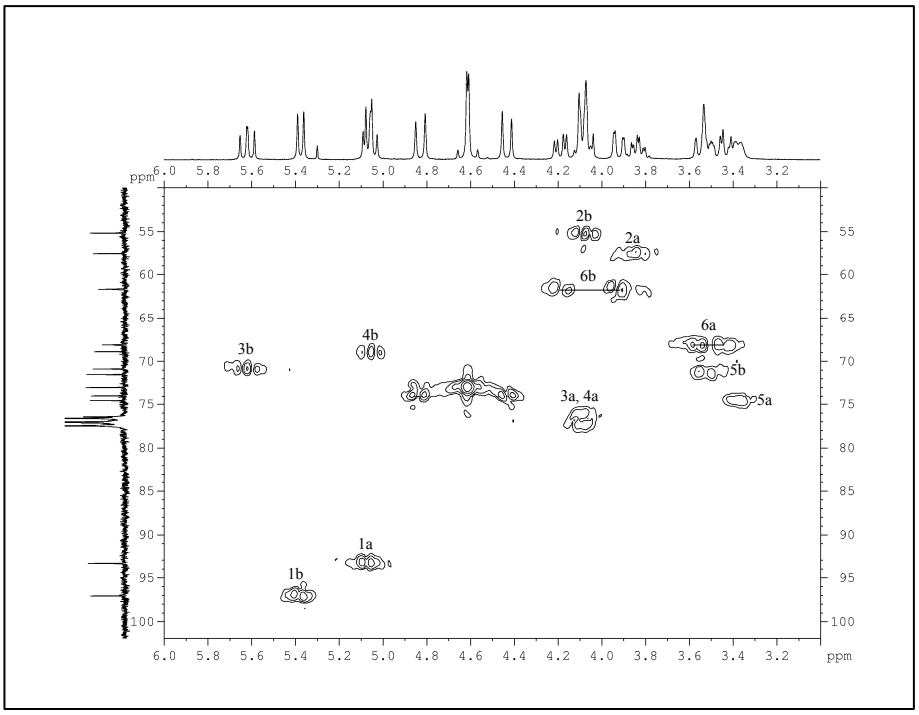
^{1}H-NMR spectrum of compound **5** (500 MHz, D_2O)



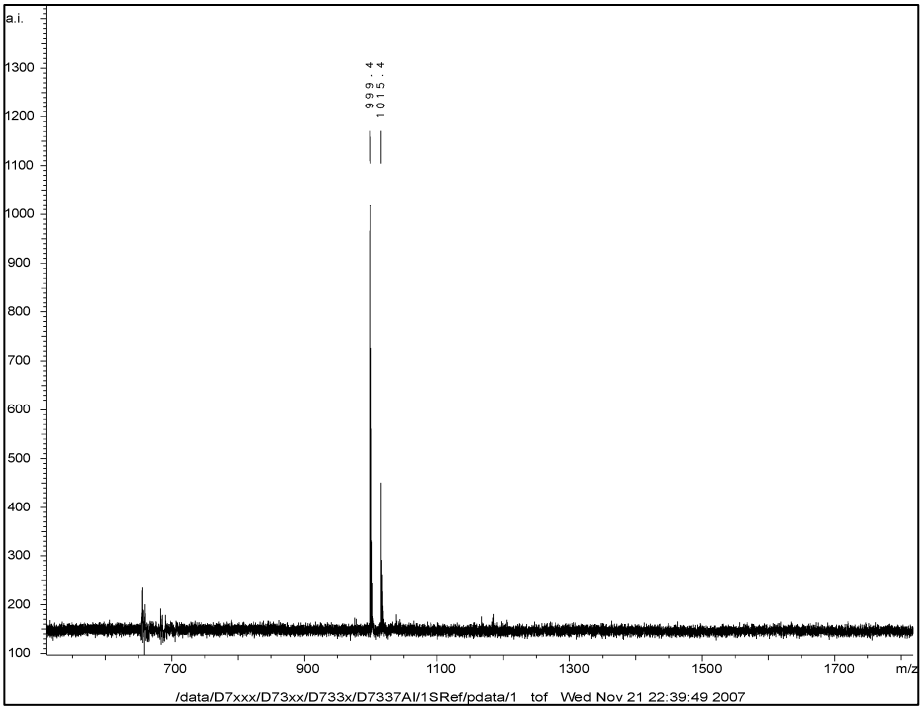
MALDI spectrum of compound **5**



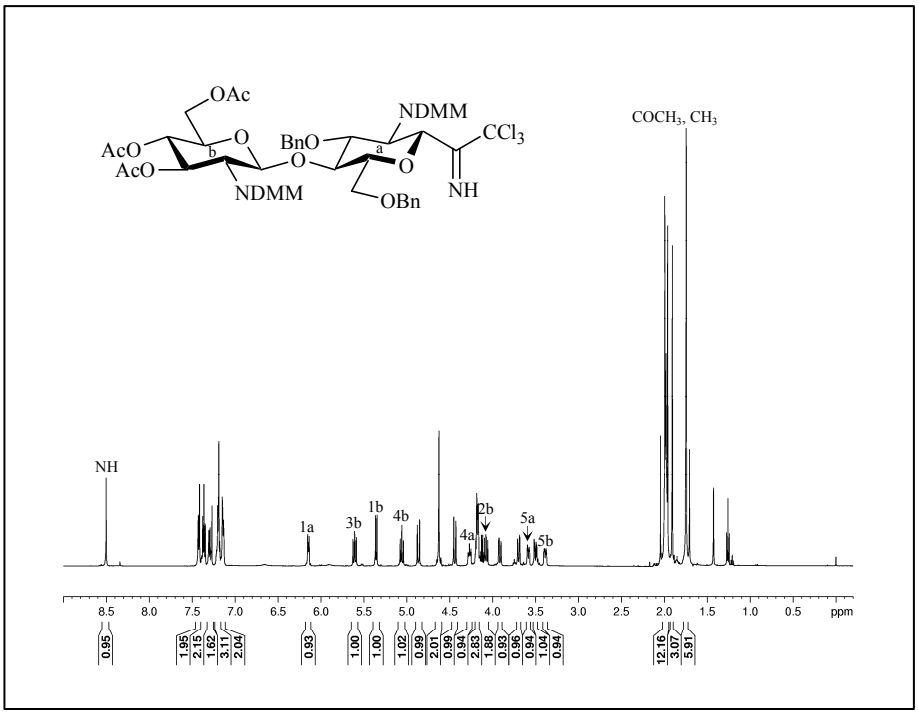
^{1}H-NMR spectrum of compound **66** (300 MHz, $CDCl_3$)



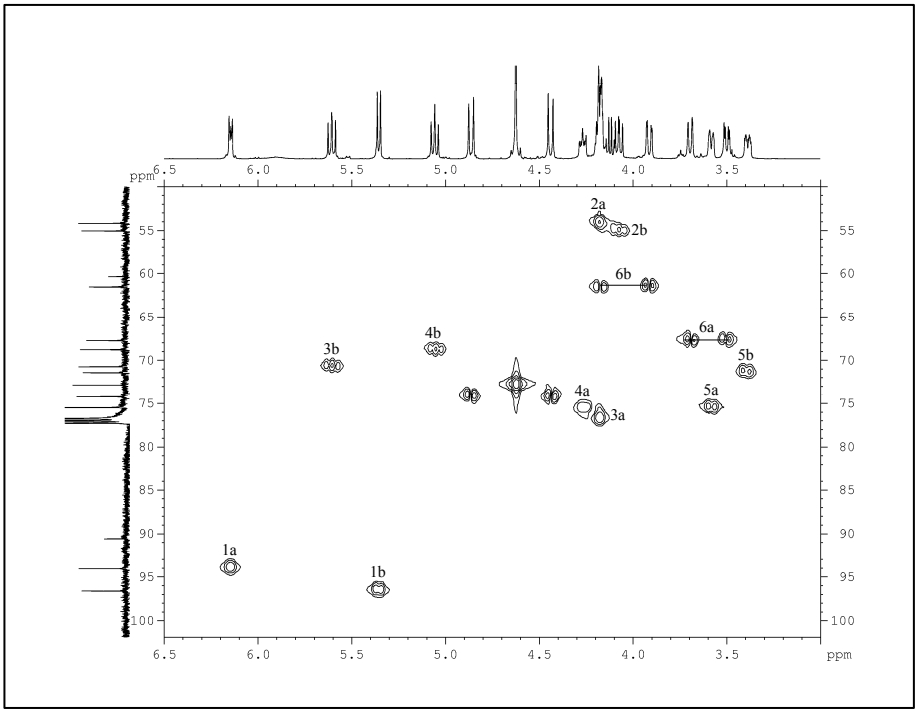
HMQC spectrum of compound **66**



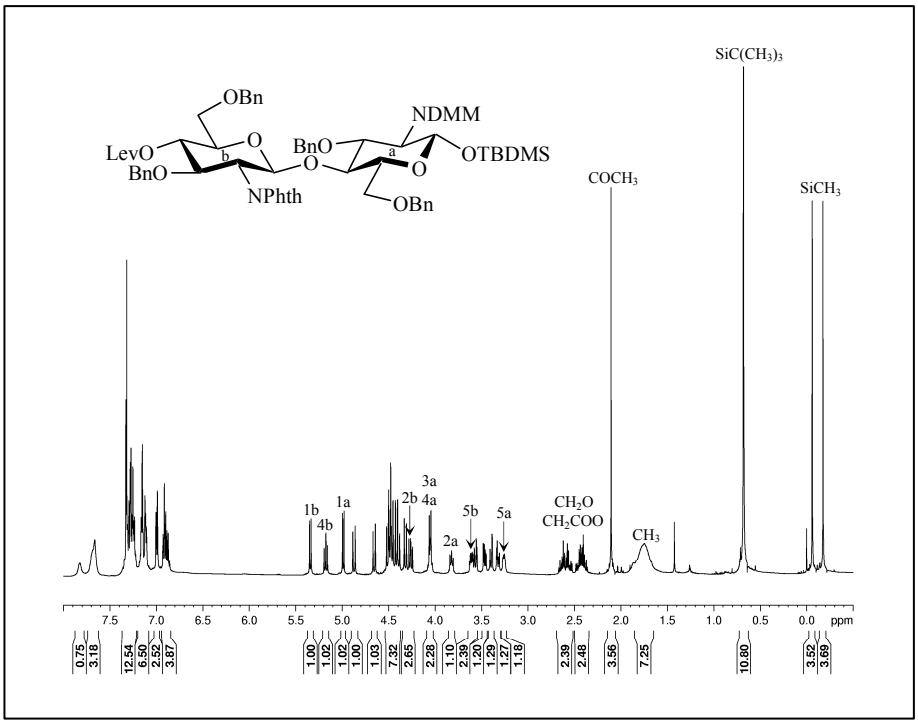
MALDI spectrum of compound **66**



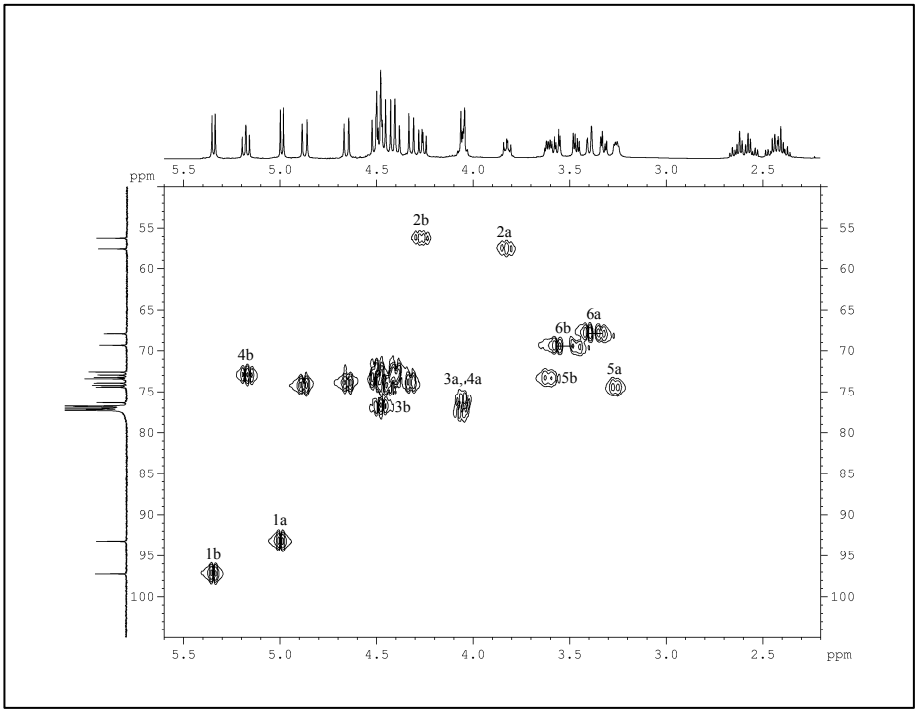
^{1}H-NMR spectrum of compound **67** (500 MHz, $CDCl_3$)



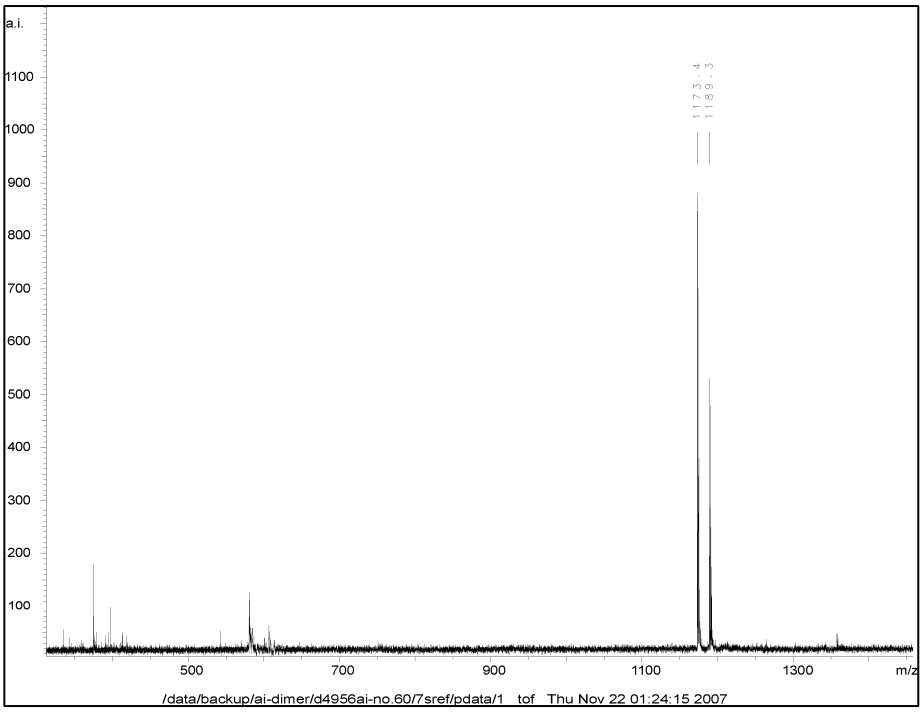
HMQC spectrum of compound **67**



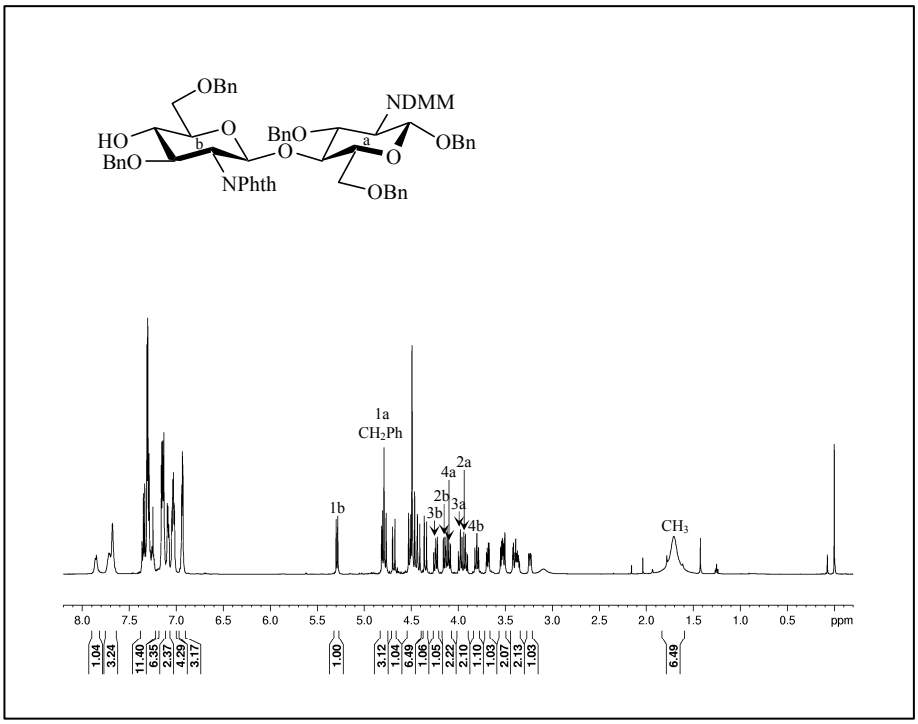
^{1}H-NMR spectrum of compound **70** (300 MHz, $CDCl_3$)



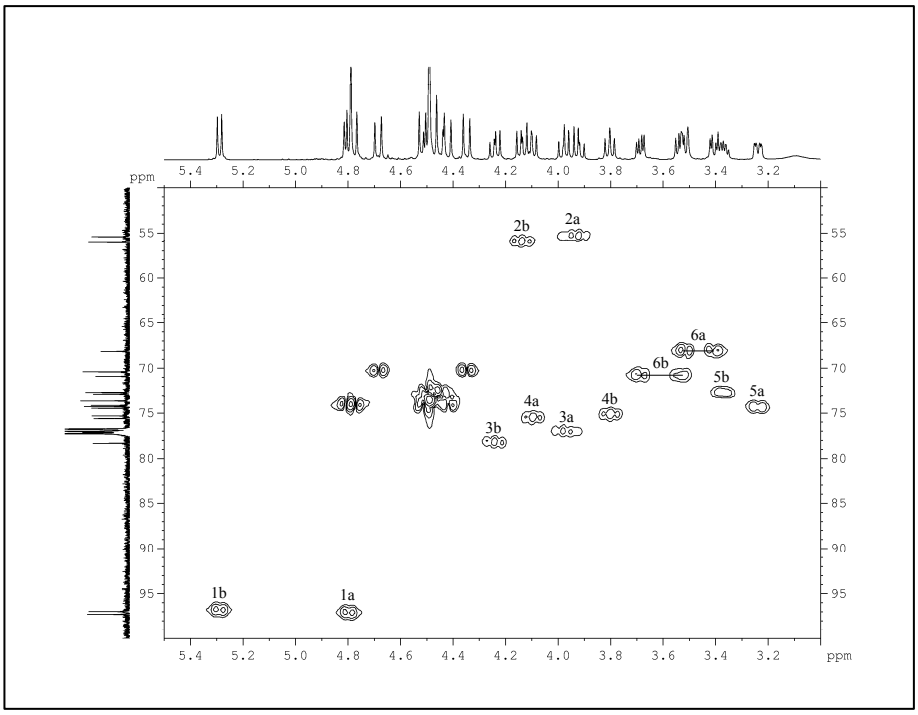
HMQC spectrum of compound **70**



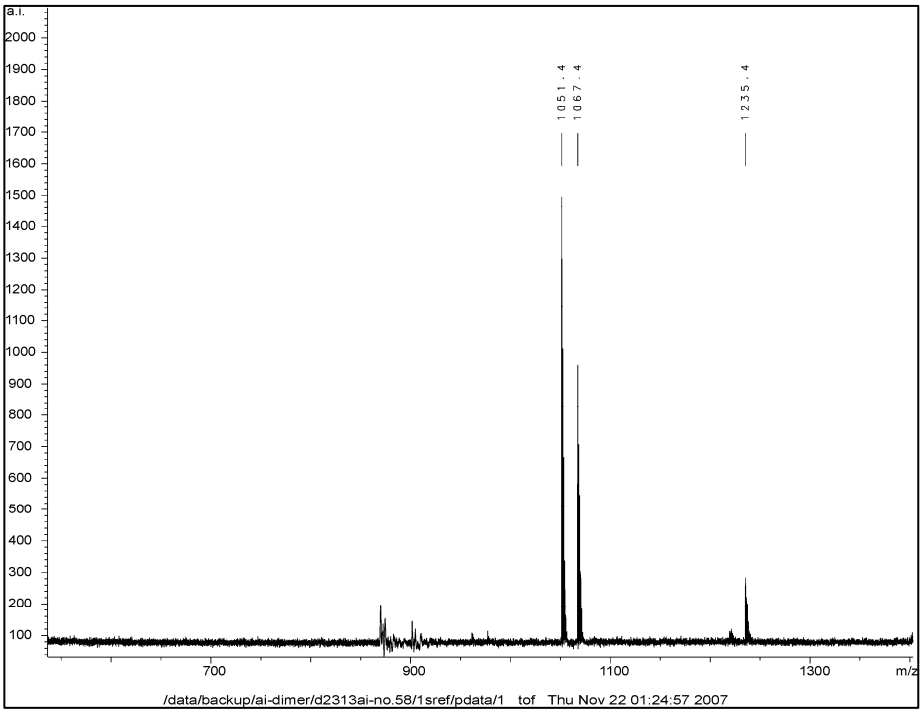
MALDI spectrum of compound **70**



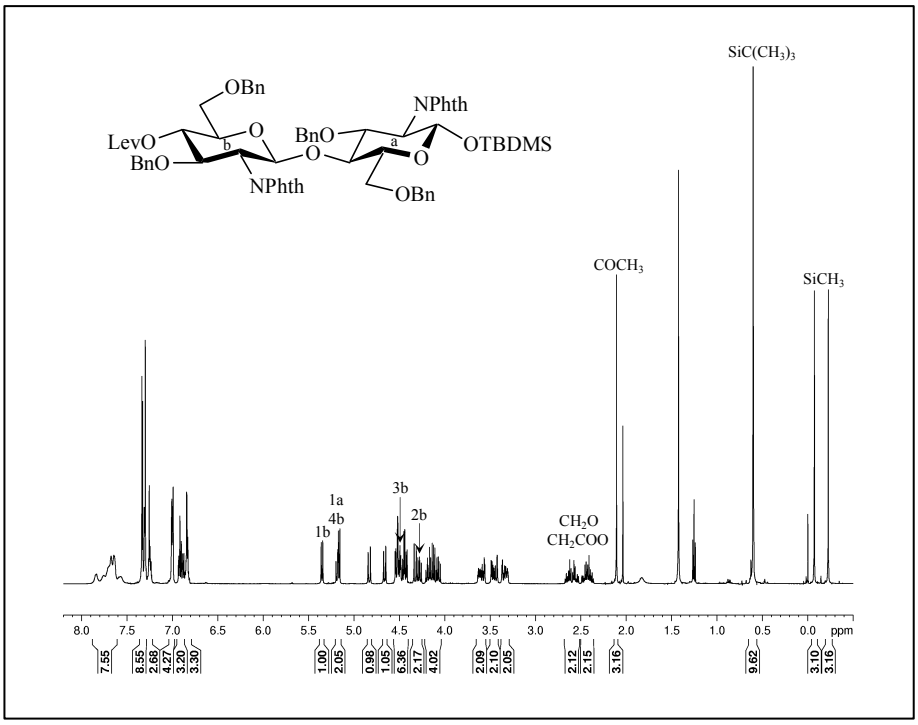
^{1}H-NMR spectrum of compound **68** (500 MHz, $CDCl_3$)



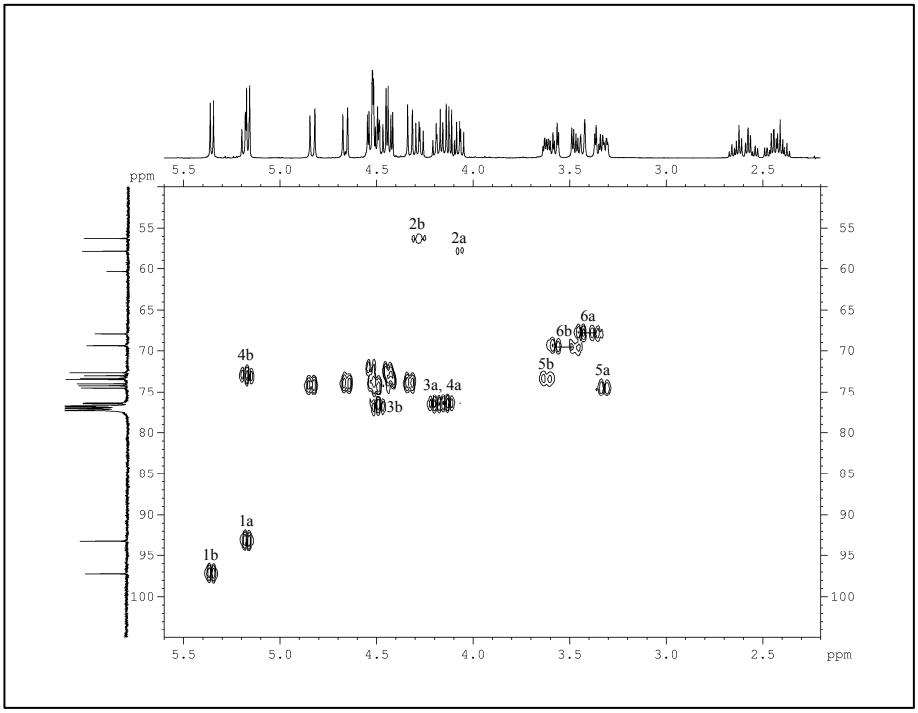
HMQC spectrum of compound **68**



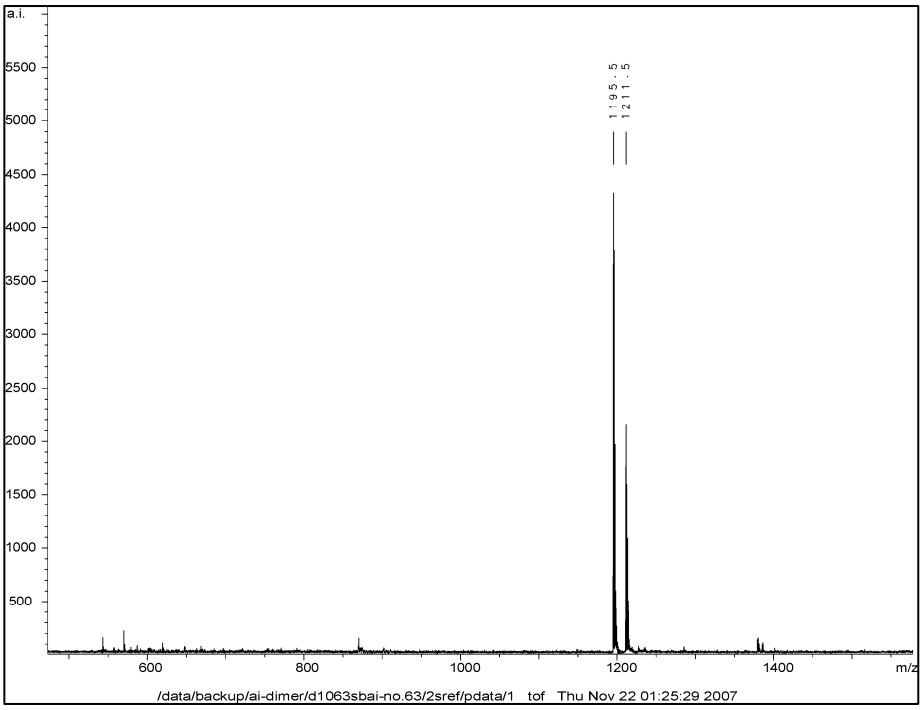
MALDI spectrum of compound **68**



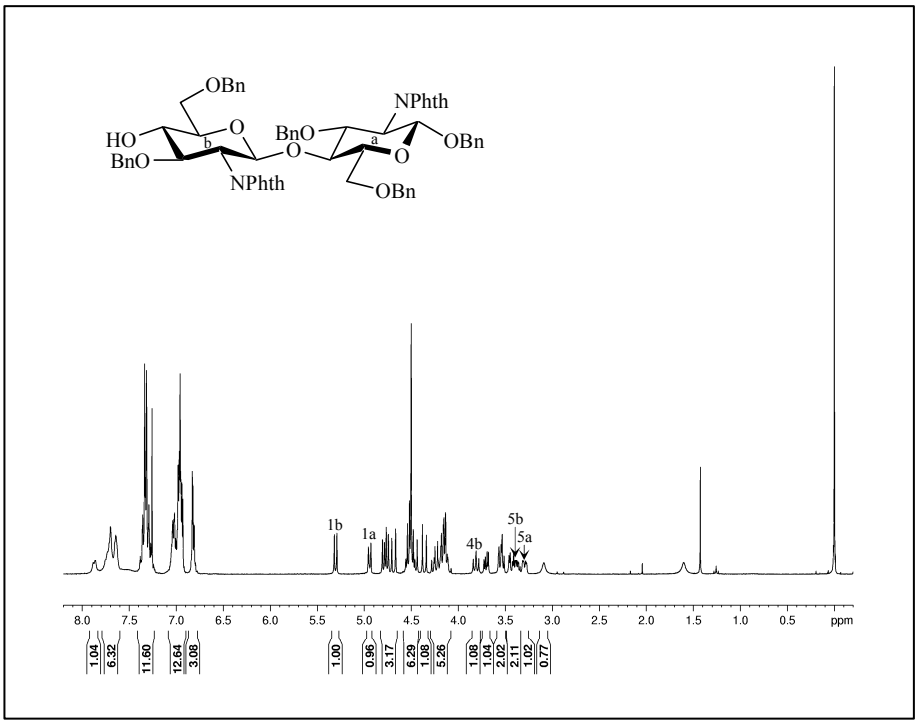
^{1}H-NMR spectrum of compound **73** (300 MHz, $CDCl_3$)



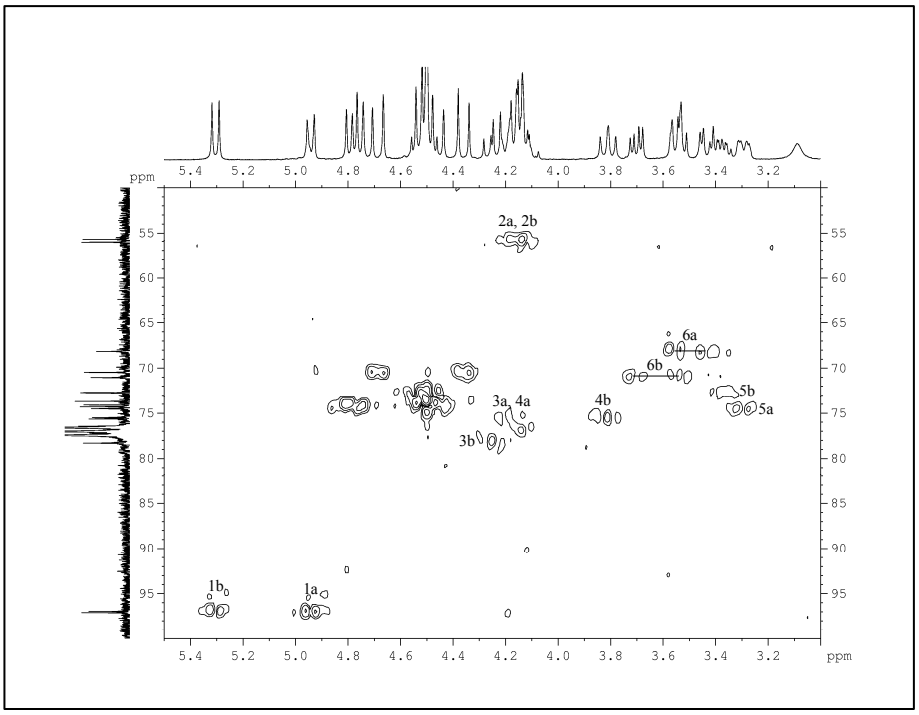
HMQC spectrum of compound **73**



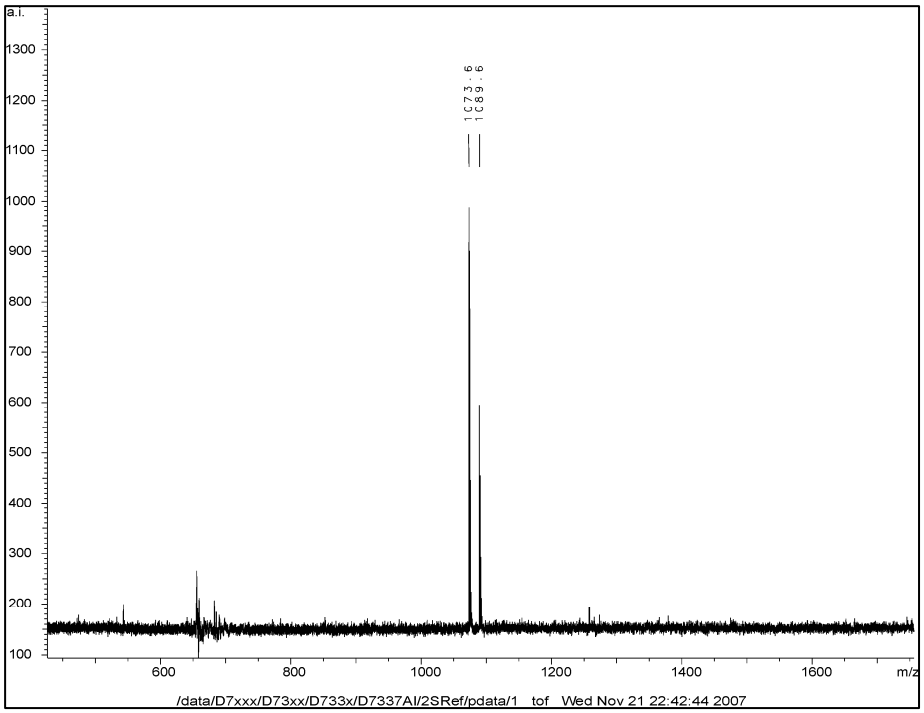
MALDI spectrum of compound **73**



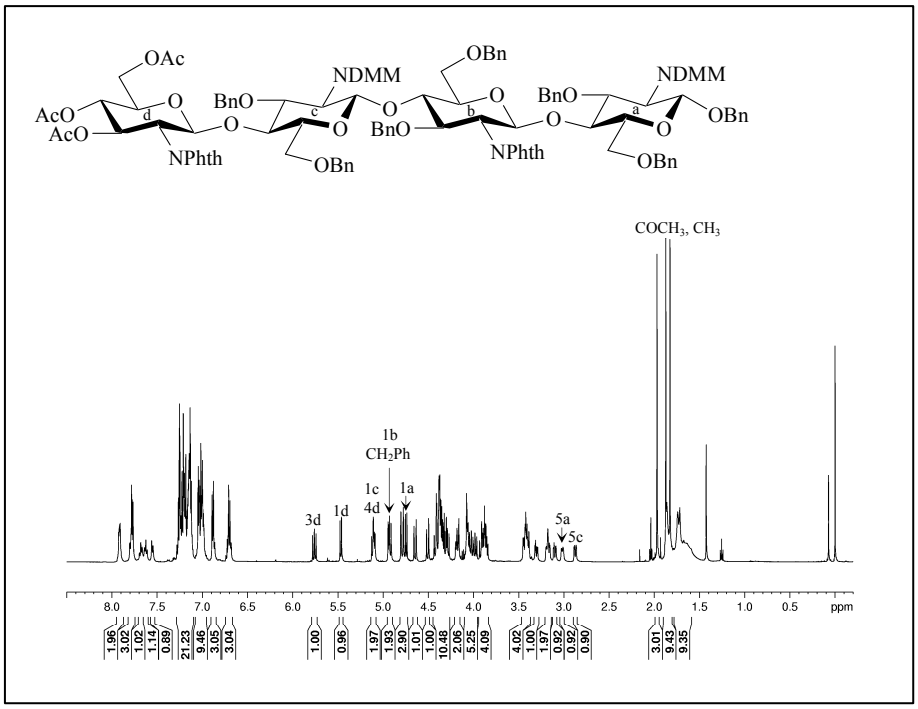
^{1}H-NMR spectrum of compound **69** (300 MHz, $CDCl_3$)



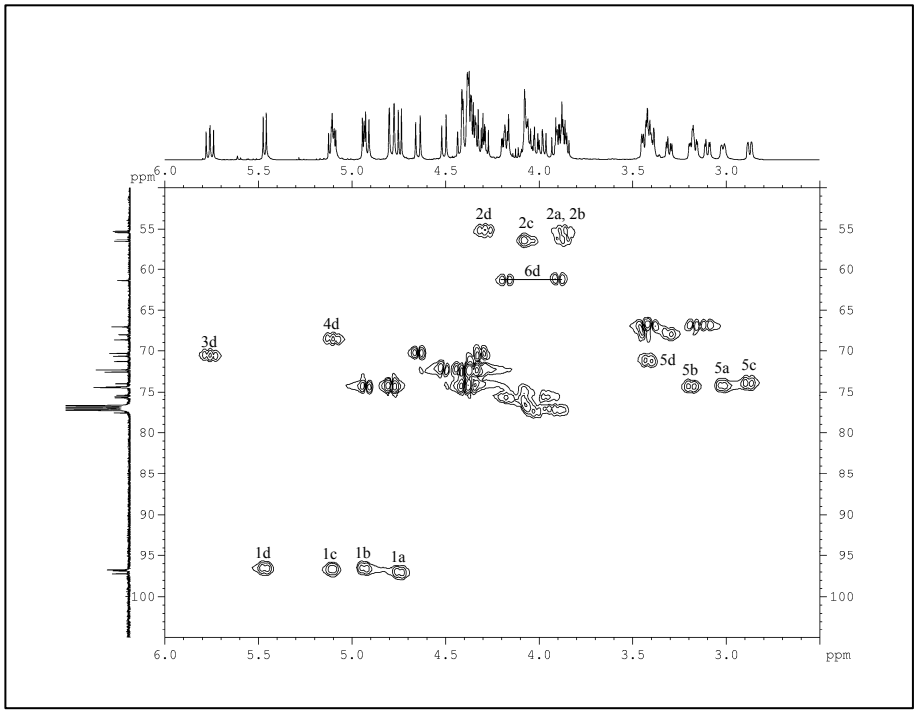
HMQC spectrum of compound **69**



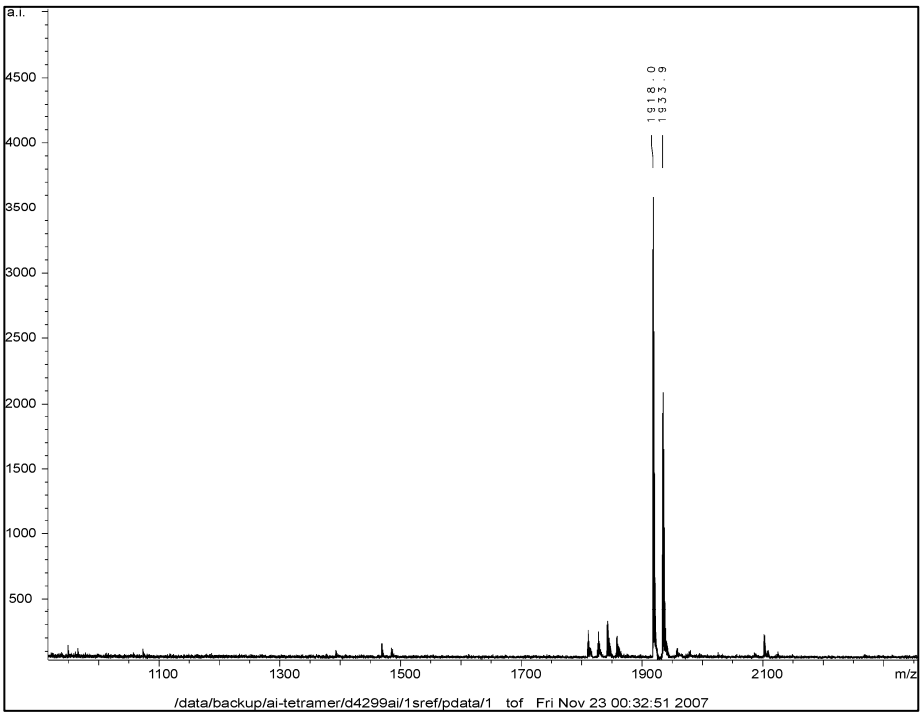
MALDI spectrum of compound **69**



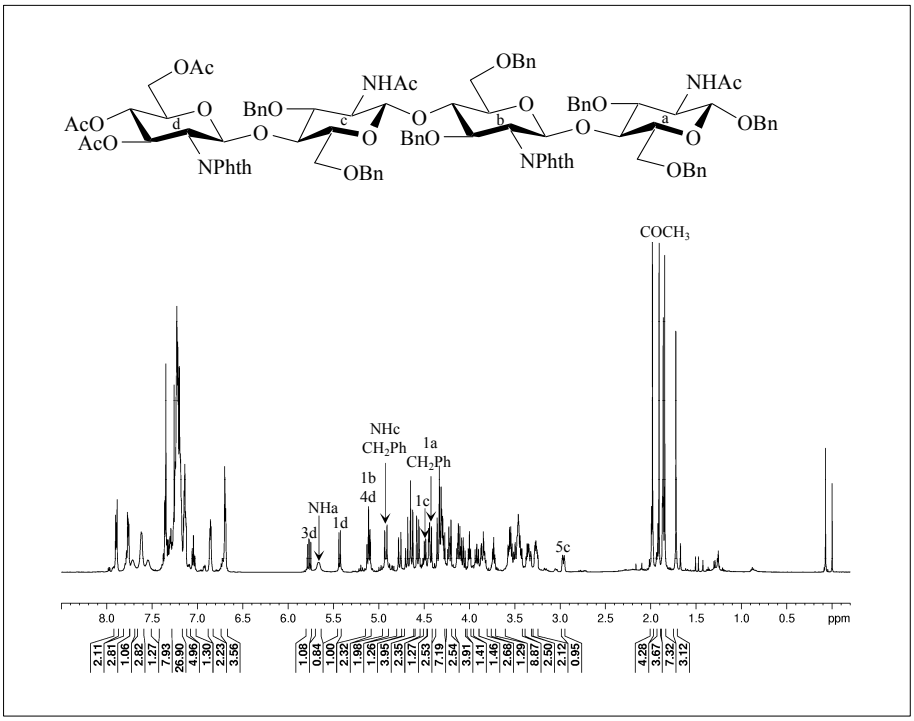
^{1}H-NMR spectrum of compound **76** (500 MHz, $CDCl_3$)



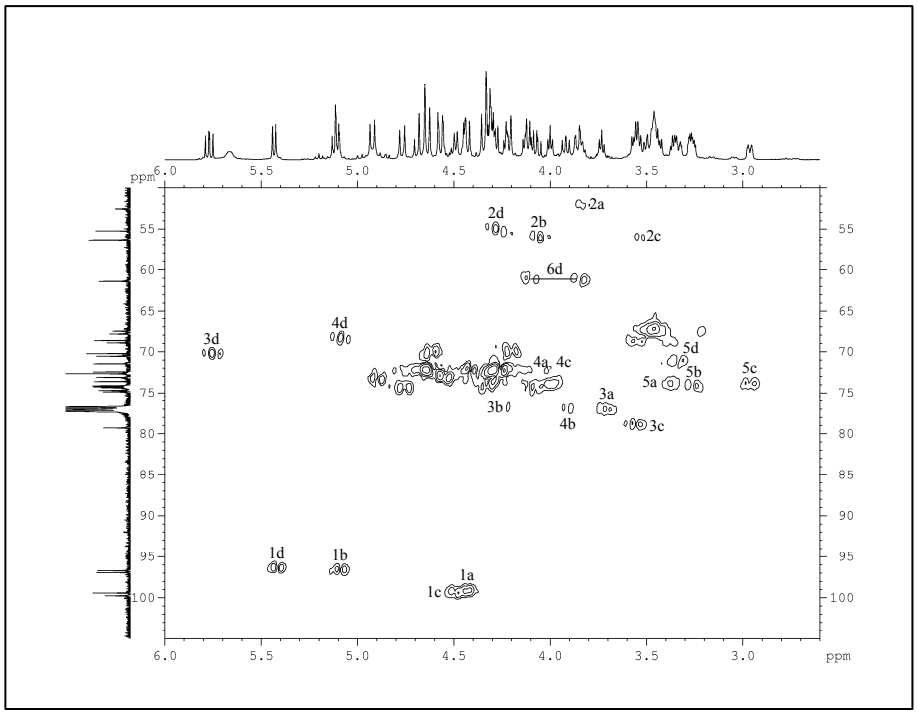
HMQC spectrum of compound **76**



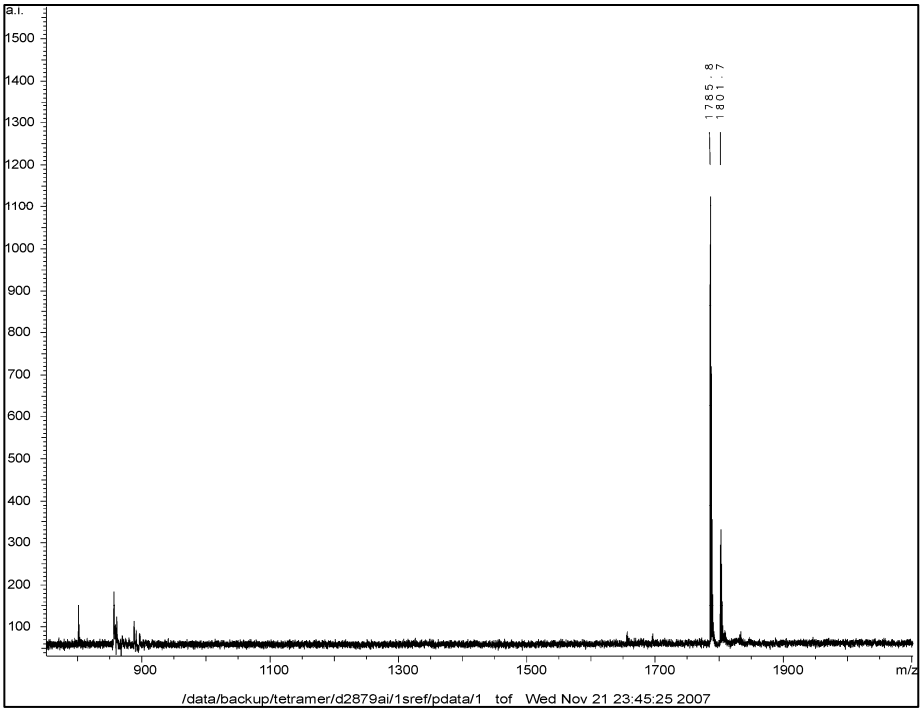
MALDI spectrum of compound **76**



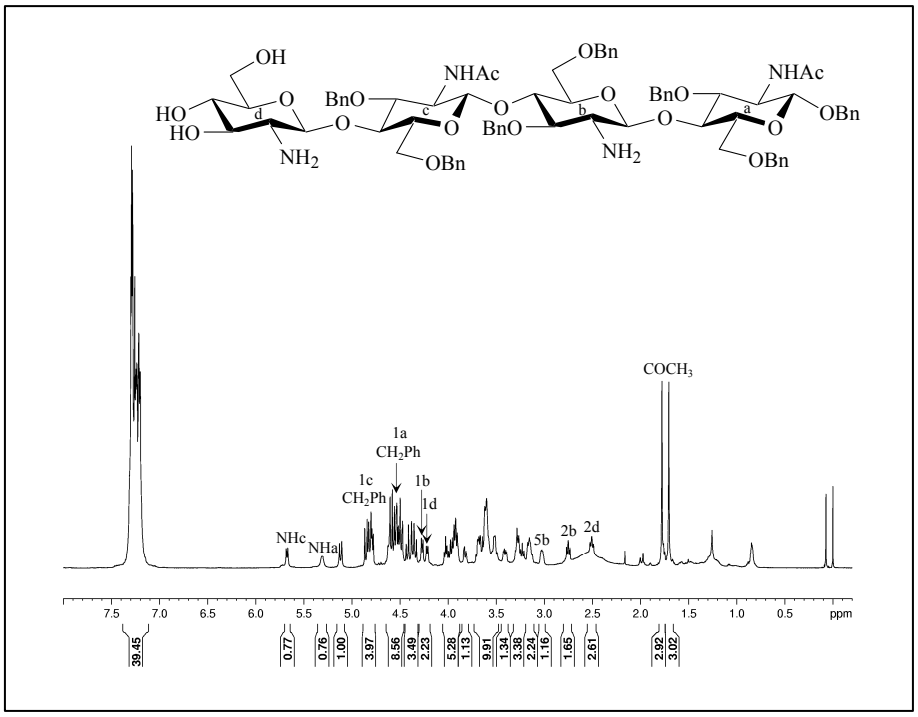
^{1}H-NMR spectrum of compound **77** (500 MHz, $CDCl_3$)



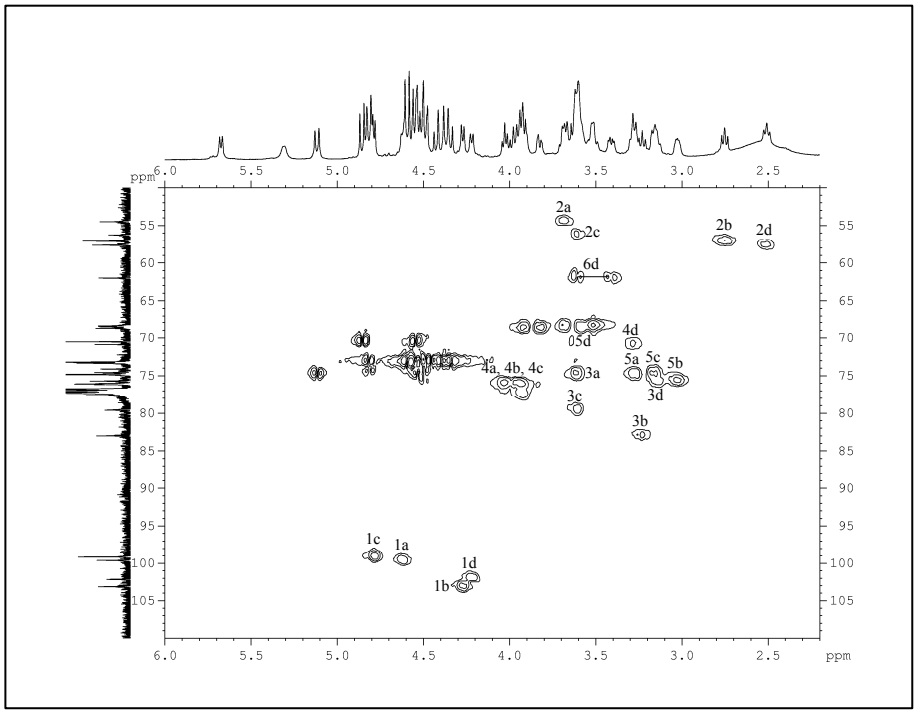
HMQC spectrum of compound **77**



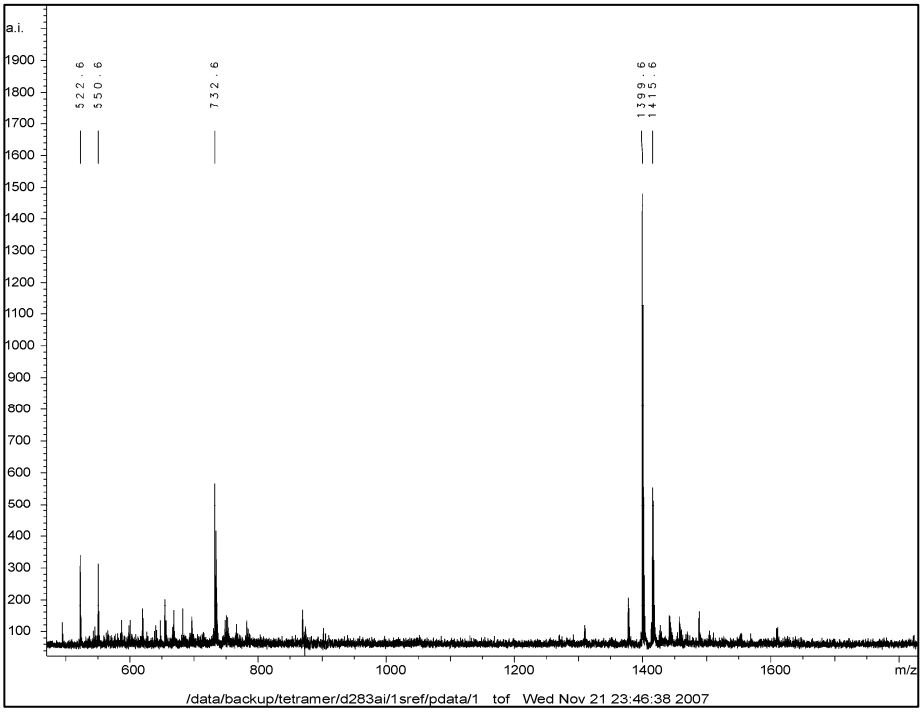
MALDI spectrum of compound **77**



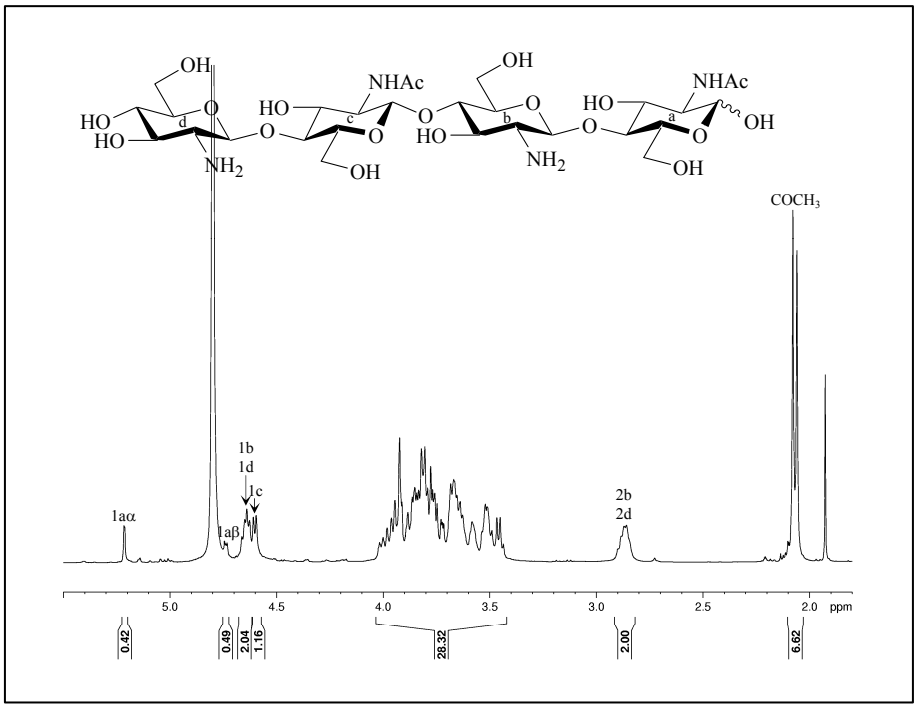
^{1}H-NMR spectrum of compound **81** (500 MHz, $CDCl_3$)



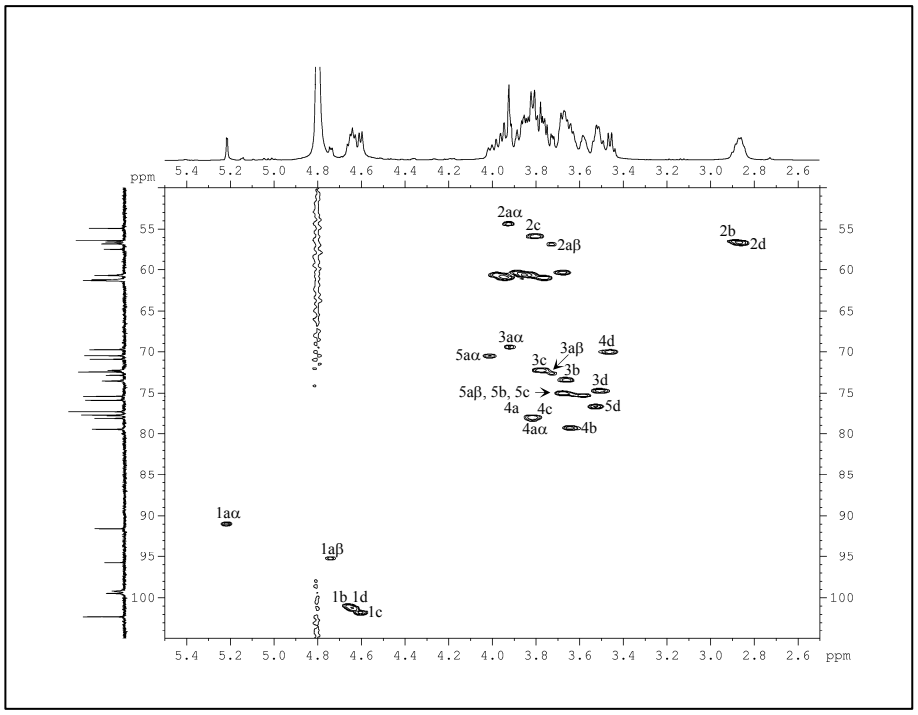
HMQC spectrum of compound **81**



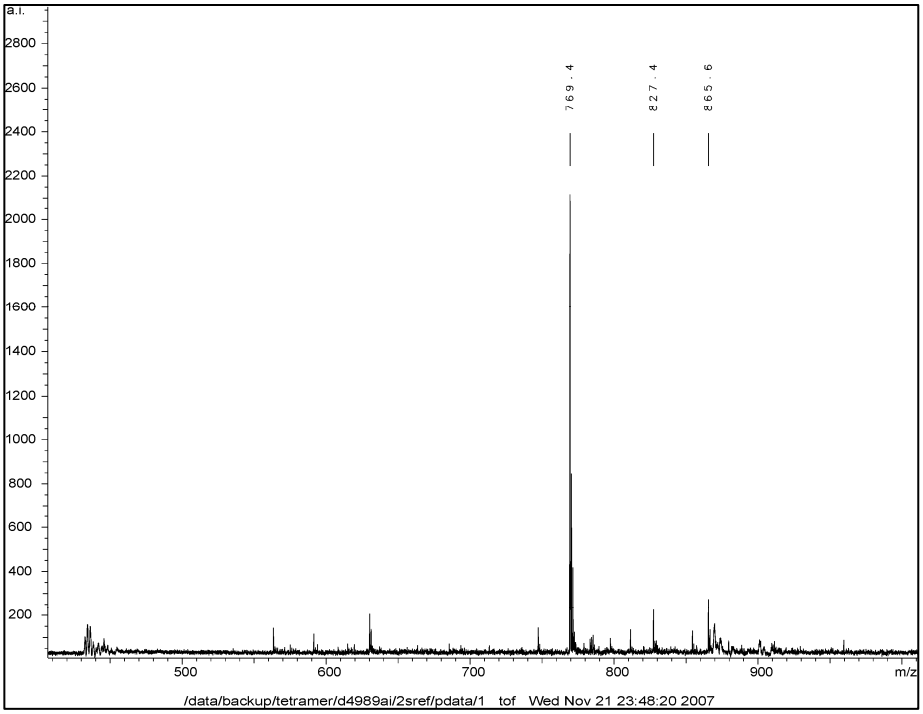
MALDI spectrum of compound **81**



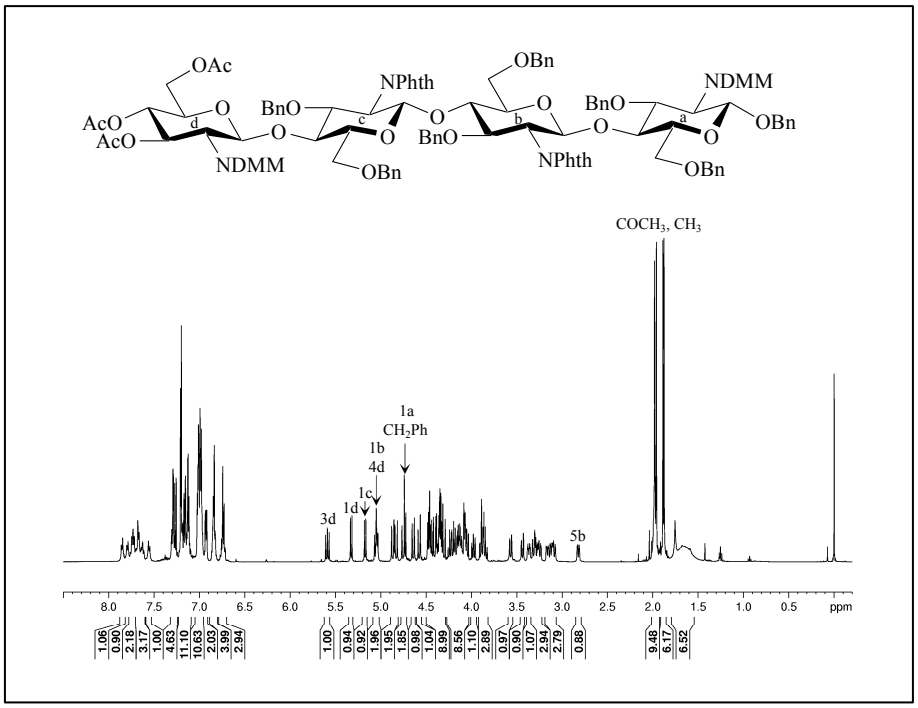
^{1}H-NMR spectrum of compound **61** (600 MHz, D_2O)



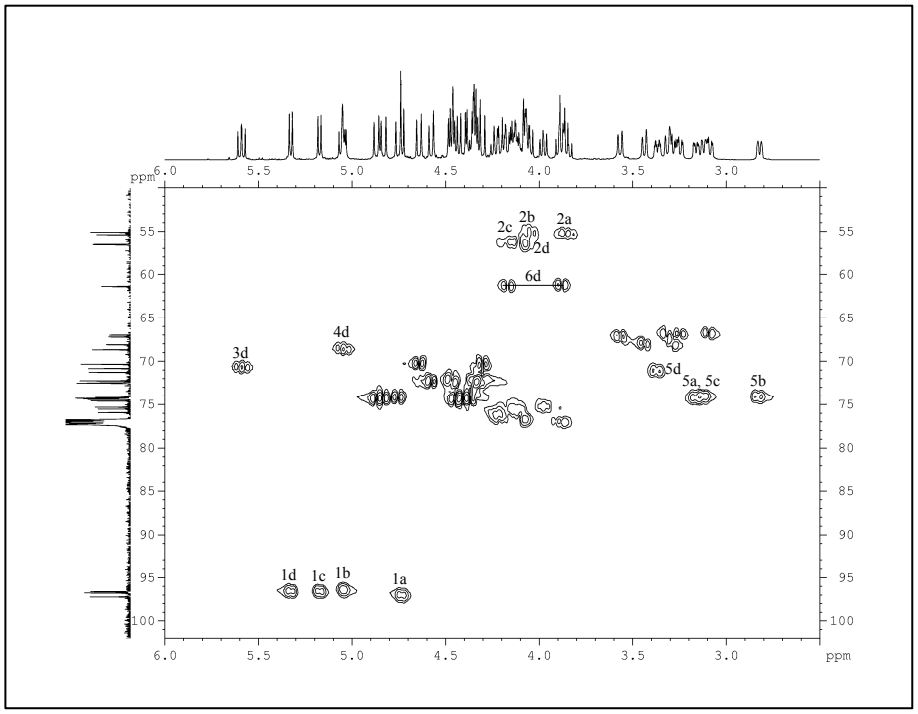
HMQC spectrum of compound **61**



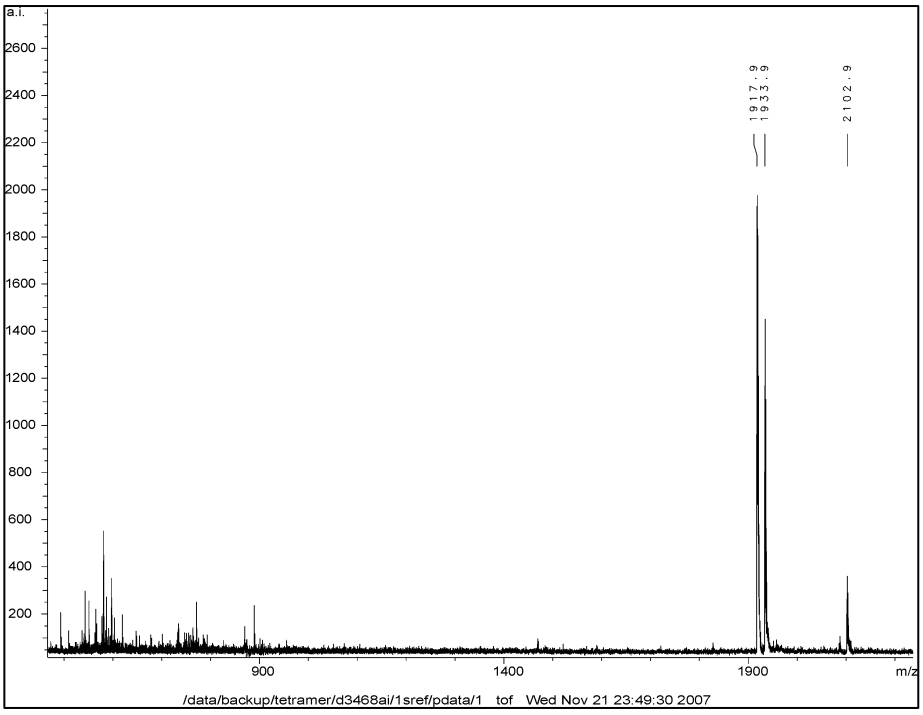
MALDI spectrum of compound **61**



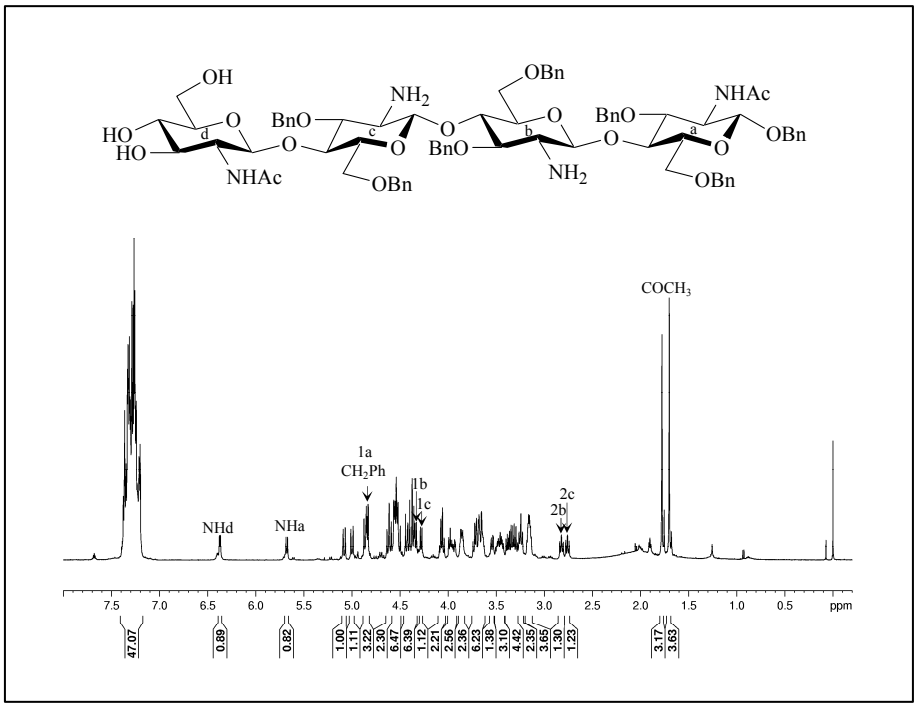
^{1}H-NMR spectrum of compound **84** (500 MHz, $CDCl_3$)



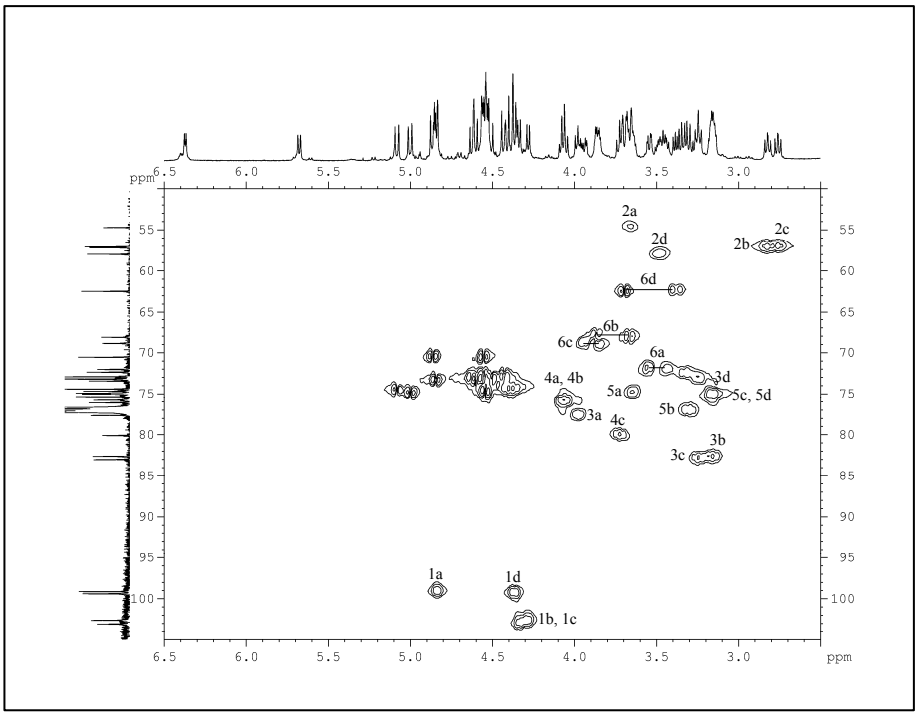
HMQC spectrum of compound **84**



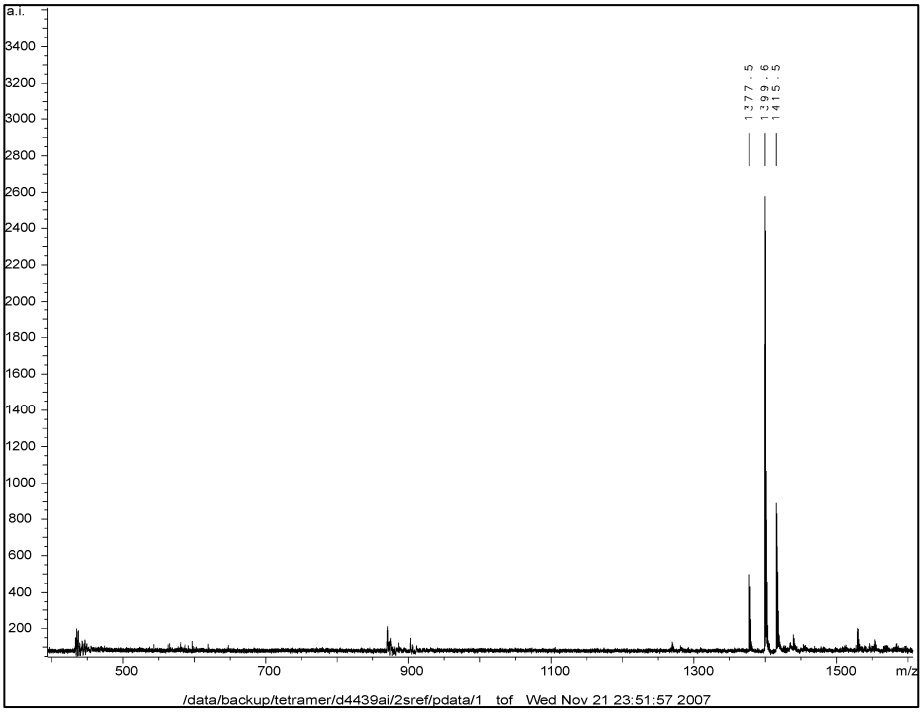
MALDI spectrum of compound **84**



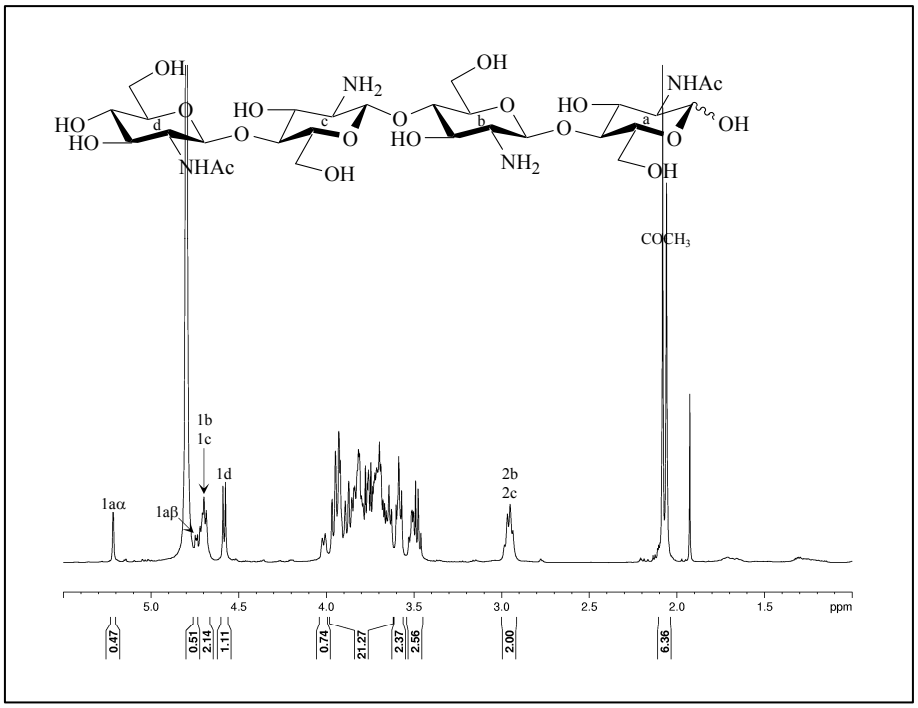
^{1}H-NMR spectrum of compound **87** (500 MHz, $CDCl_3$)



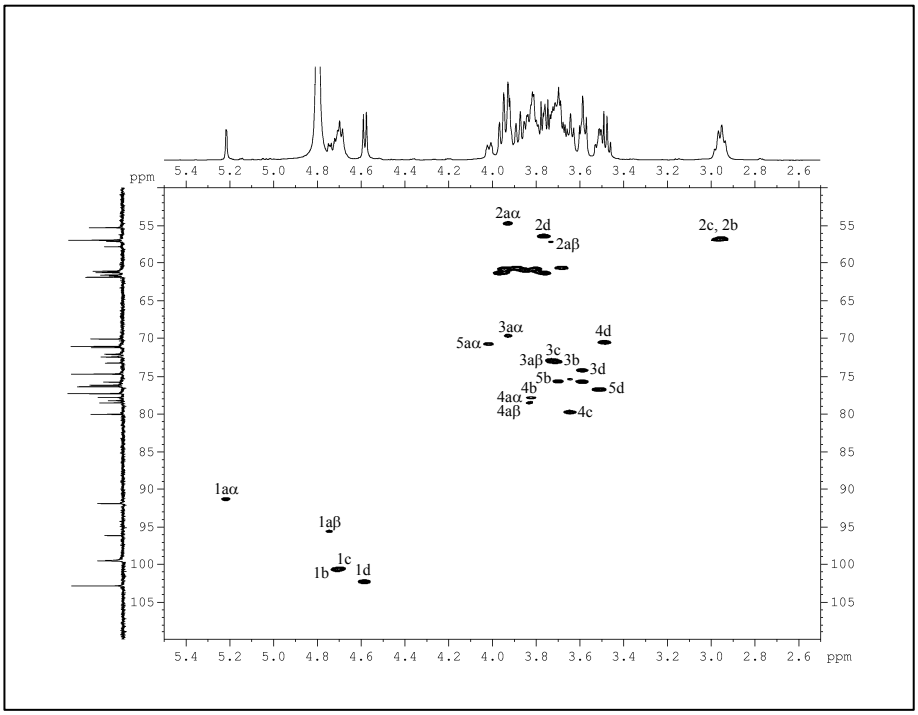
HMQC spectrum of compound **87**



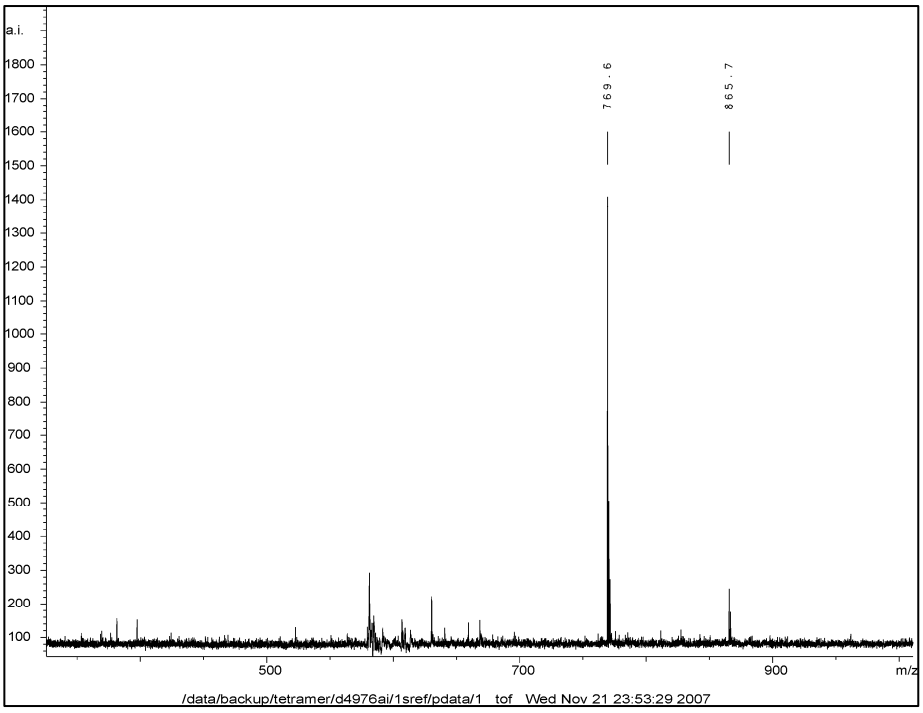
MALDI spectrum of compound **87**



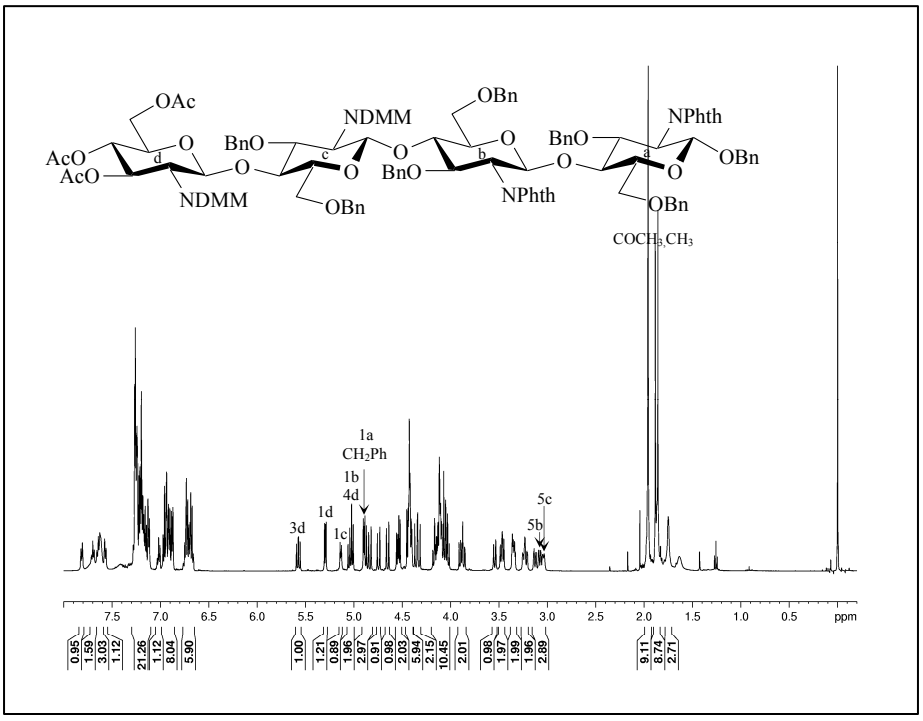
^{1}H-NMR spectrum of compound **62** (600 MHz, D_2O)



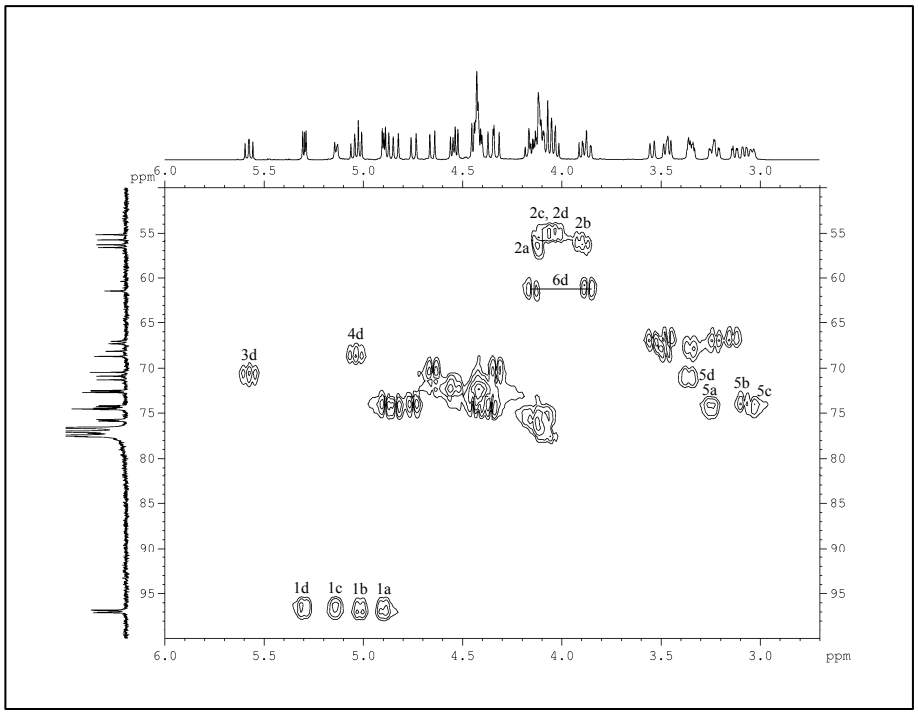
HMQC spectrum of compound **62**



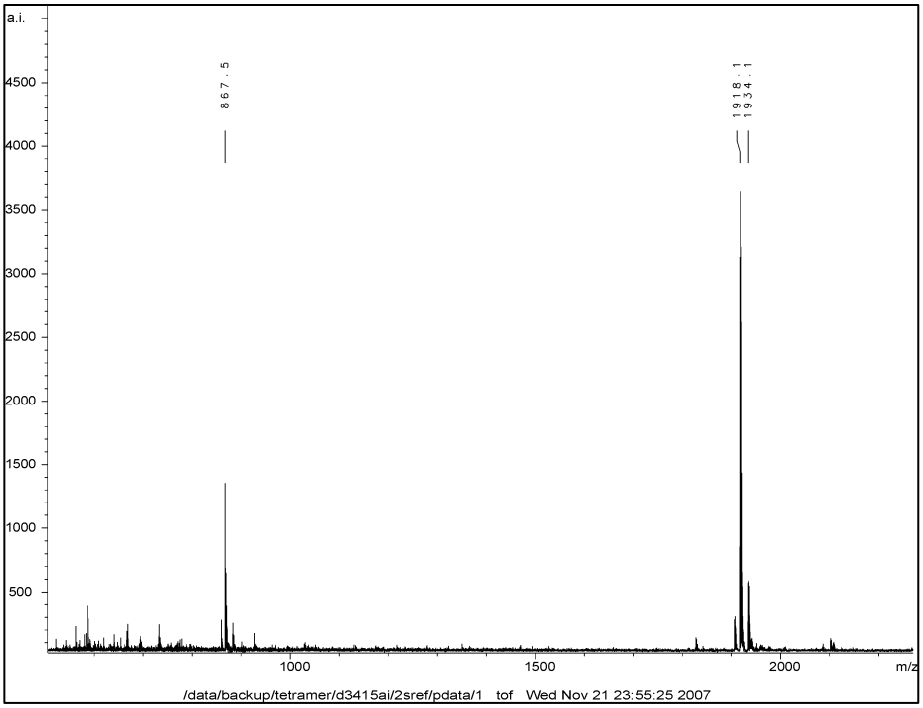
MALDI spectrum of compound **62**



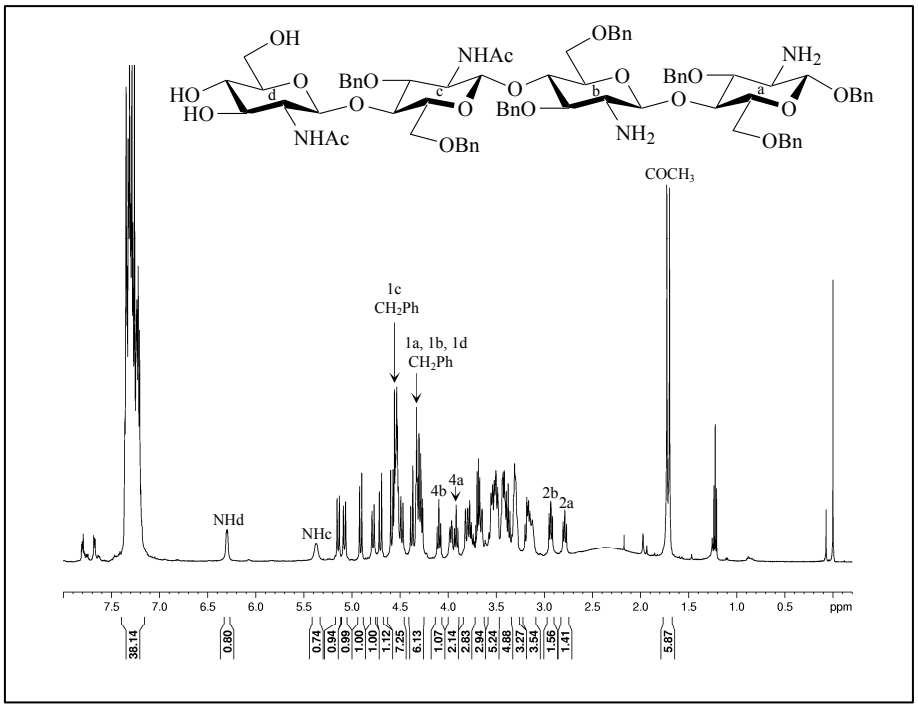
^{1}H-NMR spectrum of compound **88** (500 MHz, $CDCl_3$)



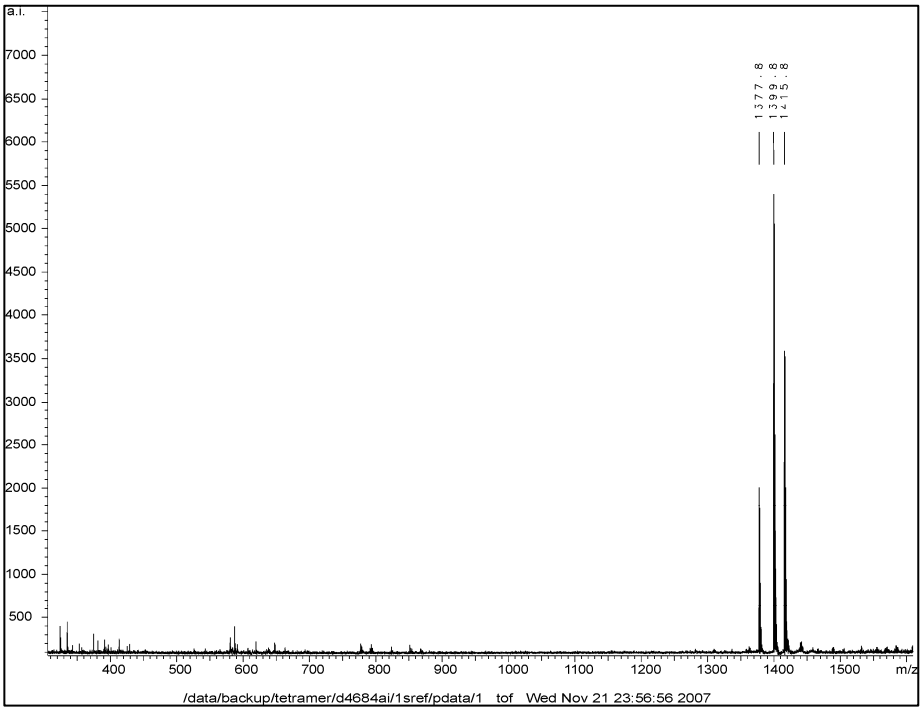
HMQC spectrum of compound **88**



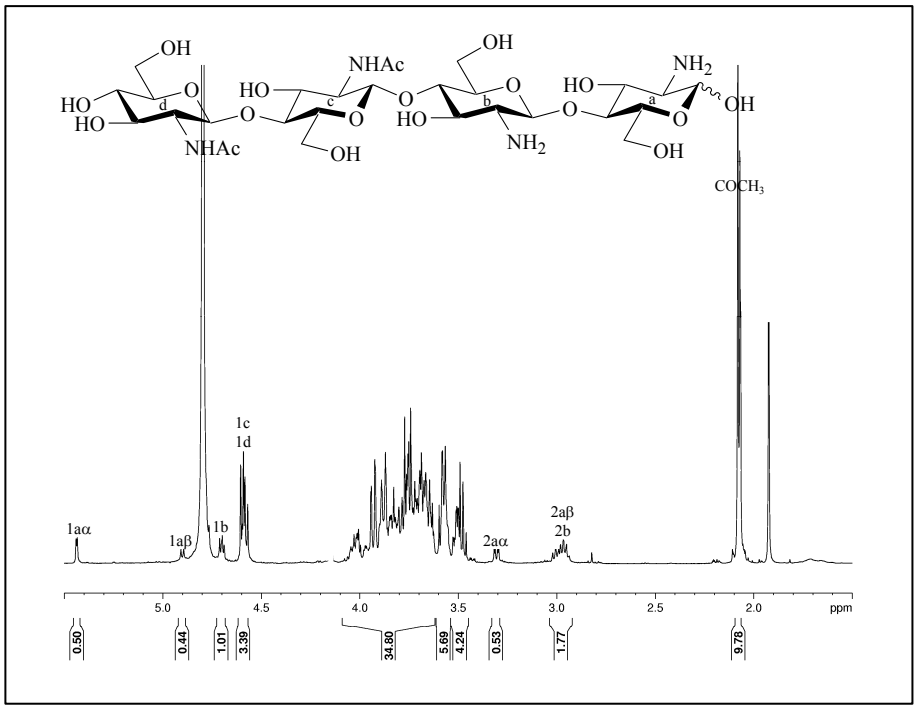
MALDI spectrum of compound **88**



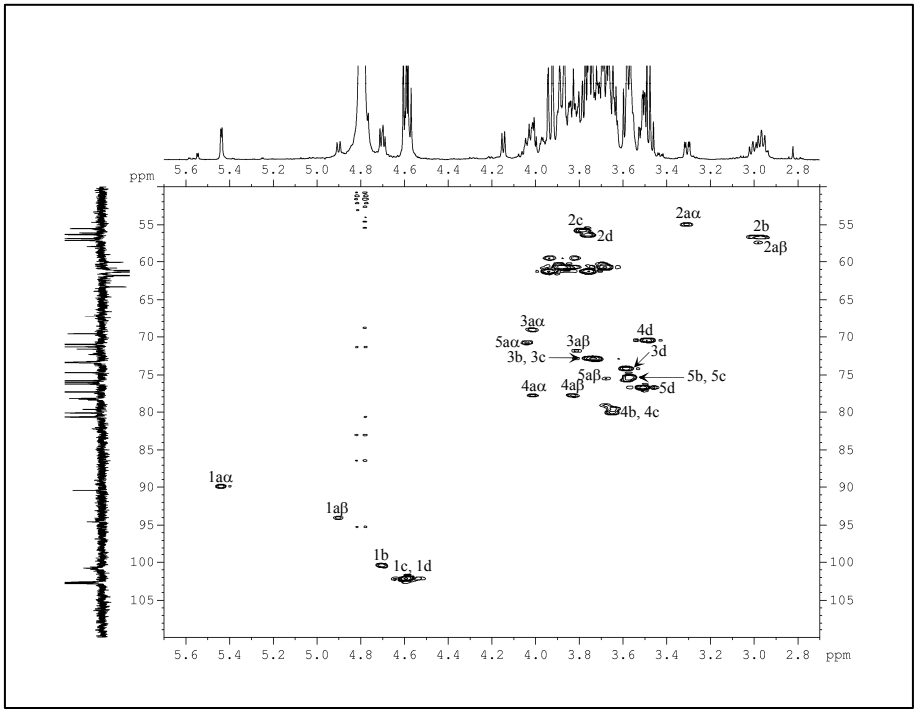
^{1}H-NMR spectrum of compound **91** (500 MHz, $CDCl_3$)



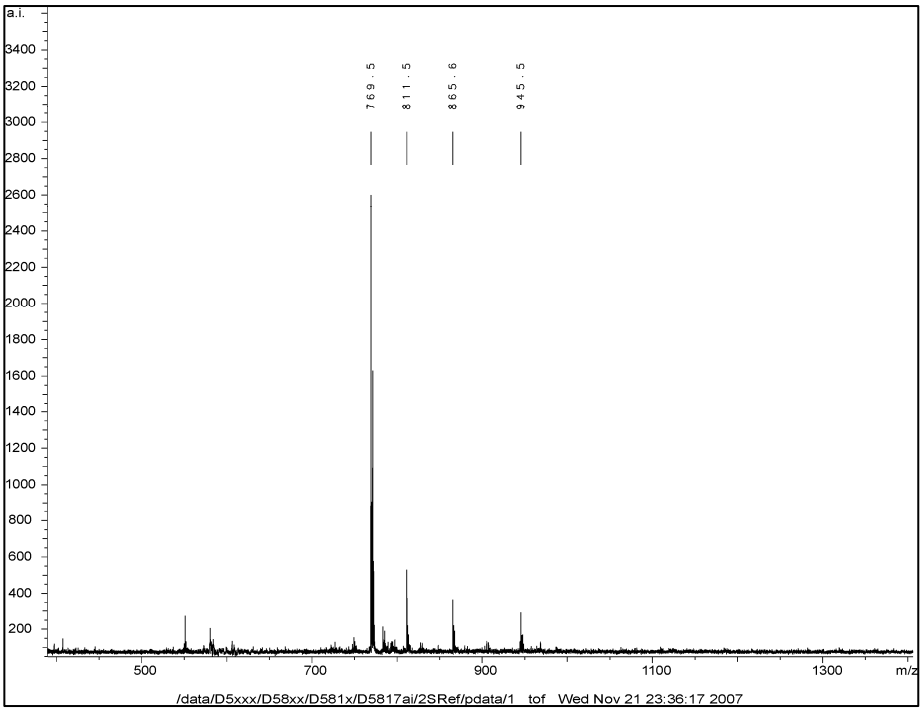
MALDI spectrum of compound **91**



^{1}H-NMR spectrum of compound **63** (600 MHz, D_2O)



HMQC spectrum of compound **63**



MALDI spectrum of compound **63**

Curriculum Vitae

Name:	Arisara Issaree
Date of birth:	11 March 1976
Place of birth:	Udonthani, Thailand
Nationality:	Thai
Marital status:	Single

Education Background

02/2004 to present:	**Ph.D. student** at University of Potsdam, Potsdam, Germany under the guidance of Prof. Dr. Martin G. Peter **Thesis title:** "Synthesis of Hetero-chitooligosaccharides"
2000-2003:	**Master of Science** (Organic Chemistry) at Mahidol University, Bangkok, Thailand under the guidance of Prof. Dr. Manat Pohmakotr **Thesis title:** "Synthetic Approach to Tetrasubstituted Dihydrofurans and Furans" **Scholarships:** - The Ministry Staff Development Project Scholarship under the organization of the Ministry of University Affairs, Thailand - Partial support from Postgraduate Education and Research Program in Chemistry (PERCH), Thailand
1994-1998	**Bechelor of Science** (Chemistry) at Khon Kaen University, Khon Kaen, Thailand

Publications

1. Pohmakotr, M.; Sampaongoen L.; Issaree, A.; Tuchinda, P.; Reutrakul V. "Vicinal dianions of diethyl α-aroylsuccinates: a general synthetic route to α-aroyl- and α-arylidene-γ-butyrolactones" *Tetrahedron Lett.* **2003**, *44*, 6717-6720.
2. Pohmakotr, M.; Issaree, A.; Sampaongoen L.; Tuchinda, P.; Reutrakul V. "Vicinal dianions of diethyl α-aroylsuccinates: preparation of functionalized-2,3-dihydrofurans and -furans, and diaxial 2,4-diaryl-3,7-dioxabicyclo[3.3.0]octanes" *Tetrahedron Lett.* **2003**, *44*, 7937-7940.
3. Issaree, A.; Peter, M. G. "Synthesis of mono-*N*-acetylated chitobioses" (manuscript submitted for publication).
4. Issaree, A.; Peter, M. G. "Synthesis of partially acetylated Chitotetraoses" (manuscript in preparation).

Poster presentations

1. 9. International SFB-Symposium, 10-11 October, 2005, Aachen, Germany
2. The 2nd Glycan Forum in Berlin, 24-25 November, 2005, Berlin, Germany
3. Issaree, A.; Peter, M. G., "Synthesis of Partially Acetylated Chitotetraoses" Poster at 10th ICCC-Euchis' 06, 6-9 September, 2006, Montpellier, France
4. Issaree, A.; Peter, M. G., "Synthesis of Partially Acetylated Chitotetraoses" Poster at 8th Tetrahedron Symposium: Challenges in Organic Chemistry, 26-29 June, 2007, Berlin, Germany