Technische Universität München
Max-Planck-Institut für Biochemie

Towards the synthesis of *V. Cholerae* O1 antigens and GQ1bα ganglioside as potential therapeutic agents. Structural Chemistry and characterization

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Alina Ariosa Alvarez

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A mi madre

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A mis padres, mi abuela y mi hermana por estar siempre a mi lado.

A mis amigos todos, por ustedes estoy aquí, para ustedes estaré siempre.

Content

Abbreviations

Ac	acetyl
ax	axial
Bn	benzyl
BSA	bovine serum albumin
Bu	butyl
Bz	benzoyl
CMP	cytidine monophosphate
COSY	correlation spectroscopy
DBU	1,8-Diazabiciclo[5.4.0]undec-7-ene
DCM	dichlormethane
DCMME	dichloromethyl methyl ether
DMAP	dimethylaminopyride
DMF	dimethylformamide
DMM	dimethylaminomaleoyl
DMP	dimethoxypropane
DMSO	dimethyl sulfoxide
DMTST	dimethyl(methylthio)sulfonium triflate
EDAC	3-ethyl-1-(dimethylaminopropyl)carbodimide
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
ELISA	enzime-linked inmunosorbent assay
eq	equatorial
Et	ethyl
EtOAc	ethyl acetate
Gal	galactose
GalNAc	N-Acetylglucosamine
Glc	glucose
GlcNAc	N-acetylgalactosamine
GSLs	glycosphingolipids
HATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronum hexafluorphosphate
HMQC	heteronuclear multiple-quantum coherence
IDCP	iodosuccinimide-perchlorate
J	vicinal coupling constant

Lac	lactose
LPS(s)	lipopolysaccharide(s)
Lys	lysine
Me	methyl
MPLC	medium-pressure liquid chromatography
MPM	p-methoxyphenylmethyl
Ms	mesyl or methanesulfonyl
Ms-MALDI	Matrix-assisted laser desorption
Neu5Ac	N-acetyl-D-neuraminic acid
NMR	nuclear magnetic resonance
OMP	meningococcal outer membrane protein
OPS/ O-PS	O-polysaccharide
PE	petroleum ether
Ph	phenyl
Piv	pivaloyl
ppm	parts per million
p-TsOH	p-toluenesulphonic acid
Py	pyride
Sn(TfO)2	tintrifluoromethane sulphonate
TBABr	tetrabutylammoniun brommide
TBAF	tetrabutylammonium fluoride
TD	T-dependent antigents
TDS	thexyldimethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoracetic acid
THF	tetrahydrofuran
TI	T-independent antigens
TLC	thin layer chromatography
TMSOTf	trimethylsilyl triflate
Ts	p-toulenesulfonyl
UV	ultraviolet
WHO	World Health Organization
δ	chemical shift

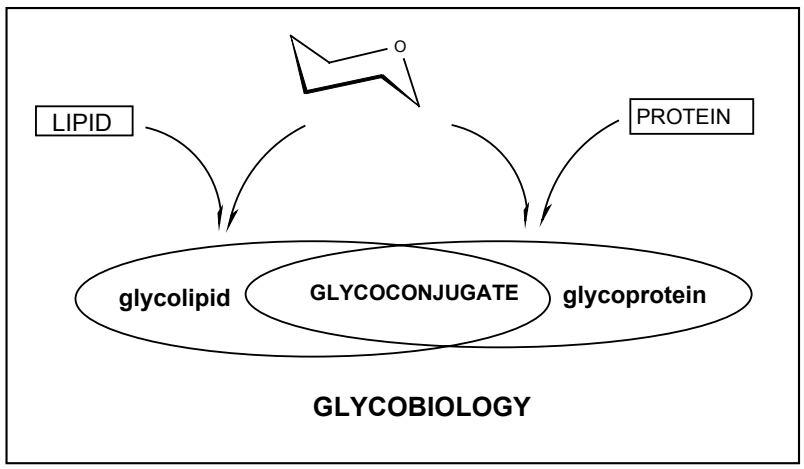
1. Introduction and Objectives

1.1 Glycobiology

Carbohydrates are playing a particularly important role in the assembly of complex multicellular organs and organisms where interactions between cells as well as with the surrounding matrix are essential.

These carbohydrates are frequently covalently bound to non-carbohydrate natural products of different kinds, and the molecules which derive from the combination of sugars with other biomolecules are called glycoconjugates. Consequently, glycobiology addresses the research in structure, biosynthesis, and biology of the sugars or glycans that are distributed in nature.[1]

A large variety of modifications of the oligosaccharides enhances the diversity of oligosaccharides found in nature, and this diversity serves to mediate specific biological functions. For example, the hydroxy groups of the different monosaccharides can be phosphorylated, sulfated, methylated, *O*-acetylated or fatty acylated; amino groups can be *N*-acetylated or *N*-sulfated, and carboxy groups are occasionally involved in lactonization with adjacent hydroxy groups. On the other hand, complex carbohydrates are linked to proteins and lipids producing a large number of different glycoconjugates called glycoproteins and glycolipids, respectively (Scheme 1). Thereby the carbohydrate moieties comprise an enormous structural variety which is used by nature to store biological information that is crucial for the development, growth, function, or survival of organisms.



Scheme1. Glycoconjugate

1.2 Glycolipids

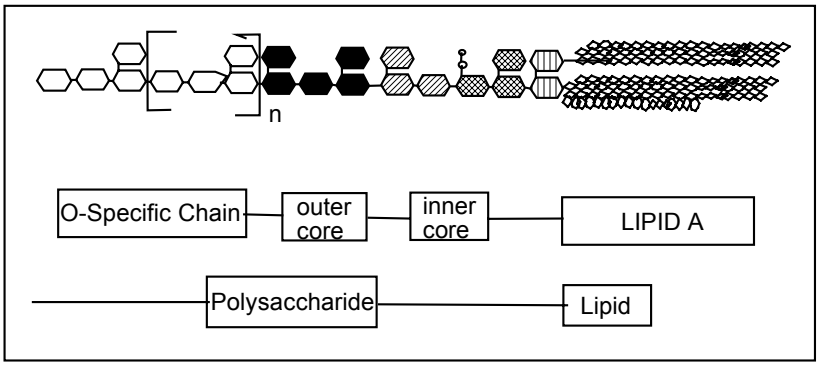
Glycolipids cover a wide range of structures and thus often overlap with other classes of lipid-containing common structural motifs, making a classification difficult and confusing. The following classification of glycolipids is based on their origin reflecting the structural complexity of glycolipids from various sources.[2]

- **Animal glycolipids.** Glycosphingolipids (often called glycolipids) are oligosaccharides usually attached via glucose or galactose to the terminal primary hydroxy group of the lipid moiety ceramide. More than 200 structurally distinct glycosphingolipids (GSLs) from a wide variety of eukaryotic sources have been reported. More complex glycosphingolipids are called gangliosides. Essentially all of the GSLs are antigenically active, and one of their biological properties is that they act as immunogens. Some of the GSLs serve as cell receptors for bacterial toxins and possibly even for bacteria and viruses.

- **Plant and algal glycolipids.** D-Galactosyl-diacylglycerols are the major components in plant lipid material. The variations found in the fatty acid and other parts of the lipid are more diverse than those found in animal glycolipids, but the carbohydrate moiety shows less variation with D-galactose being the most abundant carbohydrate residue.

- **Microbial glycolipids.** Lipopolysaccharides (LPS)[3] are the major constituent of the outer cell envelope of gram-negative bacteria and represent the first line of defense against the complement system and bacteriophages. They also contain the major antigenic determinants that distinguish various serotypes of bacteria, which are sometimes correlated with diseases. Lipopolysaccharides consist of three distinct parts which differ in their genetic determination, biosynthesis and architecture. These regions are named O-specific side chain, the core-oligosaccharide, and lipid A (Scheme 2).On the other hand, most Gram-positive bacteria, produce Lipoteichoic acid (LTA). It is an amphiphile molecule composed of a membrane lipid or glycolipid covalently linked to a polymer of either glycerol phosphate or ribitol phosphate, with varios sugars and amino acids as substitutes. Thus, LTA shares with endotoxin (lipopolysaccharide) many of its pathogenetic properties.[4]

The exact biological function of glycolipids is still the subject of much speculation. These type of molecules is associated with the biosynthesis of glycoproteins and complex polysaccharides, with the mechanism of control in the biosynthesis of proteoglycans and with inhibition of the biological activities of toxins and antiviral agents.[5]



Scheme 2. Principal structural regions of LPS molecules

1.3 Cholera

Cholera is considered responsible for at least 120 000 deaths annually and constitutes a major global public health problem. Infections with *Vibrio cholerae* are common in several regions of the world and thousands of cases of cholera are reported each year to the World Health Organization (WHO).[6] New strains of cholera continue to emerge and these may potentially cause new pandemics. Correspondingly, there is an urgent need for efficient vaccines as an additional public health tool for cholera prevention.

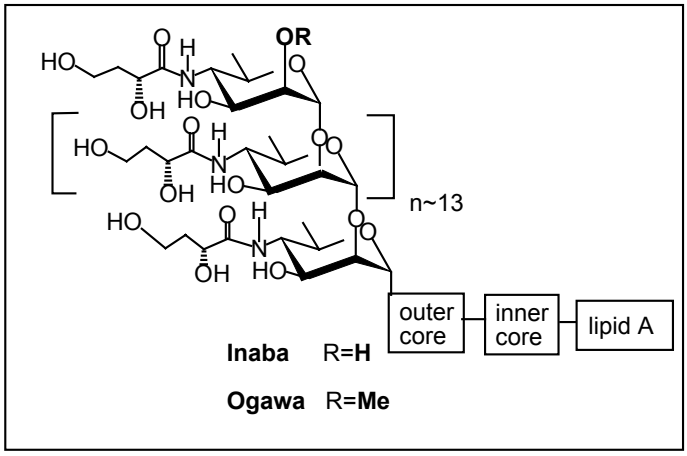
Vibrio cholerae is a non-invasive pathogen that colonises the small intestine; it comprises over 150 serogroups based upon the antigenicity of the surface polysaccharides, and until the recent identification of the O139 serogroup, [7,8,9] only the O1 strains were associated with epidemic cholera. In O1 strains the major surface polysaccharide is the lipopolysaccharide, and the O-antigen component has been shown to extend over the cell surface and the flagellum.[10]

V. cholerae O1 can be divided into two biotypes: i) classical strains which are thought to have been responsible for the first six recorded cholera pandemics, and ii) the El Tor biotype thought to have been responsible for the seventh.

1.3.1 *Vibrio Cholerae* O1- O-antigen

The serotype-specificity of Gram-negative bacteria to which *V.cholerae* O1 species belong, resides in the O-polysaccharide (or O-antigen, OPS) portion of lipopolysaccharides located on the outer membrane of smooth strains of such pathogens. The *V. cholerae* O1 strains of both biotypes have been further subdivided into three serotypes,[11] depending upon the presence and amount of particular antigens on the O-specific chain of the lipopolysaccharide. The three serotypes are designated Inaba,[12] Ogawa, [13] and Hikojima, which all share a common antigen referred to as the A antigen. There are two specific antigens, B and C, which are present in varying amounts on the different serotypes. Inaba strains express only C and A, while Ogawa strains express all three antigens A, B and C, although C is present in much reduced amounts compared to Inaba (Scheme 3). The third, the Hikojima serotype, is extremely rare and unstable and expresses elevated levels of all three antigens.[11]

The O-antigen of *V. cholerae* O1 consists of a homopolymer of 4-amino-4,6-dideoxy-mannose (perosamine) which is substituted with 3-deoxy-L-*glycero*-tetronic acid.[12,13,14] This basic structure is repeated 18 times on average and is joined to lipid A via a linker core oligosaccharide region.



Scheme 3. Ogawa and Inaba serotypes of V.Cholerae O1

The O-antigen of the LPS also appears to be the major protective antigen, and consequently a change in the O-antigen and thus in the serogroup of the organism, can potentially lead to strains with new pandemic capabilities.

1.3.2 Immunity and Vaccination

Protective immunity against cholera is mediated mainly, if not exclusively, by antibodies produced locally in the intestinal mucosa and secreted onto the gut mucosal surface. These antibodies are directed against bacterial components including cholera toxin (CT), and protect the host organism by inhibiting bacterial colonization and multiplication and by blocking the toxin action.

The immunity conferred by an attack of cholera is said to be short-lived. Little is known about the factors responsible for immunity, and vaccination has, therefore, so far been empirical. Due to its low efficacy and short duration of protection, the old parenteral vaccine is no longer recommended. Compared with the parenteral vaccine, the presently internationally available oral vaccines provide better and more long-lasting protection against cholera.[6] However, insufficient protection in children below 2 years of age excludes these newer vaccines from use in national infant immunization programmes.

Clinically useful cellular vaccines against cholera elicit relatively short-lived, anti-LPS antibodies, but they are far from satisfactory. To overcome this deficiency, there is the hope that conjugate vaccines containing a T-helper cell-directing protein component, will stimulate the production of memory cell and thus, of long-lasting protective IgG antibodies.[15]

The immunological specificity of polysaccharide antigens resides in their structures. Therefore, the two most important steps toward a better understanding of the immunology of polysaccharides are firstly to elucidate their basic structures, and secondly, to ascertain which part of the total structure (determinant or epitope) is responsible for the immunospecificity.

1.4 Carbohydrate Antigen and Antibodies

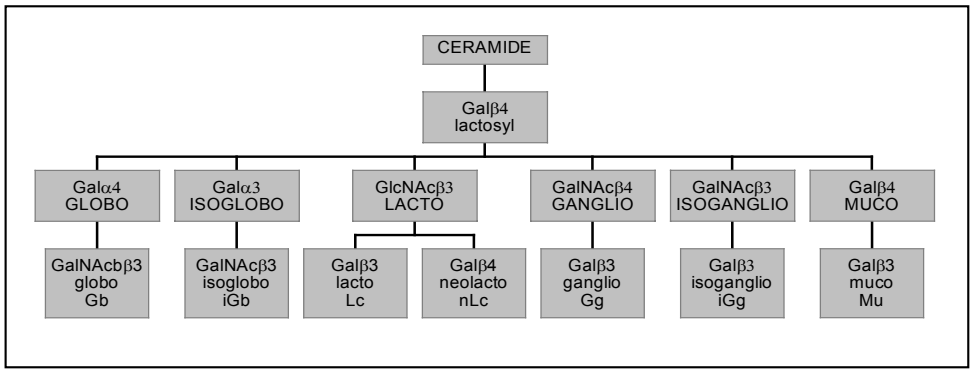
From studies with antibodies[16,17] and lectins[18] it was suggested that the size of the oligosaccharide recognition element that actually binds to a protein receptor is restricted to a disaccharide or trisaccharide unit. Such epitopes are often present as part of a larger antigenic determinant and recognition can involve small portions of the flanking residue. Carbohydrate antigens at the surfaces of bacteria, red blood cells and tumor cells may provoke a powerful immune response that can exert beneficial effects, e.g. in vaccines.[19] The relatively small area of an oligosaccharide to which the binding site is complementary, is referred to as the antigenic determinant or epitope. Since all but the simplest oligosaccharides are commercially

not available, estimating the size of an antigenic determinant requires a program of oligosaccharide synthesis. If the knowledge about the epitope size has to be implemented by the knowledge about the functional groups that are essential for recognition, then the synthetic approach involves the synthesis of an extensive panel of oligosaccharide analogues.

1.5 Gangliosides

Gangliosides differ from other glycosphingolipids as they contain an additional characteristic carbohydrate constituent, namely the sialic acid. All gangliosides have in common a β-lactosyl moiety in the ceramide. Lactosylceramides are further modified to produce a wide variety of structures glycosylated in a more complex manner. These are classified into different series depending upon the common structural elements in their carbohydrate pattern.

The classical gangliosides, i.e., the predominant species in the brain of higher animals, contain carbohydrate moieties of the ganglio-series, i.e. gangliotriaose (GalNAcβ1-4Galβ1-4Glc), gangliotetraose (Galβ1-3GalNAcβ1-4Galβ1-4Glc) and gangliopentaose (GalNAcβ1-4Galβ1-3GalNAcβ1-4Galβ1-4Glc).



Scheme 4. The series of Gangliosides

In this oligosaccharide series substitution with single or multiple α2-linked sialic acids takes place at the position 3 or 6 of galactose or at the position 6 of *N*-acetylgalactosamine residues. The sialic acid residues are linked one to another by an α(2-8) ketoside. Many of these extensions are generally similar to those of *N*- and *O*-glycans.

1.5.1 Sialic acid

The sialic acids are typically found at the outermost ends of *N*-glycans, *O*-glycans, and glycosphingolipids.[20] They are subject to a wide variety of modifications. The variability in binding type, give rise to physico-chemically distinguishable sialic acids and may thus contribute to the great diversity of biological phenomena sialic acids are involved in. Combinations of different glycosidic linkages with various positions lead to the most diverse architectures for presentation of sialic acid on the surface of glycoconjugates. Even within a particular group of glycoconjugates, a modification may be restricted to a defined sialic acid residue at a specific position of the glycan.

The presence of sialic acid in gangliosides indicates that these lipids are typical cell-surface constituents. Whilst free sialic acid in aqueous solution is present in the β-anomeric form, it is known that naturally occurring sialo-compounds contain sialic acid in the α-glycosidic linkage, except for CMP-*N*-acetylneuraminic acid. With the exception of gangliosides discovered in starfish, sialic acid substitution always takes place in branching or terminal positions of the oligosaccharide. Sialic acid residues in ganglioside may form lactones under acidic conditions. Such lactone formation occurs readily in the terminal sialic acid that is linked to another sialic acid by an α(2→8) ketoside. It is tempting to speculate that such lactonization-delactonization of gangliosides may also occur under in vivo conditions. This would result in changes of electric charge of membranes that may be of functional significance. The sialic acid of all gangliosides of human origin is *N*-acetylneuraminic acid or 9-*O*-acetyl-*N*-acetyl-neuraminic acid.

1.5.2 Biological Roles

The carbohydrate portion of the ganglioside molecules is oriented toward the outer environment, and this strategic position enhances the diversity of biological events in which gangliosides are implicated. The biological significance of gangliosides is connected with other molecules, possibly proteins that are able to interact more or less tightly with them.

The ganglioside profile depends significantly and sensitively upon the developmental state of the cell and the growth conditions. Thus cellular differentiation or dedifferentiation due to oncogenic transformation correlates with changes in gangliosides distribution. [21,22]

Studies of gangliosides have revealed that they are involved in different biological events such as interactions with I) lectins, [23,24] (ii) toxins, hormones, interferon, cell growth and differentiation factors [25]as well as iii) neurotropics agents.[26]

From studies of the changes in the ganglioside pattern there is the hope that yet unknown functional aspects of these membrane constituents might be disclosed.

1.6 Neoglycoconjugates

Natural glycoconjugates usually consist of complex and non-homogeneous carbohydrate components. This heterogeneity of the glycans in glycoconjugates makes an investigation of the biological role of the carbohydrate moiety much more difficult. Therefore it would be desirable to use glycoconjugates that contain a single carbohydrate species of well-defined structure for such studies. The term “neoglycoconjugates” defines all the hybrid structures which contain carbohydrates of known composition and defined homogeneity.[27]

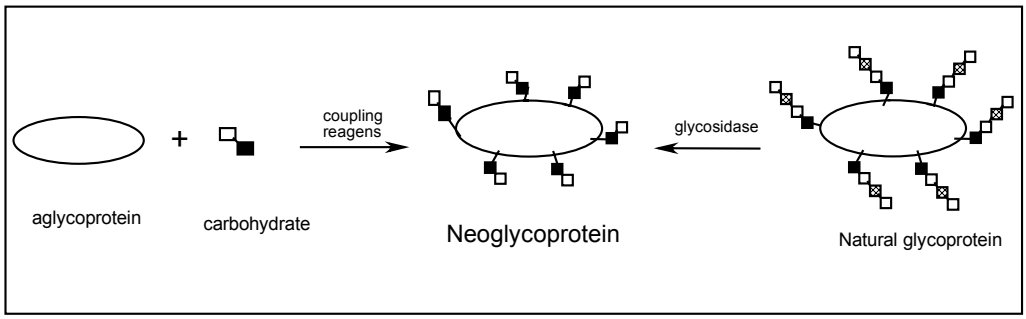
Glycolipids have an additional source of heterogeneity. While the glycoproteins differ mostly by different oligosaccharide chains on the same peptide backbone, glycolipids can vary also in their fatty acid composition. Natural glycolipids, even those with a single species of carbohydrate chain, often exhibit multiple bands or peaks because of variations in the fatty acid components. In such cases, a neoglycolipid containing a simple, well-defined lipid structure may simplify the data interpretation.[28]

On the other hand, the carbohydrate structures involved in biological activities are often limited to a terminal sugar residue or a short oligosaccharide sequence, and thus the total synthetic reproduction of the natural oligosaccharide structures is usually not required for the construction of effective neoglycoconjugates.

The synthesis of glycoconjugates has been a long-standing approach for the production of hapten-specific antibodies, but the interest increased in the early 70s when diverse strategies were developed for the covalent attachment of oligosaccharides to proteins.[29,30]

1.6.1 Synthesis of Neoglycoproteins: General consideration

Neoglycoproteins are synthesized by coupling carbohydrate molecules to proteins or by partial degradation of glycoproteins as outlined in Scheme 5. This attractive molecules or "double agents" contain both the carbohydrate-mediated function and the original biological activity of the proteins.



Scheme 5. Neoglycoprotein synthesis

Many approaches have been developed and proposed for the preparation of neoglycoproteins, and all of them take in consideration the available functional groups on proteins and the type of linkage useful for the production of conjugates. The glycosides can be *O*-, *N*- or *S*-linked, but the aglycon must be functionalized at the terminal position to enable conjugation to the protein component. The preparation of carbohydrate derivatives suitable for conjugation is one of the most important steps for the successfully synthesis of neoglycoconjugates. When the polysaccharide contains chemically reactive groups such as amino or carboxy functions that can serve for direct covalent linkage to a protein carrier, the glycosyl groups are usually attached to protein via a spacer arm. The length of the spacer can be of importance for triggering the desired biological activity; e.g. it has been observed that the spacer size significantly affects affinity for different isolectins. The clear consensus in this context is that the optimal length is reached when full accessibility is assured, but at the same time the loss of entropy upon binding is not a prohibitive cost in the binding equilibrium.

In the past a large number of neoglycoconjugates have been synthesized from synthetic oligosaccharides for use as experimental vaccines in infectious diseases[31] and cancer[32]

1.7 Objectives

From what has been discussed in the preceding sections, it is clear that the presence of defined carbohydrate units in naturally occurring structures results in dramatic effects on their physical, chemical and biological properties.

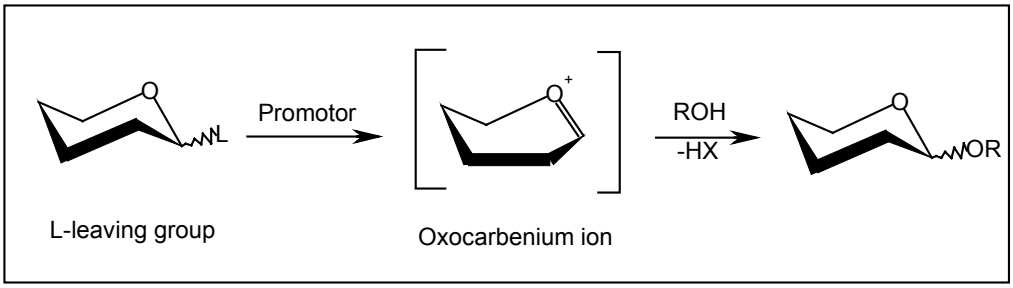
Aim of the present study was therefore the synthesis of oligosaccharide structures of natural origin, i.e. of lipopolysaccharides related to *Vibrio cholerae* O1 and gangliosides. The main attention was paid to the development of potent immunogens for generating a protective anti-*V. cholerae* O1 immune response. In this context a detailed knowledge of the mode of binding of the OPS and its homologous structures to antibodies is a prerequisite for the rational development of a medically useful immunogen. In order to gain such information, in first instance the main objectives were i) the synthesis of fragments of the OPS of both serotypes of *V. cholerae* O1 and their use in competitive antibody binding studies, and ii) their use for the synthesis of neoglycoconjugates.

The biological significance of gangliosides in various animal cells is well documented, although at the present state the exact role of gangliosides at molecular level is not understood because of the lack of detailed structure-function studies. Therefore, pure synthetic gangliosides are highly desirable molecules for studying their multifunctionality. Aim of the second part of this study was the development of innovative strategies for the synthesis of the GQ1bα ganglioside.

2. Results and Discussion

2.1 Chemical Synthesis of Oligosaccharides

Chemical and biological studies of oligosaccharides is of crucial importance to our understanding of living organisms and to biomedical research. Therefore an explosion of interest in glycoscience has taken place in the past decade that has fostered research in the field with particular attention paid to the difficulties associated with chemical syntheses of oligosaccharides. The synthetic methods available for glycosidic bond formation are becoming increasingly powerful and efficient as well evidenced by the ever increasing complexity of targeted oligosaccharides. However, even more effective methods are required. Foremost among them are the generation, accessibility, and reactivity of the key reactive intermediates in glycoside coupling, i.e. the cyclic oxocarbenium ions (Scheme 6).



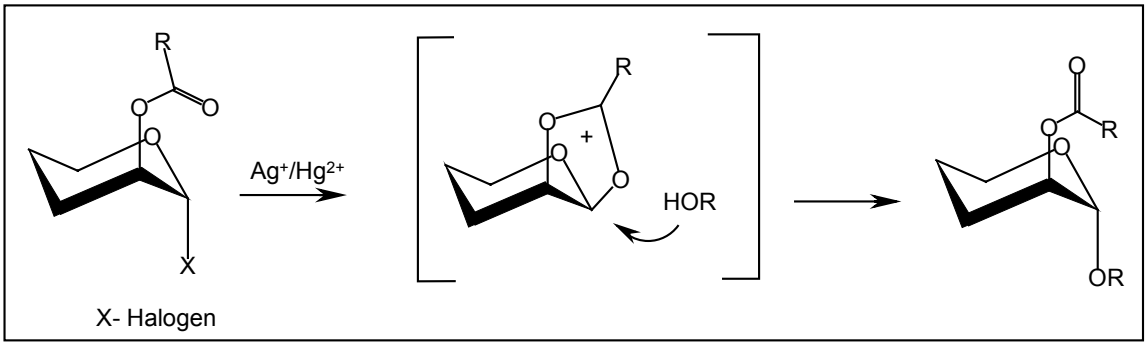
Scheme 6. Chemical O-glycoside synthesis

Much effort has been properly devoted to the development of new glycosyl donors, particularly of those where the anomeric leaving groups are halides, trichloroacetimidate, alkyl or arylthio and phosphite glycosides. These four types have been recently used in the synthesis of complex oligosaccharides consisting of at least five saccharide units.

2.1.1 Koenigs-Knorr Method

As outlined in Scheme 7, the classical Koenigs-Knorr method[33] of oligosaccharide synthesis, along with its modern variations, achieves activation through the use of glycosyl halides (bromides, chlorides) in the presence of heavy metal salts (generally silver or mercury).

Advanced modifications make use of glycosyl fluorides as donor compounds.[34] This method has been comprehensively reviewed.[35]

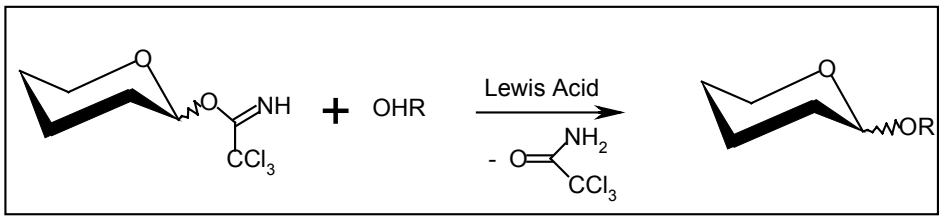


Scheme 7. Koenigs-Knorr method

Despite the efficiency of several of the variants of the Koenig-Knorr method, some disadvantages still remain: i) the glycosyl halide generation requires harsh conditions; ii) the glycosyl halides are relative unstable intermediates; iii) the glycosyl halides are high sensitive to aqueous hydrolysis; iv) the heavy metal salts used in the reactions are expensive and toxic.

2.1.2 Trichloroacetimidate Method

Glycosyl trichloroacetimidates were first developed as glycosyl donors by Schmidt and Michel in 1980[36] (Scheme 8). These are stable, frequently isolable intermediates that can be activated through acid catalysis without the use of heavy metal salts.



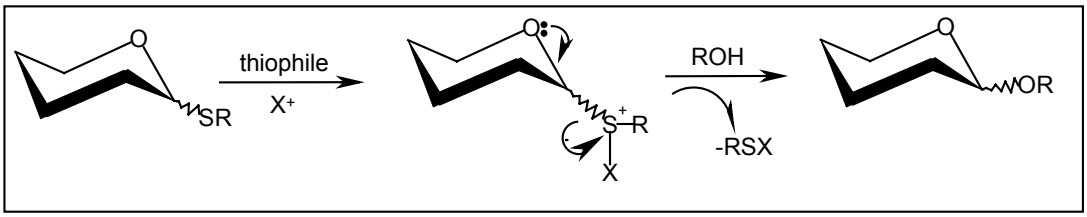
Scheme 8. Trichloroacetimidate method

This coupling method generally leads to inversion of the configuration at the anomeric center; however, the presence of 2-acyl substituent generates invariably the product of neighboring

group participation, i.e. the 1,2-*trans* compound. Furthermore, in the case of mannose and rhamnose generally the α-products are formed owing to the strong anomeric effect in these systems. An additional advantage of this method is the control of anomeric configuration of the product by selecting the required donor precursor.[37]

2.1.3 Thioglycoside Method

Thioglycosides are highly versatile and useful intermediates in oligosaccharides synthesis. A distinct advantage of these derivatives is that their thio group can be used for temporary protection, and then employed for almost any coupling procedure currently in use.[38] The mechanism of activation of this reaction is similar to that of the Koenigs-Knorr reaction as a thiophile activates the sulfur first, which is then released to give the corresponding oxonium ion. Glycosidation then follows by trapping this species with a hydroxy compound in the normal way as shown in Scheme 9.



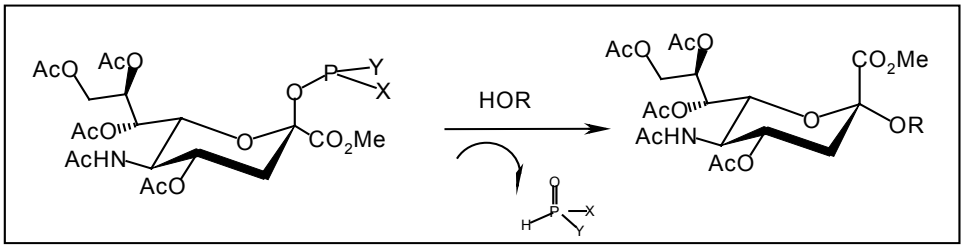
Scheme 9. Thioglycoside method

More effective activating agents introduced recently include methyl triflate, dimethyl(methylthio)sulfonium triflate (DMTST) and iodosuccinimide-perchlorate (IDCP).However all these thiophilic promoters are required in at least equimolar amounts, which can also lead to undesired side reactions.

2.1.4 Phosphite Method

The glycosyl-donating abilities of phosphites as intermediates in the synthesis of oligosaccharides were discovered by Martin and Schmidt in 1992.[39] These intermediates can be synthesized from free anomeric sugars and a phosphitylating agent in presence of Hünig´s base. This procedure is quite general since the activation of glycosyl phosphites can be

achieved by treatment with catalytic amounts of TMSOTf. [39,40] The most interesting examples so far reported are those using sialic acid phosphites as sialylating agents.

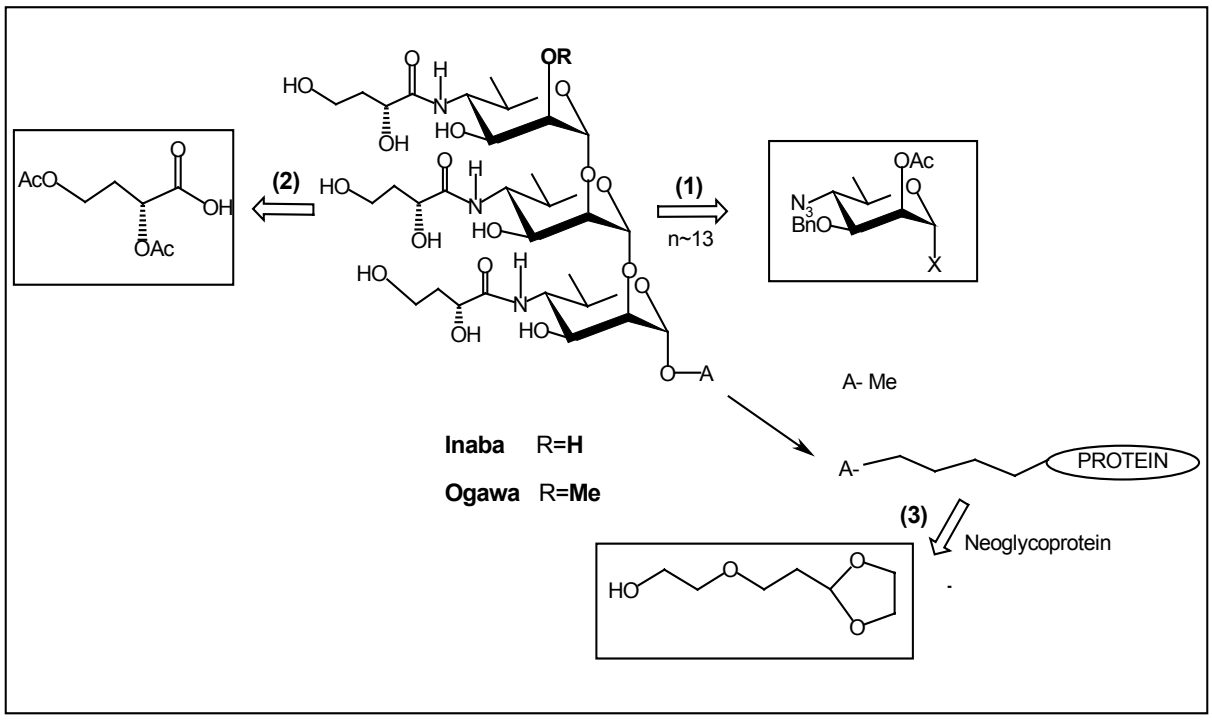


Scheme 10. Phosphite method

2.2 Synthesis of O-antigens of *V. Cholerae* O1

In view of the continued occurrence of explosive epidemics and periodic pandemic of cholera, the almost century-long effort directed towards developing a safe, practical, and effective vaccine and new, reliable diagnostic tests for this disease has not diminished. In this context, we are interested in a better understanding of the interaction of carbohydrate antigens with antibodies involving the OPS of *V.Cholerae* O1. In the initial phase the work was aimed at identifying structural requirements or molecules expected to elicit protective antibodies when linked to a suitable carrier. At first, the methyl α-glycosides of mono- and disaccharides that mimic the terminus of the O-polysaccharide of *V. Cholera*e O1 of both serotypes Inaba and Ogawa were synthesized.

2.2.1 Retrosynthetic Strategy



Scheme 11. Retrosynthetic analysis of the O-polysaccharide of V.Cholerae O1

The O-antigens of serotypes Ogawa and Inaba contain the same intracatenary monosaccharide repeating unit and consist of a relative short chain of α(1→2)-linked moieties of 4-amino-4,6-

dideoxy-D-mannose (D-perosamine), N-acylated with 3-deoxy-L-glycero-tetronic acid. The two O-polysaccharides differ in the presence of a methyl group at O-2 in the "nonreducing" α-D-perosamininyl moiety of the Ogawa strain.[41,42]

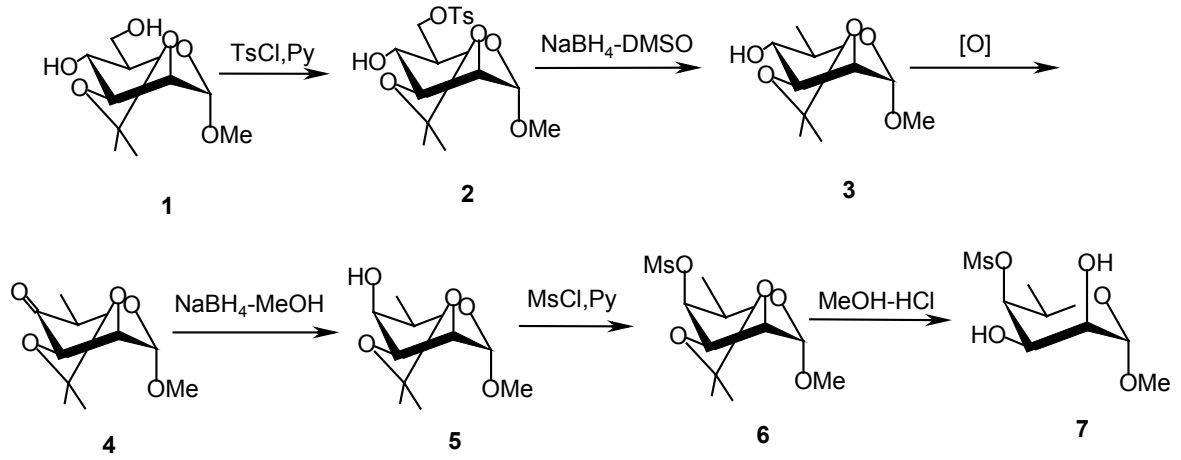
Taking into account this general structure of the O-antigens of *V.cholerae* O1, the molecule was disconnected into perosamine (cleavage 1), side chain (cleavage 2) and for the preparation of neoglycoconjugate the molecule was disconnected at the linkage with the spacer arm (cleavage 3). Correspondingly, the synthetic strategy should fulfil the following requirements:

1. Use of a suitable perosamine molecule that serves as a common precursor for the glycosyl donor and acceptor.
2. α-Selectivity in the glycosylation and possibility of reiterated reaction.
3. The side chain should possess a set of protecting groups that allow a convenient transformation into an acylation agent.
4. Accessible method for the attachment of the perosamine moiety with the side chain, useful for the preparation of oligosaccharides of large size.
5. For the preparation of a neoglycoprotein, introduction of a spacer arm compatible with the strategy of synthesis already elaborated.

2.2.2 D-Perosamine

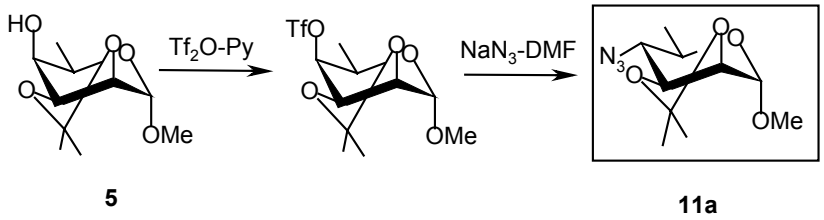
Synthesis of perosamine containing oligosaccharides was originally developed by Bundle and co-workers [43,44,45] in connection with the preparation of methyl glycosides of antigenic determinants of the Brucella A antigen. A second approach was carried out by M. Eis *et al*.[46] as part of a program to develop a new, totally synthetic brucellosis vaccine. In this study the authors described an improved route to methyl α-D-perosinamide.

We reproduced with minor changes the previously described routes to D-perosamine. Methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside **1**[47] was selectively tosylated (**2**) and then reduced with sodium borohydride in DMSO to afford the corresponding D-rhamnopyranoside (**3**) in 83% yield avoiding the trouble caused by the presence of triphenylphosphine by-products in the original procedure. Oxidation of **3** to give the 4-keto derivative **4** which was not isolated, was performed using the Swern procedure.[48] Reduction of **4** with sodium borohydride gave the talopyranoside **5** in high overall yield.



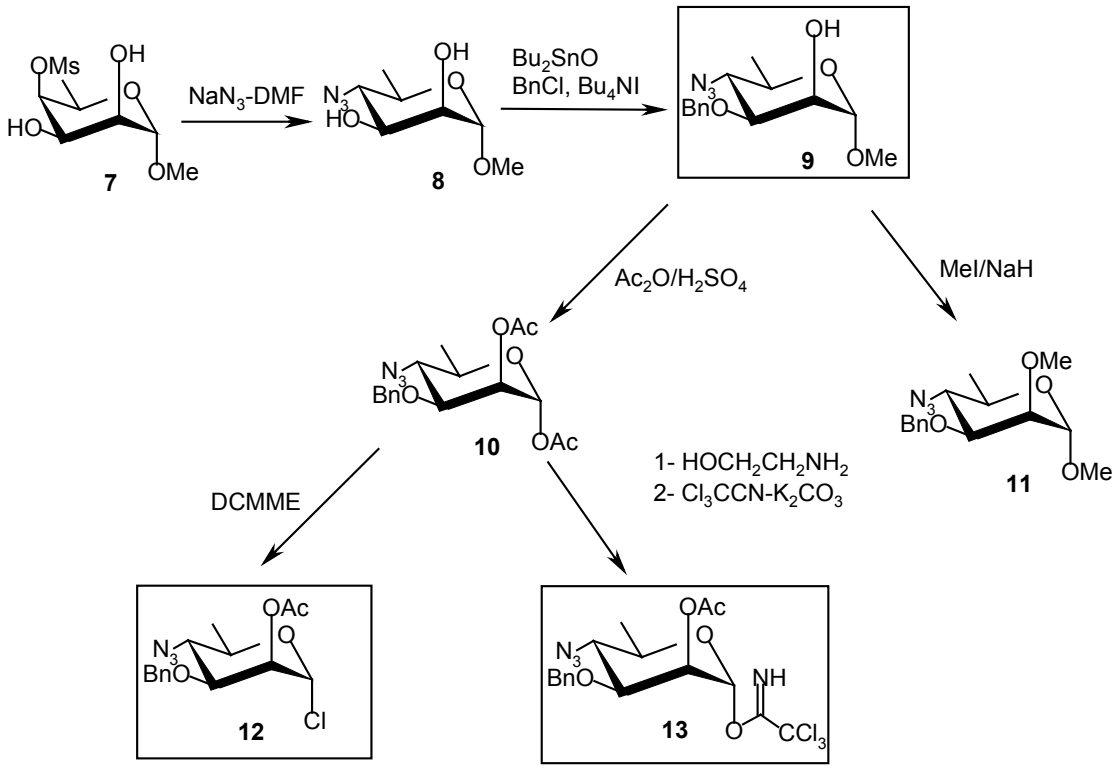
Scheme 12. Towards the synthesis of D-perosamine

We have noted that for the large scale preparation of **8**, the 4-*O*-trifluormethanesulfonyl derivative of **5** used by Bundle *et a*l [49] (Scheme 13) can be conveniently replaced with the mesyl derivatives as described by Eis *et al.*[46]



Scheme 13. Synthesis of D-perosamine using Bundle's procedure

The following steps were performed exactly as previously described (Scheme 14). Thus, after removing the adjacent isopropylidene protecting group, the resultant mesylate **7** was subjected to normal replacement with NaN_3 in DMF, to afford the azide **8** in 80%. The product **8** was then converted into the dibutylstannyl acetal derivative by employing Bu_2SnO in refluxing benzene. Treatment with BnCl-Bu_4NI in the same solvent served to selectively block HO-3 to give **9** whose methylation with MeI-NaH in DMF afforded **11** in 95% as precursor of the monosaccharide mimicking the serotype Ogawa. Thus compound **9** could serve as a bifunctional precursor for generation of either the glycosyl acceptor or, by two additional steps, the glycosyl donors **12** and **13**.



Scheme 14. Synthesis of acceptor and donors derivatives of D-perosamine

The stepwise construction of the disaccharide and trisaccharide required a glycosyl donor allowing the extension of the oligosaccharide chain at O-2 position. Such a synthon was obtained from **10** containing the 2-*O*-acetyl group at the neighboring position which in turn allowed the production of the 1,2-*trans*-glycoside by anchimeric assistance, and after glycosylation, to be removed in order to extend the chain.

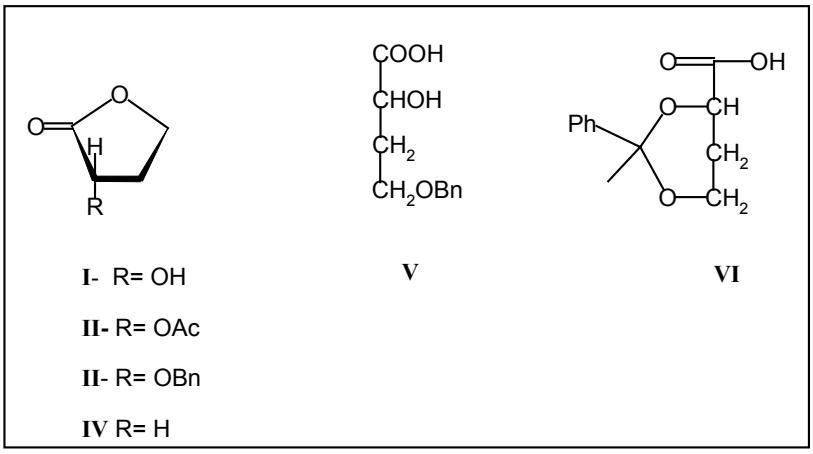
Acetolysis of **9** furnished the known derivative **10** with a small proportion of the β-anomer which was converted to the glycosyl chloride using dichloromethyl methyl ether (DCMME)[50] in presence of catalytic amounts of freshly fused zinc chloride; the glycosyl donor was obtained in high yield. With a large excess of DCMME according to the conditions routinely applied to convert acetylated methyl glycosides to the corresponding glycosyl chlorides, a number of side products were formed. When only two molar equivalents of the reagent were applied, the glycosyl donor was obtained in virtually theoretical yield. The amount of $ZnCl_2$ only affected the reaction rate, but not the eventual outcome of the reaction. The glycosyl donor **12** was characterized by NMR spectroscopy and used immediately in glycosylation

reactions for the synthesis of the methyl α-glycoside disaccharide fragments mimicking the terminus of the O-polysaccharide of *V. cholerae* O1, serotype Inaba and Ogawa.

On the other hand, for the construction of higher oligosaccharides suitable for linkage to a protein carrier, a second donor was also synthesized. In this case the new perosamine donor **13** was obtained in 84% yield from the corresponding 1,2-di-*O*-acetyl-4-azido-3-*O*-benzyl-4, 6-dideoxy-α-D-mannopyranose (**10**) by chemoselective deacetylation using ethanolamine in EtOAc followed by imidation with trichloroacetonitrile and potassium carbonate in DCM.

2.2.3 3-Deoxy-L-*glycero*-tetronic Acid

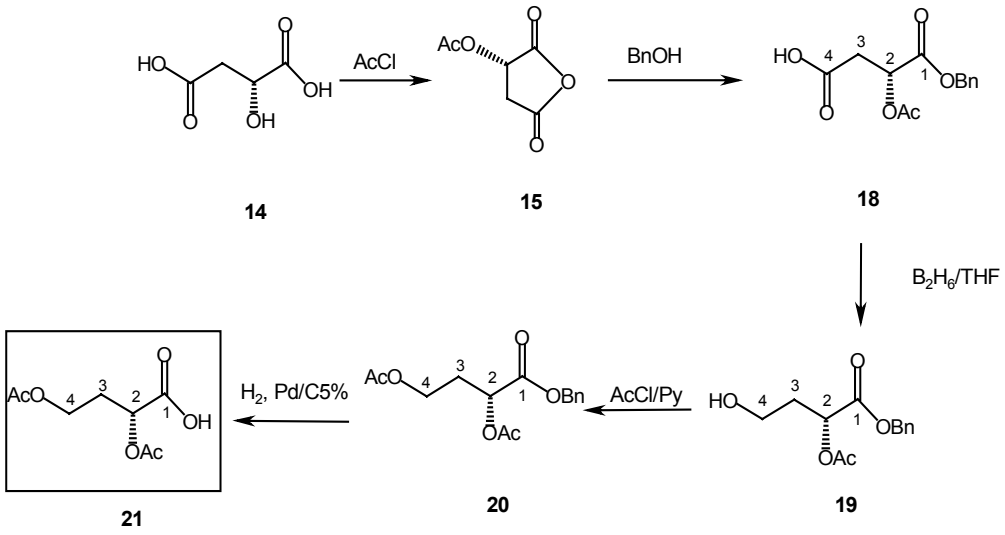
The method for 3-deoxy-L-*glycero*-tetronylation of methyl perosaminide with 3-deoxy-L-*glycero*-tetronolactone (**I**) was originally developed by Kenne *et al*.[51] The 2-deoxy analogue (**IV**) of this compound was prepared by P. Kovác *et al*. in a similar way, using γ-lactone of 4-hydroxybutyric acid for *N*-acylation.[52] But further reports by these authors [53] revealed that while 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid (**V**) showed itself to be a more powerful reagent for 3-deoxy-L-*glycero*-tetrononylation of perosamine derivatives than 3-deoxy-L-*glycero*-tetronolactone (**I**),[54] the former gave a low yield of 3-deoxy-L-*glycero*-tetronamidation in the case of higher oligosaccharides. 2,4-*O*-Benzylidene-3-deoxy-L-*glycero*-tetronic acid (**VI**) has also been used as precursor of the side chain.[55]



Scheme 15. 3-deoxy-L-glycero-tetronic acid

As part of the efforts towards the synthesis of fragments of the OPS of *V. cholerae* O1, was explored an alternative route to obtain a precursor of 3-deoxy-L-*glycero*-tetronic acid taking into consideration that it represents a basic component of the antigen structures.

Thus, 3-deoxy-L-*glycero*-tetronic acid with suitable protecting groups was prepared from the commercially available L-malic acid **14** by the sequence of reactions outlined in Scheme 16. The reaction of **14** with acetyl chloride gave the corresponding anhydride **15** that reacts directly with excess ethyl alcohol to afford the C-1 ester. The C-4 carboxylic function of **16** was then reduced with diborane in tetrahydrofuran (→**17**, 86%), but attempts to remove the ethyl ester by using either acid or basic conditions proceeded with a significant decomposition and probably the formation of several lactonic byproducts. To overcome this problem, was used the benzyl ester **18** that was obtained in a similar manner (using benzyl alcohol). After reduction (→**19**, 64% overall yield), the resulting hydroxy group were protected by acetylation to avoid lactonization giving **20** as a stable compound that can be stored for longer times and conveniently transformed into the acid only when the *N*-acylation reaction takes place. Thus, hydrogenolisis of the benzyl protecting group afforded **21** in quantitative yield, which can be used as the derivatizing reagent in the acylation of the amino groups of the perosamine moieties.



Scheme 16. Synthesis of a new precursor of the 3-deoxy-L-glycero tetronic acid

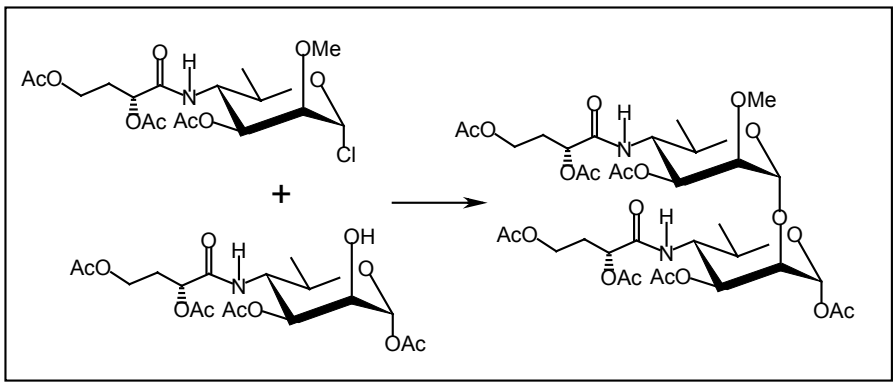
The structural assignment of the derivatives during the reaction sequences was performed by ^{1}H-and ^{13}C-NMR analysis. The data are summarized in Table 1.

Compound	C1 ppm	H2 ppm	C2 ppm	H3 ppm	C3 ppm	H4 ppm	C4 ppm
18	170.0	5.51	67.9	2.93	35.7	-	174.5
19	170.4	5.20	69.4	2.09-2.00	33.6	3.76-3.60	57.7
20	170.1	5.16	68.9	2.24-1.97	30.0	4.22-4.09	59.6
21	173.6	5.12	68.8	2.27-2.05	30.0	4.24-4.18	59.8

*Table 1. ^{1}H and ^{13}C data of the derivatives **18**, **19**, **20** and **21***

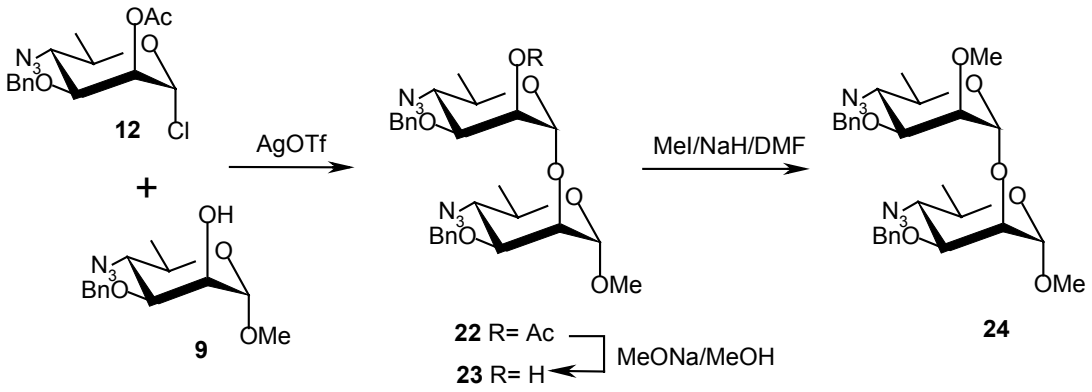
2.2.4 Preparation of the Disaccharide

Kovác and coworkers have encountered various difficulties during a recent synthesis of oligosaccharides related to O-PS of *V. cholerae* O1. [55,56] For example, migration of AcO-1 to AcO-2 and AcO-3 to AcO-2 was observed in some of the intermediates making the isolation of these products difficult. On the other hand, these authors have used an approach in the preparation of the intracatenary disaccharide repeating unit of the O-PS which was based on the use of glycosyl donors and glycosyl acceptors having the *N*-3-deoxy-L-*glycero*-tetronyl group already in place (Scheme 17). [57]



Scheme 17. Synthesis of disaccharide: Kovác strategy

In the present work was prepared the disaccharides according to Bundle and co-workers.[43] This approach uses building blocks such as **9**, where acyl migration due the use of the *O*-acetyl groups for permanent protection cannot occur. The synthesis of the disaccharide and higher oligosaccharides is based on the use of the *O*-benzyl derivatives for permanent protection and of *O*-acetyl groups for temporary protection. Correspondingly, the disaccharide derivative **22** was obtained from the 2-*O*-acetyl-4-azido-3-benzyl-4,6-dideoxy-α-D-mannopyranosyl chloride **12** and the acceptor **9** in a silver triflate promoted glycosylation reaction.



Scheme 18. Synthesis of the disaccharides: Bundle strategy

The presence of a small quantity of a transacetylated acceptor was also detected. The second disaccharide derivative **24** which mimicks the terminal portion of the serotype Ogawa was obtained after deacetylation and methylation with CH_3I-NaH in DMF in 80% yield.

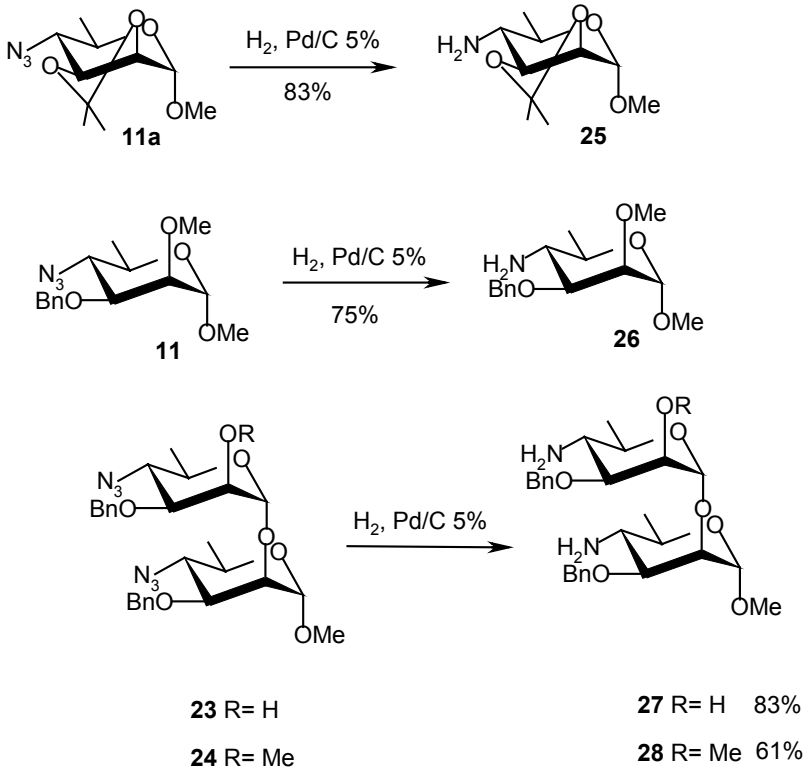
2.2.5 Reduction of the Azido Group

The azido group has been widely used in the synthesis of aminosugars as it serves as an excellent latent function and in other cases as protecting group. The azido group is stable under acid and basic conditions, but cleavage of this protecting group sometimes is difficult. To convert azido to amino functions in the presence of *O*-benzyl groups using H_2S as the reagent , Kovac *et al* applied the protocol of Peters and Bundle.[57] Comparing the outcome of this conversion in higher oligosaccharides effected by the method of Lemieux and co-workers [58] or according to Garegg and coworkers[59] (treatment in pyridine/trimethylamine at 0 °C to room temperatures) with the method applied by Peters and Bundle (treatment in

pyridine/water at 40 °C overnight), the latter procedure was found to be more efficient. While the former gave satisfactory results with mono- or disaccharides, a relatively minor change in reaction conditions made a substantial difference in the case of higher oligosaccharides.

The two different 4-amino-4,6-dideoxy-D-mannose derivatives **25** and **26** were selected for the synthesis of terminal monosaccharide derivatives as occurring in *V. cholerae* O1 LPS and two others, i.e. **27** and **28**, for the synthesis of the corresponding disaccharide elements. The azido group in derivatives **11a**, **11**, **23** and **24** were selectively hydrogenated over 5% Pd/C in ethanol for 24 h to afford the corresponding amines **25**, **26**, **27** and **28**. This transformation was assessed by ^{13}C NMR where the chemical shift of C-4 clearly confirmed the conversion from N_3 to NH_2: (**11a**→**25**, δ 64.1→54.5; **11**→**26**, δ 64.2→53.6; **23**→**27**, δ 63.8, 63.7→ 53.6, 53.2; **24**→**28**, δ 64.9, 64.8→53.7, 53.5).

It is noteworthy that under these conditions the adjacent benzyl group remained unchanged as has been reported for others derivatives.[60] This result also agrees with previous reports about prevention of *O*-benzyl ether hydrogenolysis in the presence of intramolecular amines.[61,62]



Scheme 19. Reduction of the azido group

2.2.6 Condensation and Deprotection

2.2.6.1 *N*-Acylation of D-Perosamine

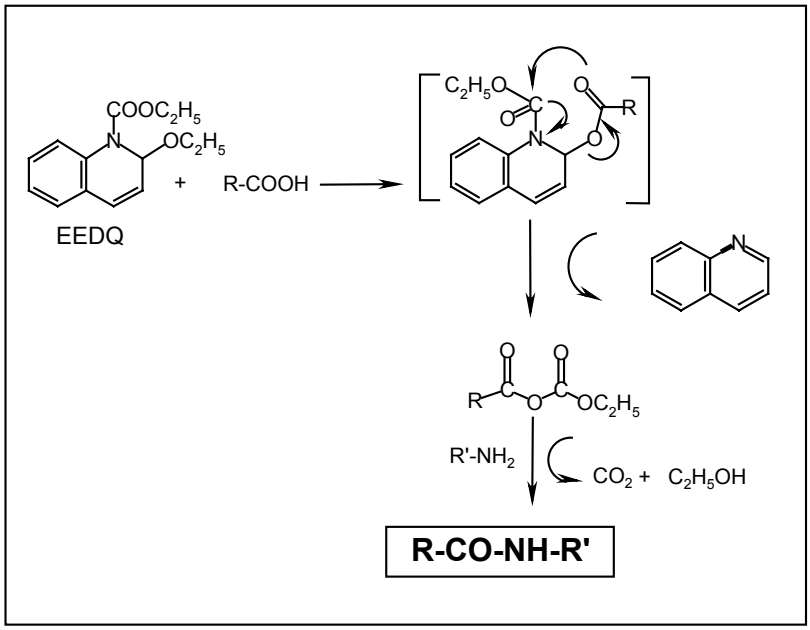
Variation of the acyl groups in homopolysaccharides composed of the same aminosugar often distinguishes LPS of one bacterial species from another. In addition to O-PS of *V.cholerae* O1, variously *N*-acylated perosamine is the key constituent of O-specific polysaccharides of many other bacterial pathogens. There, the O-PS differs from that of *V. Cholerae* O1 mainly in the nature of the *N*-acyl side chain. For example, *N*-formylated α-(1→2)-linked D-perosamine occurs in the O-specific chain of the LPS of *Yersinia Enterocolitica* serotype 09 and *Brucella abortous*.

In this context it is interesting to note that *N*-3-hydroxypropionyl-α-D-perosamine and *N*-3-hydroxypropionyl-2-*O*-methyl-α-D-perosamine were identified as constituents of lipopolysaccharides from *Vibrios* serogroup 1875, referred to as Original and Variant, respectively.[63,64] On the other hand, the O-polysaccharide chain from *V. cholerae* O-76[65] and O-144[66] are homopolymers of *N*-2-hydroxy-L- and D-propionyl-α-L-perosamine, respectiveLy.

As already discussed, the introduction of the side chain has been achieved by different ways using mainly lactone derivatives. Moreover, 3-ethyl-1-(dimethylaminopropyl)carbodiimide (EDAC) and *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronum hexafluorophosphate (HATU) have also been used as coupling agents in the acylation reaction with 2,4-O-benzylidene-3-deoxy-L-*glycero*-tetronic acid by Kovác *et al* .[67]

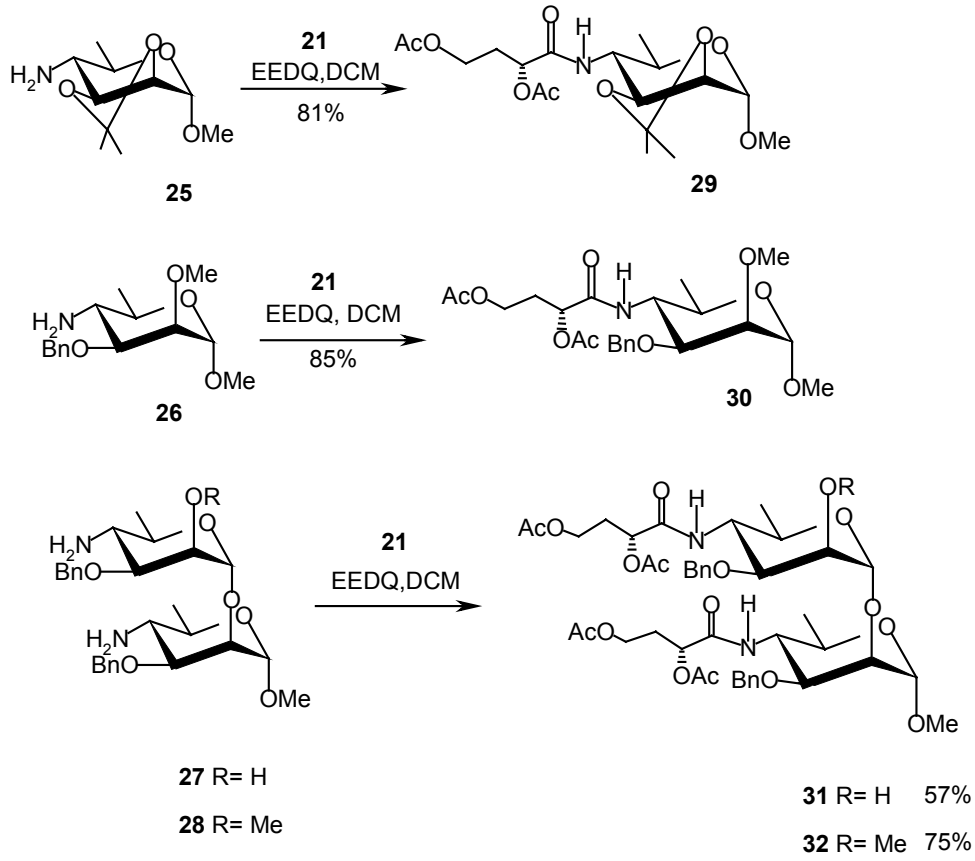
2.2.6.2 *N*-Acylation with 2,4-Di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic Acid

The availability of an efficient procedure for acylation of amine derivatives with 3-deoxy-L-*glycero*-tetronic acid was essential for an efficient synthesis of analogous oligosaccharides.



Scheme 20. Mixed anhydride methods: EEDQ

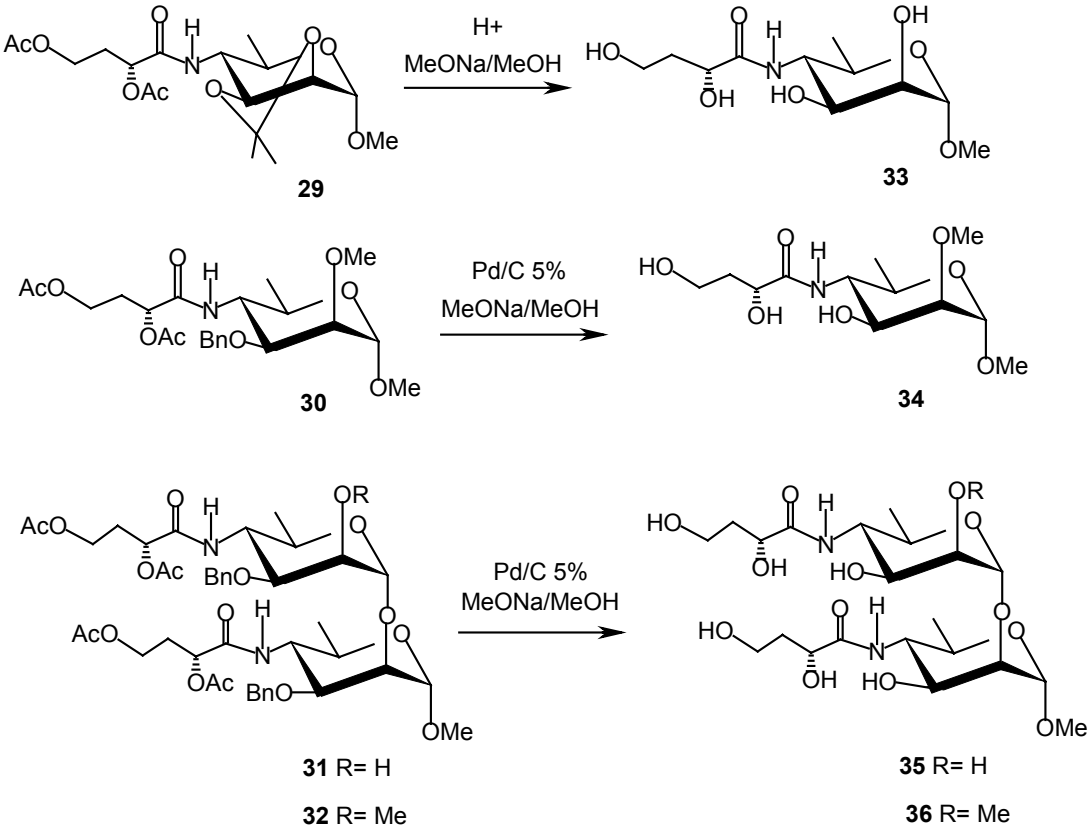
With this aim, the bimolecular reaction between the carboxy component of the side chain and the amino component of the perosamine moiety was carried out with the coupling reagent 2-ethoxy-1-ethxycarbonyl-1,2-dihydroquinoline (EEDQ). This is a stable and commercially available reagent. It is not entirely inert toward the amine, but the rate of urethane formation is low when compared with the reaction rate between the reagent and carboxylic acids. This reaction starts with the displacement of ethanol and leads, via an intramolecular attack, to the formation of a carbonic acid mixed anhydride which immediately reacts with the amine component to give the amide in good yields. Condensation with 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid (**21**) in the presence of EEDQ in dichloromethane afforded the expected amides →**29** 81%, →**30**, 85%, →**31**, 57%, →**32**, 75% (Scheme 21). Their structures were confirmed by ^{1}H-NMR analysis: the signal for the NHCO proton appeared downfield (δ 5.80-6.50 ppm; J= 5-6 Hz) as one doublet for **29** and **30,** or two doublets for **31** and **32**. On the other hand, the tetronamide H-2 appeared as a double of doublet at 5.19-5.22 ppm.



*Scheme 21. N-acylation using **21***

2.2.6.3 Deprotection

Hydrogenolysis of the benzyl groups over 5 % Pd/C or acid hydrolysis of the isopropylidene groups followed by Zemplén deacetylation afforded the deprotected methyl glycosides **33**, **34**, **35** and **36** (Scheme 22). The NMR spectra of these compounds were consistent with the assigned structures (**33**→ δ 4.76 ppm, $J_{1,2}$= 1,5 Hz; **34**→ δ 5.00 ppm, $J_{1,2}$= 1,6 Hz; **35**→ δ 4.85 ppm, $J_{1,2}$= 1.8 Hz and **36**→ δ 4.85 ppm, $J_{1,2}$= 2.0 Hz); only the α-glycosides which form the upstream end-group of the OPS of *V.cholerae* O1 were obtained.



Scheme 22. Deprotection

2.2.7 Inhibition

The agglutination of *V. cholerae* by commercial rabbit anti-Ogawa and anti-Inaba antiserum was studied using the model compounds **33→ I$_1$**, **34→ O$_1$**, **35→ I$_2$** and **36→ O$_2$** as inhibitors. Anti- Inaba antibodies did not show any appreciable affinity for these oligosaccharides and thus, no conclusion about the Inaba epitope could be derived from these experiments. However, anti-Ogawa antibodies were inhibited both by compounds **34→ O$_1$** and **36→ O$_2$** at 0.04 mmol/mL demonstrating that in this case the epitope is most likely the terminal monosaccharide containing the 2-*O*-methyl group.

New inhibition studies using other anti-Ogawa polyclonal reagents (murine) were also performed. The ability of synthetic model compounds to inhibit the reaction of mouse polyclonal antibodies was studied by an enzyme-linked inmunosorbent assay (ELISA). The antibodies obtained against the Ogawa serotype were absorbed with Inaba LPS in order to eliminate the fraction of antibodies directed to a common antigen. The reaction in ELISA between the remaining fraction and Ogawa LPS was then separately inhibited with compounds **I$_1$**, **I$_2$**, **O$_1$** and **O$_2$**.

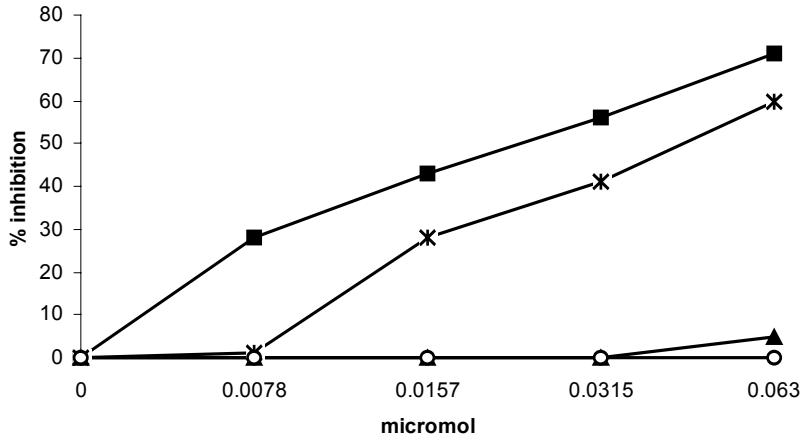


Fig. 1 Inhibition with synthetic fragments ***O₂*** *(–■–),* ***O₁*** *(–x–),* ***I₂*** *(–▲–),* ***I₁*** *(–o–) of the reaction between anti-Ogawa polyclonal serum and the homologous lipopolysaccharides*

As can be seen from Figure 1, anti-Ogawa antibodies were inhibited by the monosaccharide **O_1** and very strongly by the disaccharide **O_2.** Therefore, the 2-O-methylated terminal monosaccharide seems to be the dominant serotype-specific determinant for Ogawa strains.

It is not unusual that small determinants can dictate an immune response in mice.[68]

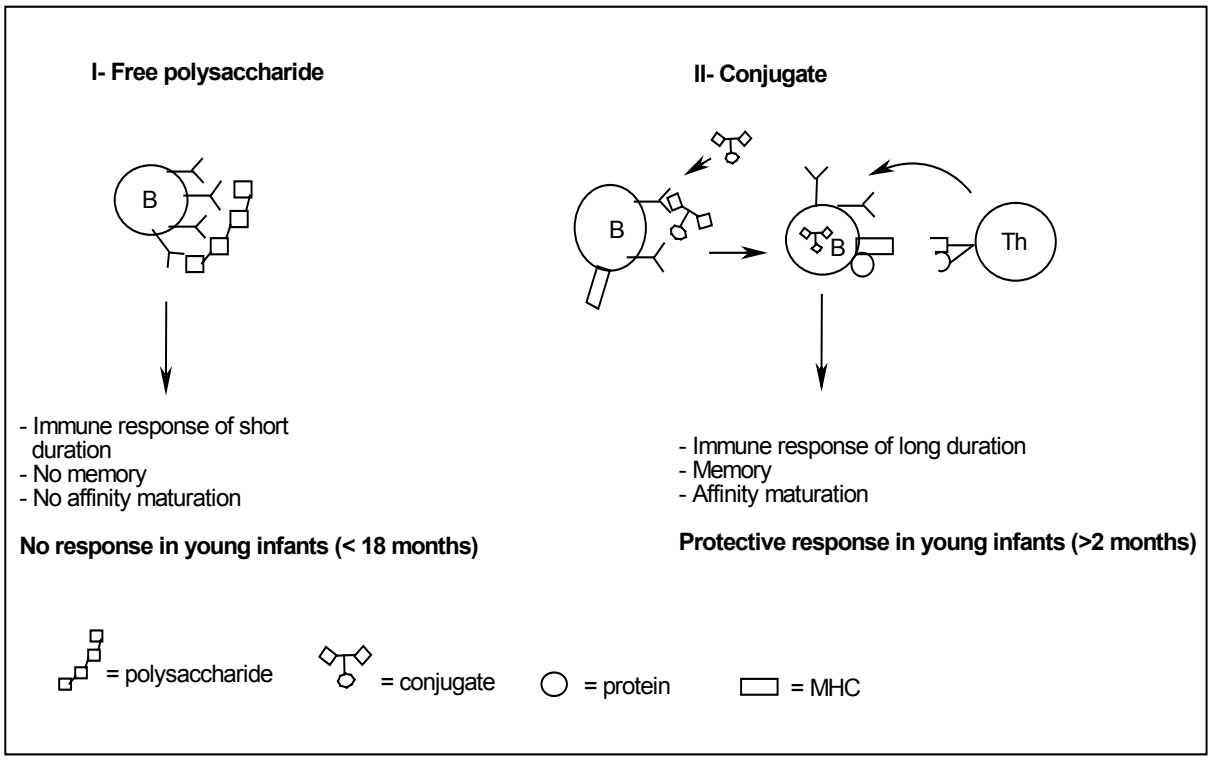
Due to these encouraging results, the synthesis of terminal trisaccharides of both serotypes attached to dioxolane-type spacer arms was attempted to demonstrate their usefulness for the preparation of neoglycoproteins.

2.3 Synthesis of Neoglycoconjugates of the Determinants of *V.cholerae* O1 Serotypes Inaba and Ogawa

2.3.1 The Rational of LPSs-Conjugates

Fully developed LPSs confer serotype-specificity and may serve as protective antigens. It has been suggested that humoral antibodies against O-specific polysaccharide (OPS) components of LPSs are "important for protection".

Plain polysaccharides are known to induce a poor response in infants, and at later ages of life the responses are generally of short duration; similarly the response cannot be boosted, and the affinity does not mature (Scheme 23). From a practical vaccine point of view these facts clearly indicate that polysaccharide antigens need to be conjugated to carrier proteins in order to produce effective vaccines.



Scheme 23. Immune response of free polysaccharide and relative conjugates

Conjugate-vaccine technology[69] has overcome some of the limitations of carbohydrates as vaccine antigens because of the T-helper cell stimulation conferred by the protein. In fact,

LPS are typically classified as T-independent antigens (TI), because they do not require mature T cell help to elicit a humoral response in vivo. TI antigens generally fail to trigger a memory response and usually do not elicit affinity maturation. These properties have precluded their use as pure carbohydrate or PS vaccines in the patients most at risk. Coneversely, conjugate vaccines consist of purified capsular polysaccharide or oligosaccharide antigens covalently linked to immunogenic carrier proteins**.** This formulation converts the polysaccharide to a TD antigen and increases its immunogenicity.[70] The principle of TI to TD conversion of immuneepitopes by conjugation to proteins was exploited to develop the first carbohydrate-based vaccine against Haemophilus influenzae type b (HIB) for use in infants.[71] It has been developed already in the 60ies for immuno responses against peptides which are also not sufficient to induce a humoral response. The ability of on antigen to induce antibodies with a cross reactive profile may be a desirable outcome in the application of polysaccharide vaccines.[19]

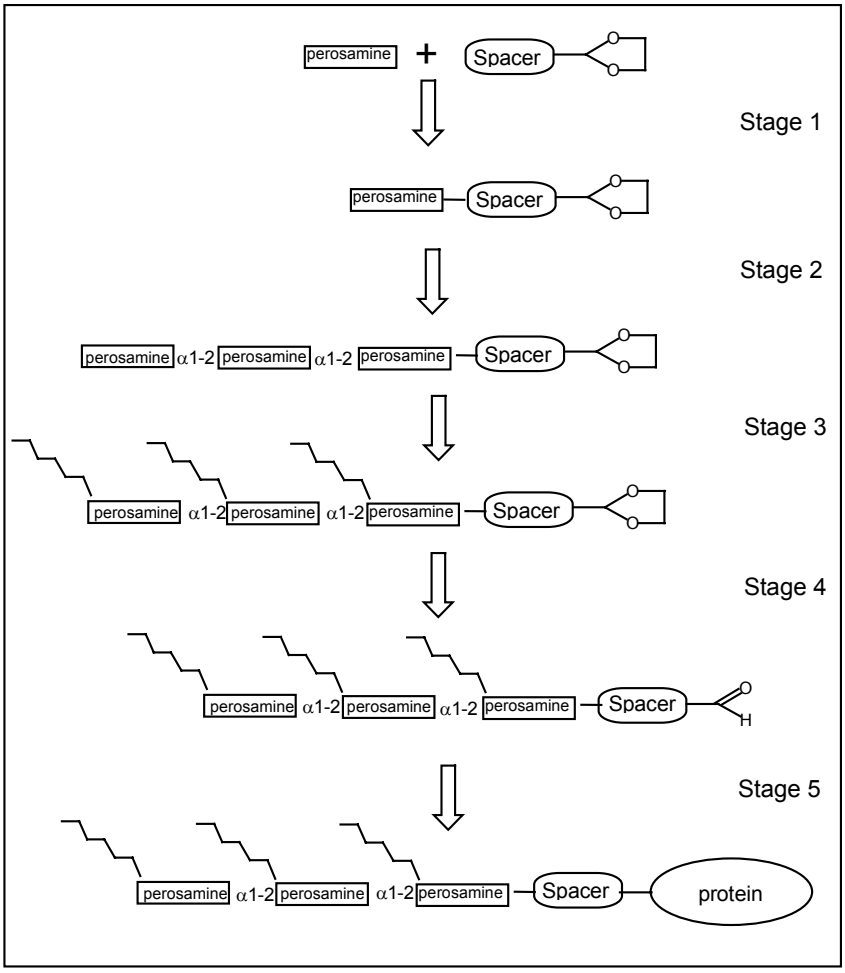
2.3.2 Synthesis of Saccharide/Protein Conjugates

Like natural saccharides, synthetic carbohydrates may be coupled to proteins. Thereby antigenicity has to be retained, and the immunogenicity of the saccharide has to improve after conjugation. Small-size saccharides may be poorly antigenic when compared to the corresponding native polysaccharides. This may not necessarily be a drawback in terms of immunogenicity if the saccharide is conjugated to protein.

Numerous existing or potential techniques are available for the conjugation of bio-organic molecules, including saccharides and proteins.[72] A large variety of these, often derived from pioneering research in affinity chromatography, have been used for the preparation of conjugate vaccines as e.g. reductive amination, amidation, and etherification reactions.[73] For this purpose, often groups with specific reactivities are introduced into synthetic carbohydrates. Usually, a spacer arm with a reactive group is coupled to the anomeric center and used as linker between the saccharide and the protein in order to avoid shielding of important saccharide epitopes by the spatial structure of the carrier molecule. A spacer can also provide greater efficiency of coupling with polysaccharides by reducing steric hindrance of activated moieties.

2.3.3 Retrosynthetic Aspects

The key stages for the construction of oligosaccharide–protein conjugates using the synthetic carbohydrate molecules is outlined in Scheme 24. It represents the strategy selected in the present study and consists of grafting a spacer arm to the monosaccharide unit (stage 1); as spacer a trisaccharide unit was prepared in a stepwise manner (stage 2) and coupled with the side chain to the monosaccharide (stage 3). Then the spacer was deprotected and the aldehyde group was unmasked (stage 4) to allow conjugation to the carrier protein (stage 5).



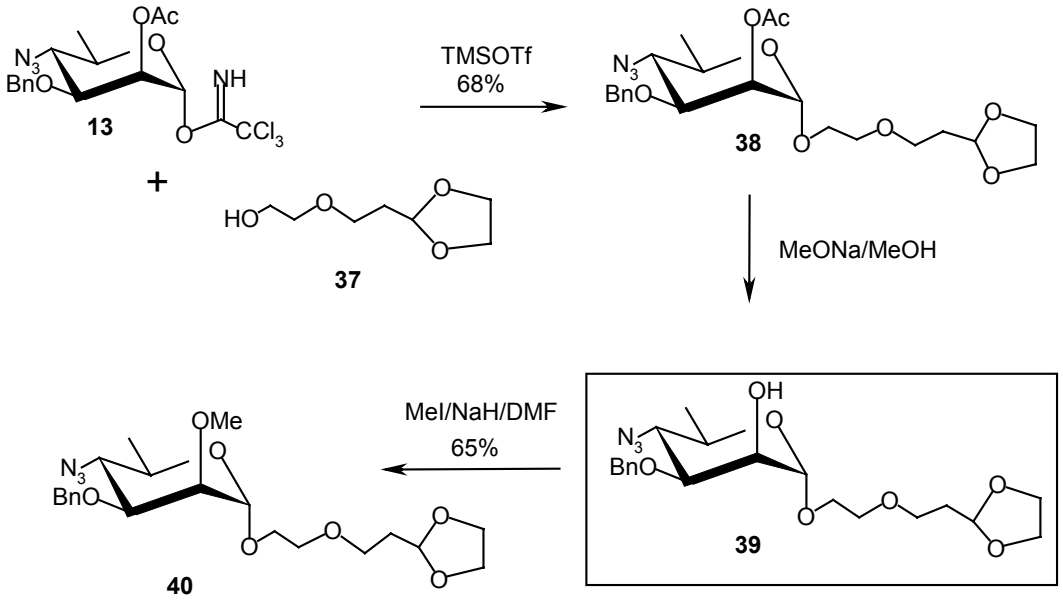
Scheme 24. Key stages of the glycoconjugates synthesis

2.3.4 Synthesis of Fragments of the O-Antigen of *V. cholerae* O1 Attachment to a Dioxolane-Type Spacer

For the synthesis of α-1,2 homopolymers containing D-perosamine an efficient synthesis of 4,6-dideoxy-4-azido-α-D-mannose in combination with a synthetic strategy based upon the use of imidate[37] to generate 1,2-*trans*-glycosidic linkages was required. By this way, well defined synthetic compounds as substitutes of polysaccharides of bacterial origin would become accessible for their covalent attachment to proteins and their assays in serological tests and eventually as vaccines.

For this purpose, a method was chosen that takes advantage of TMSOTf-promoted glycosylation using imidate sugars as glycosyl donors. This procedure has been shown to be particularly useful in the synthesis of oligosaccharides to avoid the main disadvantages of the Koenigs-Knorr method that needs at least stoichiometric amounts of the promoters, as well as the thermal instability of the glycosyl halides.[33] On the other hand, the usefulness of a dioxolane-type spacer has been previously demonstrated in the synthesis of other neoglycoconjugates.[74] The heterobifunctional spacer carries an hydroxy group for its grafting to the oligosaccharide and a masked aldehyde function for condensation with amino groups on the protein surface. Due to its stability during many chemical manipulations, the dioxolane spacer can be introduced in the initial synthetic step.

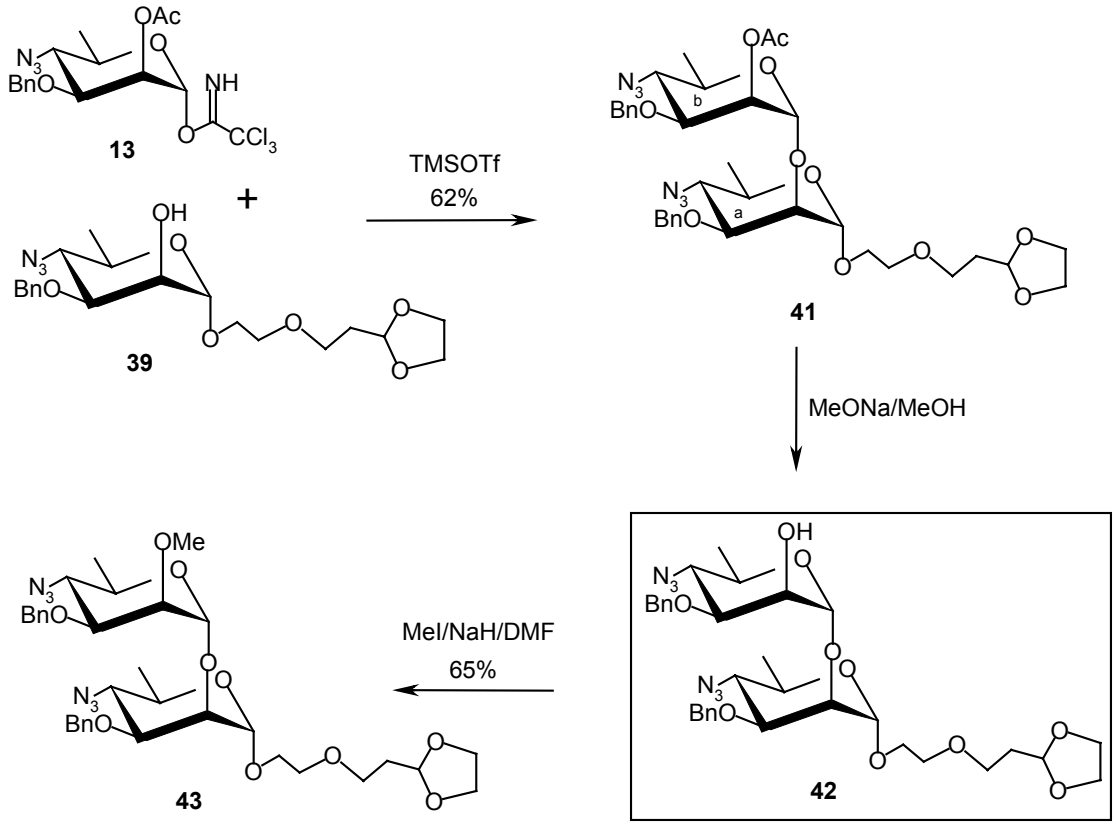
By this strategy a stepwise construction of higher oligosaccharides suitable for conjugation to protein is possible using almost the same approach as for the preparation of analogous methyl glycosides, i.e. by simple aglycon exchange from methyl to a linker spacer in the first step (Scheme 25).



Scheme 25. Monosaccharides attachment to a dioxolane-type spacer

The reaction of **13** as the key glycosyl donor with 4-(1,3-dioxolan-2-yl)-3-oxabutanol **37** proceeded smoothly in the presence of trimethylsilyl triflate affording the spacer-arm perosaminide **38** in 68% yield. The presence of the spacer in compound **38** and throughout the synthetic sequence was confirmed by the ^{1}H NMR spectra (triplets at 5.00-5.15 ppm related to the 5a-H and the signals at 102 and 65 ppm in the ^{13}C NMR spectra, corresponding to the dioxolane carbons). After de-*O*-acetylation of compound **38** (→**39**), the resulting free hydroxy group was either methylated to give the terminal Ogawa monosaccharide **40** in 65% yield or glycosidated with the same perosamine donor **13** in the presence of trimethylsilyl triflate as catalyst to afford the corresponding disaccharide **41** in 62% yield (Scheme 26).

The NMR data and $^{3}J_{1,2}$ coupling constant values indicated the presence of the 1,2-trans mannopyranosidic linkage:^{1}H-NMR [δ = 4.85 ppm, 1b-H, $J_{1b,2b}$ = 1.3 Hz; δ = 4.76 ppm, 1a-H, $J_{1a,2a}$ = 1.5 Hz] and confirmed the structure of disaccharide **41**.

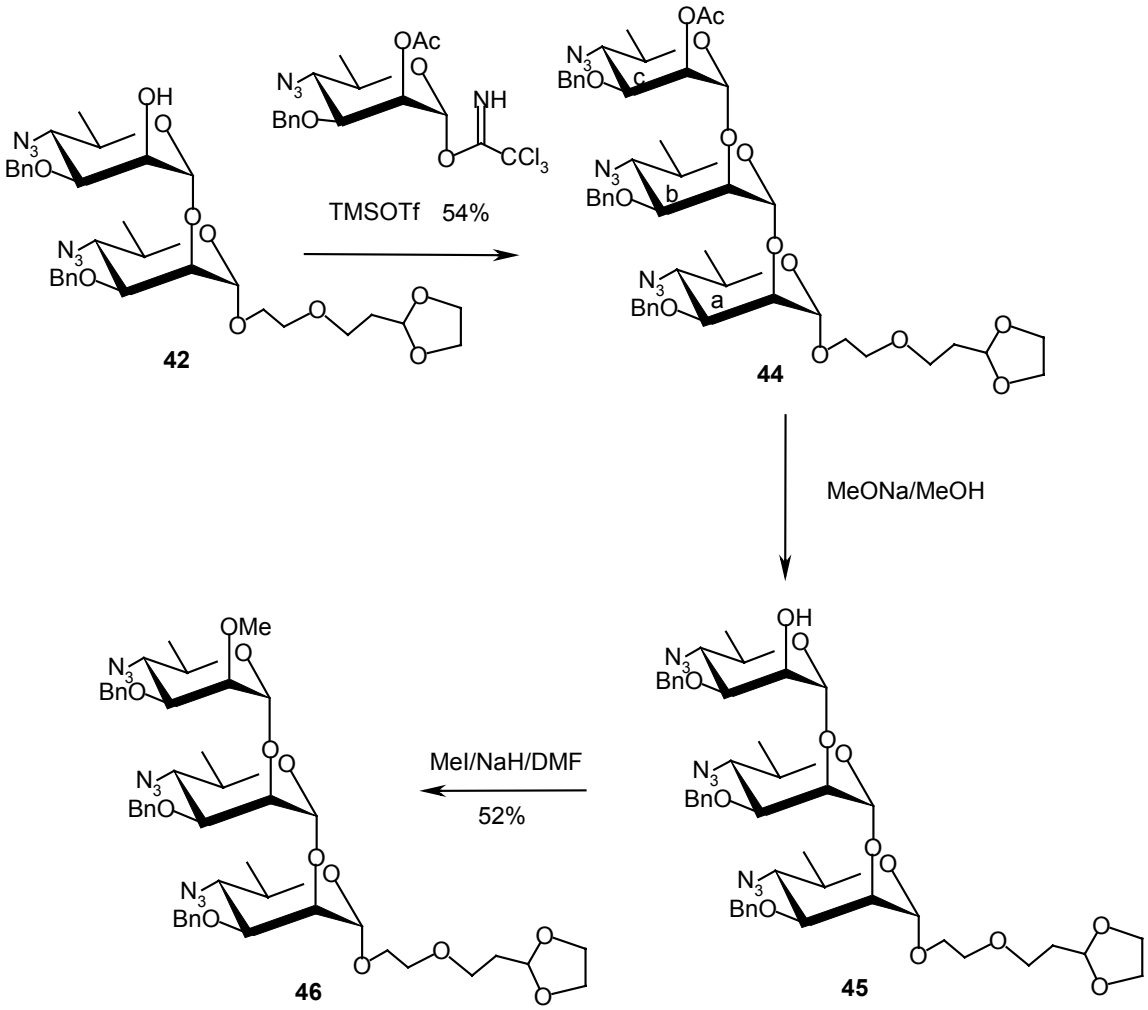


Scheme 26. Synthesis of disaccharides attachment to a dioxolane-type spacer

A migration of the acetyl group as previously detected when coupling was performed using a glycosyl halide and silver triflate, was not observed under these conditions. Deacetylation of **41** under Zemplén conditions[75] (→ **42**) followed by methylation gave the terminal Ogawa disaccharide **43** in 65% yield.

In analogous manner, the spacer-arm linked trisaccharides were synthesized (Scheme 27). The disaccharide acceptor **42** was coupled to the same glycosyl donor **13** under standard conditions (TMSOTf, DCM) giving the desired compound **44** in 54% yield. The new α-linkage was easily demonstrated by the presence in the ^{1}H NMR spectrum of a new doublet at $\delta = 4.77$ ppm ($J_{1c,2c} = 1.5$ Hz) corresponding with the anomeric proton.

As for the mono- and disaccharides, compound **44** was transformed into the 2-hydroxy - derivative **45** under Zemplén conditions.

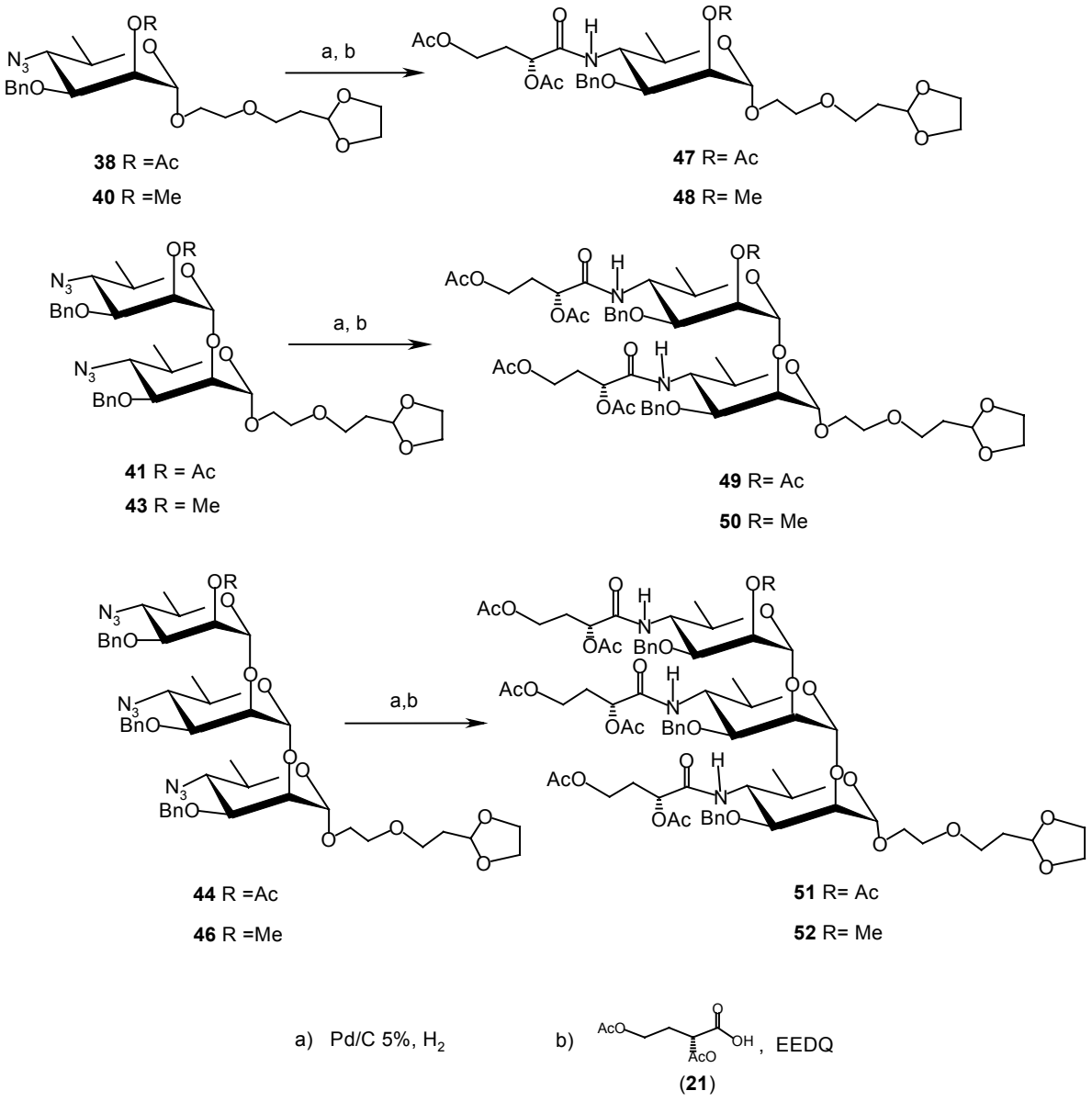


Scheme 27. Synthesis of trisaccharides attachment to a dioxolane-type spacer

Compound **45** is an important intermediate for the synthesis of trisaccharide fragments of *V.cholerae* O1 in both the Ogawa and Inaba series, as well as for synthesis of other members (tetra-, penta-, hexa-saccarides etc.), since it allows chemical manipulation (methylation or chain extension) at position O-2 in the upstream terminal moiety. Introduction of the methyl group at this position was carried out using the same procedure as used for the preparation of mono and disaccharide fragments (→ **46**).

2.3.5 Attachment of 2,4-Di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic Acid

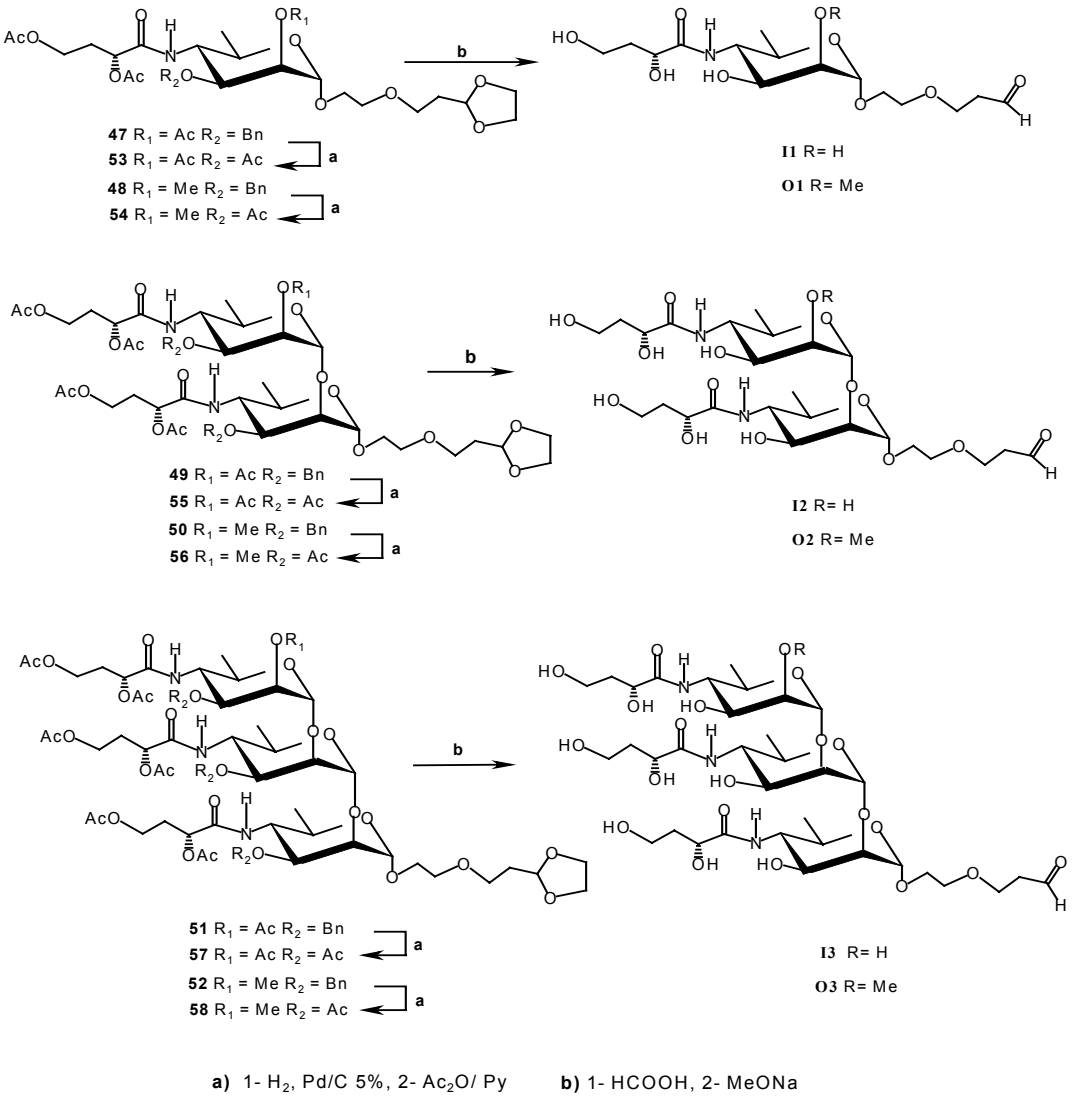
The previously described selective reduction of the azido function in the presence of benzyl groups was used for monosaccharides **38** and **40**, disaccharides **41** and **43**, and the trisaccharides **44** and **46,** respectively. For the attachment of the side-chain, the amine derivatives thus obtained were treated with 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid (**21**) in the presence of EEDQ in dichloromethane. The amides **47**, **48**, **49**, **50**, **51** and **52** were formed in yields of 55-85% and were structurally characterized by ^{1}H NMR spectra (doublets in the δ = 6-7 ppm region corresponding with the new NHCO linkage).



*Scheme 28. N-acylation using **21***

2.3.6 Deprotection and Functionalization

Hydrogenolysis of the benzyl groups proceeded successfully in the presence of Pd/C 5% for the amides **47**, **48**, **49**, **50**, **51** and **52**. Then re-*O*-acetylation gave the per-*O*-acetylated mono- (**53**, **54**), di- (**55**, **56**) and trisaccharides (**57**, **58**) in 82 - 88% yields. Deprotection of the aldehyde function was performed in standard manner with formic acid by TLC monitoring (aniline phthalate positive spot). The aldehyde group was further assessed by ^{1}H-NMR (δ = 9.75-9.72, s, 1H, HC=O). Careful deacetylation with 0.1 M sodium methoxide in methanol at 0 ^{o}C afforded the free monosaccharides (**I1**, **O1**) disaccharides (**I2**, **O2**) and trisaccharides (**I3**, **O3**) with the aldehyde-functionalized spacer.



Scheme 29. Deprotection and activation of aldehyde group

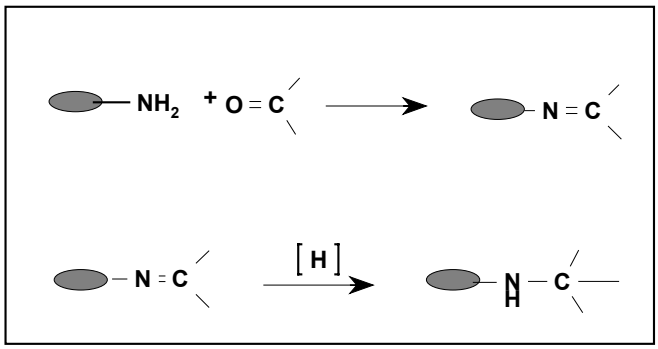
2.3.7 Conjugation

2.3.7.1 Reductive Amination

Ideal methods for coupling sugars to proteins should be such that (1) the coupling proceeds efficiently in a moderate pH range, (2) that the products formed retain the original overall charge distribution with minimal additional hydrophobicity, and (3) that the newly formed linkage is characterized by reasonable chemical stability.

Among the various methods used for modification of proteins, reductive amination[76] is still the most frequently used strategy for glycoprotein preparation. It is known that this procedure preserves the protein structure close to is native state. Moreover, it is suitable for the preparation of neoglycoconjugates with very high saccharide/protein ratios.

Two separate reactions are involved: an amino group and a carbonyl compound undergo condensation to an imine and the imine which is unstable, is usually reduced to the stable amine (Scheme 30).

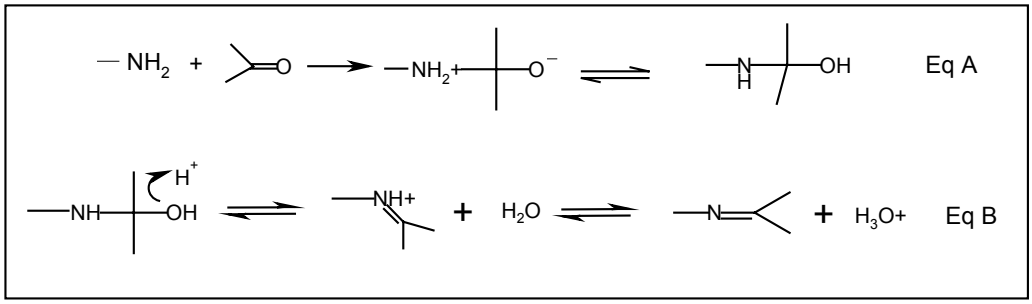


Scheme 30. Reductive Amination

For simple aliphatic aldehydes and ketones, and strongly basic amines such as the ε-amino groups of the lysine residues of the carrier proteins, the reaction to imines is rapid and reversible. Since condensation of an aldehyde with ε-amino groups of lysine residues in proteins followed by reduction converts primary amino groups into secondary ones, the net charge of the proteins remains virtually unchanged.

Rates of imine formation reflect both pH dependencies (Eqs A and B) and are usually maximal around 4 to 5. The nucleophilic attack (Eq A) is rate-limiting at lower pH values,

where amines are largely protonated, while dehydration (Eq B) which is acid-catalyzed, is rate-limiting at higher pH values.

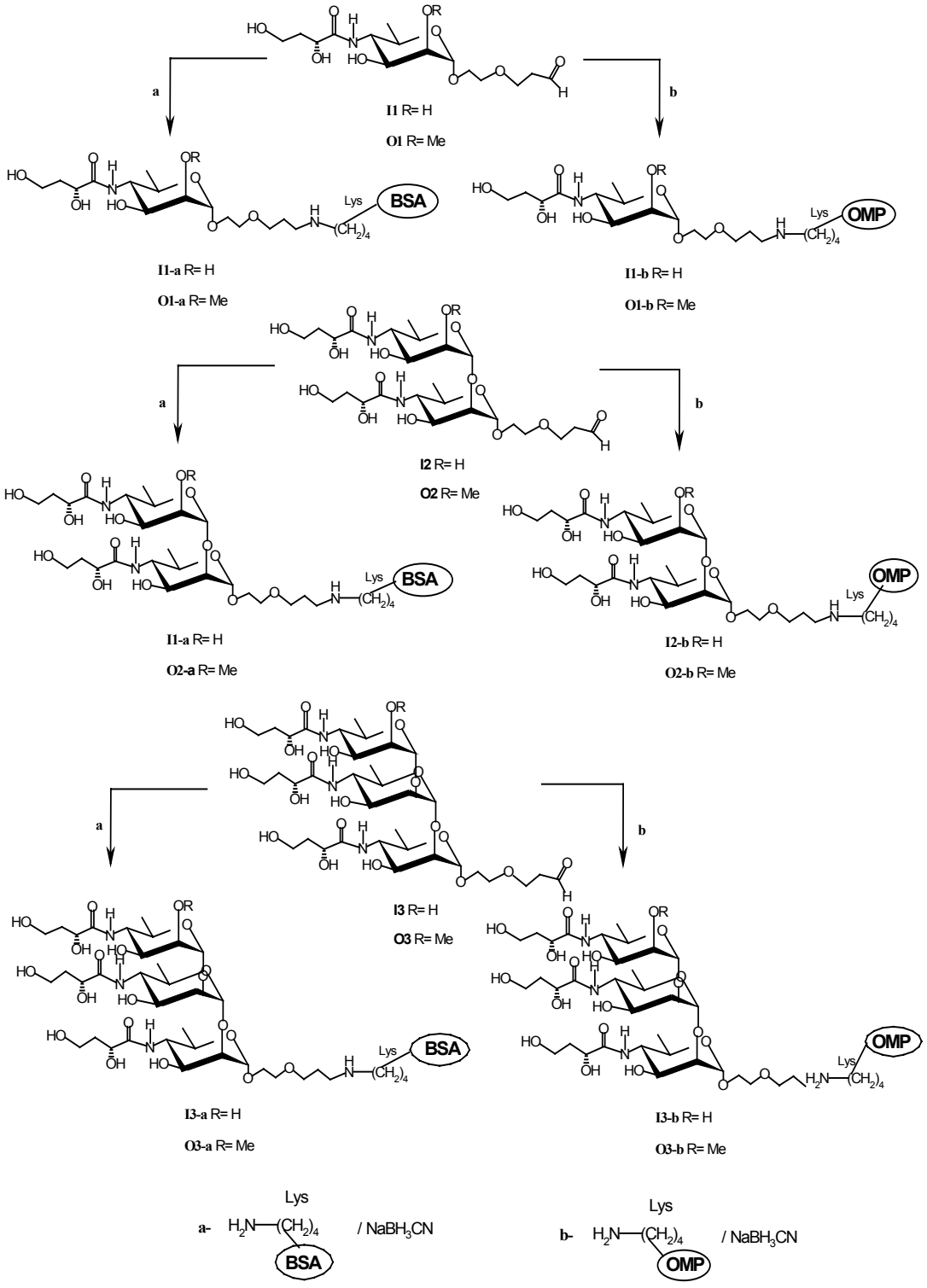


Scheme 31. Imine formation

Reduction of imines with sodium borohydride or sodium cyanoborohydride involves the transfer of a hydride ion to the protonated imines; with cyanoborohydride, for example, reactions are usually optimal around pH 7-8 where the protonated imine is abundant. Other reducing agents are usually either too unstable in aqueous solution or require conditions unsuitable for most proteins. Sodium cyanoborohydride is more stable than sodium borohydride at low pH and allows reaction to be conducted at neutral pH or lower; due to the much slower reduction of free carbonyl compounds, their incorporation into proteins via imine formation is usually improved. The greater stability of the reagents allows for longer reaction times and the use of a wider pH range: this may be especially important for less reactive carbonyl compounds.

2.3.7.2 Preparation of Conjugates Using Synthetic Fragments

The final stage was the preparation of the neoglycoconjugates using the derivatives **I1**-**O3** (Scheme32).



Scheme 32. Preparation of conjugates

Coupling of the derivatives **I1-O3** with BSA via reductive amination led to the conjugates (→ **I1-a**, **O1-a**, **I2-a**, **O2-a**, **I3-a**, **O3-a**) with incorporation levels of 22-34 mol of oligosaccharides per mol of BSA (se Table 2). In order to obtain neoglycoproteins with vaccine potential, the same reaction was performed with the highly immunogenic Meningococcal Outer Membrane Protein complex (OMP). In this case carbohydrate/protein ratios of 1/5-10 (mg/mg) were obtained **(→ I1-b**, **O1-b**, **I2-b**, **O2-b**, **I3-b**, **O3-b**) confirming the efficiency of the conjugation procedure through dioxolane-type spacer arms (Table 2).

compound	hapten/BSA (mol/mol)	OMP/hapten (mg/mg)
I1	**I1-a**→ 34	**I1-b**→ 12
O1	**O1-a**→ 27	**O1-b**→ 7
I2	**I2-a**→ 28	**I2-b**→ 5
O2	**O2-a**→ 23	**O2-b**→ 8
I3	**I3-a**→ 24	**I3-b**→ 9
O3	**O3-a**→ 22	**O3-b**→ 6

Table 2. Carbohydrate (hapten)/protein ratios obtained by conjugation of the saccharides to BSA and OMP

The BSA-glycoconjugates were used for characterization of the molecular specificity of anti-Ogawa and anti-Inaba antibodies. Conversely, the immunogenicity of saccharaides/OMP conjugates and their use as potential vaccines is presently under investigation in laboratory animals.

2.3.8 Biological Results

Synthetic oligosaccharides fragments (**I1-a→ O3-a**) reflecting the structural difference between Ogawa and Inaba O-antigens were used to study the specificity of monoclonals directed against *Vibrio cholerae* O1 LPS -Inaba and Ogawa respectively.

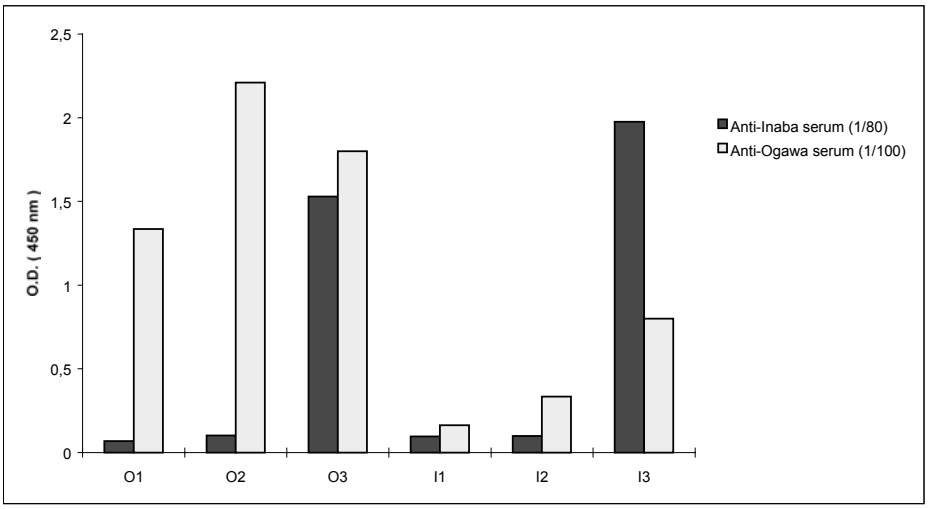


Fig 2 Binding affinities of mono-, di and trisaccharides as mimics of the Ogawa and Inaba O-antigen, for anti-Ogawa and anti Inaba antibodies.

As shown in Figure 2 only minor differences are observed in the binding affinities of mono-, di- and trisaccharide of the Ogawa serotype, clearly indicating a specificity for the terminal residue of the Ogawa O-PS. On the other hand, synthetic oligosaccharide fragments related to the structure of Inaba O-antigens, possess weak affinity for both Ogawa and Inaba organisms. Indeed, the mono- and disaccharide failed to show interaction with antibodies in the ELISA system employing either LPS-Ogawa or LPS-Inaba as ligands. However, the behavior of the trisaccharide was completely different. In this case the synthetic fragment exhibited high affinity for both antibodies; as expected, this affinity was one order of magnitude lower for the anti-Ogawa-antibody.

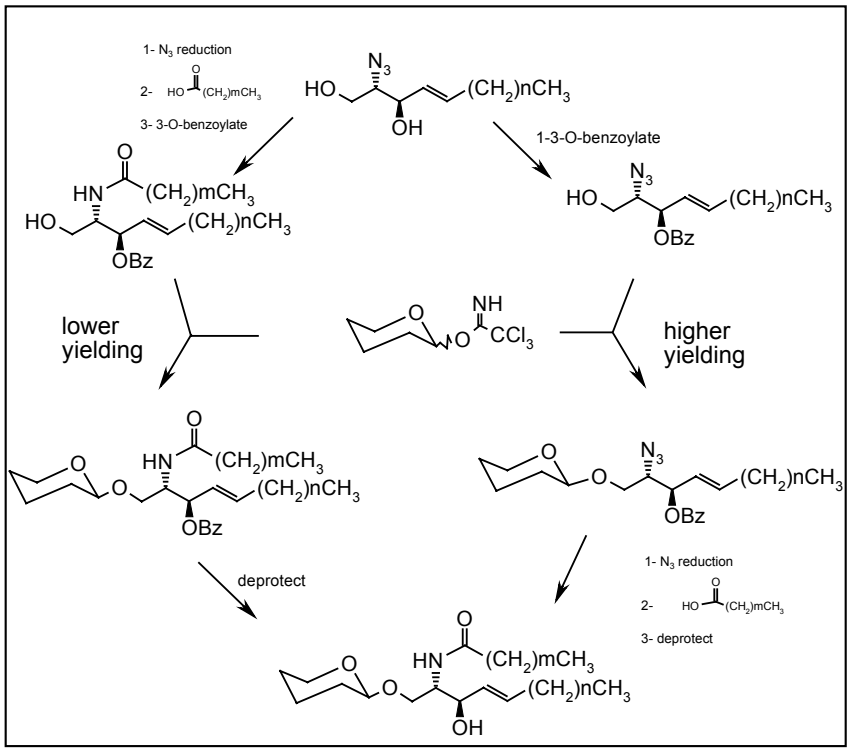
These experimental results are consistent with the hypothesis that probably the trisaccharide fragment constitutes the minimum fragment of the antigen epitope shared by the O-polysaccharides of both Inaba and Ogawa strains. Moreover, tha data confirm that the 2-O-methylated terminal monosaccharide represents the dominant serotype-specific determinant for Ogawa strain.

In summary, it is clear that some of the compounds synthesized in this study are related to structural features of important antigenic epitopes of the O-polysaccharide of *V. cholerae* O1 antigen and may be useful molecules for characterization of these antigens.

2.4 Synthesis of Ganglioside: Strategies and Problems

Independently of the complexity of ganglioside structures, three main units have to be combined synthetically, i.e. the ceramide residue, the lactose moiety and the oligosaccharide constituent. Thus, the synthetic strategy for gangliosides generally applied is based on glycosylation of different building blocks of a lactosyl acceptor followed by coupling with azidosphingosine or ceramide derivatives in the last step. For the introduction of the ceramide moiety, early syntheses were based on Koenig-Knorr methods. Presently, a strategy based on glycosyl trichloroacetimidate donors represents the most widely applied procedure for the synthesis of complex glycosphingolipids.

As shown in Scheme 33, pathway I allows for an easy preparation of the glycosphingolipid upon the coupling step by simple deacylation, but it suffers from low yields in the coupling reaction, a fact that has been attributed to steric effects or problems resulting from chelation to the catalyst.[77] On the other hand, pathway II leads to high yields in the coupling reaction, but various steps are required for the introduction of the N-linked fatty acid residue. The coupling of ganglioside derivatives with protected azidosphingosine derivatives rather than with protected ceramide derivatives is more commonly used, particularly if a series of fatty acyl analogs is required.[78,79]

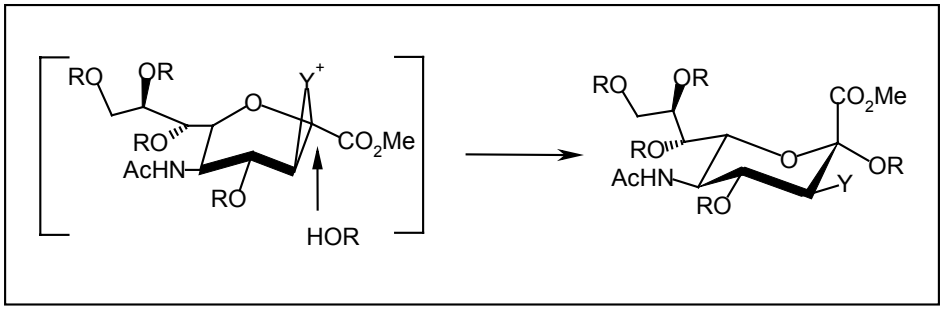


Scheme 33. Ceramide and azidosphingosine glycosidation procedures

Sialylation, i.e. glycosylation with *N*-acetyl-neuraminic acid donors, has been recognized as the most challenging task in carbohydrate chemistry, especially when a second sialic acid moiety has to act as acceptor. The main difficulties encountered in such syntheses are the following: *i)* the C-2 carbon to which sugar residues have to be linked, is quaternary and carries an electron withdrawing carboxylate group, *ii*) substitution reactions at the anomeric position are disfavoured sterically as well as electronically, and *iii)* sialic acid possesses a C-3 deoxy structure that readily leads to 1,2-elimination reactions of active species. Moreover, the classical stereocontrol tactics of glycoside synthesis such as in situ anomerization or neighboring acyloxy group participation, can not be applied for this particular type of glycoside.

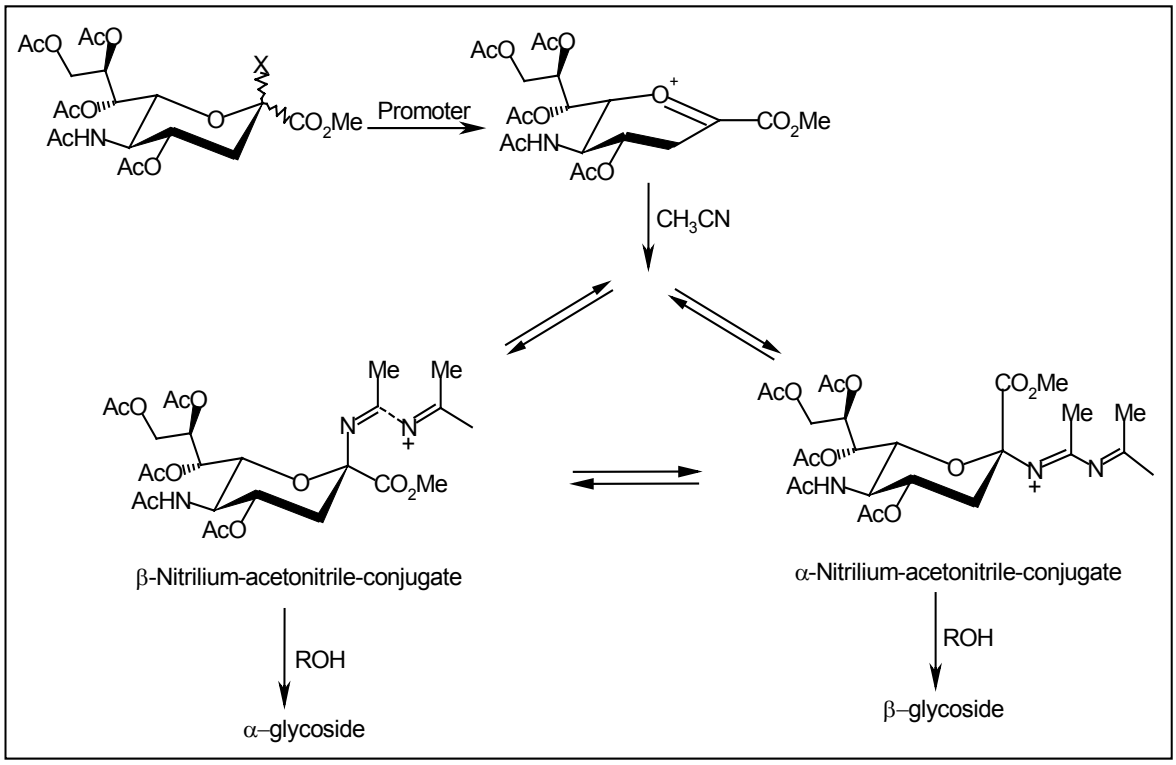
Since β-glycosides of sialic acid have so far not been found in nature, α-sialylation is required. This is rather difficult to accomplish in high yields, a problem that has been extensively studied over the years. From this research suitable donors for α-sialylation have evolved as follows:

- Optimization of the anomeric leaving group, where β-phosphites[39] and α/β-thioglycosides[80] led to the best results. However, such sialyl donors are prone to elimination forming a glycal whenever poorly reactive acceptors are to be sialylated. Moreover, these donors yield α/β product mixtures.
- Introduction of an auxiliary 3-thio-substituent to suppress glycal formation and to improve the α/β selectivity. The most successful procedures use donors bearing neighboring group participation as the phenylthio-,[81,82,83] phenylseleno[84] and phenoxy-thiocarbonyl-groups.[85] There is the consensus that such substituents decrease the acidity of the H-3 proton and generate a reactive intermediate that can only be attacked by the acceptor molecule from the α-side of the donor (Scheme 34). The anchimerically assisting group is removed from the product by a radical reaction in practically quantitative manner .



Scheme 34. Glycosylation of sialic acid aided by stereocontrolling auxiliaries

- The logical consequence was then to combine the best anomeric leaving group with the best auxiliary substituent. In this context, Schmidt *et al.*[86] have reported the efficient use of sialyl phosphites for high-yields and α-selective sialylation, in which a combination of TMSOTf as promoter and CH_3CN as suitable solvent is also highlighted.



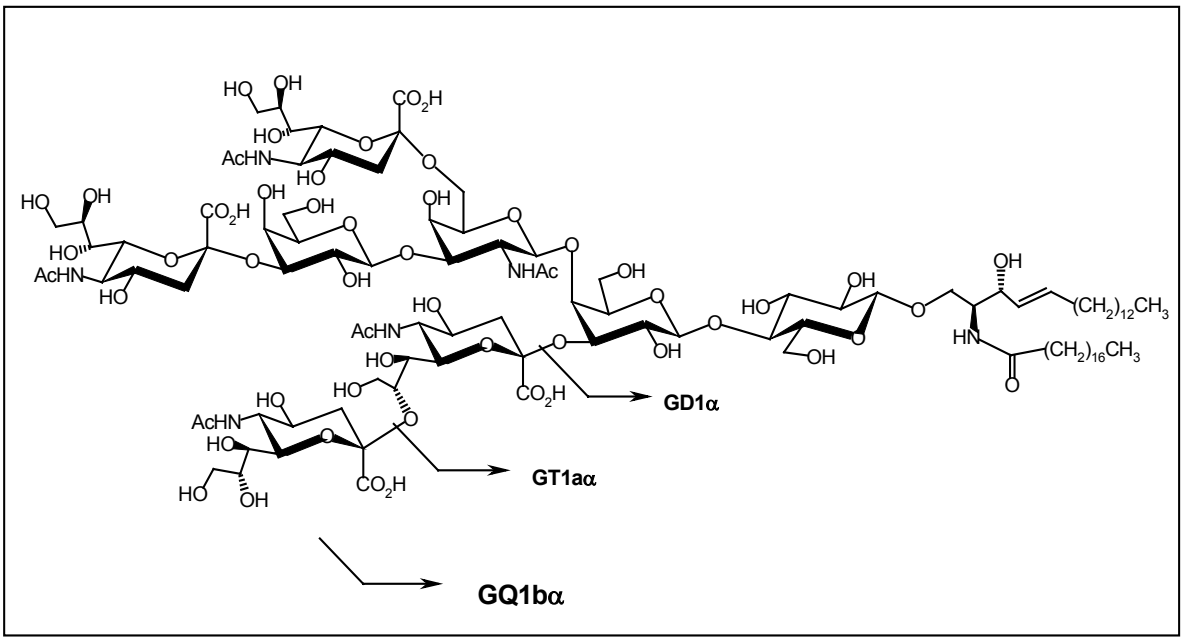
Scheme 35. Nitrile effect in the glycosilation reaction

In O-glycosylation reactions the nitrile solvents favor under kinetically controlled conditions the equatorial glycoside, i.e. the α-product in the case of neuraminic acid, and when used for phosphite-activated neuraminic acid derivatives, lead to improved α-selectivity. Obviously with nitriles as solvents a simple acid-sensitive leaving group is required.

2.5 Approach to the synthesis of GQ1bα

The biological importance of gangliosides in various animal cells is now well documented. However, the current scenario of gangliosides is not completely clear as their structure-function relationships at the molecular level remain largely unknown. Since biologically derived gangliosides are available only in limited quantity, several research groups have initiated efforts to provide chemically synthesized pure gangliosides and their analogues to be used in biological studies.

The α-polysialogangliosides such as GD1α, GT1aα and GQ1bα, represent a new series of gangliosides containing Neu5Ac linked to C-6 of GalNAc of the gangliotetraosyl backbone[41,87] (Scheme 36). Since these gangliosides are found only in a particular region of the brain as components of a defined tissue, little is know about their physiological functions and possibly they may serve as ligands for some neural proteins.

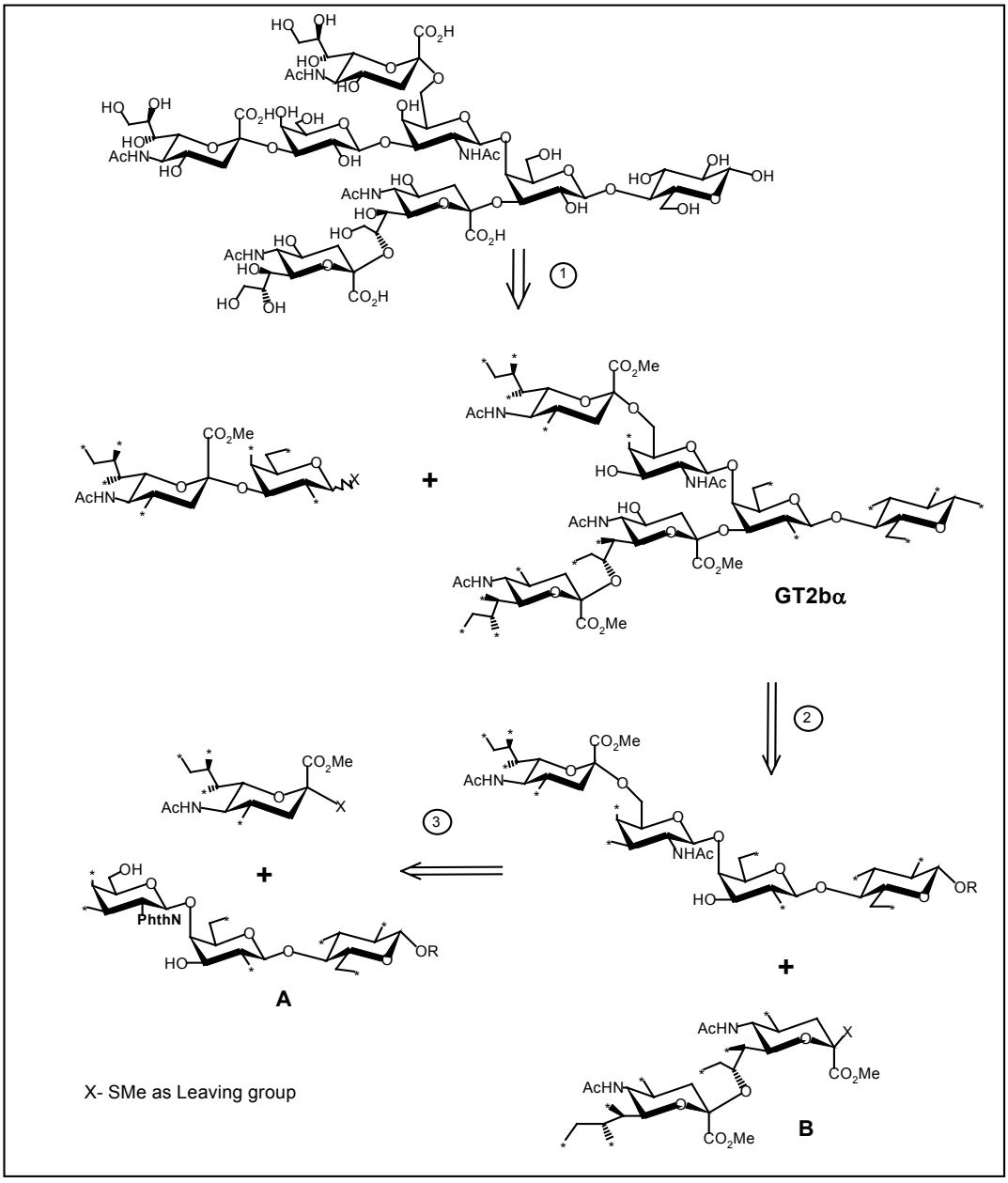


Scheme 36. Structures of α-series polysialogangliosides

The ganglioside GQ1bα (see Scheme 36) is one of the most complex structures among these classes of natural products and first preliminary studies for the total synthesis of the GQ1bα structure are reported in the second part of the thesis work.

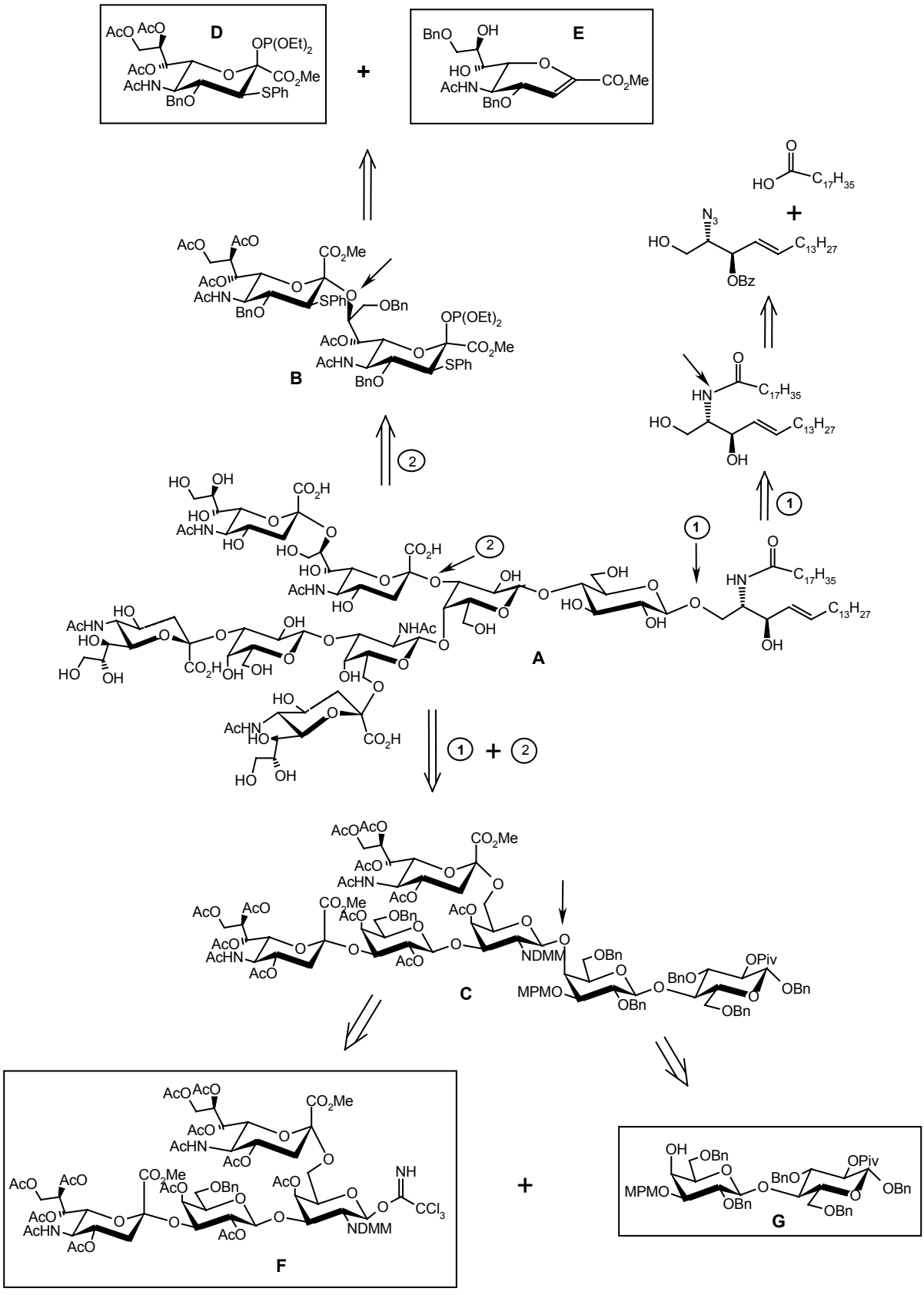
2.5.1 Previous Syntheses

A first approach to the synthesis of GQ1bα ganglioside has been reported by Hasegawa *et al*. [88,89] The strategy was based on the use of a GT2bα acceptor as key intermediate which was prepared by successive sialylation of the gangliotriaosyl acceptor **A** (Scheme 37). The sialylα(2→8)sialyl donor derivative **B** used in this strategy was obtained by limited acid hydrolysis of colominic acid and the galactosamine moiety was protected at the amino function as phthaloyl derivative.



Scheme 37. Hasegawa strategy

2.5.2 Retrosynthetic Analysis



Scheme 38. Retrosynthesis of GQ1bα

With a retrosynthetic analysis illustrated in Scheme 38 a new strategy was designed. The octasaccharide **A** was disconnected into the Neu5Acα(2→8)Neu5Ac donor **B** and the hexasaccharide **C** (GD1α derivative). A second disconnection of the Neu5Acα(2→8)Neu5Ac donor **B** affords the sialyl donor **D** and the acceptor **E**. On the other hand, for the preparation of hexasaccharide **C**, two different building blocks should be synthesized, the tetrasaccharide donor **F** and the lactose moiety **G** as a key glycosyl acceptor.

Such a synthetic strategy would allow the use of new methods developed in Schmidt's laboratory for the efficient regio- and stereoselective synthesis of gangliosides:

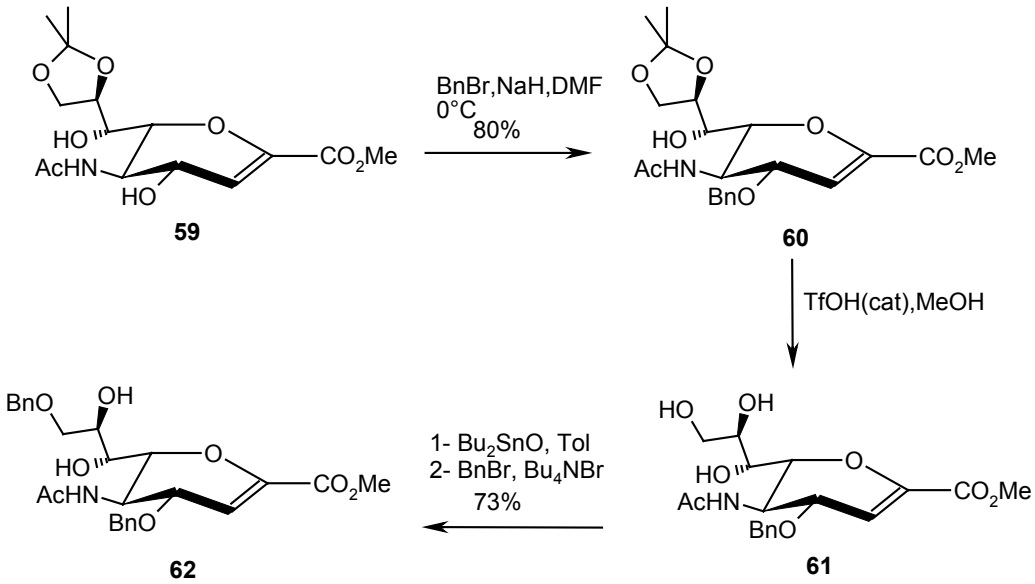
- the azidosphingosine methodology for the introduction of ceramide moiety;[78]
- the lactose derivative **G** which allows for derivatization at the 3b and 4b positions, and which contains a pivaloyl group at the 2a position to prevent the formation of an orthoester side product in glycosylation reactions;[90]
- the use of donor **D** which carries a phosphite as leaving group and a phenylthio group at 3 position to ensure the α-stereoselectivity in the sialylation reaction;[39]
- the extension of this methodology to the synthesis of the Neu5Acα(2→ 8)Neu5Ac donor **B** and the glycosylation of the 3b position of the lactose with this new donor;
- the use of the DMM protecting group for the galactosamine moiety which allows β-stereoselectivity in the glycosylation reaction and can be removed under relatively mild conditions;[91]
- the use of the efficient trichloroacetimidate method[37] to generate very reactive glycosyl donors which can be activated using catalytic amounts of acid.

In a first stage of this synthetic route the disaccharide donor **B** was synthesized and analyzed in its efficiency for glycosylation of the 3b position of lactose.

2.5.3 Synthesis of the Neu5Acα (2→8) Neu5Ac donor

2.5.3.1 Acceptor Synthesis

The acceptor **62** was designed in a way to combine a good reactivity of the 8-O-position for attachment of the sialic acid donor and to allow further transformation of the molecule through the double bond into a new donor for a subsequent sialylation reaction. This compound is readily obtained from the known 3-*O*-benzyl-8,9-*O*-isopropylidene-2,3-dehydroneuraminic acid derivative **60**[79] (Scheme 39) which after acid-catalysed removal of the *O*-isopropylidene group (→**61**) and regioselective 9-*O*-benzylation via an 8,9-stanylidene acetal generates the desired compound **62** in good overall yield.

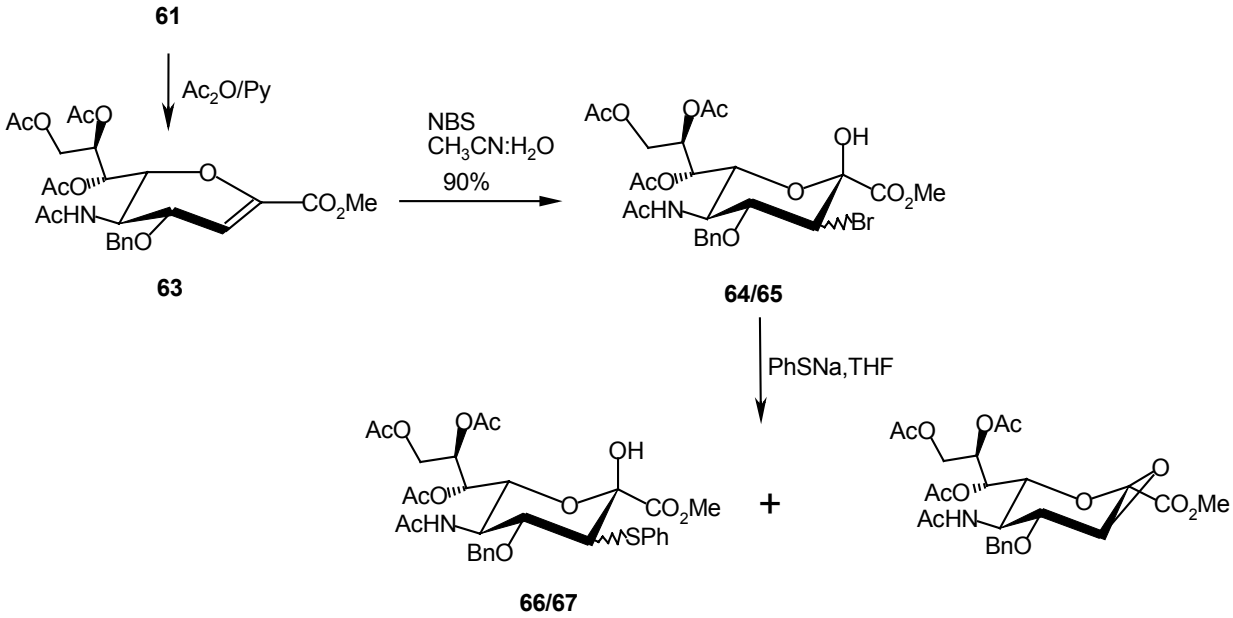


*Scheme 39. Synthesis of sialyl acceptor **62***

The structural assignment of compound **62** was readily achieved by ^{13}C-NMR data:
δ 138.0, 137.5 (2C-ipso-Bn); 52.4 (CO_2Me); 72.5 (C-4); 69.2 (C-7); 68.9 (C-8), 71.8 (C-9).

2.5.3.2 Donor Synthesis

For the synthesis of sialyl donor **68** the same intermediate **61** was used as starting material which after *O*-acetylation afforded **63** in 96 % yield. In order to functionalize the C-2 and C-3 properly, **63** was treated with *N*-bromosuccinimide in acetonitrile/water at 60 °C to produce a mixture of bromohydrins (→ **64**/**65**). The mixture thus obtained was directly transformed with sodium thiophenolate in THF into the 3-phenylthio derivatives **66**/**67** and a small amount of a side-product which exhibited a similar Rf as **66** and led to difficult purification of the desired compound by normal flash chromatography. After meticulous separation of the reaction mixture, the 2,3-epoxide was identified as the side-product (Scheme 40).

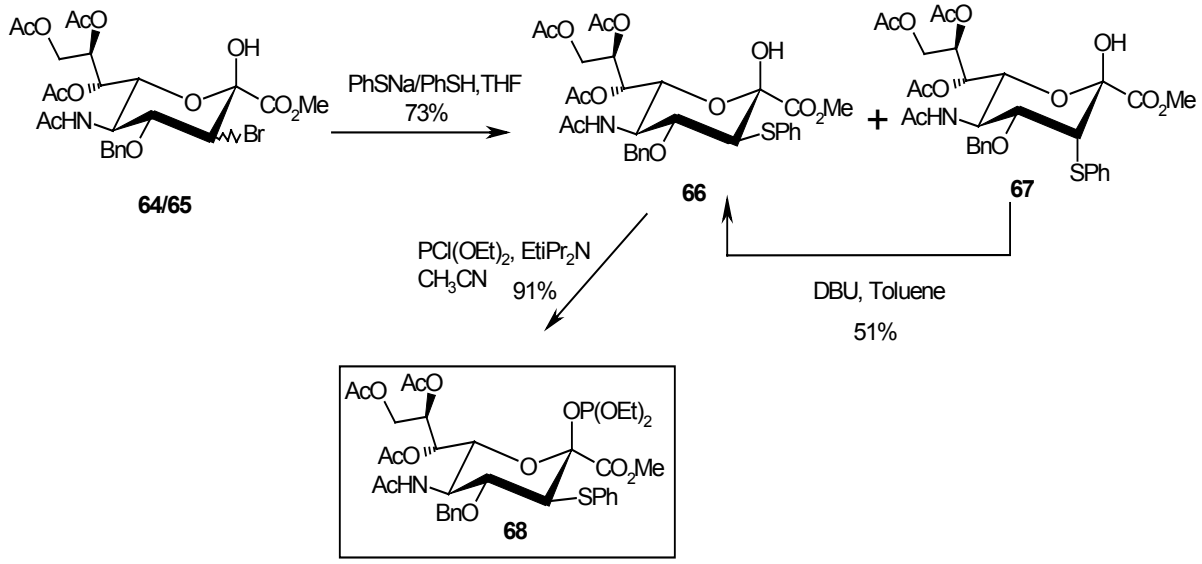


Scheme 40. Transformation of the bromohydrins using PhSNa/THF

Obviously, under the reaction conditions used in addition to the nucleophilic substitution of the bromide by the thiophenolate, a base-catalyzed epoxide formation is taking place. To prevent this side-reaction, the reaction was performed using a mixture of PhSNa/PhSH where the thiophenol should quench the base generated in the reaction, thus avoiding the formation of epoxide as side-product. The new system sodium thiophenolate/thiophenol in THF furnished a mixture of products in which the corresponding 3-phenylthio equatorial adduct **66** was found to predominate largely over the axial compound **67**. Moreover, **66** was readily separated by silica gel chromatography. Treatment of **67** with DBU led to partial epimerization at C-3, thus generating additional key intermediate **66**. The new improved

procedure permitted to obtain the desired stereoisomer **66** in relatively good yield with only a small amount of the undesired α-epimer **67**.

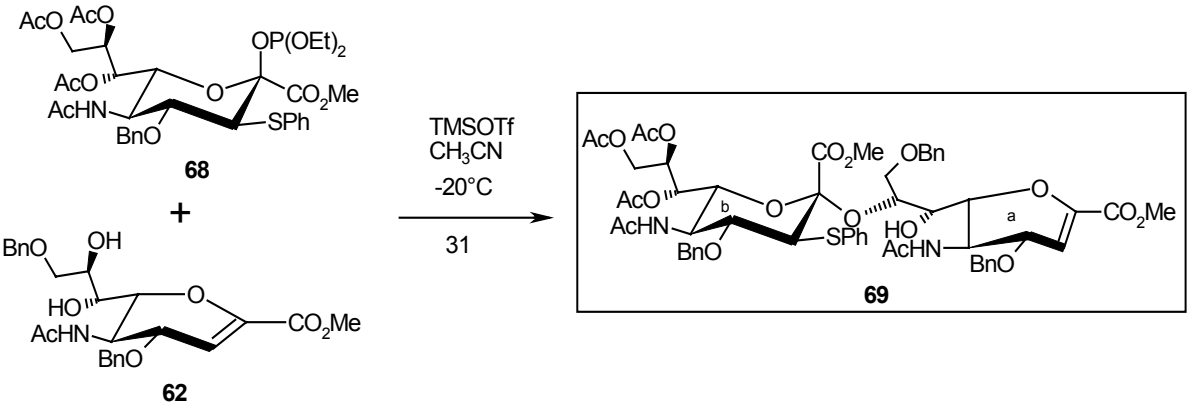
Subsequent conversion of the hemiketal **66** into the sialyl donor **68** was easily achieved using the well known phosphite method.[39]



*Scheme 41. Synthesis of the sialyl donor **68***

2.5.3.3 Sialylation of acceptor 62 with donor 68

The synthesis of the disaccharide **69** was accomplished as outlined in Scheme 42. Reaction of **68** with the neuraminic acid acceptor **62** at –20 °C in the presence of catalytic amounts of trimethyl trifluoromethanesulfonate (TMSOTf, 0.1eq) in MeCN as solvent produced the expected α-sialoside compound **69** in 31 % yield after chromatographic purification (unoptimized yield).

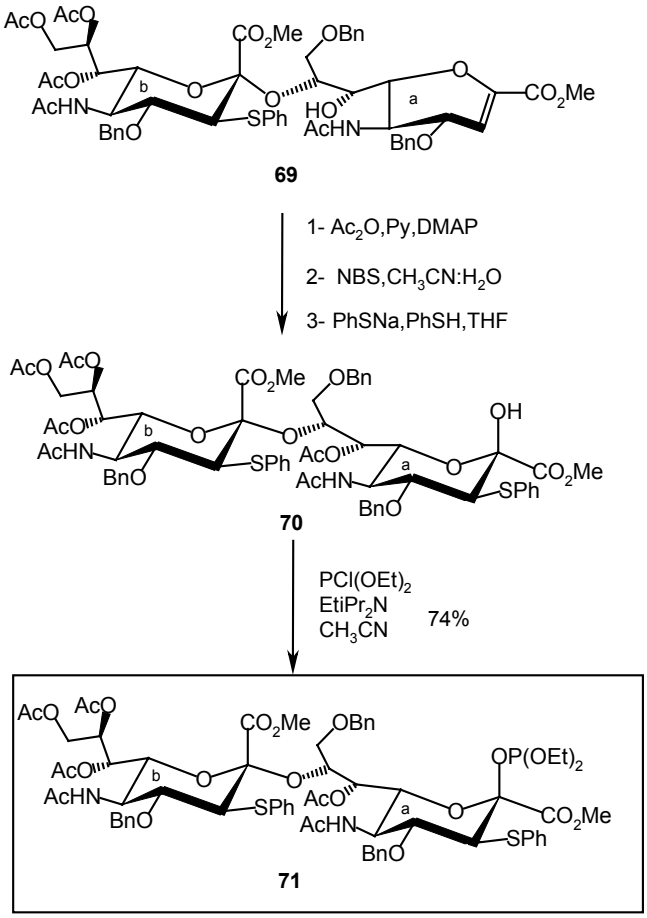


*Scheme 42. Synthesis of the Neu5Acα(2-8)Neu5Ac derivative **69***

The NMR-spectra were consistent with the structure of **69**. The stereochemistry of the glycosidic linkage was determined by measuring the long-range ^{13}C-^{1}H coupling constants: $J_{C(C-1)-H(H-3ax)}$ = 8.4 Hz corresponds to the α-linkage. The undesired β-glycoside was not observed.

2.5.3.4 Synthesis of the Neu5Acα (2→8) Neu5Ac donor 71.

The α(2→8)-linked disaccharide **69** possesses at the reducing end a 2,3-dehydro structure which permits further transformation of this derivative into the new donor **71** by the identical procedure used for the synthesis of the sialic donor **68**. Correspondingly, the 7-position of **69** was acetylated and transformed into a mixture of bromohydrines via *in situ* HOBr addition. Then, regioselective introduction of the phenylthio group at C-3 position using PhSNa/PhSH gave **70** in 55% yield; ^{1}H-NMR data: δ =3.69, 3.45 ppm (3a-H, $J_{3a,4a}$=10.5 Hz, 3b-H, $J_{3b,4a}$= 10.0 Hz). Finally the hemiacetal **70** was converted into the novel phosphite donor **71** as indicated by the ^{1}H-NMR data: δ = 3.89, 3.49 (2s, 6H, 2 OCH_3), 1.35 (2t, 6H, CH_3CH_2).



*Scheme 43. Synthesis of the novel donor **71***

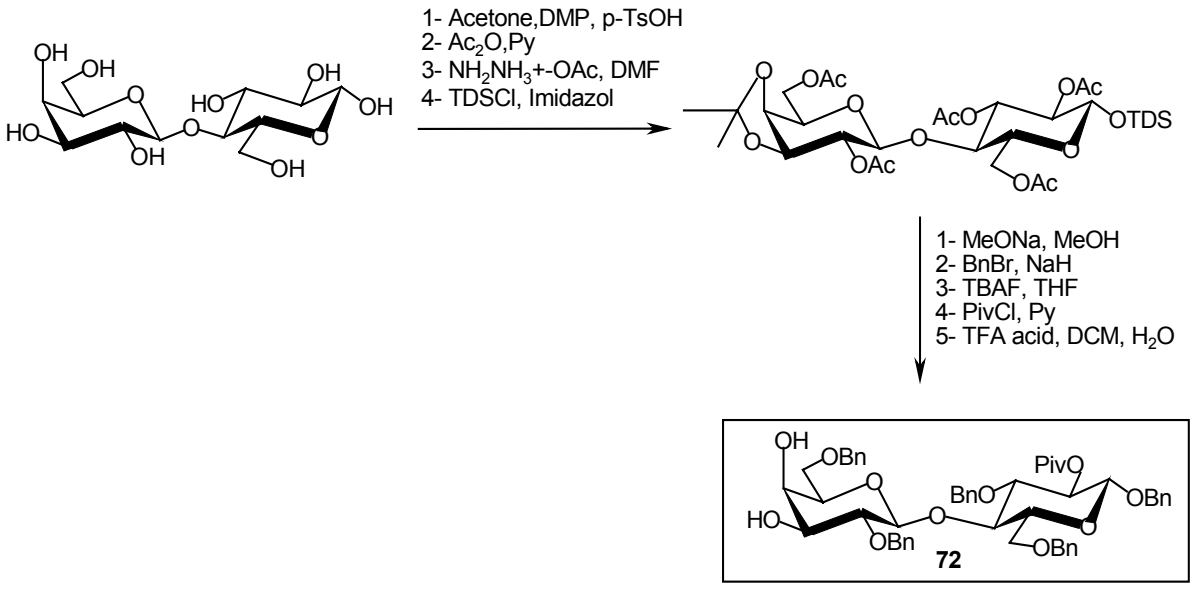
The logical next step was to test the efficiency of the new donor **71** in the sialylation reaction.

2.5.4 Sialylation with the novel donor 71

The sialyl donor **71** was expected to have the following properties:

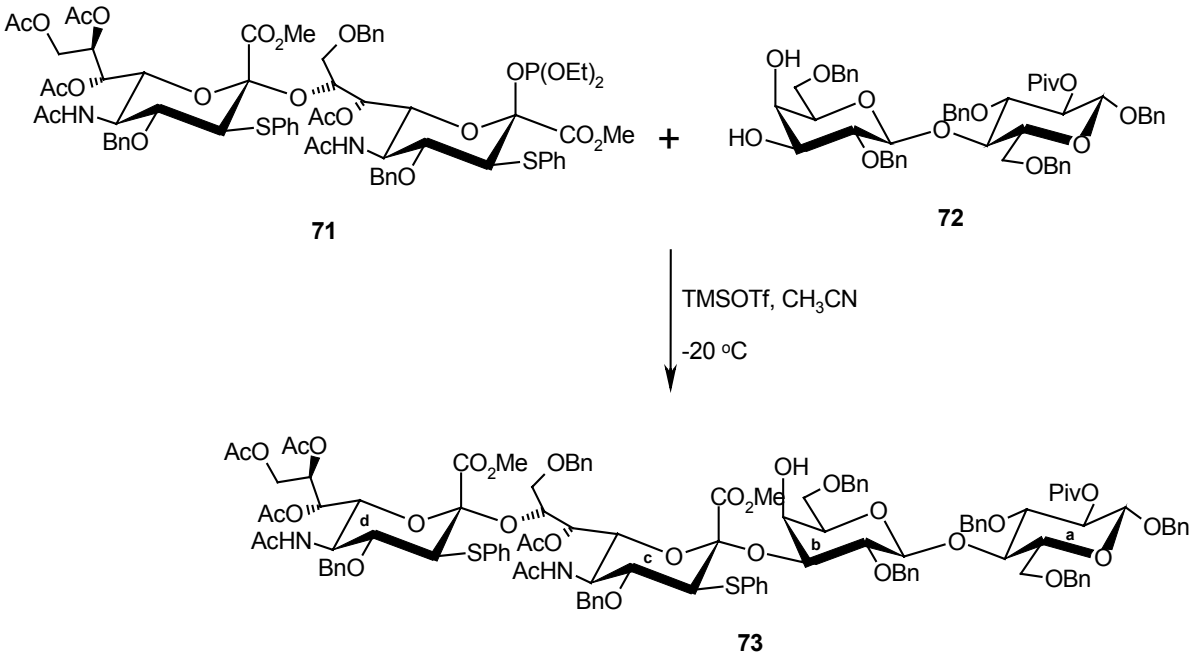
- convenient activation by the use of catalytic amounts of an acidic promoter;
- high and predictable α-stereoselectivity.

In view of these properties the sialylation reaction was carried out using the well known lactose acceptor **72**. This building block was already synthesized by Lasaletta *et al.*[90] employing the reaction sequence shown in Scheme 44.



*Scheme 44. Synthesis of the lactose derivative **72** following a procedure from Lasaletta et al*

The sialylation reaction with the new donor **71** was performed under standard conditions (CH_3CN, -20°C) in the presence of TMSOTf as promoter (Scheme 45). The desired GD3 ganglioside derivative **73** was obtained without further optimizattion in 31% yield and the structure was confirmed by NMR analysis: δ 3.32, 3.24 ppm (3d-H, $J_{3d,4d}$= 9.9 Hz; 3c-H, $J_{3c,4c}$= 9.8 Hz) , 4.39 (1a-H, $J_{1a,2a}$= 7.8 Hz), 4.17 (1b-H, $J_{1b,2b}$= 7.7 Hz). The α-stereoselectivity was based on the intense heteronuclear three-bond correlation between C-1c and H-3c ($^3J_{C\text{-}1c,\ H\text{-}3c}$ = 7.2 Hz) which is typical for an α-configuration at C-2. In addition the regioselectivity of the new tetrasaccharide was confirmed by ^{13}C-NMR: δ 76.8 ppm (C-3b) and 67.0 ppm (C-4b) which clearly indicated O-glycosylation of the 3b-position.

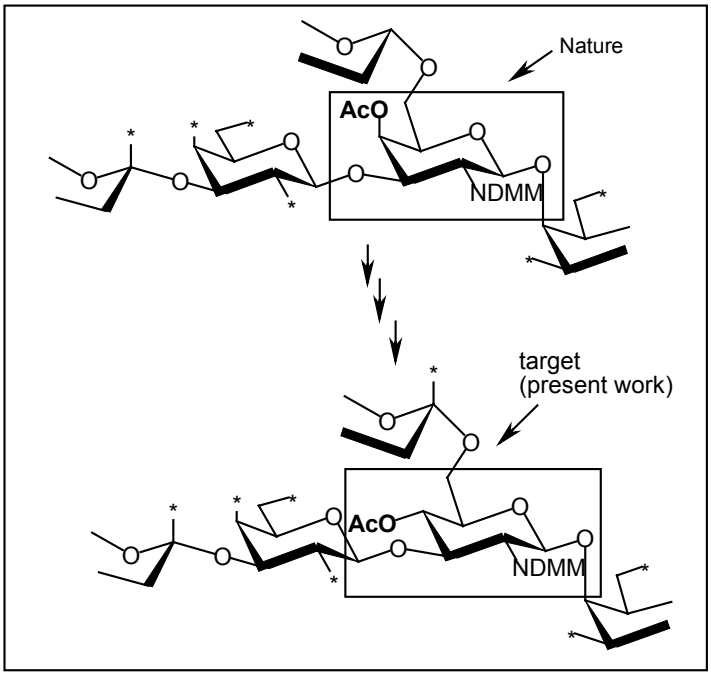


*Scheme 45. Synthesis of GD3 derivative using a new donor **71***

This experiment clearly demonstrated that the Neu5Acα (2→8) Neu5Ac donor **71** can be employed for a successful α-selective sialylation and confirmed the versatility of the strategy that combines the use of sialyl phosphite donors and neighboring group participation at 3-position for high-yields and α-selective sialylation.

2.5.5 Approaches for an efficient synthesis of the hexasaccharide

With the synthetic strategy for the disaccharide donor **71** well established, the attention was focussed on the preparation of the challenging hexasaccharide (Building block **C**). The design of an efficient and novel methodology that allows the construction of this important molecule was the highlight of the work. Due to the complex structure of this building block, which contains very expensive intermediates, and the little information about the synthetic strategy of this important molecule, a new target was selected. To analyze the feasibility of the synthetic route, the synthesis of a hexasaccharide bearing as key building block the sequence Galα(1→3)GlcNHAc instead of Galα(1→3)GalNHAc as present in the natural ganglioside was attempted.



Scheme 46. Design of a new target

Although facilitating the synthesis, this slight modification was expected to still provide valuable information for the elaboration of an efficient route for the desired ganglioside. On the other hand, oligosaccharides containing this building block are also of general importance in glycoconjugate synthesis; they have been found in *N*- and *O*-glycosidic units of glycoproteins.

The presence of the β(1→4) linkage-containing lactose to GalNHAc and GlcNHAc are a frequently occurring structural unit in nature. Various biological functions are apparently attributed to these structures.

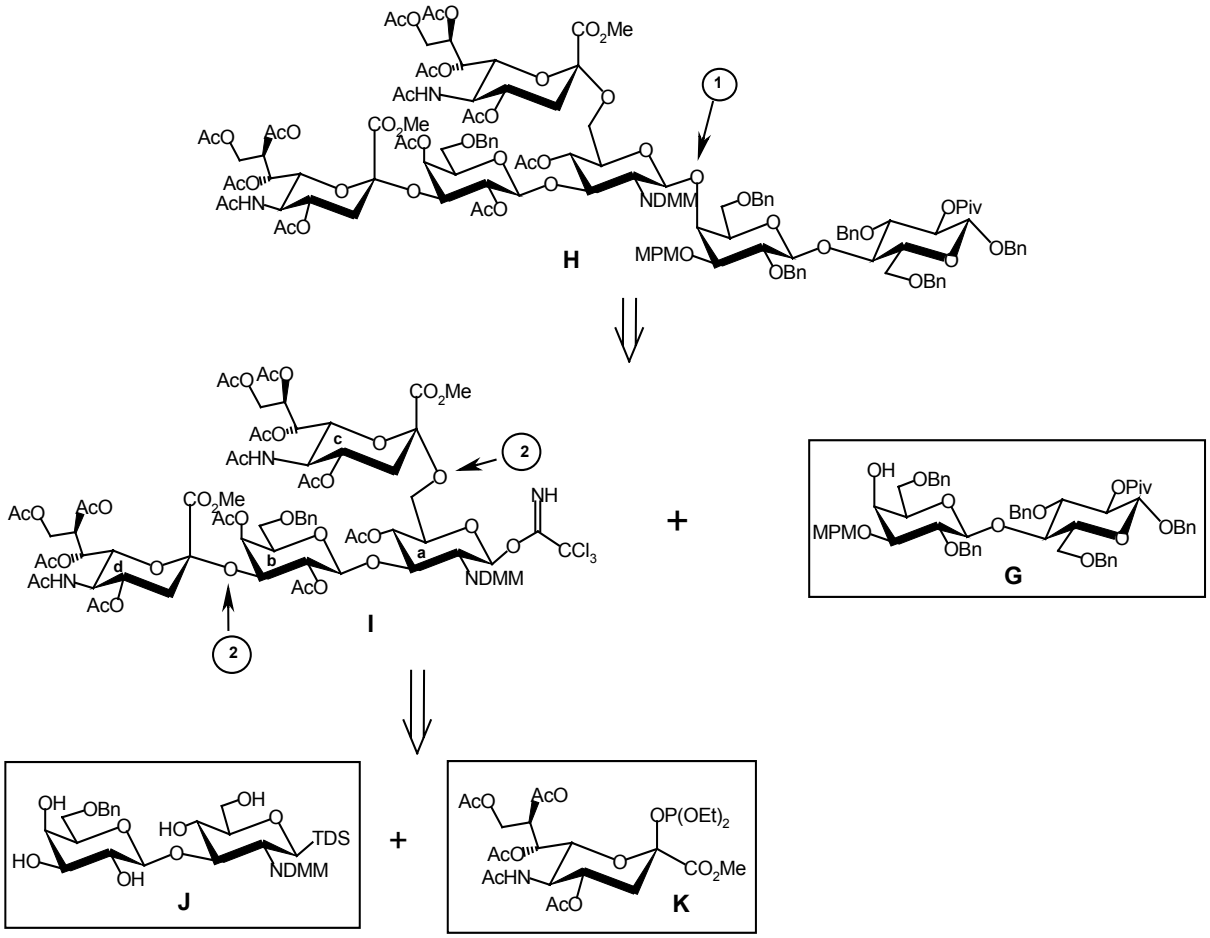
The crucial point in the synthesis of these molecules is the availability of versatile donor and acceptor building blocks that possess suitable protection, particularly for the amino group. Various protecting groups have been employed for such purposes [92,93]. Recently, Schmidt *et al.* [91] have proposed the dimethylmaleoyl (DMM) group as a new protecting group for glucosamine and galactosamine. This group was employed for the protection of 2-amino-2-deoxy sugars taking into consideration the following reasons:

- ease of introduction and cleavage;
- high reactivity of glycosyl donors and acceptors because of the electronwithdrawing group;
- excellent neighbouring participating group (enhanced β-glycoside formation);
- stability to acids and non nucleophilic bases;

- compatibility with an array of different functional groups and protecting groups;
- facile structural assignment by NMR.

The successful results obtained on the synthesis of biologically important oligosaccharides, [94] [95]using donors and acceptors possessing this protecting group, prompted us to extend the applicability of this strategy to the construction of the ganglioside GQ1bα. In Scheme 47 a retrosynthetic analysis of the target molecule **H** is shown.

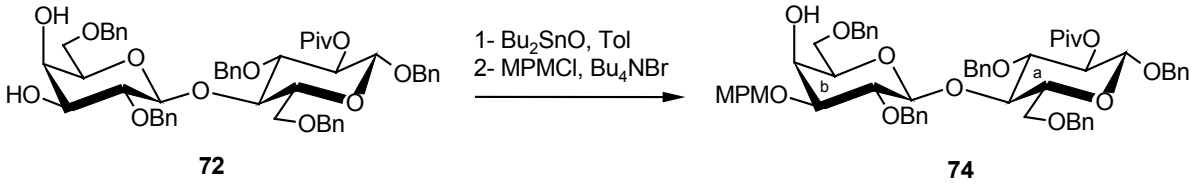
A new 3b-protected lactose derivative **G** was selected as a key glycosyl acceptor due to the higher reactivity of this position compared with the 4 position. For the preparation of the new donor **I** containing two sialic acid residues α-linked at 3b position of Gal and 6a position of GlcNHAc, respectively, a further disconnection was carried out giving the known acceptor **J** and the universal phosphite donor **K**.



*Scheme 47. Retrosynthetic analysis of hexasaccharide **H***

2.5.5.1 Synthesis of the lactose acceptor

The benzylated lactose acceptor **74** was synthesized starting from known lactose derivative **72** by selective introduction of the p-methoxybenzyl (MPM) group at the 3b position. This protecting group was selected to enhance the reactivity of the poorly reactive axial hydroxy group at the 4b position. Refluxing **72** and Bu_2SnO in toluene with azeotropic removal of the water for 5h and then addition of MPMCl and TBABr and refluxing the mixture for additional 3h, resulted in complete transformation of the starting material into the desired acceptor **74** as a faster moving spot on t.l.c (Scheme 48). The presence of the MPM group at 3-position of the galactose unit was confirmed by ^{1}H-NMR; as expected, chemical shifts appeared at δ 3.77 ppm (s, 3H, OMe) and δ 6.85 ppm (d, 2H, MPM) denoting the introduction of a new ether bond on the molecule. The ^{13}C-NMR spectrum completed the structural assignment of compound **74**; the signal at δ 80.5 ppm corresponding to the C-3b proved the O-alkylation of this position.



72 **74**

Scheme 48. Synthesis of the new lactose acceptor 74

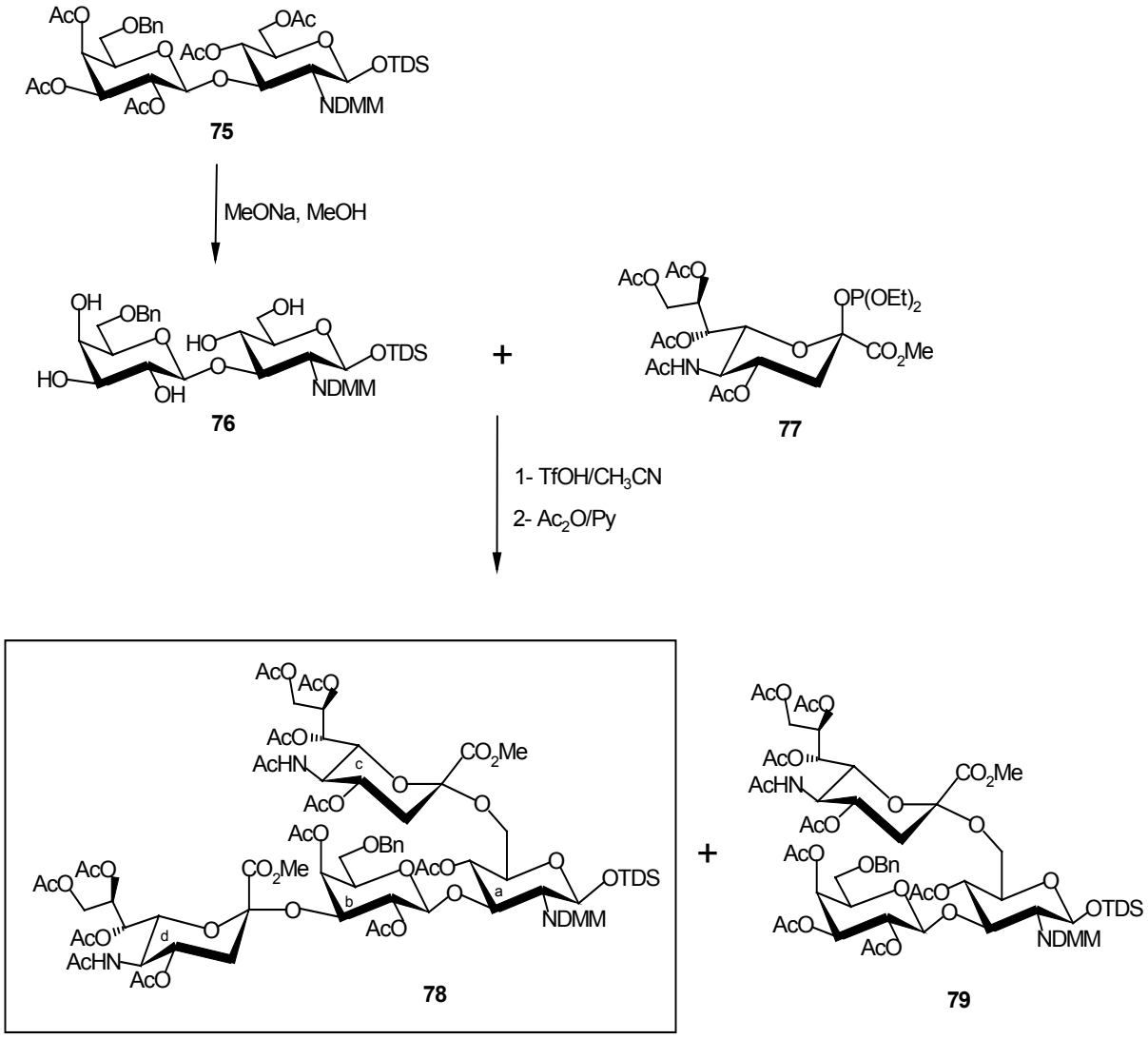
With the lactose acceptor **74** available the synthesis of the tetrasaccharide donor **I** was attempted as a new challenge.

2.5.5.2 Synthesis of the tetrasaccharide donor

The synthesis of the tetrasaccharide donor (building block **I,** Scheme 47) was designed starting from the known disaccharide **75** and the sialyl phosphite donor **77**. Thus, the disaccharide **75** which was readily synthesized using the procedure established by Schmidt *et al*.[95] was deacetylated under Zemplén conditions to give the new acceptor **76**. Then, a regioselective disialylation for the 6a and 3b position of the suitably protected disaccharide **76** was performed applying a novel strategy which made use of both the direct and inverse procedure.[96]

Reaction of sialyl donor **77** (4eq) with acceptor **76** in MeCN at –40 ^{o}C in the presence of catalytic amounts of TfOH afforded a complex mixture of α/β anomers which was difficult to

separate by normal procedures at this stage. The main side-product observed was the glycal. Direct acetylation of the reaction mixture and further purification by MPLC gave the desired disialyl tetrasaccharide **78** and the monosialyl derivative **79** at a 1:2.5 ratio accompanied by small amounts of other undesired stereoisomers (Scheme 49).



*Scheme 49. Synthesis of the tetrasaccharide **78***

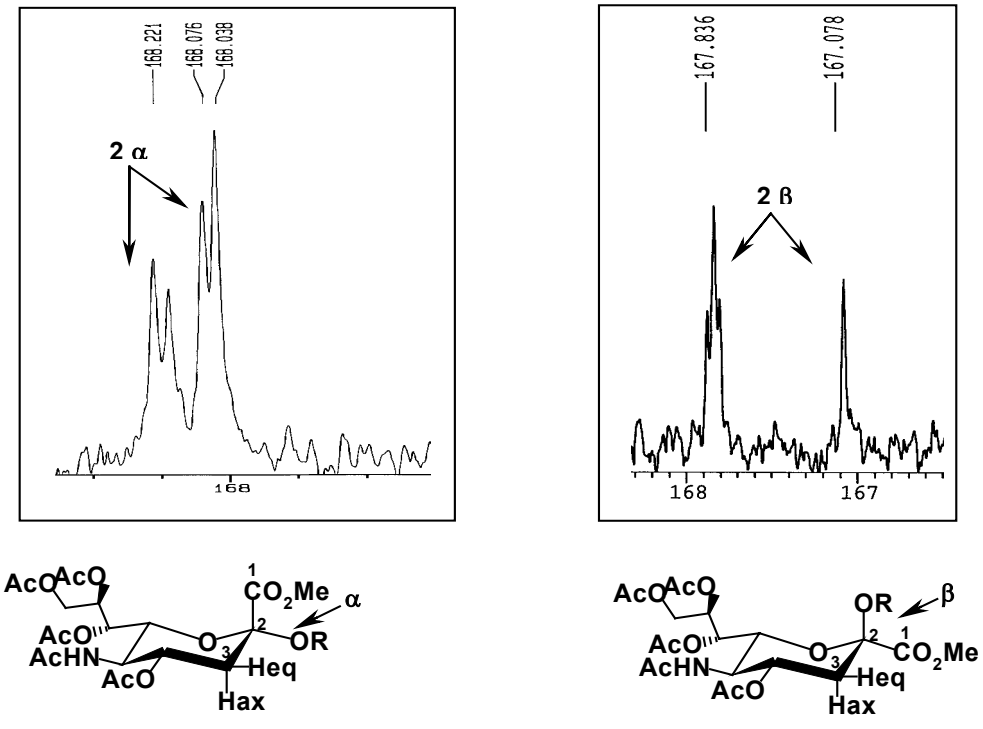
The configuration at C-2 of the sialic acid residues **c** and **d** in **78**, could not be unequivocally assigned only by ^{1}H-NMR data. However, the interproton couplings and the chemical shifts of the equatorial protons of the respective sialyl residues (3c-H_{eq} and 3d-H_{eq}), HMQC spectra and the gated decoupled ^{13}C-NMR spectra contributed to the structural assignment of both anomers (Table 3).

Glycoside	3c-Heq/3d-Heq (ppm)
α (∅)	2.62
β (∅)	2.51
78 α	2.56/2.52
78 β	2.48/2.21

*Table 3 Comparison of 1H-NMR data (600MHz) of compound **78** with average values of α- and β- glycosides of neuraminic acid as determined by Hasegawa*

Earlier NMR-studies of sialyl glycosides have shown that the equatorial H-3 proton of α-glycosides appears at lower field in the spectrum than that of β-glycosides.[97]

On the other hand, an intense heteronuclear three-bond correlation between C-1 and 3-Hax ($^3J_{C\text{-}1,\ Hax\text{-}3c,}$ = 5.8 Hz, $^3J_{C\text{-}1,\ Hax\text{-}3d}$ = 6.3 Hz) is typical for an α-configuration at C-2 and a small coupling constant value ($^3J_{C\text{-}1,\ Hax\text{-}3c,}$<1.0 Hz) for a β-configuration at C-2 (Fig 3).



$^3J_{C\text{-}1}$, $H_{eq\text{-}3}$<1.0 Hz;

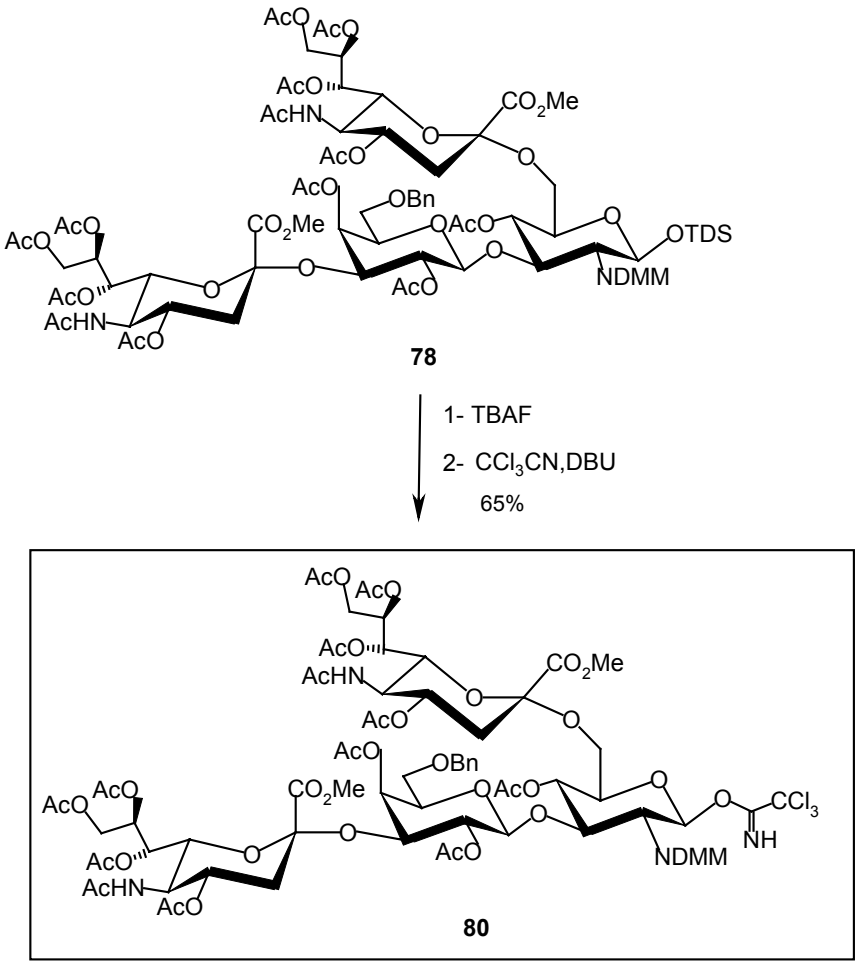
$^3J_{C\text{-}1}$, $H_{ax\text{-}3}$ = 5.8 Hz; $^3J_{C\text{-}1}$, $H_{ax\text{-}3}$= 6.3 Hz;

$^3J_{C\text{-}1}$, $H_{eq\text{-}3}$<1.0 Hz;

$^3J_{C\text{-}1}$, $H_{ax\text{-}3}$<1.0 Hz;

*Fig 3. Gated-decoupled spectra of compound **78***

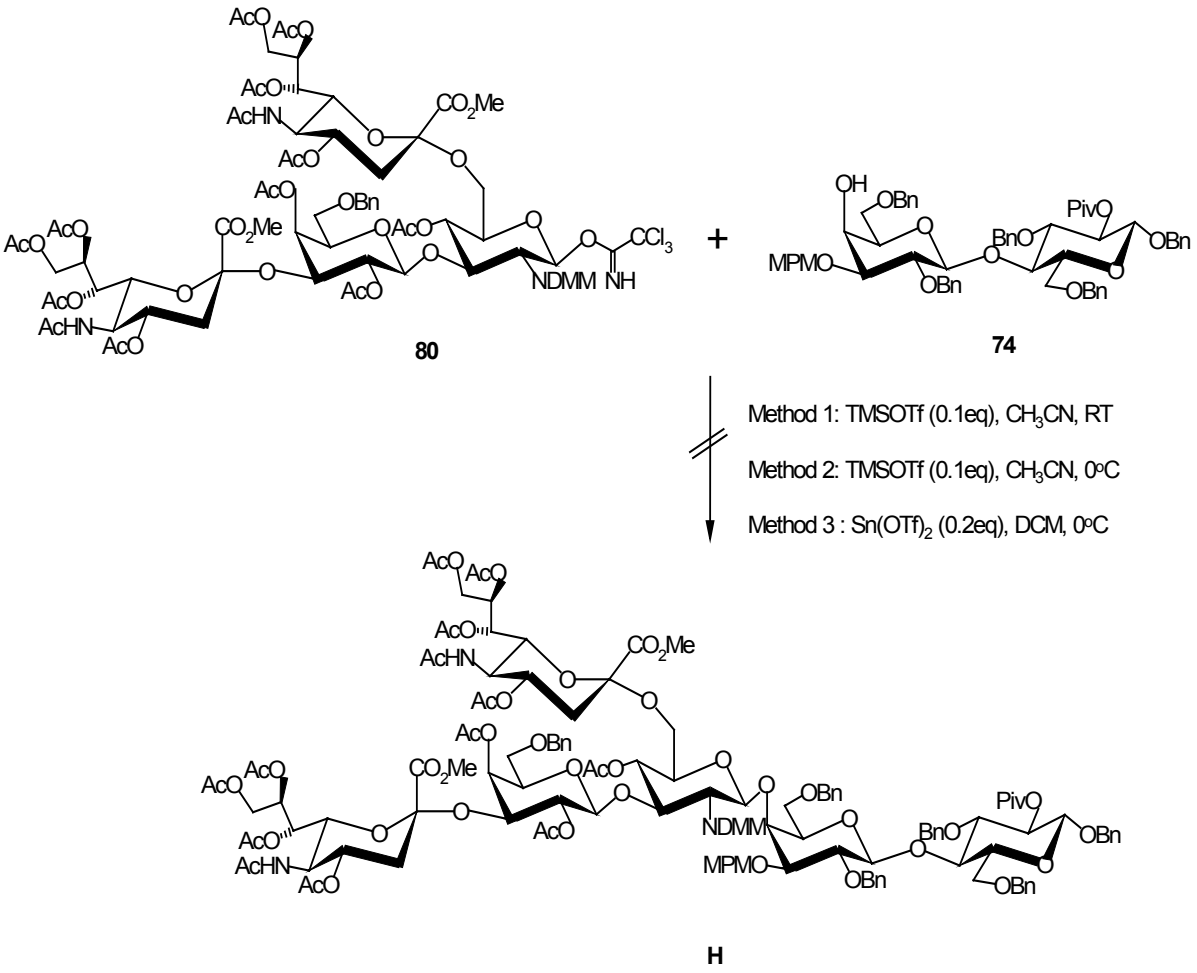
To obtain the donor **80** suitable for the sialylation reaction, the TDS protection was removed under mild reaction conditions using tetrabutylammonium fluoride solution in THF and after work up, the hemiacetal was reacted with CCl_3CN in the presence of DBU to produce the trichloroacetimidate **80** in 65 % yield (Scheme 50). As already found for *N*-DMM protected glucosamine and galactosamine, only the β-trichloroacetimidate was obtained ($J_{1a,2a}$=10.4 Hz).



*Scheme 50. Synthesis of the donor **80***

2.5.5.3 Assembly of the Hexasaccharide

With the key intermediates (**80** and **74**) synthesized and characterized, the attention was directed to the assembly of the hexasaccharide. For this purpose various attempts were performed (Scheme 51), but no product could be isolated from the reaction mixture. The reason for this failure might be due to steric hindrance of the donor **80** and particularly the low reactivity of the 4-O-position of the lactose **74**.



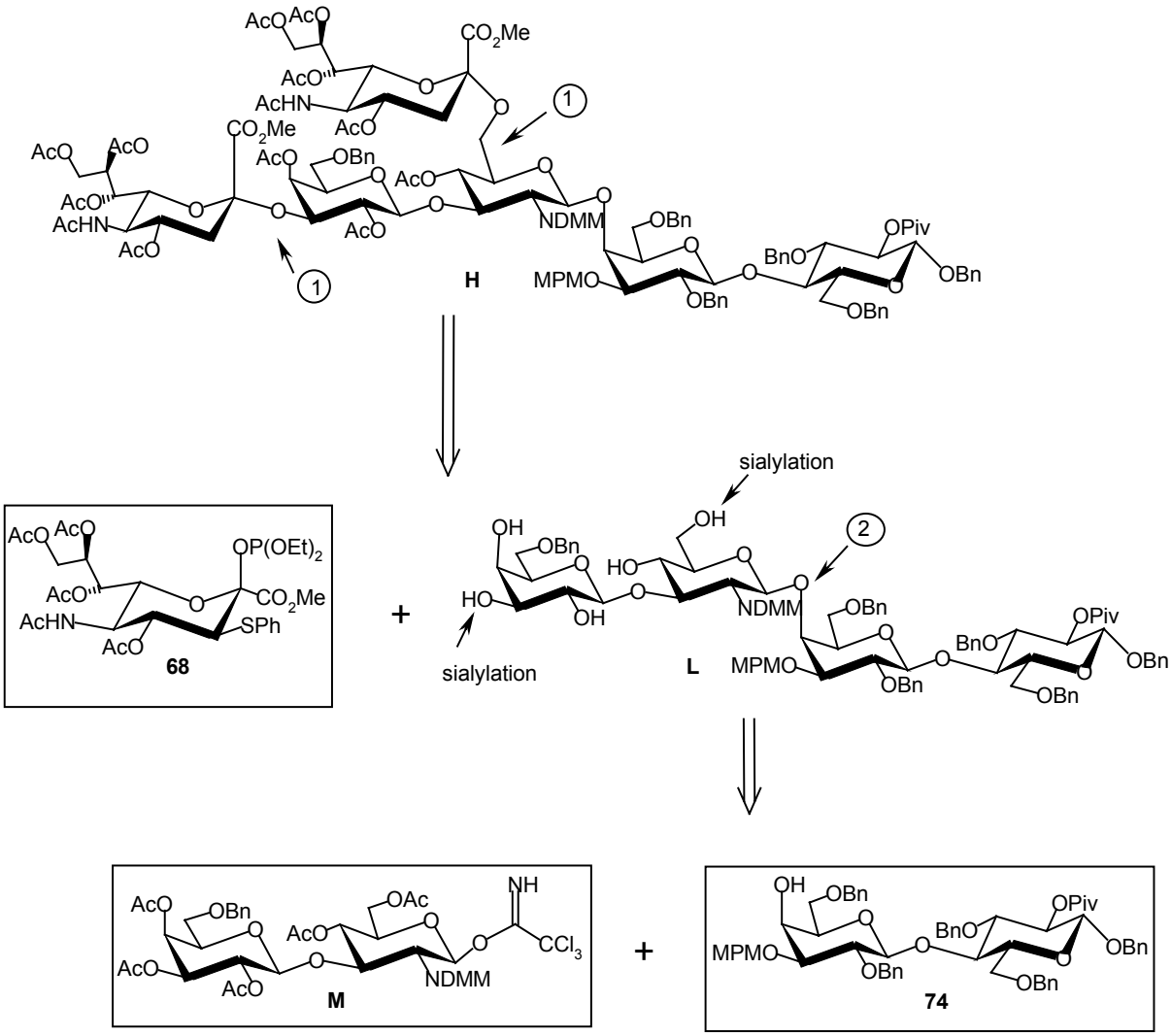
*Scheme 51. Attempts of synthesis of hexasaccharide **H***

Thus, the most important conclusions drawn from these experiments were the following:

- the novel methodology for the regioselective introduction of sialic acid moieties, opened a new way towards the synthesis of complex molecules (gangliosides) containing this residue on their structures;
- for the synthesis of the hexasaccharide a new strategy that avoids the introduction of a large building block to the unreactive 4b position of the lactose residue, has to be developed.

2.5.6 New Approach towards the synthesis of the hexasaccharide

A new alternative route for the synthesis of the desired hexasaccharide was explored by a new retrosynthetic analysis of this molecule as shown in Scheme 52.



Scheme 52. New retrosynthetic analysis of the hexasaccharide

The advantages of this new strategy should be the following:

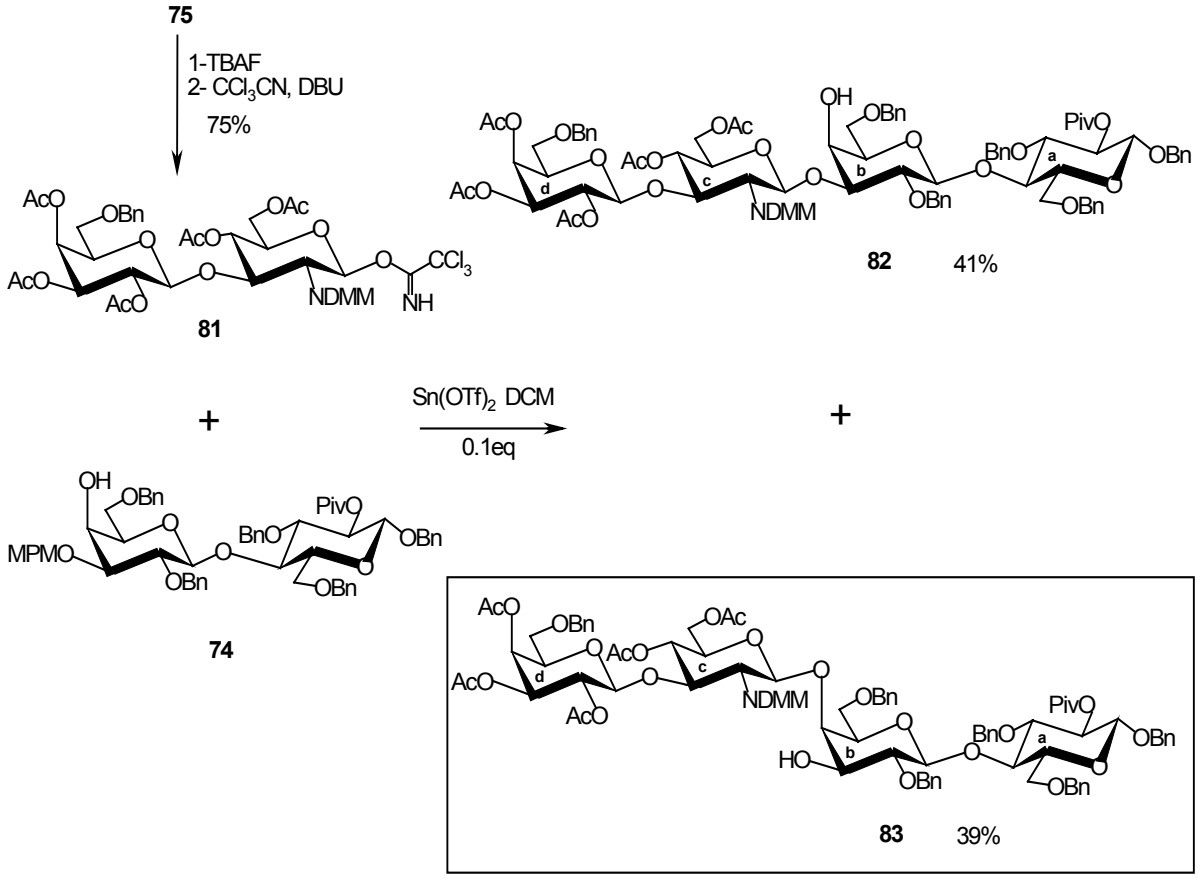
- it permits to use of already synthesized key intermediaries (**68** and **74**);
- the introduction of the disaccharide **M** into the 4b position of the lactose moiety **74** should be easier than that of the tetrasaccharide **80**;
- it permits to test the effectiveness of the disialylation reaction using a more complex molecule (tetrasaccharide **J**);
- the sialyl donor **68** is introduced which is known to enhance the stereoselectivity of the reaction, thus avoiding big purification problems at the end of the synthesis.
- introduction of the sialic acid residue at the very end of the synthesis represents the most promising aspect of the new strategy due to the high price of this molecule.

To analyze this new synthetic route the building block **L** had to be prepared.

2.5.6.1 Synthesis of the tetrasaccharide (building block L)

Initially the known disaccharide **75** was converted into a new donor **81** under standard conditions previously described for the tetrasaccharide **78** (TDS removal and introduction of the imidate leaving group). Significant signals in the ^{1}H-NMR spectra of **81** were one-proton doublet signal at δ 6.2 ppm ($J_{1a,2a}$ = 9.9 Hz, 1a-H) and one-proton singlet signal at δ 8.6 ppm (C=NH) which indicated the imidate to be in β configuration.

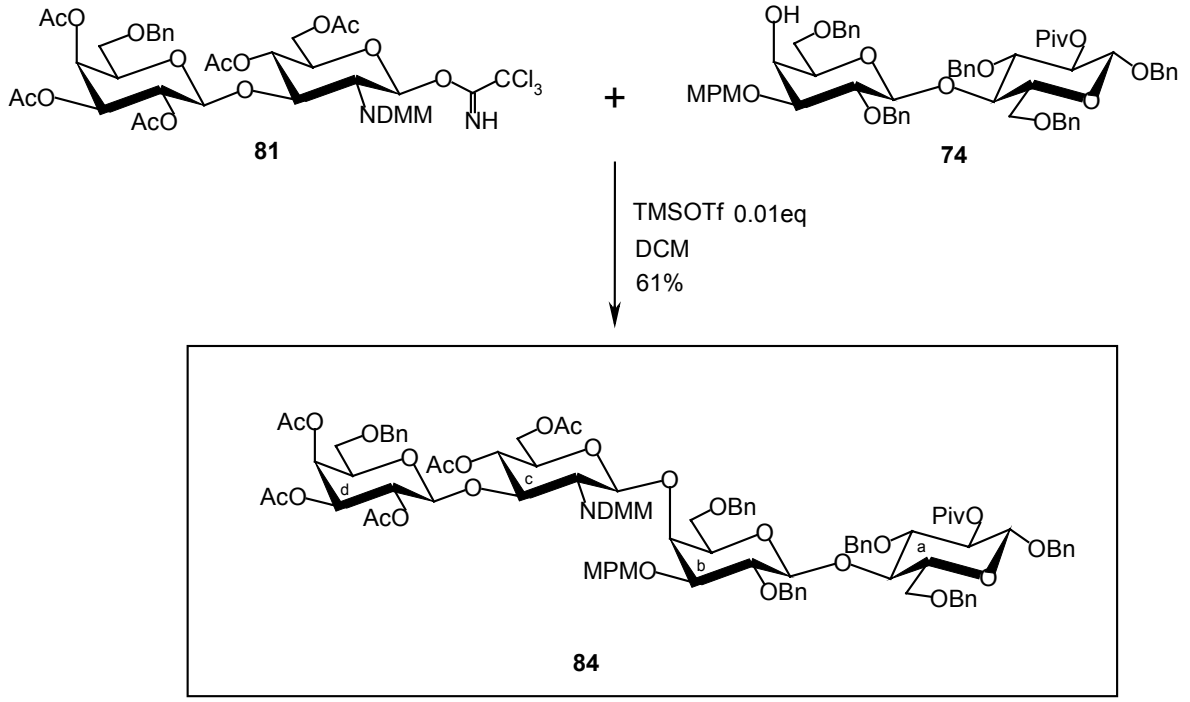
Coupling of the suitably protected lactose acceptor **74** with the new donor **81** was carried out in the presence of $Sn(OTf)_2$ (0.1eq) as catalyst and DCM as solvent; a total transformation of the starting material was observed. However, an unexpected result was obtained after chromatographic purification of the reaction mixture. Two different compounds were isolated almost in the same quantities and the analysis of the NMR-spectra indicated a mixture of the tetrasaccharides **82** and **83** (Scheme 53). The β (1→3) linkage of residue **c** to derivative **82** was assessed by ^{1}H-NMR (δ 5.13 ppm , $J_{1c,2c}$= 8.5 Hz) and ^{13}C-NMR (δ 83.9 ppm,C-3b), but the data indicated that the newly formed glycosidic linkage was at the 3-position and no at the desired 4-position. The tetrasaccharide **83** containing the β(1→4) linkage was also identified; the ^{1}H-NMR spectrum shows a doublet at δ 5.08 ppm ($J_{1c,2c}$= 8.6 Hz) that confirms the β stereoselectivity of the new bond formed and the ^{13}C-NMR data: with δ 75.7 ppm (C-4b) proved the position of the new linkage formed.



*Scheme 53. Glycosilation reaction using the donor **81** and the acceptor **74***

Based on these results we concluded that under these glycosylation reaction conditions the reactivity of the donor **81** was not sufficient for the formation of the desired tetrasaccharide **83**. Again the known low reactivity of the 4b-position of the lactose was responsible for the results. The reaction occurs very slowly allowing the hydrolysis of the p-methoxybenzyl group (MPM) under acid conditions. Thus, the 3,4b-O-unprotected lactose **72** is formed and the attack of the donor **81** can occur at both positions. To explain the similar yield of both compounds, it was assumed that the less hindered lactose **72** allows the introduction of donor **81** at both positions.

This unexpected result was reason to investigate another promoter system that would avoid the hydrolysis of the p-methoxybenzyl group and allow regioselective glycosylation at the desired 4b-O-position. Investigation of the reaction using DCM at 0^{o}C and TMSOTf (0.01eq) furnished the desired tetrasaccharide **84** as the sole stereoisomer in acceptable yield 61% (Scheme 54).



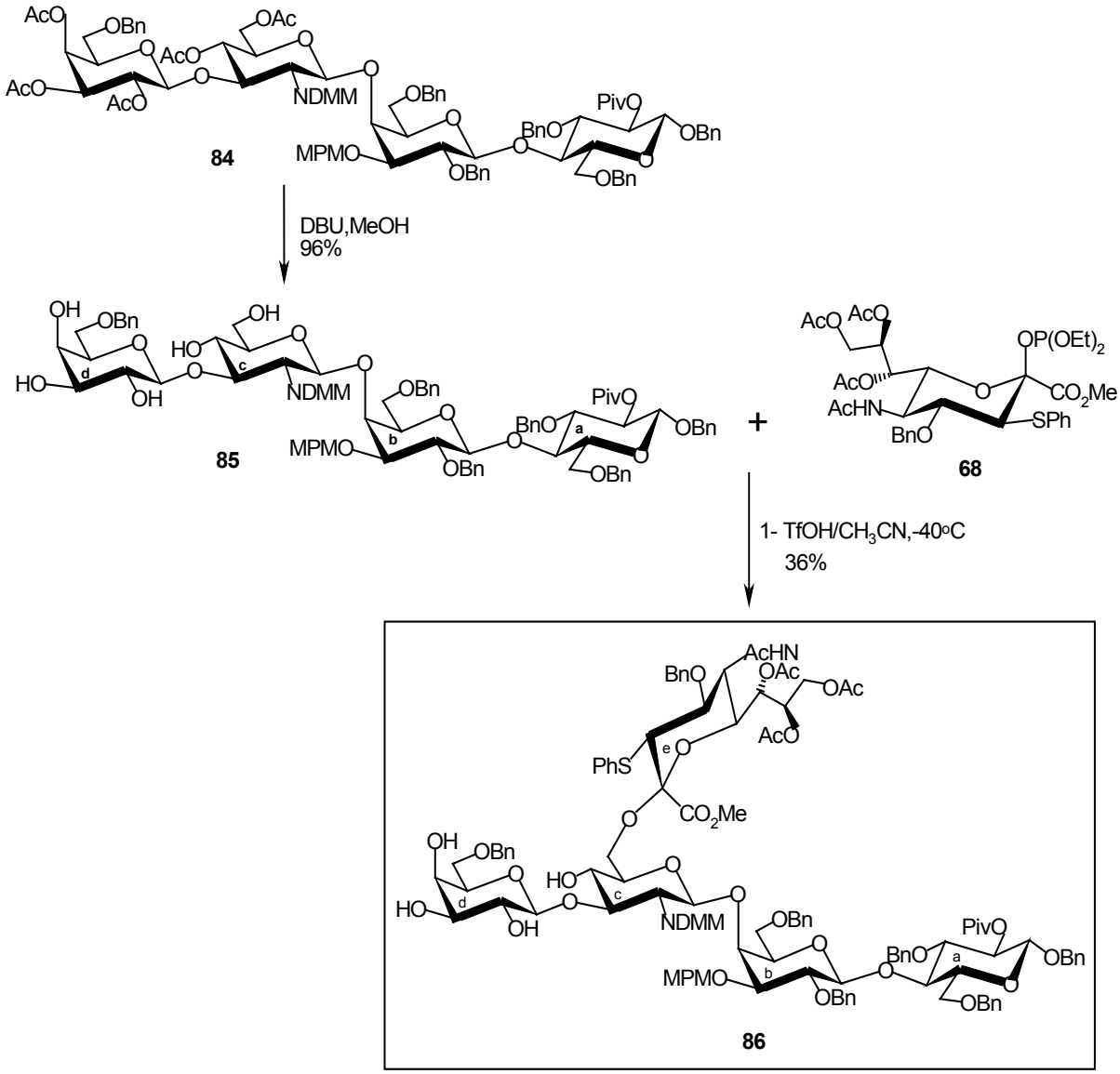
*Scheme 54. Synthesis of the tetrasaccharide **84***

Under these conditions no β(1→3) linkage was detected, and the structure of the compound **84** was assessed by ^{1}H-NMR: δ 4.98 ppm (d, $J_{1c,2c}$ =8.3 Hz, 1c-H) and δ 3.78 ppm (s, CH_3O, MPM).

2.5.6.2. Regio- and stereoselective sialylation (I)

Upon the successful synthesis of the tetrasaccharide **84**, the attention was directed to the conversion of this building block into a new acceptor **85** suitable for further sialylation reaction at the 6c-O and 3d-O-positions (Scheme 55). Therefore, regioselective removal of the acetyl groups in the presence of the pivaloyl group was accomplished using DBU in methanol giving the desired acceptor **85** in a high overall yield. In the next step we exploited the advantages offered by the disialylation reaction using the new tetrasaccharide acceptor **85** and the known sialyl donor **68**, bearing a thiophenyl group as anchimerically assisting group at the β-face of the 3-position that should avoid the formation of an undesired mixture of stereoisomers. Sialylation of the tetrasaccharide derivative **85** was performed using the same procedure employed for the synthesis of **78**. A combination of the direct and inverse procedure in the presence of catalytic amounts of TfOH was expected to give the α-sialoside derivative. The complete transformation of the starting material on the t.l.c confirmed the formation of new products. After separation of the reaction mixture by MPLC, the main

compound was identified as the pentasaccharide **86** containing a sialic acid moiety with an α (2→6) linkage (Scheme5).



*Scheme 55. Sialylation using the acceptor **85***

This result is the logical consequence of the higher reactivity of the primary 6c-OH group compared with the secondary 3d-OH group. The second fraction obtained by the purification was the desired hexasaccharide according to analysis by MS-MALDI: *m/z* = 2803.2 [M+Na^+]; but due to the small amount obtained and the additional contamination by other side-products, no further characterization was possible (Fig 4).

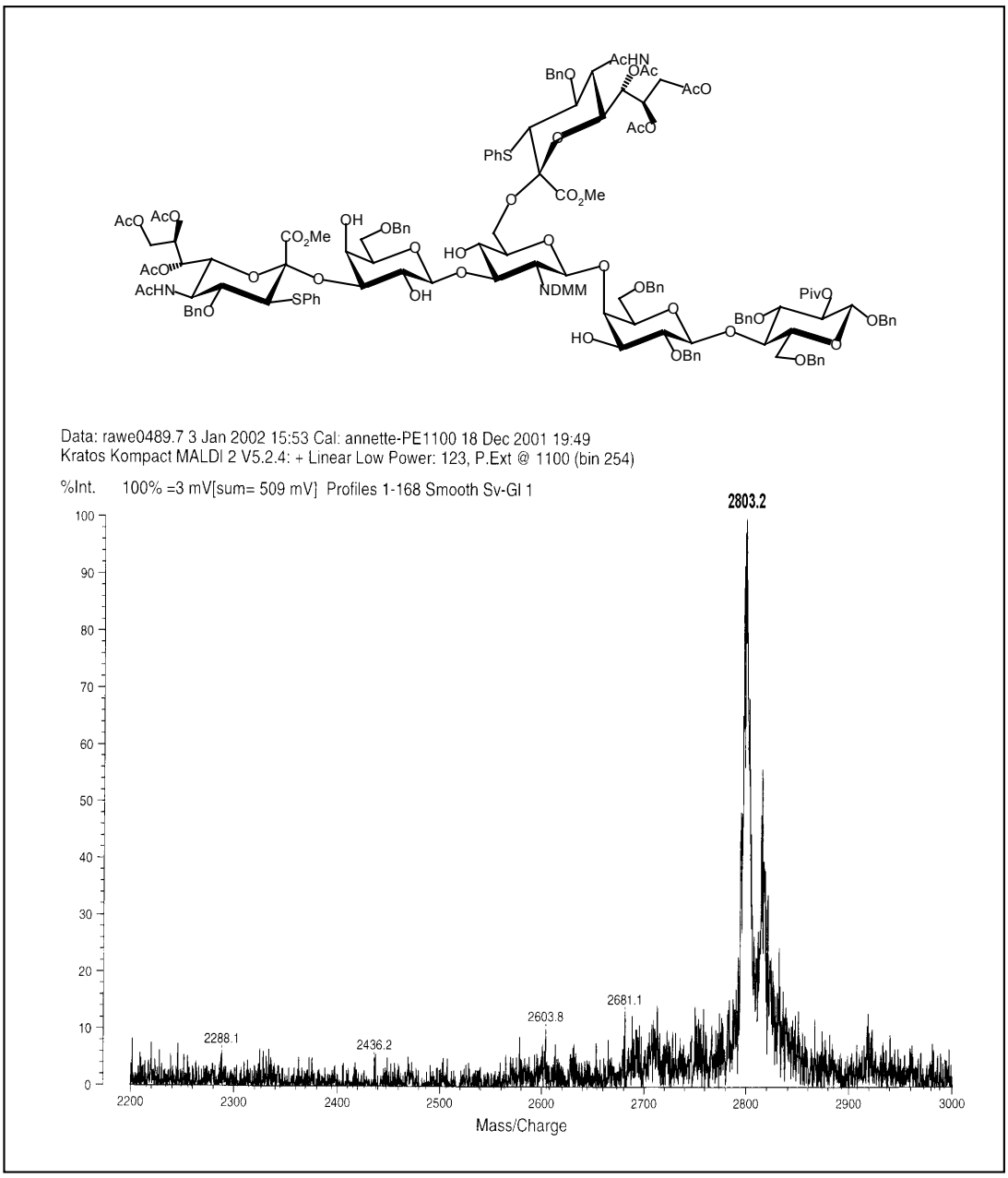


Fig 4. MS-MALDI of the hexasaccharide

Therefore, a new sialylation attempt was carried out using the tetrasaccharide **83** as acceptor precursor.

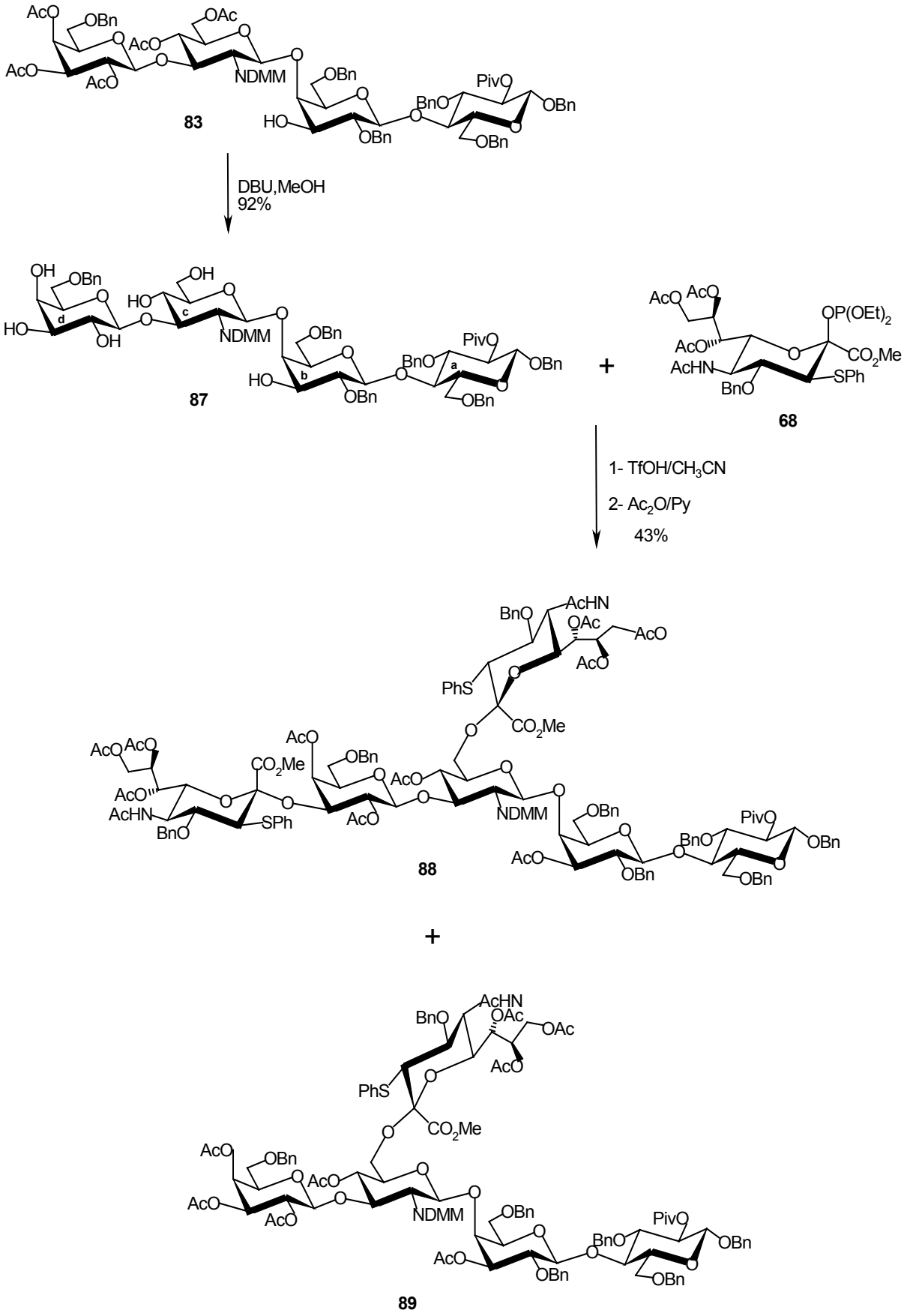
2.5.6.3 Regio-and stereoselective sialylation (II)

The conversion of the tetrasaccharide **83** into the new acceptor **87** was accomplished using the same procedure established for the preparation of **85**. However, this new derivative **87** creates a new challenge, since not only the 6c and 3d position of this molecule are potentially sialylated, but also the 3b position. A tris-sialylation reaction represents without any doubt a big task so far not studied.

For this purpose a final glycosylation reaction of the two key intermediates **87** and **68**, following the procedure already mentioned, was attempted. In this reaction 6 equiv of the sialyl donor **80** per acceptor **87** were used in order to ensure the desired transformation. After 30 min, complete conversion of the starting material into faster moving spots on the t.l.c. very close to each other, confirmed the formation of at least two new products. The reaction mixture could not be quantitatively separated at this stage even using MPLC techniques; due to the difficult and laborious separation of the components, the mixture was *O*-acetylated and then purified. The main compounds isolated from this purification were **88** and **89** (Scheme 56). Although the desired hexasaccharide **88** was obtained in significantly minor quantity than the pentasacharide **89**, the average yield was higher compared with the one obtained using the tetrasaccharide acceptor **85**. The structural assignment of compound **88** was obtained by NMR analysis: δ = 3.02–3.17 ppm (2d, H-3e, 3f-H, $J_{3,4}$ = 7.7 Hz), δ= 3.98-4.19 ppm (m, 4H, 9e-H, 9f), 4.23 (d, 1H, 1d-H), 4.31 (d, 1H, 1b-H), 4.46 (d, 1H, 1a-H), 5.14 (d, 1H, 1c-H).

However, no tri-sialylated derivative could be detected in the reaction mixture, a fact that is not unexpected because of the higher steric hindrance of the 3b-O-position compared with the 3d- and 6c-O-positions.

In conclusion, the synthesis of the hexasaccharide **88**, constituted an appreciable innovation in the synthesis of sialo derivatives and might be of interest for the design of the GQ1αb ganglioside and related analogues.



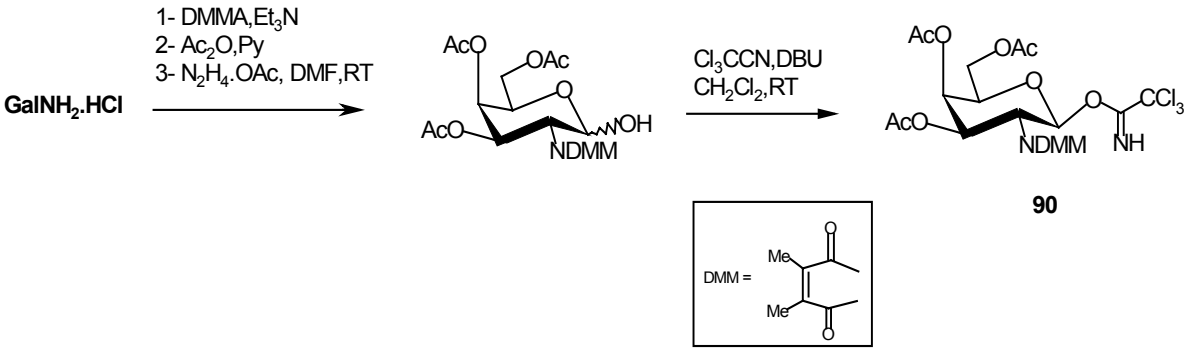
*Scheme 56. Synthesis of the hexasaccharide **88***

2.6 Towards an efficient synthesis of complex molecules containing GalNAcβ(1→4)Lac linkage. Use of DMM as Nitrogen protecting group

In the preceeding sections a new methodology was described for the synthesis of GQ1bα ganglioside using Galβ(1→3)GlcNAc as key intermediate. A number of important structures were successfully prepared which can be used directly for the synthesis of gangliosides. Encouraged by these results, a more demanding glycosylation using GalNAc derivatives as glycosyl donor was studied. For this purpose a series of molecules containing as a common feature the natural GalNAcβ(1→4)Lac linkage, were selected. A systematic study of the influence of donor structures on the efficiency of glycosylation for the well known unreactive 4b-OH-position of the lactose is highly desirable in order to implement synthetic strategies for various gangliosides as well as glycoconjugates.
The targets chosen are partial gangliosidic structures that could serve as key intermediates in the synthesis of other members of the ganglio-series.

2.6.1 Introduction of GalNDMM

The first structure addressed was the trisaccharide GalNAcβ(1→4)Galβ(1→4)Glc. It is the simplest motive of all structures in the ganglio-series and it is the basis for all more complex molecules. Initially the glycosyl donor **90** was prepared from galactosamine hydrochloride over four steps following a published procedure (Scheme 57).[91]

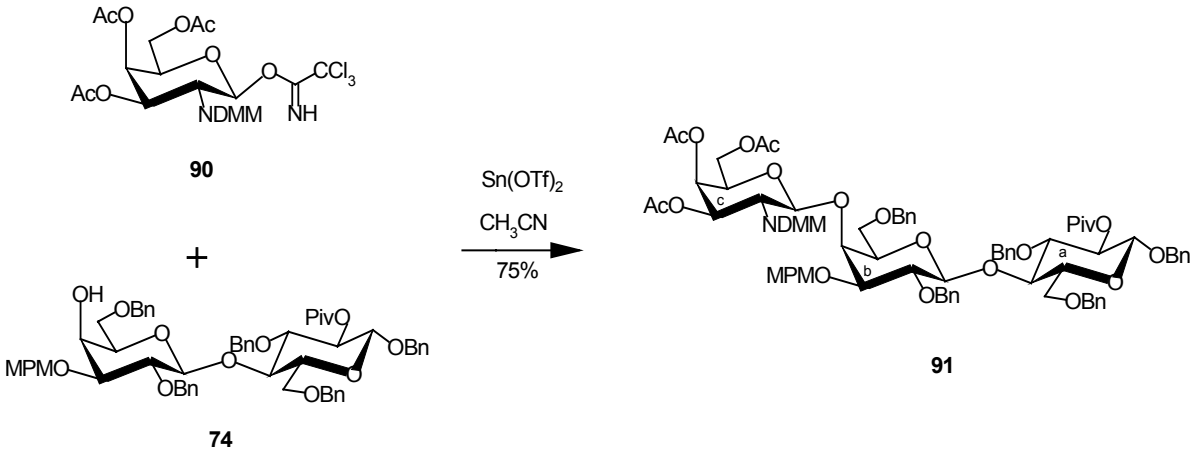


*Scheme 57. Synthesis of the donor **90***

As a first test case the glycosylation reaction of standard 4b-OH unprotected lactose acceptor **74** and the donor **90** was performed in acetonitrile at room temperature with

$Sn(OTf)_2$ activation. The glycosylation product obtained was the β-linked trisaccharide **91**, which was isolated in 75% yield (Scheme 58).

The ^{1}H-NMR spectrum confirmed the structure of compound **91**. The β-configuration of the GalNDMM residue **c** appeared as a doublet at δ=5.17 ppm (1c-H, $J_{1,2}$= 8.4 Hz), thus having a big coupling constant with 2c-H.



Scheme 58. Synthesis of the trisaccharide GalNDMMβ(1-4)Galβ(1-4)Glc

2.6.2 Introduction of Neu5Acα(2→6)GalNDMM

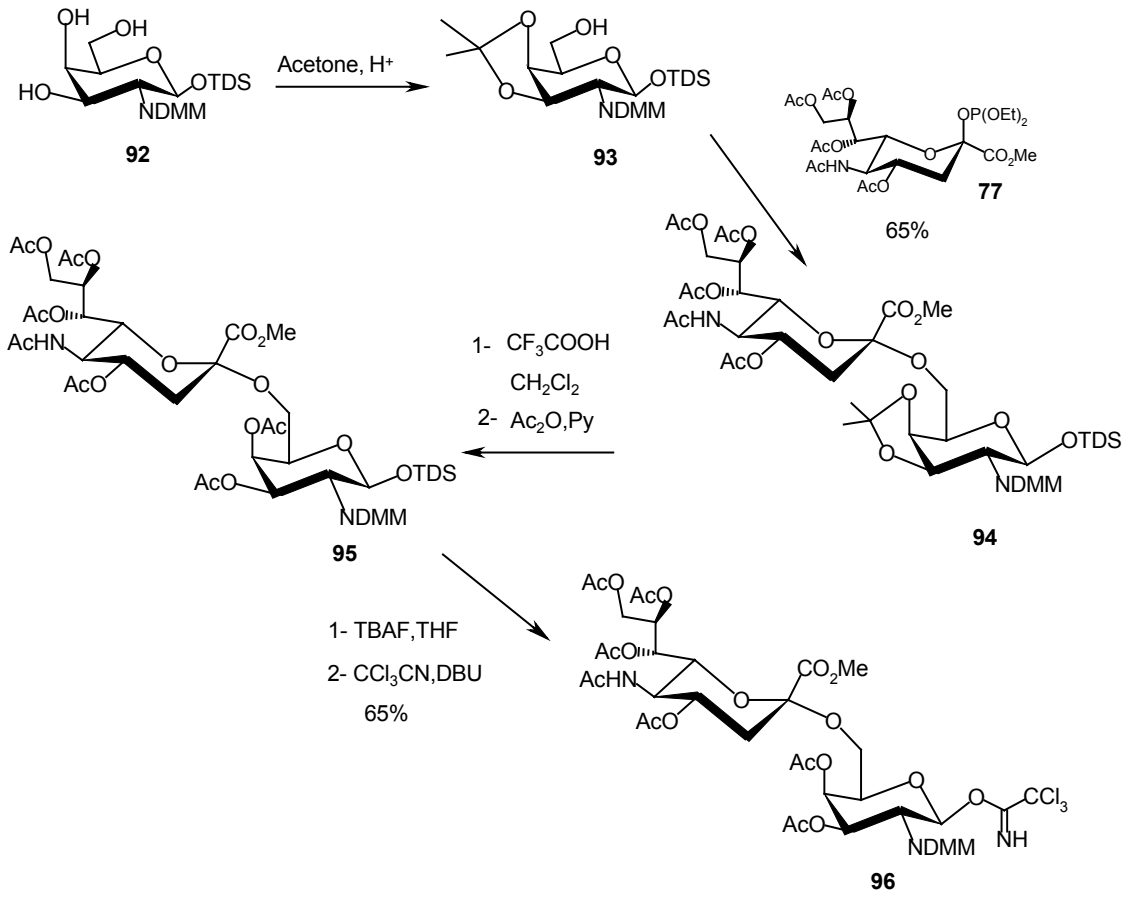
Because oligosaccharides containing this building block occur frequently in nature, the synthesis of this structure was also investigated. The α(2→6) linkage is an example of a "side chain" terminal sialic acid and has also been found in *N*- and *O*-glycosidic oligosaccharide units of glycoproteins.

First, a suitably protected donor **96** had to be prepared. To obtain an acceptor suitable for the sialylation reaction, the triol **92** was selectively protected at the positions 3 and 4 using the isopropylidene moiety. The acceptor **93** was elongated by a sialic acid residue at position 6. In this reaction neuraminic acid phosphite **77** was coupled at –40 °C in the presence of CH_3CN using TMSOTf activation. This reaction afforded a mixture of α- and β isomers at a 4:1 ratio accompanied by the protected 2-deoxy-2,3-dehydroneuramic acid ester. The mixture was separated by flash chromatography and the α-glycosidic-linked disaccharide **94** was obtained in 65% yield (Scheme 59). The α-glycosidic linkage of neuraminic acid was confirmed by comparing the chemical shifts of 3b-H_e and 4b-H, and the differences of the chemical shift of 9-H and 9'H with average values determined by Hasegawa *et al* [97] (Table 4).

Glycoside	3-Heq (ppm)	4-H (ppm)	Δσ [9-H-9'-H] (ppm)
α(∅)	2.62	4.87	0.21
β(∅)	2.51	5.27	0.70
94	2.57	4.84	0.19

*Table 4 Comparison of 1H-NMR data (600MHz) of compound **94** with average values of α-and β- glycosides of neuraminic acid as determined by Hasegawa*

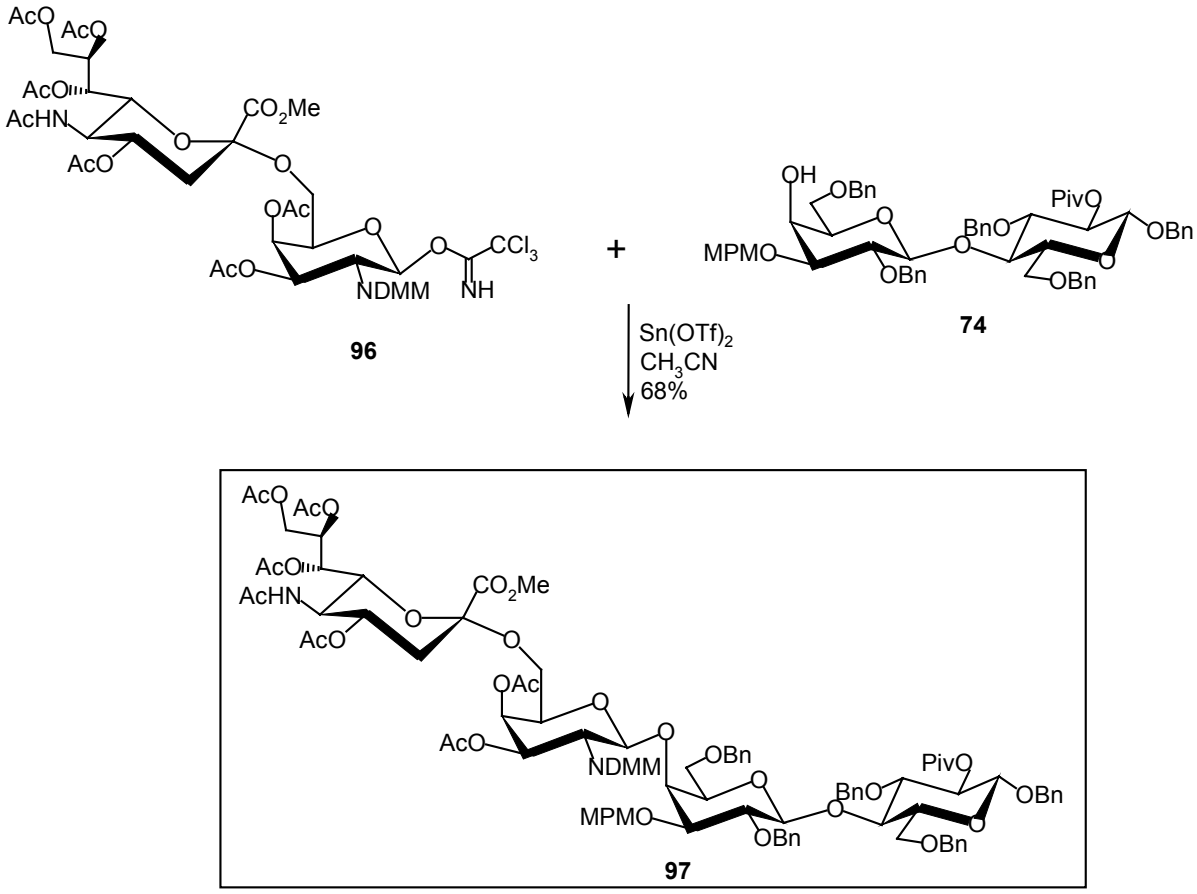
Transformation of the disaccharide **94** into the new donor **96** was achieved in several steps. First, the 3,4-O-isopropylidene group was cleaved by acids, and the free hydroxy groups were acetylated (→ **95**). Then removal of the silyl ether with TBAF in acetic acid and subsequent reaction of the resulting hemiacetal with CCl_3CN in the presence of catalytic amounts of DBU produced the trichloroacetimidate **96** in 65% yield (Scheme 59).



*Scheme 59. Synthesis of the donor **96***

The optimised reaction conditions from the previous experiments were again applied. Thus 68% of the desired tetrasaccharide **97** was formed (Scheme 60). The new β linkage could

be readily confirmed from the coupling of the anomeric proton (δ 5.27 ppm, $J_{1c,2c}$ = 8.4 Hz) typical for β-glycosides.



Scheme 60. Synthesis of the tetrasaccharide Neu5Acα(2-6)GalNDMMβ(1-4)Lac

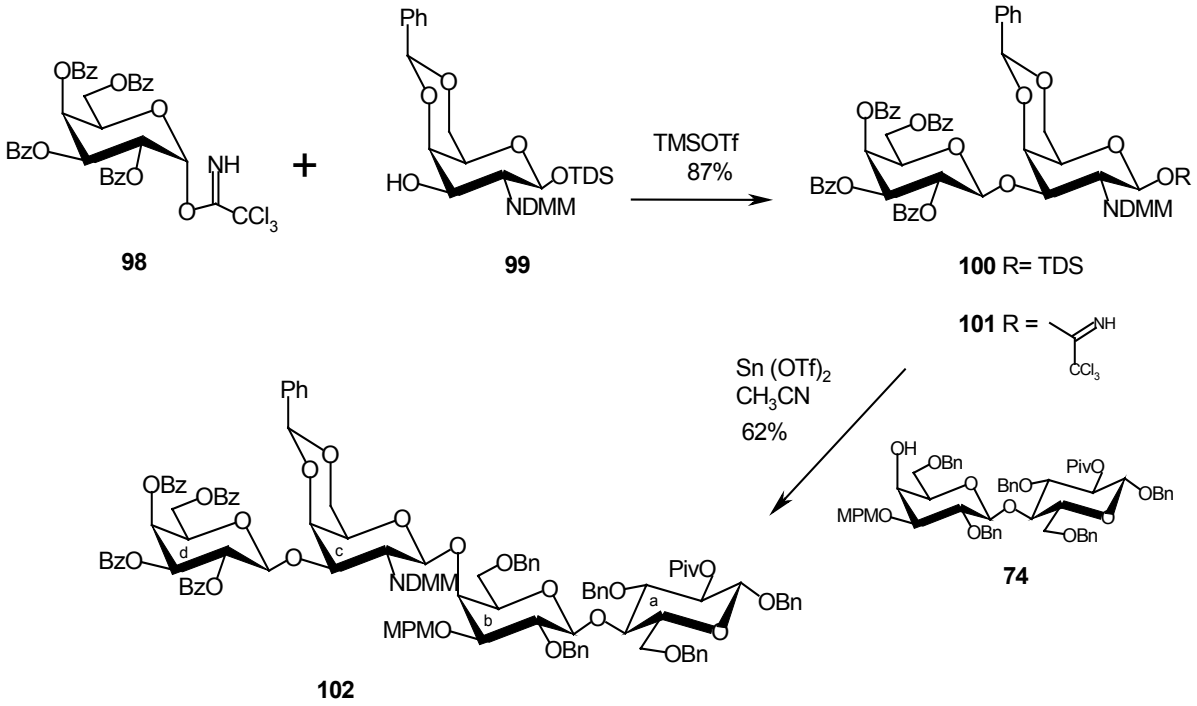
The moderate yield of the desired tetrasaccharide **97** (68 %) is however, rather appreciable taking into account the generally low nucleophilicity of the axial 4-hydroxy group of galactose and the relative bulkiness of the donor **96**.

2.6.3 Introduction of Galβ(1→3)GalNDMM

The sequence Galβ(1→3)GalNAcβ(1→4)Galβ(1→4)Glc is a frequently found as part of glycoconjugates including various gangliosides and it represents a key building block in the synthesis of compounds of the ganglio-series. The disaccharide building block **100** was obtained in 87% yield from the donor **98** and acceptor **99** by glycosylation in the presence of TMSOTf as promoter to produce the desired β-linked disaccharide. The β-glycosidic linkage was confirmed by the coupling of the anomeric proton ($J_{1b,2b}$ = 7.8 Hz). Conversion

of the selectively protected disaccharide **100** to the corresponding donor **101** was effected in two steps in one-pot procedure as has been described before (Scheme 61).

To obtain the desired tetrasaccharide **102**, the reaction between glycosyl donor **101** and lactose acceptor **74** was performed. It proceeded smoothly under the standard conditions already established giving **102** in 62 % yield. The β linkage of residue **c** was derived from NMR spectroscopic data (^{1}H-NMR: $J_{1c,2c}$ = 8.4 Hz).



Scheme 61. Synthesis of the tetrasaccharide Galβ(1-3)GalNDMMβ(1-4)Lac

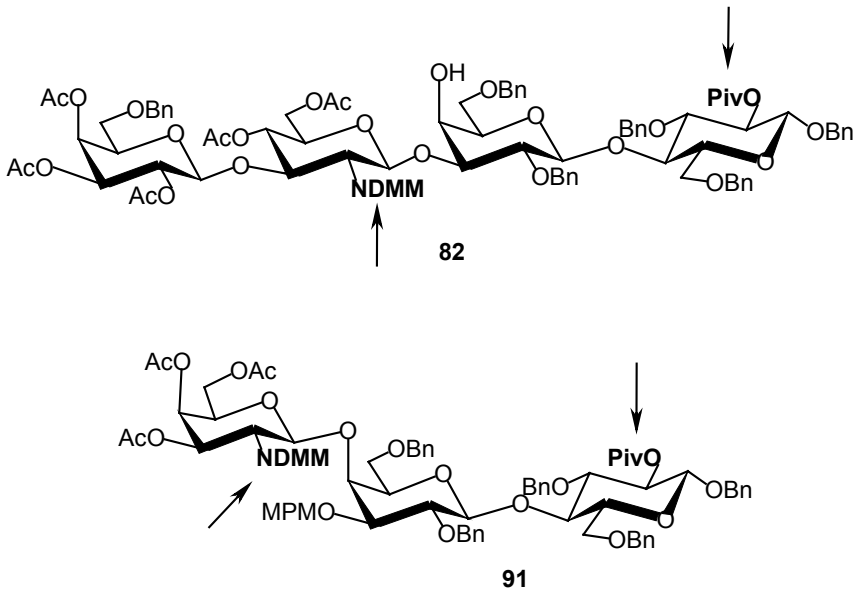
With the synthesis of the building blocks **91**, **97** and **102** it was shown that the conceptually new approach using DMM group as protecting group of the galactosamine moiety can be applied efficiently and in a straightforward manner to ganglioside synthesis. All reactions proceeded smoothly at room temperature to completeness within 15 min-1h. Uniformly, in all cases studied the desired β-glycosides were isolated in good yields.

2.6.4 Deprotection of the Dimethylmaleoyl (DMM) protecting group

Having demonstrated the efficiency of this strategy using the excellent properties of the DMM protecting group in glycosyl donors and glycosyl acceptors, it was evident that the liberation of the amino group and generation of the N-acetyl function was the next important step to take into consideration.

The DMM protecting group could be removed in one step by sodium hydroxide and then mild acid treatment as described previously.[94] However, the reaction conditions (time, solvents, work up) might change from one molecule to the other depending upon the complexity, protecting group pattern, solubility etc. The successful cleavage of the DMM group in the presence of a neuraminic acid in the molecule was already studied by Schmidt *et al*.[98]but so far, not in molecules containing the pivaloyl group.

In order to extend the use of DMM group as amino protecting group and to evaluate its properties under standard reaction conditions as well as its compatibility with the general approach already discussed, the two representative molecules **82** and **91** were studied (Scheme 62).

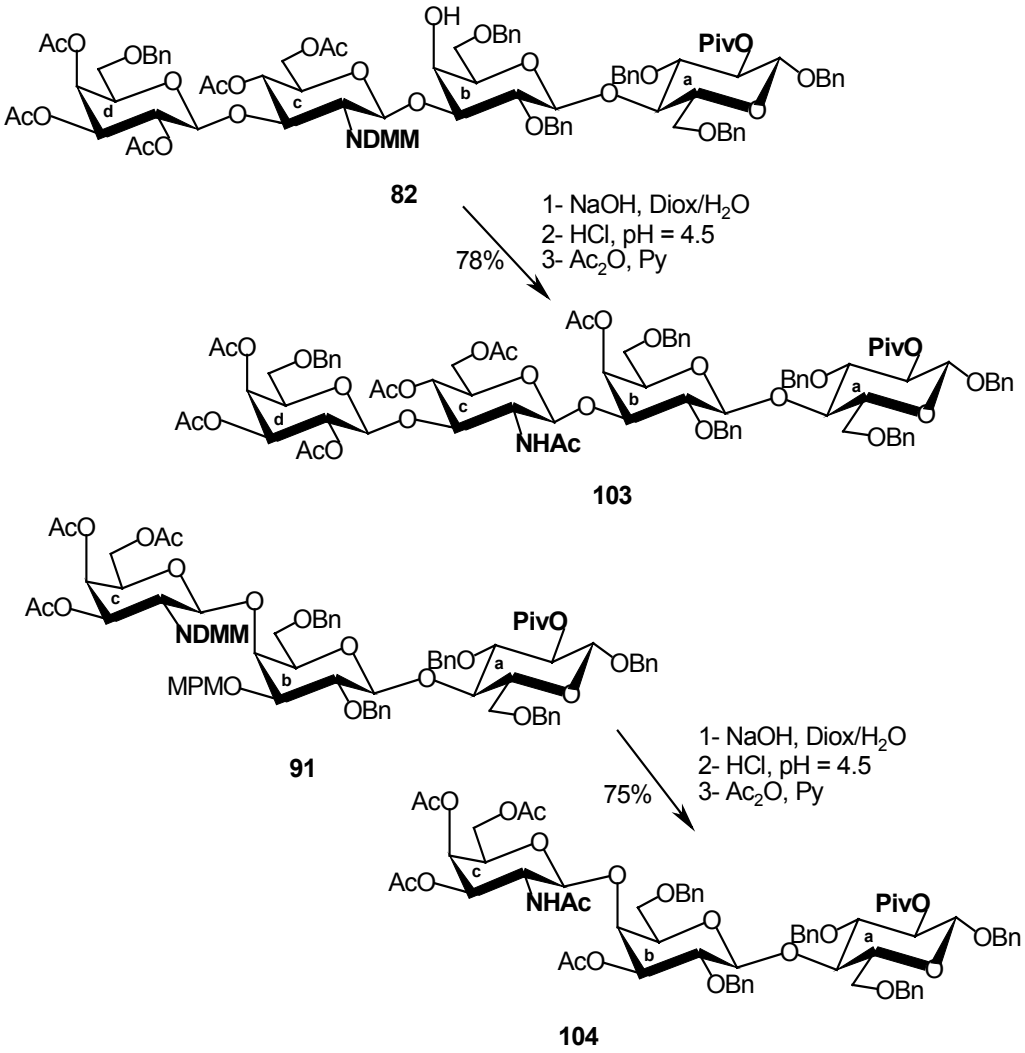


Scheme 62. Molecules used on the deprotection of DMM protecting group

Removal of the DMM in the presence of a Piv protecting group is highly desirable, since the latter group should remain unchanged until the end of the reaction sequences for the reasons discussed in Section 2.5.2, i.e. high stereoselectivity of the glycosylation reaction with ceramide avoiding the formation of orthoester.

2.6.4.1 Removal of the dimethylmaleoyl group in the presence of pivaloyl derivatives

Compounds **82** and **91** were treated under standard conditions (1 M NaOH followed by 1 M HCl until pH = 4.5 and acetylation). The reaction proceeded smoothly following the normal procedure, and transformation of *N*-DMM into NHAc may be easily controlled by TLC (disappearance of the starting material spot and formation of a single slower moving spot corresponding to the desired NHAc derivatives). In the examined cases it was observed that **103** and **104** were obtained in similar overall yields (70-80%) as was already observed for others oligosaccharides.[91,94,95]



Scheme 63. Removal of the DMM group in the presence of Piv

The structural assignment of the products was performed by NMR:

derivative	^{1}H-NMR: **2c**-H (ppm)	^{13}C-NMR: C-**2c** (ppm)
82 (NDMM)	4.38	51.7
103 (NHAc)	3.73	52.7
91 (NDMM)	4.09	55.1
104 (NHAc)	3.38	57.0

*Table 5. NMR data of **103** and **104***

From this experiment it was concluded that DMM as protecting group might be employed in sequences containing the demanding lactose unit **74**.

In conclusion, the general strategy elaborated in this section can be applied for the synthesis of gangliosides containing GalNAc and GlcNAc moieties, thus extending the scope of DMM group as protecting group for the synthesis of these complex molecules.

3. Experimental Part

3.1 General

Solvents were purified and dried according to the standard procedures. The boiling range of the petroleum ether used was 35°C-65°C.

Thin layer chromatography (TLC): silica gel 60 F_{254} plastic plates (E. Merck, layer thickness 0.2 mm); detected by UV absorption and by treatment with one of the following reagents followed by heating at 120°:

- Mostaine [ammonium molibdate (20 g) and cerium (IV) sulfate (0.4 g) in 10% aq. sulfuric acid (400 mL)].
- 15% aq.sulfuric acid.

Preparative flash chromatography: silica gel 60 (30-60 μm, J.T.Baker) at a pressure of 0.2-0.4 bar.

Middle pressure chromatography: silica gel Lichropre S &0 (15-25 μm, E. Merck) at a pressure of 5-10 bar.

Optical rotation: were measured at 25°C with a POLAMATA automatic polarimeter using a 5cm 5mL cell or Perkin-Elmer polarimeter 241/MS.1dm cell.

NMR spectra: measured on the following instruments

- Brucker AC 250= (250MHz)
- Brucker DRX-600 (600MHz)

Tetramethylsilane (0.00 ppm) or the signal of the deuterated solvents was used as internal standard. The chemical shifts were given in ppm and coupling constants in Hz.

Explanation to ^{1}H NMR data : s= singulet, bs= broad singulet, d= doublet, t= triplet, m= multiplet, dd= doublet doublet. In the case of oligosaccharide the monosugar unit was indicated by a, b, c...., beginning with the sugar at right end.

The assignment of the resonances was determined using multi-dimensional NMR techniques (COSY, HMQC)

MALDI-MS: The mass spectra were measured with a KRATOS Analytical Kompact MALDI 1 spectrometer using 2,5 di-hydroxy benzoic acid (DHB)as matrix.

Concentrations: were conducted under reduced pressure at 40°C

3.2 Experimental

Methyl 2,3-O-isopropylidene-α-D-mannopyranoside (1).-

Compound **1** was synthesized following a published procedure. The physical data are in accordance with published values.[47]

Synthesis of the D-Perosamine derivatives (2-10).-

The compounds **2, 3, 6, 7, 8, 9** were synthesized following the Eis *et a.l* procedure.[46]
The compounds **4, 5, 11a, 10** and **12** were synthesized following the Bundle *et a.l* procedure.[49]
The physical data are in accordance with published values

Methyl 4-azido-3-O-benzyl-4,6-dideoxy-2-O-methyl-α-D-mannopyranoside (11).-

To a solution of **9** (0.05 g, 0.170 mmol) in N,N-dimethylformamide (0.5 mL) was added sodium hydride (8 mg, 0.340 mmol, oil dispersion) at 0°C. After 30 min, methyl iodide (13 μL, 0.204 mmol) was added at 0°C and the mixture was stirred for an additional period of 15 min at 25 °C. Methanol was added to destroy the excess of sodium hydride. After evaporation *in vacuo* a solution of the residue in chloroform (5 mL) was washed with water (2 mL), dried (Na_2SO_4) and evaporated. Column chromatography (toluene) afforded **11**, isolated as a syrup (0.049 mg, 95%): $[\alpha]_D$ +105.30° (c 1.1, dichloromethane); R_f 0.56 (3:1 hexane-ethyl acetate); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.68 (s, 1H, 1-H), 3.71 (dd, 1H, 3-H), 3.5-3.48 (m, 2H, 2, 4-H), 3.47 (s, 3H, OCH_3), 3.46-3.44 (m, 1H, 5-H), 3.32 (s, 3H, OCH_3), 1.31 (d, 3H, 6-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ 137.7 (C ipso), 128.4, 127.9, 127.8 (C_6H_5), 98.4 (C-1), 78.2 (C-3), 76.4 (C-2), 71.9 ($PhCH_2$), 66,9 (C-5), 64.2 (C-4), 59.3 (OCH_3-C-2), 54.8 (OCH_3-C1), 18.4 (C-6).

2-O-Acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl chloride (12).-

Compound **12** was synthesized following a published procedure. The physical data are in accordance with published values.[49]

2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl Trichloroacetimidate (13).-

2-Aminoethanol (0.06 mL) was added to a solution of 1,2-di-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranose **10** (188 mg, 0.5 mmol) in ethyl acetate (3 mL). After the solution had been stirred for 4 h, TLC (dichloromethane/acetone 8:1 v/v) showed complete transformation of the starting diacetate into a more polar compound (R_f 0.7). The solution was washed with water until the washings were neutral and the organic phase was dried (anhydrous Na_2SO_4), filtered, concentrated and dried *in vacuo*.
The resulting syrup was stirred in the presence of K_2CO_3 (0.142 g) and trichloroacetonitrile (0.2 mL) in dry dichloromethane (3 mL) for 24 h. Then the suspension was filtered through Celite and the filtrate concentrated *in vacuo* to give **13**, isolated as a syrup (0.202 g, 84%): R_f 0.65 (dichloromethane/acetone 4:1 v/v); $[\alpha]_D$ -47.0^o (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.29 (s, 1H, $NHCCl_3$), 7.40-7.20 (m, 5H, Ph), 6.28 (d, 1H, 2-H), 5.50 (d, 1H, $J_{1,2}$ =1.6 Hz, 1-H), 4.60 (s, 2H, $PhCH_2$), 3.89 (dd, 1H, 3-H), 3.75 (m, 1H, 5-H), 3.5 (dd, 1H, 4-H), 2.25 (s, 3H, CH_3CO), 1.49 (d, 3H, 6-H).

L-malic acid (14).-

This compound was purchased from Merck.

2-O-acetyl-L-malic anhydride (15).-

Compound **15** was synthesized following a published procedure. The physical data are in accordance with published values.[99]

(S)-3-Acetoxy-3-ethoxycarbonyl propionic acid (16).-

To a solution of freshly prepared 2-acetyl-L-malic anhydride **15** (1 g, 7.46 mmol) was dissolved in ethanol (6 mL) and stirred for 24 h at room temperature. The mixture was concentrated and distillated at 80-105 ^{o}C to afford **16** (1.20 g, 79 %) as a low melting solid; $[\alpha]_D$-20.6^o (c 1, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.45 (t, 1H, $J_{2,3}$ = 6.0 Hz, 3-H), 4.23 (q, 2H, OCH_2), 2.94 (d, 1H, 2-H), 2.15 (s, 3H, CH_3CO), 1.28 (t, 3H, CH_3); ^{13}C NMR

(250 MHz, CDCl3): δ 174.7 (C-1), 170.0 and 168.7 (C=O), 68.0 (C-3), 61.9 (OCH_2), 35.8 (C-2), 20.4 (CH_3CO), 13.9 (CH_3).
Anal. Calcd for $C_8H_{12}O_6$ (204.1): C, 46.07; H, 5.93. Found: C, 46.74; H, 5.78.

Ethyl 2-O-acetyl-3-deoxy-L-glycero tetronate (17).-

Compound **16** (0.2 g, 0.98 mmoL) was dried *in vacuo* for 2 h. A solution of borane tetrahydrofuran complex (1M, 3.4 mL, 3.43 mmol) was added under argon and the mixture was stirred for 1 h. The reaction was quenched with ethanol (1 mL), concentrated and co-concentrated with ethanol (2 mL) and toluene (2 x 2 mL) to afford **17**, isolated as a syrup (0.161 g, 86 %): $[\alpha]_D$ -25.5° (c 0.9, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.16 (dd, 1H, 2-H), 4.22 (dd, 2H, OCH_2), 3.76 (m, 2H, -CH_2OH), 2.15 (s, 3H, CH_3CO), 2.08 (m, 2H, 3-H), 1.28 (t, 3H, CH_3); ^{13}C NMR (250 MHz, $CDCl_3$): δ 170.6 (2C=O), 69.7 (C-2), 61.6 (OCH_2), 58.2 (CH_2OH), 33.9 (C-3), 20.6 (CH_3CO), 14.1 (CH_3).
Anal. Calcd for $C_8H_{14}O_5$ (190.14): C, 50.51; H, 5.93. Found: C, 50.20; H, 5.86.

(S)-3-Acetoxy-3-benzyloxycarbonyl propionic acid (18).-

To a solution of freshly prepared 2-acetyl-L-malic anhydride **15** (3g, 22.4 mmol) in dry dichloromethane (3 mL) was added benzyl alcohol (2.3 mL, 2.23 mmol). The mixture was stirred for 24 h and concentrated. Column chromatography (2:1 hexane-ethyl acetate) of the residue afforded **18**, isolated as a syrup (2.5 g, 83 %): $[\alpha]_D$-28.5° (c 1, chloroform); R_f 0.5 (3:1 chloroform-acetone); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.30-7.35 (m, 5H, C_6H_5), 5.51 (t, 1H, 3-H), 5.18 (s, 2H, $PhCH_2$), 2.93 (d, 2H, 2-H), 2.12 (s, 3H, CH_3CO); ^{13}C NMR (250 MHz, $CDCl_3$):δ 174.5 (C-1), 170.0 and 168.6 (C=O), 134.9 (Ph), 128.6, 128.5 and 128.1 (C_6H_5), 67.9 (C-3), 67.5 ($PhCH_2$), 35.7 (C-2), 20.4 (CH_3CO).
Anal. Calcd for $C_{13}H_{14}O_6$ (266.1): C, 58.64; H, 5.30. Found: C, 58.50; H, 5.65.

Benzyl 2-O-acetyl-3-deoxy-L-glycero tetronate (19).-

Compound **18** was reduced as for the preparation of **17**. Column chromatography (1:1 hexane-ethyl acetate) of the residue afforded **19**, isolated as a syrup (0.183 g, 64 %): $[\alpha]_D$ -51.8° (c 1.08, chloroform); R_f 0.42 (1:1 hexane-ethyl acetate); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.32 (s, 5H, C_6H_5), 5.20 (dd, 1H, 2-H), 3.76-3.60 (m, 2H, CH_2OH), 2.10 (s, 3H, CH_3CO),

2.09-2.0 (m, 2H, 3-H); ^{13}C NMR (250 MHz, $CDCl_3$):δ 170.4, 170.2 (2C=O), 135.0 (C ipso), 128.4, 128.2, 127.9 (C_6H_5), 69.4 (C-2), 66.9 ($PhCH_2$), 57.7 (CH_2OH), 33.6 (C-3) and 20.3 (CH_3CO).

Benzyl 2,4-di-O-acetyl-3-deoxy-L-glycero tetronate (20).-

To a solution of **19** in pyridine (50 mL) cooled to 0-5°C was added acetic anhydride (10 mL). The resulting solution was stirred for 24 h, concentrated and co-concentrated with toluene (3 x 10 mL). Column chromatography (1:1 hexane-ethyl acetate) of the residue afforded **20**, isolated as a syrup (3.60 g, 83 %): $[\alpha]_D$ -29.7° (c 1, chloroform); R_f 0.5 (2:1 hexane-ethyl acetate); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.35 (s, 5H, C_6H_5), 5.18 (s, 2H, $PhCH_2$), 5.16 (dd, 1H, 2-H), 4.22-4.09 (m, 2H, 4-H), 2.13 and 2.00 (2s, 6H, CH_3CO), 2.24-1.97 (m, 2H, 3-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ 170.6, 170.1, 169.5 (C=O), 135.0 (C ipso), 128.4, 128.3, 128.0 (C_6H_5), 68.9 (C-2), 67.1 ($PhCH_2$), 59.6 (C-4), 29.9 (C-3) 20.5 (CH_3CO).
Anal. Calcd. for $C_{15}H_{18}O_6$ (294.2) : C, 61.21; H, 6.16. Found: C, 61.23; H, 6.46.

2,4-di-O-Acetyl-3-deoxy-L-glycero-tetronic acid (21).-

A suspension of **20** (0.3 g, 1.09 mmol) and 5% Pd/C (45 mg) in ethanol (5 mL) was stirred under hydrogen for 20 h, filtered and concentrated to yield **21** (0.208 g, 94 %): $[\alpha]_D$-23.2° (c 1.1, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.12 (broad s, 1H, COOH), 5.12 (q, 1H, 2-H), 4.24-4.18 (m, 2H, 4-H), 2.27-2.05 (m, 2H, 3-H), 2.15 and 2.05 (2s, 6H, CH_3CO); ^{13}C NMR (250 MHz, $CDCl_3$): δ 173.6 (COOH), 171.2 and 170.6 (C=O), 68.7 (C-2), 59.8 (C-4), 29.9 (C-3), 20.6 and 20.4 (CH_3CO).
Anal. Calcd for $C_8H_{12}O_6$ (204.1): C, 47.06; H, 5.93. Found: C, 47.75; H, 5.67.

Methyl 2-O-(2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (22).-

Compound **22** was synthesized from **12** and **9** following a published procedure. The physical data are in accordance with published values.[49]

Methyl 4-azido-2-O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (23).-

Compound **23** was synthesized following a published procedure. The physical data are in accordance with published values.[49]

Methyl (4-azido-3-O-benzyl-4,6-dideoxy-2-O-methyl-α-D-mannopyranosyl)- (1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (24).-

To a solution of **23** (90 mg, 1.6 mmol) in dry methanol (1 mL), sodium methoxide was added to pH 9 and the mixture was stirred for 30 min. The resulting solution was neutralized with Dowex-50 (H+) resin, filtered and concentrated. The residue was methylated as for the preparation of **11** using methyl iodide (12 μL, 1.9 mmol), NaH (8 mg, 3.2 mmol, 80% oil dispersion) in N,N-dimethylformamide (1 mL). Column chromatography (20:1 toluene-acetone) of the residue afforded **24**, isolated as a syrup (74 mg, 80 %): $[\alpha]_D$ +58.5^o (c 1.06, chloroform); R_f 0.56 (20:1 toluene-acetone); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.37-7.26 (m, 10H, $2C_6H_5$) 4.89 (d, 1H, $J_{1b,2b}$ = 1.8 Hz, 1b-H), 4.70 (dd, 2H, $PhCH_2$), 4.63 (dd, 2H, $PhCH_2$), 4.49 (d, 1H, $J_{1a,2a}$ = 1.6 Hz, 1a-H), 3.92 (dd, 1H, $J_{2a,3a}$ = 2.4 Hz, 2a-H), 3.71 (dd, 1H, $J_{3a,4a}$ = 9.7 Hz, 3a-H), 3.49 (m, 1H, 5b-H), 3.47 (t, 1H, $J_{4,5}$ = 8.5 Hz, 4b-H), 3.45 (m, 1H, 5a-H), 3.31 (s, 3H, OCH_3), 3.22 (t, 1H, $J_{4,5}$ = 9.7 Hz, 4a-H), 3.18 (s, 3H, OCH_3), 1.31 (d, 3H, 6a-H), 1.30 (d, 3H, 6b-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ 99. (C-1b), 99.0 (C-1a), 78.2 (C-3b), 77.0 (C-3a), 76.4 (C-2b), 73.4 (C-2a), 67.7 (C-5b), 66.8 (C-5a), 64.4 (C-4a), 64.1 (C-4b), 58.8 (OCH_3), 54.8 (OCH_3), 18.5(C-6a, 6b).
Anal. Calcd for $C_{28}H_{36}O_7N_6$ (568.4): C, 59.14; H, 6.38; N, 14.78. Found: C, 59.42; H, 6.18; N, 14.85.

Methyl 4-amino-4,6-dideoxy-2,3-O-isopropylidene-α-D-mannopyranoside (25).-

A solution of **11a** (660 mg, 3.04 mmol) in ethanol (5 mL) was stirred in the presence of 5 % Pd/C (200 mg) under hydrogen. After 24 h, TLC (4:1 ethyl acetate-methanol) revealed complete conversion of the starting material into a new compound . The mixture was filtered , and the filtrate was concentrated and purified by column chromatography on silica gel (hexane-ethyl acetate 1:4 v/v) to give the title compound **25** isolated as a syrup (492 mg, 83%): (R_f 0.33, ninhydrine positive, 1:3 hexane-ethyl acetate); ^{1}H NMR (250 MHz, $CDCl_3$):

δ = 4.88 (s, 1H, 1-H), 4.06 (d, 1H, 2-H), 3.85 (dd, 1H $J_{2,3}$ = 5.5 Hz, $J_{3,4}$ = 8.5 Hz, 3-H), 3.50 (dq, 1H $J_{4,5}$ = 10.4 Hz, $J_{5,6}$ = 6.4 Hz, 5-H), 3.35 (s, 3H, OCH_3), 2.60 (dd, 1H, 4-H), 1.5 (s, 3H, CH_3), 1.3 (s, 3H, CH_3), 1.2 (d, 3H, 6-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ 190.0 (CMe_2), 97.9 (C-1), 56.4 (C-4), 54.5 (OCH_3), 28.0, 26.1 ($2CH_3$), 17.3 (C-6).
Anal. Calcd for $C_{10}H_{19}NO_4$ (217.2): C, 55.28; H, 8.81. Found: C, 55.63; H, 9.12.

Methyl 4-amino-3-O-benzyl-4,6-dideoxy-2-O-methyl-α–D-mannopyranoside (26).-

A solution of **11** (70 mg, 0.23 mmol) in ethanol (3mL) was hydrogenated as for the preparation of **25**. Work-up then gave **26** (48 mg, 75 %): (R_f 0.35, ninhydrine positive, 1:3 hexane-ethyl acetate); ^1H NMR (250 MHz, $CDCl_3$): δ = 7.40-7.20 (m, 5H, C_6H_5), 4.75 (d, 1H, $J_{1,2}$ = 1.1 Hz, 1-H), 4.63 (dd, 2H, $PhCH_2$), 3.47 (s, 3H, OCH_3), 3.34 (s, 3H, OCH_3), 2.94 (dd, 1H, $J_{3,4}$ = 9.6 Hz, $J_{4,5}$ = 9.6 Hz, 4-H), 1.25 (d, 3H, 6-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ 138.0 (C ipso), 128.5 - 127.8 (C_6H_5), 98.6 (C-1), 79.5 (C-3), 76.5 (C-2), 71.4 ($PhCH_2$), 59.1 (OCH_3-C2), 54.7 (OCH_3-C1), 53.5 (C-4), 18.0 (C-6).

Methyl (4-amino-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-4-amino-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (27).-

A solution of **23** (40 mg, 0.079 mmol) in ethanol was hydrogenated as for the preparation of **25**. Work up then gave **27** (30 mg, 83%): (R_f 0.60, ninhydrine positive, 1:4 methanol-ethyl acetate.); ^1H NMR (250 MHz, $CDCl_3$): δ 7.38-7.20 (m, 10H, $2C_6H_5$), 5.02 (d, 1H, $J_{1,2}$ = 2.0 Hz, 1b-H), 4.70 (d, 1H, $J_{1,2}$ = 2.0 Hz, 1a-H), 4.09 (d, 1H, 2b-H), 3.90 (d, 1H, 2a-H), 3.69-3.67 (m, 2H, 3a,3b-H), 3.52-3.51 (m, 2H, 5a,5b-H), 3.32 (s, 3H, OCH_3), 2.90 (dd, 1H, 4b-H), 2.85 (dd, 1H, 4a-H), 1.35, 1.30 (2d, 6H, 6a, 6b-H); ^{13}C NMR (250 MHz, CDCl3): δ 137.7-137.5 (C ipso), 128.5, 128.0, 127.9 (C_6H_5), 101.0, 100.2 (C-1a, 1b), 54.7 (OCH_3), 53.6, 53.2 (C-4a, 4b), 18.2, 18.0 (C-6a, 6b).

Methyl-(4-amino-3-O-benzyl-4,6-dideoxy-2-O-methyl-α-D-mannopyranosyl) -(1→2)-4-amino-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyde (28).-

A solution of **24** (52 mg, 0.1 mmol) in ethanol was hydrogenated as for the preparation of **25**. Work up then gave **28** isolated as a syrup (29 mg, 61 %): (R_f 0.43, ninhydrine positive 1:4 methanol-ethyl acetate); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.40-7.25 (m, 10H, $2C_6H_5$), 5.02 (d, 1H, 1b-H), 4.73 (dd, 2H, $PhCH_2$), 4.70 (d, 1H, PhCH), 4.65 (d, 1H, 1a-H), 4.55 (d, 1H, CHPh), 3.30 (s, 3H, OCH_3), 3.29 (s, 3H, OCH_3), 2.98-2.80 (m, 2H, 4a, 4b-H) 1.33-1.29 (2d, 6H, 6a, 6b-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ 137.9-137.7 (C ipso) 128.5-127.8 (C_6H_5), 100.3, 99.1 (C-1a, 1b) 58.9 (OCH_3-C2), 54.6 (OCH_3-C1), 53.7-53.5 (C-4a, 4b) 18.1-18.1 (C-6a, 6b).

Methyl 4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)- 4,6-dideoxy- 2,3-O-isopropylidene-α-D-mannopyranoside (29).-

To a solution of **25** (228 mg, 0.97 mmol) and **21** (398 mg, 1.94 mmol) in dichloromethane (4 mL) was added EEDQ (479 mg, 1.94 mmol) and the mixture stirred for 15 min. Then, TLC (1:3 hexane-ethyl acetate) showed the absence of **25** (R_f 0.33) and a new spot (R_f 0.50). After evaporation, column chromatography (1:1 hexane-ethyl acetate) of the residue afforded **29**, isolated as a syrup (335 mg, 81 %): $[\alpha]_D$-37.7^o (c 1.06, dichloromethane); R_f 0.50 (3:1 ethyl acetate-hexane); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.37 (d, 1H, NHCO), 5.23 (dd, 1H, 2b-H), 4.85 (s, 1H, 1a-H), 4.23-4.07 (m, 3H, 3a, 2a, 4b-H), 3.86 (dd, 1H, 4a-H), 3.74 (dq, 1H, 5a-H), 3.4 (s, 3H, OCH_3), 2.35-2.16 (m, 1H, 3b-H), 2.15 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 1.53 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.24 (d, 3H, 6a-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 170.8, 169.5, 169.1 (C=O), 109.5 (C ipso), 98.2 (C-1a), 74.7 (C-3a), 74.3 (C-2a), 71.0 (C-2b), 65.8 (C-5a), 59.9 (C-4b), 54.9 (OCH_3), 52.8 (C-4a), 30.7 (C-3b), 27.4, 25.9 ($2CH_3$), 20.7 (CH_3CO), 18.2 (C-6a).

Anal. Calcd for $C_{18}H_{29}O_9N$ (403.3): C, 53.59; H, 7.24; N, 3.47. Found: C, 53.32; H, 7.82; N, 3.22.

Methyl 4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-2-O-methyl-α-D-mannopyranoside (30).-

The reaction was performed as for the preparation of **29** using **26** (54 mg, 0.19 mmol), **21** (0.75 mg, 0.37 mmol), EEDQ (93 mg, 0.37 mmol) and dichloromethane (1 mL). Column chromatography (1:1 hexane-ethyl acetate) of the residue afforded **30**, isolated as a syrup (76 mg, 85 %): m.p. 161.1-163.6°C; $[\alpha]_D$ +3.17° (c 0.63, chloroform); R_f 0.55 (4:1 ethyl acetate-hexane); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.2-7.5 (m, 10H, $2C_6H_5$), 5.90 (d, 1H, NHCO), 5.17 (dd, 1H, 2b-H), 4.73 (d, 1H, 1a-H), 4.68-4.45 (2d, 2H, $PhCH_2$), 4.10 (m, 2H, 4b-H), 3.88 (m, 3H, 3a, 4a, 5a-H), 3.59 (s, 1H, 2a-H), 3.30 and 3.50 (2s, 6H, OCH_3), 2.15 (m, 2H, 3b-H), 2.10 and 2.00 (2s, 6H, CH_3CO), 1.2 (d, 3H, 6a-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 170.8, 169.5 and 169.2 (C=O), 138.1 (C ipso), 128.4-127.7 (C_6H_5), 98.7 (C-1a), 76.1 (C-2a), 75.6 (C-3a), 71.1 (C-2b and $PhCH_2$), 67.3 (C-5a), 60.0 (C-4b), 59.3 (C-4a), 59.2 (OCH_3-C2a), 54.3 (OCH_3-C1a), 30.9 (C-3b), 20.8 and 20.7 (CH_3CO), 18.01 (C-6a).
Anal. Calcd for $C_{23}H_{33}O_9N$ (467.3): C, 59.09; H, 7.11; N, 3.00. Found: C, 58.90; H, 7.35; N, 2.91.

Methyl [4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-6- dideoxy-α-D-mannopyranosyl]-(1→2)-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (31).-

The reaction was performed as for the preparation of **29** using **27** (70 mg, 0.139 mmol), **21** (113 mg, 0.55 mmol), EEDQ (136 mg, 0.55 mmol) dichloromethane (2 mL). Column chromatography (1:4 hexane-ethyl acetate) of the residue afforded **31**, isolated as a syrup (300 mg, 57 %): $[\alpha]_D$ +49° (c 1, chloroform); R_f 0.33 (2:1 dichloromethane-acetone); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.40-7.20 (m, 10H, $2C_6H_5$), 6.11, 5.91 (2d, 1H, NHCO), 5.20, 5.18 (2dd, 1H, 2c,2d-H), 4.98 (d, 1H, $J_{1b,2b}$ = 1.8 Hz, 1b-H), 4.70 (d, 1H, $J_{1,2}$ = 1.8 Hz, 1a-H), 4.68 (d, 1H, J = 11.3 Hz, $PhCH_2$), 4.62 (d, 1H, J = 11.5 Hz, PhCH), 4.52 (d, J = 11.5 Hz, PhCH), 4.46 (d, 1H, J = 11.3 Hz, $PhCH_2$), 4.22-3.98 (m, 9H, 2a, 2b, 3a, 4a, 4b, 4c, 4d-H), 3.90-3.60 (m, 3H, 3a, 5a, 5b-H), 3.30 (s, 3H, OCH_3), 2.1-2.3 (m, 4H, 3c, 3d-H), 2.05-1.9 (m, 12H, CH_3COO), 1.2, 1.0 (d, 3H, 6a,6b-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 170. 9, 169. 7, 169.6 (C-1c, 1d, $4CH_3CO$), 137.5 (C ipso), 128.5-127.8 (C_6H_5), 101.0, 100.1 (C-1a,1b), 68.0, 67.8 (C-2c,2d), 60.0, 59.9 (C-4c,4d), 55.1 (OCH_3), 52.3, 52.2 (C-4a,4b), 30.9, 30,8 (C-3c,3d), 20.8, 20.7, 20.6 (CH_3CO), 17.9, 17.8 (C-6a, 6b).

Anal. Calcd for $C_{43}H_{58}O_{18}N_2$ (890.6):C, 59.03; H, 6.68; N, 3.20. Found: C, 58.75; H, 6.80.; N, 3.29.

Methyl 4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-2-O-methyl-α-D-mannopyranosyl]-(1→2)-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α–D-mannopyranoside (32).-

The reaction was performed as for the preparation of **29** using **28** (70 mg, 0.135 mmol), **21** (110 mg, 0.54 mmol), EEDQ (134 mg, 0.54 mmol) and dichloromethane (2 mL). Column chromatography (1:3 hexane- ethyl acetate) of the residue afforded **32**, isolated as a syrup (90 mg, 75 %): $[\alpha]_D$-17.8^o (c 1, dichloromethane); R_f 0.73 (2:1 dichloromethane-acetone); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.42-7.25 (m, 10H, $2C_6H_5$), 5.88, 5.85 (2d, 1H, NH, NH'), 5.20 (dd, 2H, 2c, 2d-H), 4.96 (d, 1H, $J_{1b,2b}$ = 2.5 Hz, 1b-H), 4.72 (d, 1H, J = 11.2 Hz PhCH), 4.66 (d, 1H, $J_{1a,2a}$ = 2.5 Hz, 1a-H), 4.66 (d, 1H, J = 11.2 Hz, PhCH), 4.20 - 4.0 (m, 8H, 3b, 4a, 4b, 2b, 4c, 4d-H), 3.83-3.60 (m, 4H, 2a, 3a, 5a, 5b-H), 3.33, 3.30 (2s, 6H, OCH_3), 2.4-2.2 (m, 4H, 3c, 3d-H), 2.1, 2.08, 2.0, 1.9 (4s, 4 x 3H, CH_3CO), 1.29, 1.19 (2d, 6H, 6a, 6b-H); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 170.9, 169.8, 169.4 (C=O), 138.0, 137.9 (C ipso), 128.5-127.6 (C_6H_5), 100.1, 99.6 (C-1a, 1b), 60.0, 59.9 (C-4c, 4d), 59.1, 55.0 (OCH_3), 52.4, 52.0 (C-4a, 4b), 31.0, 30.8 (C-3c, 3d), 20.9, 20.8, 20.7 (CH_3CO), 18.0 (C-6a, 6b).
Anal. Calcd for $C_{44}H_{60}O_{17}N_2$ (888.6): C, 59.45; H, 6.80; N, 3.15. Found: C, 59.19; H, 7.19; N, 3.21.

Methyl 4-(3-deoxy-L-glycero-tetronamidol)-4,6-dideoxy-α-D-mannopyranoside (33).-

A solution of **29** (272 mg, 0.674 mmol) in trifluoroacetic acid 90 % was stirred at ambient temperature for 15 min. Then TLC (3:1 ethyl acetate-hexane) showed the reaction to be complete. The mixture was concentrated and co-concentrated with toluene (3 x 5 mL). To a solution of the crude in dry methanol (2 mL), sodium methoxide was added to pH 9 and the mixture was stirred for 15 min. The resulting solution was neutralized with Dowex-50 (H+) resin, filtered and concentrated. The residue was dissolved in water and freeze dried to gave **33** (163 mg, 87 %): $[\alpha]_D$ +20^o (c 1, H_2O); R_f 0.33 (5:1 ethyl acetate-methanol); ^{1}H NMR (250 MHz, D_2O): δ = 4.76 (s, 1H, $J_{1,2}$ = 1.5 Hz, 1-H), 4.35 (dd, 1H, 2b-H), 4.00(d, 1H, 2a-H), 3.98-3.88 (m, 3H, 3a, 4a, 5a-H), 3.83 (t, 2H, 4b-H), 3.46 (s, 3H, OCH_3), 2.20 - 2.02 (m, 1H, 3b-H), 2.02-1.84 (m, 1H, 3b-H), 1.26 (d, 3H, 6a-H); ^{13}C NMR (250 MHz, D_2O): δ = 102.1 (C-1a, J

$_{C1H1}$ = 170.6 Hz), 70.3 (C-2a), 70.2 (C-2b), 69.1 (C-3a), 68.4 (C-5a), 59.0 (C-4b), 55.9 (OCH_3), 54.0 (C-4a), 37.1 (C-3b), 18.0 (C-6a).

Methyl4-(3-deoxy-L-glycero-tetronamidol)-4,6-dideoxy-2-O-methyl-α-D-mannopyranoside (34).-

A solution of **30** (154 mg, 0.329 mmol) in ethanol (2 mL) was stirred in the presence of 5% Pd/C (0.1 mg) under hydrogen. After 24 h, TLC (1:4 hexane-ethyl acetate) revealed complete conversion of the starting material (R_f 0.9) into (R_f 0.21). The mixture was filtered, concentrated and dissolved in dry methanol (1 mL), sodium methoxide was added to pH 9 and the mixture was stirred for 15 min. The resulting solution was neutralized with Dowex-50 (H+) resin, filtered and concentrated. The residue was dissolved in water and freeze dried to give **34** (0.090 mg, 93 %): $[\alpha]_D$ +16.6^o (c 0.96, Methanol); R_f 0.36 (5:1 ethyl acetate-methanol); ^{1}H NMR (250 MHz, D_2O): δ = 5.00 (d, 1H, $J_{1a,2a}$ = 1.6 Hz, 1a-H), 4.38 (dd, 1H, 2c-H), 4.04 (dd, 1H, 3a-H), 3.93 - 3.81 (m, 4H, 5a, 4a, 4b-H), 3.67 (dd, 1H, 2a-H), 3.59 and 3.51 (2s, 2 x 3H, OCH_3), 2.20 - 2.05 (m, 1H, 3b-H), 2.02 - 1.85 (m, 1H, 3b-H), 1.3 (d, 1H, 6a-H); ^{13}C NMR (D_2O): δ 178.4 (C=O), 98.9 (C-1a), 80.2 (C-2a), 70.2 (C-2b), 68.9 (C-3a), 68.3 (C-5a), 60.1 (OCH_3-C2a), 59.1 (C-4b), 56.0 (OCH_3-C1a), 54.5 (C-4a), 37.2 (C-3b), 18.0 (C-6a).

Methyl [4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-(3-deoxy-L-glycero-tetronamidol)-4,6-dideoxy-α-D-mannopyranoside (35).-

The reaction was performed as for the preparation of **34** using **31** (70 mg, 0.08 mmol), Pd/C 5% (40 mg) and ethanol (2 mL) to afford **35** (35 mg, 83 %): $[\alpha]_D$ +1.0^o (c 1, H_2O): ^{1}H NMR (250 MHz, D_2O): δ = 5.10 (s, 1H, 1b-H), 4.85 (d, 1H, $J_{1,2}$=1.8 Hz, 1a-H) , 4.35 (dd, 2H, 2c,2d-H) , 4.15 (dd, 1H, 2b-H), 4.10-3.85 (m, 7H, 3a, 3b, 2a, 4a, 5a, 5b-H), 3.80-3.70 (m, 2H, 4c, 4d-H), 3.95 (s, 3H, CH_3), 2.15-2.00 (m, 2H, 3c, 3d-H), 2.00-1.80 (m, 2H, 3c, 3d-H), 1.30; 1.25 (2d, 6H, 6a, 6b-H); ^{13}C NMR (250 MHz, D_2O): δ = 178.5 (C-1c, 1d), 103.3, 100.7 (C-1a, 1b), 78.9 (C-2b), 70.3 (C-2a), 70.1 (C-2c, 2d), 69.2 (C-2b), 68.7, 68.6, 68.4 (C-3a, 3b, 5a, 5b), 59.0 (C-4c, 4d), 56.1 (CH_3O), 54.1 (C-4a), 53.9 (C-4b), 37.1 (C-3d), 18.0 (C-6a, 6b).

Methyl [4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-2-O-methyl-α-D-manno-pyranosyl]-(1→2)-4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (36).-

The reaction was performed as for the preparation of **34** using **32** (30 mg, 0.034 mmol), Pd/C 5% (15 mg) and ethanol (1 mL) to afforded **36** (18 mg, 90 %): $[\alpha]_D$ +1.1^o (c 1, H_2O); ^{1}H NMR (250 MHz, D_2O): δ = 5.22 (d, 1H, $J_{1b,2b}$ = 1.7 Hz, 1b-H) , 4.85 (d, 1H, $J_{1a,2a}$ = 2.0 Hz, 1a-H), 4.35 (dd, 2H, 2c, 2d-H), 4.15 (dd, 1H, 3b-H), 4.10-3.95 (m, 6H, 2a, 5b, 3b, 4a, 4b, 5b-H) , 3.85-3.7 (m, 3H, 2b, 4c, 4d-H), 3.55, 3.45 (2S, 6H, CH_3O), 2.1 (m, 2H, 3c, 3d-H), 1.9 (m, 2H, 3c, 3d-H), 1.3;1.2 (2d, 6H, 6a, 6b-H); ^{13}C NMR (250 MHz, D_2O): δ = 178.5,178.4 (C-1c, 1d), 100.2, 100.7 (C-1a, 1b), 80.1 (C-2b), 79.1 (C-2a), 70.1 (C-2c, 2d), 69.1 (C-3a), 68.6 (C-3b ,5a, 5b), 59.9 (CH_3O), 58.9 (C-4c, 4d), 56.1 (CH_3O), 54.3 (C-4b), 54.1 (C-4a), 37.1 (C-3c, 3d), 18.0 (C-6a, 6b).

Inhibition reaction.

Procedure 1. Agglutination was performed in plates using commercial Rabbit polyclonal antibodies against Ogawa or Inaba antigens (Wellcome Laboratories) and isolates of *Vibrio cholerae* from different Latino American countries previously serotyped at the Cuban National Referent Laboratory.

Procedure 2. Pool of sera obtained from male BALB/C mice immunized, as have been described [100]with ***V. cholerae*** O1 serotype Ogawa (strain E 7946) was absorbed with 0.25 mg /mL of LPS serotype Inaba (strain 569 B) by incubation at 37 ^{o}C for 2 h to eliminate cross reactions. The strains were from the Finlay Institute collection (Havana, Cuba).
Compounds **33**, **34**, **35**, and **36** were dissolved in H_2O, adjusted to the following concentration: 0.063, 0.0316, 0.0157 and 0.0078 μmol and added to the pool of absorbed sera diluted 1/1000 in skim milk 1% in phosphate-buffered saline (PBS, Ph 7,4)-Tween 20. The mixture was incubated for 2 h at 37 ^{o}C and then analyzed by ELISA. Briefly, the 96 well flat-bottom microdilution plates (Flow Laboratories) were coated with 0,1 mL of LPS (27 μg/mL, from both serotypes) in carbonate/bicarbonate buffer overnight at 37 ^{0}C. After the plates were washed 4 times with H_2O-Tween, skim milk was added at a concentration of 2 % in PBS and the plates were kept at 37 ^{o}C for 1 h. After the skim milk solution was discarded, the samples were added and incubated for 1 h at 37 ^{o}C. The plates were washed as describe above, 100 μl of Peroxidase-conjugated rabbit anti-mouse immunoglobulin (Sigma) diluted 1/1000 in skim

milk 1 % / PBS was added and incubated for 1 h at 37 ^{o}C. Then, the plates were washed and 100 µl of substrate solution (H_2O_2-o-phenylendiamine in phosphate-citrate buffer, Ph 5,0) was added to each well and incubated for 15 min at room temperature, in the dark. The reaction was stopped with 100 µl of 2,5 N sulfuric acid. The optical density at 450 nm was measured in an ELISA reader (Multiskan, Titertek)

4-(1,3-dioxolane-2-yl)-3-oxabutanol (37).-

Compound **37** was synthesized following a published procedure. The physical data are in accordance with published values.[101]

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-O-Acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (38).-

A solution of **13** (378 mg, 0.81 mmol) and 4-(1,3-dioxolan-2-yl)-3-oxabutanol (**37**) (132 mg, 0.89 mmol) in anhydrous dichloromethane (5 ml) was stirred in the presence of molecular sieves (4Å, 315 mg) at rt for 5 min. Trimethylsilyl triflate (0.059 mL, 0.32 mmol) was added dropwise. After 15 min, the reaction was quenched with triethylamine. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography (hexane/ethyl acetate 5:1 v/v) to give **38**, isolated as a syrup (251 mg, 68%): $[\alpha]_D$ +83^{o} (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.40-7.20 (m, 5H, Ph), 5.45 (dd, 1H, $J_{2,3}$ = 3.3 Hz, 2-H), 5.10 (t, 1H, dioxolane), 4.87 (d, 1H, $J_{1,2}$ = 1.5 Hz, 1-H), 4.64 (dd, 2H, $PhCH_2$), 4.06-3.90 (m, 4H, $2CH_2$ dioxolane), 3.90-3.85 (m, 5H, $2CH_2$ spacer and 3-H), 3.70-3.65 (m, 1H, 5-H), 3.54 (d, 2H, CH_2 spacer), 3.48 (dd, 1H, 4-H), 2.19 (s, 3H, CH_3CO), 1.47 (d, 1H, $J_{5,6}$ = 6.5 Hz, 6-H).
Anal. Calcd. for $C_{21}H_{29}O_8N_3$ (451.5): C, 55.87; H, 6.47; N, 9.31. Found: C, 56.01; H, 6.52; N, 9.10.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-4-Azido-3-O-benzyl-4,6-dideoxy-α-D-manno-pyranoside (39).-

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-4-Azido-3-O-benzyl-2-O-methyl-4,6-dideoxy-α-D-mannopyranoside (40).-

To a solution of **38** (0.33 mg, 0.73 mmol) in methanol (2 mL) was added methanolic sodium methoxide (3 mL) and the reaction was stirred for 1 h at rt. The reaction mixture was neutralized with Dowex-50 (H^+) resin, then filtered and the filtrate concentrated to give 4-(1,3-dioxolan-2-yl)-3-oxabutyl 4-azido-3-O-benzyl-4,6-dideoxy-α-D-manno-pyranoside **39** as a syrup.

Compound **39** (0.3 g, 0.7 mmol) was dissolved in N,N-dimethylformamide (5 mL) and sodium hydride (35 mg, 1.4 mmol) was added at 0 ^oC. After 30 min, methyl iodide (0.05 mL, 0.8 mmol) was added at 0 ^oC and the mixture was stirred for an additional 24 h at 25 ^oC. Methanol was then added to destroy the excess sodium hydride. The resulting solution was concentrated and the residue purified by column chromatography (hexane/ethyl acetate 2:1 v/v). Compound **40** was isolated as a syrup (0.29 g, 65%): $[\alpha]_D$ +92^o (c 1.0, chloroform); ^1H NMR (250 MHz, $CDCl_3$) δ = 7.40-7.29 (m, 5H, Ph), 5.05 (t, 1H, dioxolane), 4.86 (s, 1H, $J_{1,2}$ = 1.8 Hz, 1-H), 4.68 (s, 2H, $PhCH_2$), 3.97-3.95 (m, 2H, CH_2 spacer), 3.93 (dd, 1H, 3-H), 3.89-3.78 (m, 2H, CH_2 spacer), 3.75-3.62 (m, 6H, $2CH_2$ spacer, 2-H and 5-H), 3.55 (dd, 1H, 4-H), 3.53-3.50 (d, 2H, CH_2 spacer), 3.50 (s, 3H, OCH_3), 1.26 (d, 3H, $J_{5,6}$ = 6.3 Hz, 6-H).

Anal. Calcd. for $C_{20}H_{29}O_7N_3$ (423.4): C, 56.72; H, 6.90; N, 9.92. Found: C, 56.40; H, 6.87; N, 10.01.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-(2-O-Acetyl-4-azido-3-O-benzyl-4,6-di-deoxy-α-D-mannopyranosyl)-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (41).-

A solution of compound **39** (100 mg, 0.24 mmol) and trichloroacetimidate **13** (137 mg, 0.29 mmol) in dry dichloromethane (10 mL) was stirred in the presence of molecular sieves (4Å, 0.5 g) for 15 min. The mixture was cooled to 0 ^oC under nitrogen, then trimethylsilyl triflate (0.008 mL) was added and the mixture was stirred while the temperature was allowed to rise slowly (0 $^oC \rightarrow$ 25 ^oC). TLC (hexane/ethyl acetate 2:1 v/v) showed the presence of one major product (R_f 0.59). The mixture was diluted with dichloromethane (10 mL), neutralized with triethylamine, filtered and the filtrate was concentrated. The residue was purified by column

chromatography (hexane/ethyl acetate 6:1 v/v) to afford **41**, isolated as a syrup (103 mg, 62%): $[\alpha]_D$ +42° (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.40–7.25 (m, 10H, 2Ph), 5.47 (dd, 1H, $J_{2b,3b}$ = 3.5 Hz, H-2b), 5.15 (t, 1H, dioxolane), 4.85 (d, 1H, $J_{1b,2b}$ = 1.3 Hz, H-1b), 4.76 (d, 1H, $J_{1a,2a}$ = 1.5 Hz, H-1a), 4.75-4.60 (2dd, 4H, 2PhCH$_2$), 4.00-3.92 (m, 3H, 2-H and CH$_2$ spacer), 3.89-3.82 (m, 2H, CH$_2$ spacer), 3.80-3.75 (m, 2H, 3a, 3b-H), 3.75-3.65 (m, 4H, 2CH$_2$ spacer), 3.60-3.50 (m, 2H, 5a, 5b-H), 3.55 (d, 2H, CH$_2$ spacer), 3.45-3.32 (m, 2H, 4a, 4b-H), 2.28 (s, 3H, CH$_3$CO), 1.45-1.32 (2d, 6H, 6a, 6b-H).
Anal. Calcd. for $C_{34}H_{44}O_{11}N_6$ (712.7): C, 57.29; H, 6.22; N, 11.79. Found: C, 57.42; H, 6.14; N, 11.86.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-(4-Azido-3-O-benzyl-4,6-di-deoxy-α-D-mannopyranosyl) -(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-man-nopyranoside (42).-

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-(4-Azido-3-O-benzyl-2-O-methyl-4,6-di-deoxy-α-D-mannopyranosyl)-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (43).-

To a solution of **41** (0.172 g, 0.24 mmol), in methanol (2 mL) was added methanolic sodium methoxide (3 mL) and the reaction was stirred for 1 h at rt. The reaction mixture was neutralized with Dowex-50 (H^+) resin, then filtered and the filtrate concentrated to give 4-(1,3-Dioxolan-2-yl)-3-oxabutyl-(4-Azido-3-O-benzyl-4,6-di-deoxy-α-D-manno- pyranosyl)-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside **42** as a syrup.
Compound **42** was transformed into **43** using the same procedure described for the preparation of **40**. Compound **43** was isolated as a syrup (95 mg, 65%); $[\alpha]_D$ + 51° (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.45-7.30 (m, 5H, Ph), 5.05 (t, 1H, CH dioxolane), 4.93 (d, 1H, $J_{1b,2b}$ = 1.6 Hz, 1b-H), 4.87 (d, 1H, $J_{1a,2a}$ = 1.7 Hz, 1a-H), 4.83-4.59 (2dd, 4H, PHCH$_2$), 4.05-3.85 (m, 5H, 2CH$_2$ dioxolane and 2a-H), 3.80-3.65 (m, 6H, 3a, 3b, 2CH$_2$ spacer-H), 3.55 (d, 2H, CH$_2$ spacer), 3.55-3.48 (m, 3H, 4a, 5a, 5b-H), 3.40 (dd, 1H, 2b-H), 3.25 (dd, 1H, 4b-H), 3.05 (s, 3H, OCH$_3$), 1.38, 1.25 (2d, 6H, 6a, 6b-H).
Anal. Calcd. for $C_{33}H_{44}O_{10}N_6$ (684.7): C, 57.88; H, 6.48; N, 12.27. Found: C, 57.92; H, 6.40; N, 12.21.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-(2-O-Acetyl-4-azido-3-O-benzyl-4,6-di-deoxy-α-D-mannopyranosyl)-(1→2)-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (44).-

Compounds **42** (1 mmol) and trichloroacetimidate **13** (1.5 mmol) in presence of TMSOTf (0.1 mmol), was transform as described for the preparation of **41**. The residue was purified by column chromatography (hexane/ethyl acetate 8:1 v/v) to afford **44**, isolated as a syrup (54 % yield): $[\alpha]_D$ +112° (c 1.1, chloroform); ^{1}H NMR (250 MHz, CDCl3) δ = 7.46–7.28 (m, 15H, 3Ph), 5.43 (dd, 1H, $J_{2c,3c}$ = 3.4 Hz, 2c-H), 5.18 (t, 1H, dioxolane), 4.80 (d, 1H, $J_{1b,2b}$ = 1.3 Hz, 1b-H), 4.79 (d, 1H, $J_{1c,2c}$ = 1.5 Hz, 1c-H), 4.51-4.75 (m, 7H, 3PhCH_2, 1a-H), 4.00-3.92 (m, 2H,CH_2 spacer), 3.89-3.82 (m, 4H, CH_2 spacer, 2b, 2a-H), 3.80-3.75 (m, 3H, 3a, 3b, 3c-H), 3.73-3.60 (m, 4H, 2CH_2 spacer), 3.60-3.55 (m, 3H, 5a, 5b, 5c-H), 3.55 (d, 2H, CH_2 spacer), 3.42-3.28 (m, 3H, 4a, 4b, 4c-H), 2.04 (s, 3H, CH_3CO), 1.35-1.28 (3d, 9H, 6a, 6b, 6c-H).
Anal. Calcd. for $C_{47}H_{59}O_{14}N_9$ (974.0): C, 57.96; H, 6.11; N, 12.94. Found: C, 57.89; H, 5.98; N, 12.90.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (45).-

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-(4-azido-3-O-benzyl-2-O-methyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (46).-

To a solution of **44** (123 mg, 0.12 mmol), in methanol (2 mL) was added methanolic sodium methoxide (3 mL) and the reaction was stirred for 1 h at rt. The reaction mixture was neutralized with Dowex-50 (H^+) resin, then filtered and the filtrate concentrated to give **45** as a syrup.
Compound **45** was transformed into **46** using the same procedure described for the preparation of **43** giving **46** (52 % yield); $[\alpha]_D$ +42° (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.60-7.26 (m, 15H, 3Ph), 5.18 (t, 1H, dioxolane), 4.93-4.80 (2d, 1H, $J_{1b,2b}$ = 1.5 Hz, 1b, 1c -H), 4.71 (d, 1H, $J_{1a,2a}$ = 1.6 Hz, 1a-H), 4.69-4.52(m, 6H, 3PhCH_2), 4.05-3.82 (m, 2H,CH_2 spacer), 3.93-3.78 (m, 5H, CH_2 spacer, 2a, 2b, 2c-H), 3.72-3.65 (m, 3H, 3a, 3b, 3c-

H), 3.75-3.65 (m, 4H, $2CH_2$ spacer), 3.60-3.52 (m, 3H, 5a, 5b, 5c-H), 3.51 (d, 2H, CH_2 spacer), 3.50-3.31 (m, 3H, 4a, 4b, 4c-H), 3.20 (s, 3H, OMe), 1.40-1.28 (3d, 9H, 6a, 6b, 6c-H). Anal. Calcd. for $C_{46}H_{59}O_{13}N_9$ (946.0): C, 58.40; H, 6.29; N, 13.33. Found: C, 58.36; H, 6.32; N, 13.40.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-O-Acetyl-3-O-benzyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-α-D-mannopyranoside (47).-

A solution of azide **38** (0.130 g, 0.2 mmol) in absolute ethanol (2 mL) was stirred in the presence of Pd/C (5%, 63 mg) under a hydrogen atmosphere. After 2 h, TLC (hexane/ethyl acetate 1:1 v/v) revealed complete conversion of the starting material into a new compound (R_f 0.0, ninhydrin test). The mixture was filtered and the filtrate was concentrated and dried *in vacuo*.

To a solution of the resulting amine (0.10 g, 0.23 mmol) and 2,4-di-O-acetyl-3-deoxy-L-*glycero*-tetronic acid **21** (0.095 g, 0.47 mmol) in dichloromethane (0.5 mL) was added EEDQ (0.116 g, 0.47 mmol) and the resulting solution was stirred for 15 min, when TLC (hexane/ethyl acetate 3:1 v/v) showed complete conversion of the starting material. The reaction mixture was concentrated and the residue purified by column chromatography on silica gel (hexane/ ethyl acetate 4:1 v/v) to give the title compound **47**, isolated as a syrup: (0.120 g, 85%); $[\alpha]_D$ +63° (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.4–7.2 (m, 5H, Ph), 6.13 (d, 1H, $J_{4,NH}$ 9.6 Hz, NHCO), 5.39 (d, 1H, $J_{2,3}$ 3.3 Hz, 2a-H), 5.18 (dd, 1H, H-2b), 5.03 (t, 1H, dioxolan), 4.82 (s, 1H, $J_{1,2}$ = 1.9 Hz, 1a-H), 4.65, 4.37 (dd, 2H, $PhCH_2$), 4.14-4.08 (m, 2H, 4b-H), 4.03 (m, 1H, 4a-H), 3.98-3.95 (m, 4H, $2CH_2$ dioxolane), 3.87-3.86 (m, 2H, 3a, 5a-H), 3.80 (m, 2H, CH_2 spacer), 3.69-3.60 (m, 2H, CH_2 spacer), 3.55 (dd, 2H, CH_2 spacer), 2.15, 2.10, 2.05 (3s, 9H, $3CH_3CO$), 2.15-2.10 (m, 2H, H-3b), 1.28 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6a).
Anal. Calcd for $C_{29}H_{41}N_1O_{13}$ (611.6): C, 56.94; H, 6.70; N, 2.29. Found: C, 56.77; H, 6.61; N, 2.31.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 3-O-Benzyl-2-O-methyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-α-D-mannopyranoside (48).-

A solution of azide **40** (0.140 g, 0.32mmol) in absolute ethanol (2 mL) was stirred in the presence of Pd/C (5%, 70 mg) under a hydrogen atmosphere. After 2 h, TLC (hexane/ethyl acetate 1:1 v/v) revealed complete conversion of the starting material into a new compound

(R_f 0.0, ninhydrin test). The mixture was filtered and the filtrate was concentrated and dried *in vacuo*.

Treatment of the resulting amine (0.20 mmol) and 2,4-di-O-acetyl-3-deoxy-L-*glycero*-tetronic acid **21** (0.40 mmol) as for the preparation of **47** gave, after chromatography using (hexane/ethyl acetate 1:1 v/v) as eluent, compound **48**, isolated as a syrup: (0.158 g, 82%); $[\alpha]_D$ + 98° (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.43-7.26 (m, 5H, Ph), 6.28 (d, 1H, $J_{4,NH}$ = 9.6 Hz, NHCO), 5.23 (dd, 1H, 2b-H), 5.15 (t, 1H, CH dioxolane), 4.92 (s, 1H, $J_{1,2}$ = 1.3 Hz, 1a-H), 4.46 (dd, 2H, $PhCH_2$), 4.18 (m, 2H, 4b-H), 4.09 (dd, 1H, 4a-H), 4.05-3.91 (m, 4H, $2CH_2$ dioxolane), 3.88-3.80 (m, 2H, 3a, 5a-H), 3.75-3.66 (m, 4H, $2CH_2$ spacer), 3.64 (dd, 1H, 2a-H), 3.55 (d, 2H, CH_2 spacer), 3.56 (s, 3H, OCH_3), 2.26-2.17 (m, 2H, H-3b), 2.15-2.05 (2s, 6H, $2CH_3CO$), 1.29 (d, 3H, $J_{5,6}$ = 6.3 Hz, 6a -H).

Anal. Calcd for $C_{28}H_{41}N_1O_{12}$ (583.6): C, 57.62; H, 7.08; N, 2.40. Found: C, 57.64; H, 7.12; N, 2.36.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-[2-O-Acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (49).-

A solution of azide **41** (0.189 g, 0.26 mmol) in absolute ethanol (3 mL) was stirred in the presence of Pd/C (5%, 95 mg) under a hydrogen atmosphere. After 2 h, TLC (hexane/ethyl acetate 1:1 v/v) revealed complete conversion of the starting material into a new compound (R_f 0.0, ninhydrin test). The mixture was filtered and the filtrate was concentrated and dried *in vacuo*.

Treatment of the resulting amine (0.18 mmol) and 2,4-di-O-acetyl-3-deoxy-L-*glycero*-tetronic acid **21** (0.72 mmol) as for the preparation of **47** gave, after chromatography using (hexane/ethyl acetate 1:1 v/v) as eluent, compound **49**, isolated as a syrup, (0.160 g, 59%); $[\alpha]_D$ +37° (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.46-7.29 (m, 10H, Ph), 6.16 (d, 1H, $J_{4,NH}$ = 9.8 Hz, NHCO), 5.99 (d, 1H, $J_{4,NH}$ = 9.5 Hz, NHCO), 5.59 (dd, 1H, $J_{2b,3b}$ = 3.6 Hz, 2b-H), 5.28 (dd, 1H, 2c-H), 5.15 (t, 1H, CH dioxolan), 4.98 (s, 1H, $J_{1b,2b}$= 1.9 Hz, 1b-H), 4.85 (s, 1H, $J_{1,2}$ = 1.8 Hz, 1a-H), 4.60, 4.50 (dd, 4H, $2PhCH_2$), 4.28-4.05 (m, 5H, 4c, 4d, 2a, 4a, 4b-H), 4.08-3.95 (m, 4H, $2CH_2$ dioxolan), 3.95-3.78 (m, 6H, 3a, b, 5a, 5b-H, CH_2 spacer), 3.69-3.50 (m, 2H, CH_2 spacer), 3.58 (d, 2H, CH_2 spacer), 2.15-2.00 (m, 4H, 3c, 3d-H), 2.2-2.0 (5s, 15H, $5CH_3CO$), 1.27 (2d, 6H, 6a, 6b-H).

Anal. Calcd for $C_{50}H_{63}O_{21}N_2$ (730.7): C, 82.28; H, 9.38; N, 3.83. Found: C, 82.29; H, 9.20; N, 3.90.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-O-[4-(2,4-di-O-Acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-2-O-methyl-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (50).-

A solution of azide **43** (0.146 g, 0.212 mmol) in absolute ethanol (2 mL) was stirred in the presence of Pd/C (5%, 70 mg) under a hydrogen atmosphere. After 2 h, TLC (hexane/ethyl acetate 1:1 v/v) revealed complete conversion of the starting material into a new compound (R_f 0.0, ninhydrin test). The mixture was filtered and the filtrate was concentrated and dried *in vacuo*.

Treatment of the resulting amine (0.20 mmol) and 2,4-di-O-acetyl-3-deoxy-L-*glycero*-tetronic acid **21** (0.80 mmol) as for the preparation of **47** gave, after chromatography using (hexane/ethyl acetate 1:1 v/v) as eluent, the compound **50**, isolated as a syrup: (0.117 g, 55%); $[\alpha]_D$ +42° (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.48-7.20 (m, 10H, Ph), 6.26 (d, 1H, $J_{4,NH}$ = 9.6 Hz, NHCO), 5.98 (d, 1H, $J_{4,NH}$ = 9.4 Hz, NHCO), 5.24 (dd, 1H, 2c,d-H), 5.19 (t, 1H, CH dioxolan), 5.07 (s, 1H, $J_{1b,2b}$ = 1.5 Hz, H-1b), 4.88 (s, 1H, $J_{1,2}$ = 1.7 Hz, H-1a), 4.60, 4.50 (2dd, 4H, $PhCH_2$), 4.25-4.08 (m, 5H, 4d, 4c, 2a, 4a, 4b-H), 4.08-3.90 (m, 4H, $2CH_2$ dioxolan), 3.96-3.75 (m, 6H, 3a, 3b, 5a, 5b-H, CH_2 spacer), 3.75-3.66 (m, 3H, 2b-H, CH_2 spacer), 3.58 (dd, 2H, CH spacer), 3.35 (s, 3H, OMe), 2.15-2.20 (m, 4H, 3d, 3c-H), 2.25-2.09 (4s, 12H, $4CH_3CO$), 1.26 (2d, 6H, 6a, 6b-H).

Anal. Calcd. for $C_{49}H_{68}O_{20}N_2$ (1005.1): C, 58.55; H, 6.82; N, 2.79. Found: C, 58.39; H, 6.93; N, 2.85.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-[2-O-Acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-[4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (51).-

A solution of azide **44** (60 mg, 0.16 mmol) in absolute ethanol (2 mL) was stirred in the presence of Pd/C (5%, 30 mg) under a hydrogen atmosphere. After 2 h, TLC (hexane/ethyl acetate 1:1 v/v) revealed complete conversion of the starting material into a new compound

(R_f 0.0, ninhydrin test). The mixture was filtered and the filtrate was concentrated and dried *in vacuo*.

Treatment of the resulting amine (0.20 mmol) and 2,4-di-O-acetyl-3-deoxy-L-*glycero*-tetronic acid **21** (0.120 mmol) as for the preparation of **47** gave, after chromatography using (hexane/ethyl acetate 1:1 v/v) as eluent, the compound **51**, isolated as a syrup: (0.158 g, 48%); $[\alpha]_D$ +37° (c 1.0, chloroform); ^{1}H NMR (250 MHz, CDCl3) δ 7.50-7.32 (m, 15H, Ph), 6.16 (m, 2H, 2NHCO), 6.02 (d, 1H, $J_{4,NH}$ = 9.9 Hz, NHCO), 5.72 (dd, 1H, $J_{2c,3c}$ 3.6 Hz, 2c-H), 5.30-5.17 (m, 3H, 2d, e, f-H), 5.13 (d 1H, CH dioxolan), 5.01 (s, 1H, $J_{1c,2c}$ = 1.9 Hz, 1c-H), 4.85 (s, 1H, $J_{1a,2a}$ = 1.8 Hz, 1a-H), 4.80-4.40 (dd, 6H, 3PhCH$_2$), 4.30-4.00 (m, 6H, 4d, 4e, 4f, 2a, 4a, 4c-H), 4.00-3.95 (m, 4H, 2CH$_2$ dioxolan), 3.96-3.70 (m, 6H, 3a, 3b, 3c, 5a, 5b-H, CH$_2$ spacer), 3.65-3.47 (m, 2H, CH$_2$ spacer), 3.46 (d, 2H, CH$_2$ spacer), 2.15-2.00 (m, 6H, 3d, 3e, 3f-H), 2.2-2.0 (m, 35H, 7CH$_3$CO), 1.30-1.18 (3d, 9H, 6a, 6b, 6c-H).

Anal. Calcd. for $C_{71}H_{90}O_{29}N_3$ (1151.7): C, 74.05; H, 7.88; N, 3.65. Found: C, 73.99; H, 7.91; N, 3.60.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-[4-(2,4-di-O-Acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-2-O-methyl-4,6-dideoxy-α-D-mannopyranosyl] -(1→2)-[4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (52).-

A solution of azide **46** (50 mg, 0.13 mmol) in absolute ethanol (2 mL) was stirred in the presence of Pd/C (5%, 30 mg) under a hydrogen atmosphere. After 2 h, TLC (hexane/ethyl acetate 1:1 v/v) revealed complete conversion of the starting material into a new compound (R_f 0.0, ninhydrin test). The mixture was filtered and the filtrate was concentrated and dried *in vacuo*.

Treatment of the resulting amine (0.20 mmol) and 2,4-di-O-acetyl-3-deoxy-L-*glycero*-tetronic acid 21 (0.120 mmol) as for the preparation of **47** gave, after chromatography using (hexane/ethyl acetate 1:1 v/v) as eluent, compound **52**, isolated as a syrup (50%); $[\alpha]_D$ +146° (c 0.8, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.50-7.10 (m, 15H, Ph), 6.27-6.23 (2d, 2H, $J_{4,NH}$ = 9.9 Hz, NHCO), 5.92 (d, 1H, $J_{4,NH}$ = 9.8 Hz, NHCO, 5.22 (d 1H, CH dioxolan), 5.17 (s, 1H, $J_{1b,2b}$ = 1.5 Hz, 1b-H), 4.86 (s, 1H, $J_{1a,2a}$ =1.7 Hz, 1a-H), 4.60, 4.50 (dd, 4H, 2PhCH$_2$), 4.20-4.00 (m, 5H, 4d, 4e, 4f, 2a, 4a, 4b-H), 4.08-3.90 (m, 4H, 2CH$_2$ dioxolan), 3.94-3.7 (m, 6H, 3a, 3b, 3c, 5a, 5b, 5c-H, CH$_2$ spacer), 3.72-3.53 (m, 3H, 2c-H, CH$_2$ spacer),

3.50 (dd, 2H, CH spacer), 3.30 (s, 3H, OMe), 2.11-2.22 (m, 6H, 3d, 3e, 3f-H), 2.30-2.10 (m, 18H, $6CH_3CO$), 1.20-1.16 (3d, 9H, 6a, 6b, 6c-H).
Anal. Calcd. for $C_{70}H_{95}O_{28}N_3$ (1426.1): C, 74.05; H, 7.88; N, 6.71. Found: C, 74.01; H, 7.93; N, 6.82.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2,3-di-O-Acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-α-D-mannopyranoside (53).-

The protected amide **47** (50 mg, 0.08 mmol) in absolute ethanol was hydrogenated over Pd/C (5%, 45 mg). After 24 h, TLC (hexane/ethyl acetate 1:2 v/v) showed complete conversion into a new compound (R_f 0.2). The reaction mixture was filtered and the filtrate concentrated. A solution of the residue in pyridine (1 mL) was cooled to 0-5 ^oC and acetic anhydride (0.4 mL) was added. The resulting solution was stirred for 2 h, then concentrated and co-concentrated with toluene (3 x 10mL). Column chromatography (hexane/ethyl acetate 1:1 v/v) of the residue afforded **59** as a syrup (34 mg, 90%): $[\alpha]_D$ +33^o (c 1.0, chloroform); ^1H NMR (250 MHz, $CDCl_3$) δ 6.25 (d, 1H, $J_{4,NH}$ =9.5 Hz, NHCO), 5.32 (dd, 1H, $J_{3,4}$ =10.3 Hz, 3a-H), 5.15 (dd, 1H, $J_{2,3}$ =3.3 Hz, 2a-H), 5.11-5.05 (m, 2H, CH dioxolane and 2b-H), 4.85 (d, 1H, $J_{1,2}$ 1.7 = Hz, 1a-H), 4.28-4.19 (m, 1H, 4a-H), 4.15-4.10 (m, 2H, 4c-H), 4.08-3.96 (m, 4H, $2CH_2$ dioxolane), 3.85-3.75 (m, 4H, $2CH_2$ spacer), 3.8 (m, 1H, 5a-H), 3,55 (d, 2H, CH_2 spacer), 2.15 (m, 2H, C-3c), 2.15-2.05 (4s, 12H, $4CH_3CO$), 1.26 (d, 3H, $J_{5,6}$ 6.4 Hz, H-6).
Anal. Calcd for $C_{24}H_{37}O_{14}N_1$ (563.6): C, 51.15; H, 6.62; N, 2.48. Found: C, 51.31; H, 6.79; N, 2.55.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 3-O-Acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-2-O-methyl-α-D-mannopyranoside (54).-

Compound **54** was prepared from compound **48** (0.025 g, 0.04 mmol) by the same procedure as described for **53**. The yield of **54** isolated as a syrup was 0.020 g (88%); $[\alpha]_D$ +37^o (c 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 6.36 (d, 1H, $J_{4,NH}$ 9.5 Hz, NHCO), 5.27 (dd, 1H, $J_{3,4}$ = 10.5 Hz, H-3a), 5.15-5.10 (m, 2H, CH dioxolan and H-2b), 4.88 (d, 1H, $J_{1,2}$ = 1.4 Hz, 1a-H), 4.28-4.15 (m, 3H, 4a, 4b-H), 4.08-3.95 (m, 4H, $2CH_2$ dioxolan), 3.85 (m, 2H, CH_2 spacer), 3.85-3.70 (m, 3H, 5a-H, CH_2 spacer), 3.60 (d, 2H, CH_2 spacer), 3.55 (dd, 1H, 2a-H), 3.50 (s, 3H, OCH_3), 2.15 (m, 2H, 3b-H), 2.15-2.05 (3s, 9H, $3CH_3CO$), 1.26 (d, 3H, $J_{5,6}$ = 6.3 Hz, 6a-H).

Anal. Calcd for $C_{27}H_{37}O_{13}N_1$ (583.6): C, 55.56; H, 6.39; N, 2.40. Found: C, 55.48; H, 6.11; N, 2.32.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-[2,3-di-O-Acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-acetyl-4,6-dideoxy-α-D-mannopyranoside (55).-

The title compound **55** was prepared from compound **49** (0.035 g, 0.034 mmol) by the same procedure as described for **53**. The compound **55**, was obtained as syrup (0.026 g, 82%); $[\alpha]_D$ +48° (c 1.0, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.35 (d, 1H, $J_{4,NH}$ =9.6 Hz, NHCO), 6.08 (d, 1H, $J_{4,NH}$ =9.7 Hz, NHCO), 5.46 (d, 1H, $J_{2,3}$ =3.5 Hz, H-2b), 5.24 (m, 2H, 3a, 3b-H), 5.18-5.00 (m, 3H, 2c, 2d-H, CH dioxolan), 4.95 (s, 1H, $J_{1,2}$ =1.3 Hz, 1a-H), 4.88 (s, 1H, $J_{1b,2b}$ =1.5 Hz, 1b-H), 4.35-4.18 (m, 6H, 4c, 4d, 4a, 4b-H), 4.03-3.95 (m, 5H, 2a-H and $2CH_2$ dioxolan), 3.95-3.78 (m, 6H, 5a, 5b-H and $2CH_2$ spacer), 3.59 (dd, 2H, CH_2 spacer), 2.38-2.32 (m, 4H, 3c, 3d-H), 2.35-2.00 (7s, 21H, $7CH_3CO$), 1.28 (2d, 6H, 6a, 6b-H).
Anal. Calcd for $C_{40}H_{59}O_{23}N_2$ (935.9): C, 51.33; H, 6.35; N, 2.87. Found: C, 51.51; H, 6.49; N, 2.94.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-[3-O-Acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-2-O-methyl-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-3-O-acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (56).-

The compound **56** was prepared from the compound **50** (0.028 g, 0.040 mmol) by the same procedure as described for **53**. Compound **56**, was isolated in a yield of 0.030 g (0.021g, 85%); $[\alpha]_D$ +25° (c 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.45 (d, 1H, $J_{4,NH}$ = 9.7 Hz, NHCO), 6.33 (d, 1H, $J_{4,NH}$ = 9.5 Hz, NHCO), 5.38 (d, 1H, 3b-H), 5.22 (dd, 1H, 3a-H), 5.15-5.10 (m, 3H, 2c, 2d-H, CH dioxolan), 5.10 (s, 1H, $J_{1,2}$ = 1.7 Hz, H-1a), 4.86 (s, 1H, $J_{1,2}$ = 1.5 Hz, 1b-H), 4.33-4.08 (m, 8H, 4c, 4d, 4a, 4b-H, $2CH_2$ dioxolan), 4.00-3.85 (m, 5H, 2a-H and $2CH_2$ spacer), 3.85-3.60 (m, 6H, 5a, 5b, 2b-H), 3.55 (d, 2H, CH_2 spacer), 3.50 (s, 3H, OCH_3), 2.28-2.00 (m, 4H, 3c, 3d-H), 2.20-2.00 (6s, 18H, $6CH_3CO$), 1.24-1.16 (2d, 6H, 6a, 6b-H).
Anal. Calcd for $C_{39}H_{60}O_{22}N_2$ (908.9): C, 51.53; H, 6.65; N, 3.08. Found: C, 51.21; H, 6.69; N, 3.13.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-[2,3-di-O-Acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-[3-O-acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-3-O-acetyl-4,6-dideoxy-α-D-mannopyranoside (57).-

The title compound **57** was prepared from compound **51** (0.040 g, 0.035 mmol) by the same procedure as described for **53**. The compound **57** was obtained as syrup, (0.037 g, 82%); $[\alpha]_D$ +21° (c 1.1, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.32 (d, 1H, $J_{4,NH}$ = 9.5 Hz, NHCO), 6.08 (2d, 2H, 2NHCO), 5.46 (d, 1H, $J_{2,3}$ = 3.5 Hz, 2c-H), 5.39-5.24 (m, 3H, 3a, 3b, 3c-H), 5.20 (d, 1H, 2b-H), 5.16-5.00 (m, 4H, 2e, 2f, 2d, CH dioxolane-H), 4.97 (s, 1H, $J_{1a,2a}$ =1.3 Hz, 1a-H), 4.88 (s, 1H, $J_{1b,2b}$ = 1.5 Hz, 1b-H), 4.38-4.08 (m, 6H, 4d, 4e, 4f, 4a, 4b,4c-H), 4.03-3.95 (m, 5H, 2a-H and $2CH_2$ dioxolan), 3.92-3.75 (m, 7H, 5a, 5b, 5c-H and $2CH_2$ spacer), 3.57 (dd, 2H, CH_2 spacer), 2.42-2.30 (m, 6H, 3d, 3e, 3f-H), 2.35-2.00 (7s, 21H, $7CH_3CO$), 1.28 (3d, 9H, 6a, 6b, 6c-H).

Anal. Calcd for $C_{56}H_{81}O_{32}N_3$ (1308.2): C, 51.41; H, 6.21; N, 3.21. Found: C, 51.45; H, 6.17; N, 3.27.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl-[3-O-Acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-2-O-methyl-4,6-dideoxy-α-D-mannopyranosyl]- (1→2)- 3-O-acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-3-O-acetyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (58).-

The title compound **58** was prepared from compound **52** (0.035 g, 0.02 mmol) by the same procedure as described for **53**. Compound **58**, was isolated as syrup (0.025g, 80%); $[\alpha]_D$ +47° (c 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.43-6.38 (2d, 2H, 2NHCO), 6.35 (d, 1H, $J_{4,NH}$ = 9.5 Hz, NHCO), 5.54-5.46 (m, 2H, 3a, 3b-H), 5.32 (dd, 1H, 3C-H), 5.20-5.05 (m, 4H, 2d, 2e, 2f-H, CH dioxolan), 5.01 (s, 1H, $J_{1,2}$ 1.7 = Hz,1a-H), 4.83-4.78 (m, 2H, $J_{1,2}$ = 1.5 Hz, 1b,1c-H), 4.60-4.08 (m, 13H, 4d, 4e, 4f, 4a, 4b, 4c-H, $2CH_2$ dioxolan), 4.00-3.75 (m, 5H, 2a-H and $2CH_2$ spacer), 3.75-3.60 (m, 6H, 5a, 5b,5c, 2b-H), 3.58 (d, 2H, CH_2 spacer), 3.53 (s, 3H, OCH_3), 2.28-2.21 (m, 6H, 3d, 3e, 3f-H), 2.20-1.86 (m, 18H, $6CH_3CO$), 1.28-1.18 (3d, 9H, 6a, 6b, 6c-H).

Anal. Calcd for $C_{55}H_{88}O_{31}N_3$ (1281.2): C, 51.56; H, 6.92; N, 3.27. Found: C, 51.48; H, 6.96; N, 3.33.

General Procedure for Deprotection and Conjugation with Proteins.

The oligosaccharide derivatives (**53**-**58**) were dissolved in formic acid at rt and the solution was stirred for 2 h. TLC (hexane/ethyl acetate 1:6 v/v) indicated the disappearance of the starting material and the appearance of a new spot (aniline phthalate positive). The acid was removed with nitrogen gas at rt and toluene was coevaporated several times from the residue; ^{1}H NMR ($CDCl_3$) δ 9.75-9.72 (s, 1H, HC=O).

The residues (free of formic acid) were dissolved in a solution of sodium methoxide in anhydrous methanol (0.1 M, pH = 9) at 0 ^{o}C. After 45 min, TLC (ethyl acetate/methanol 20:1 v/v) revealed the complete de-O-acetylation of the starting material. The mixture was neutralized with acetic acid, concentrated at room temperature and used directly for conjugation (→**I1-O3**).

The oligosaccharide content was estimated before and after conjugation using the phenol-sulfuric acid method, with 4-(1,3-dioxolan-2-yl)-3-oxabutyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside **I1** as standard. A solution of the oligosaccharide (0.023 mmol) in a phosphate buffer (150 μL, 0.2M pH = 8) was added to a solution of BSA (9.1 mg, 1.34 x 10^{-4} mmol) in the same buffer (150 μL) or OMP (9.1 mg, 1mL buffer). Sodium cyanoborohydride (1.79 mg, 0.029 mmol) was added and the resulting suspension was stirred at 35 ^{o}C for 48 h. After several dyafiltrations, the final products were analyzed by their carbohydrate and protein content.

Methyl 5-Acetamido-2,3,5-trideoxy-8,9-O-isopropylidene-D-glycero-D-galacto-non-2-enopyranosonate (59).-

Compound **59** was synthesized following a published procedure.[85]

Methyl 5-Acetamido-4-O-benzyl-2,3,5-trideoxy-8,9-O-isopropylidene-D-glycero-D-galacto-non-2-enopyranosonate (60).-

Compound **60** was synthesized following a published procedure.[79]

Methyl 5-Acetamido-4-O-benzyl-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonate (61).-

Compound **60** (7 g, 16.5 mmol) was suspended in methanol pa (150 mL) and trifluormethanosulphonic acid (0.3 mL) was added. The reaction was monitored by TLC toluene:acetone 2:3. When all the starting material was transformed into a single slower moving spot, the reaction was neutralized with triethylamine and concentrated to dryness, to afford crude **61** (6.0 g, 95%); as a white powder, which is pure enough for its use in the next reactions. $[\alpha]_D$ = + 34 (c = 1.2, $CHCl_3$);^{1}H-NMR (250 MHz, MeOD): δ =1.98 (s, 3H, AcNH), 3.55-3.70 (m, 2H, 7, 9-H), 3.80 (s, 3H, OCH_3), 3.80-3.95 (m, 2H, 8, 9-H), 4.20 (m 2H, 5, 6-H), 4.40 (m, 1H, 4-H), 4.65 (dd, 2H, CH_2Ph), 6.10 (d, 1H, $J_{3,4}$ = 2.3 Hz, 3-H), 7.30-7.40 (m, 5H, Ph), 8.30 (b, 1H, NH).

Methyl 5-Acetamido-4,9-di-O-benzyl-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonate (62).-

Compound **61** (3 g, 7.5 mmol) was suspended in toluene:methanol 10:1 (100 ml), containing tributyltin oxide (2.5 g, 10.9 mmol). The mixture was refluxed with continuous azeotropic removal of water for 12h, then the solvent were removed and the residue was coevaporated with toluene (3×10 mL) and dried under vacuum. The residue was dissolved in absolute toluene (30 mL) and benzyl bromide (27 mL, 22.6 mmol) and tetrabutylammonium bromide (1.2 g, 3.7 mmol) were successively added. The solution was stirred at 60^{o}C for 4h. The mixture was then let to reach room temperature and concentrated. Column chromatography of the residue using the solvent system (toluene–acetone 3:1-2:1) afforded compound **62** (2.5 g, 73 %) as a white powder. $[\alpha]_D$ = + 42 (c = 1.1, $CHCl_3$): ^{1}H-NMR (600 MHz, $CDCl_3$): δ =1.89 (s, 3H, AcNH), 3.51 (dd, 1H, $J_{7,8}$= 8.7 Hz, $J_{7,OH}$= 3.5 Hz, 7-H), 3.56 (dd, 1H, $J_{9',8}$ =6.2 Hz, 9-H), 3.69(s, 3H, OCH_3), 3.74 (dd, 1H, $J_{9,9'}$= 9.7, $J_{9,8}$= 3.1 Hz, 9-H), 4.02 (m,1H, 8-H), 4.10(d, 1H, 6-H), 4.16 (dd, 1H, 5-H), 4.18 (dd, 1H, 4-H), 4.43 (m, 4H, CH_2Ph, C*H*HPh, OH), 4.60 (d,

1H, *CH*HPh), 5.66 (d, 1H, J= 7.0 Hz, NH), 6.00 (d, 1H, $J_{3,4}$ = 2.5 Hz, 3-H), 7.29-7.19 (m, 10H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 47.9 (C-5), 52.4 (OMe), 68.9 (C-8), 69.2(C-7), 71.8 (C-9), 72.5 (C-4), 77.1 (C-6), 107.9 (C-3), 145.5 (C-2), 162.3 (C-1).

$C_{26}H_{32}O_8N$ (486.6) MS (MALDI, positive mode, Matrix:DHB): m/z = 509 $[M+Na]^+$

Methyl 5-Acetamido-7,8,9-tri-O-acetyl-4-O-benzyl-2,3,5-trideoxy-D-glycero-D-galacto-non-2 -enopyranosonate (63).-

Compound **61** (6 g, 15.1mmol) was dissolved in pyridine (80 mL) containing dimethylaminopyridine (100 mg) and acetic anhydride was added with external cooling (cold water). The solution was stirred overnight at room temperature. TLC toluene:acetone 1:1. The solvents were evaporated and the residue was purified on a silica gel column using toluene:acetone 3:1 as eluant to give compound **63** (8.3 g, 96%). $[\alpha]_D$ = -13 (c = 1.2, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$): δ 2.11-1.89 (4s, each 3H, AcO, AcHN), 3.85 (s, 3H, OMe), 4.10-4.20 (m, 2H, 9, 5-H), 4.30 (m, 2H, 9'-H), 4.40 (dd, 1H, 6-H), 4.50 (dd, 1H, 4-H), 4.61 (dd, 2H, CH_2Ph), 5.40 (m, 1H, 8-H), 5.56 (t, 1H, J=6.4 Hz, 7-H), 5.90 (d, 1H, NH), 6.12 (d, 1H, $J_{3,4}$= 3.1 Hz, 3-H), 7.11-7.32 (m, 5H, Ph).

Methyl 5-Acetamido-7,8,9-tri-O-acetyl-4-O-benzyl-3-bromo-3,5-dideoxy-D-glycero-α-D-galacto-no-nulopyranosonate (64/65).-

Compound **63** (5.7g, 10.9 mmol) was dissolved in a 4:1 acetonitril:water mixture (125 mL) and heated at 80°C, then N-bromosuccinimide (3.4 g, 19.3 mmol) was added and the solution was refluxed with stirring at this temperature for 20 min. TLC toluene:acetone 1:1. The starting material was transformed in a 5:1 mixture of diasteromeric trans-bromohydrines, being the slower moving spot the major one (diaxial). The solvents were removed under vacuum, and the syrup so obtained was used in the next step without further purification.

64 (diaxial): ^{1}H-NMR (250 MHz, $CDCl_3$): δ 1.90 (s, 3H, AcNH), 2.00-2.20 (3s, 12H, 4AcO), 3.70 (m, 1H, 5-H), 3.85 (s, 3H, OMe), 4.20 (dd, 1H, 6-H), 4.40 (d, 1H, *CH*HPh), 4.50-4.65 (m, 3H, 4-H, 9b, *CH*HPh), 4.75 (d, 1H, $J_{3,4}$=3.8 H, 3-H), 4.8 (dd, 1H, 9a-H), 5.25 (m, 1H, 8-H), 5.35 (dd, 1H, 7-H), 5.65 (b, 1H, OH), 5.90 (d, 1H, NH) 7.00-7.40 (m, 5H, Ph).

65 (equatorial): ^{1}H-NMR (250 MHz, $CDCl_3$): δ 1.90 (s, 3H, AcNH), 2.00-2.20 (3s, 12H, 4AcO), 3.90 (s, 3H, OMe), 4.00-4.20 (m, 1H, 9b, 6, 4, 5-H) 4.40 (m, 1H, $J_{3,4}$=11.1 Hz, 3-H),

4.45 (dd, 1H, 9a-H), 4.75 (dd, 2H, CH_2Ph), 5.15 (m, 1H, 8-H), 5.30 (dd, 1H, 7-H), 5.90 (b, 1H, NH), 7.40 (m, 5H, Ph).

Methyl 5-Acetamido-7,8,9-tri-O-acetyl-4-O-benzyl-3- thiophenyl-3,5-dideoxy-D-glycero-α-D- galacto-nonulopyranosonate (66/67).-

The crude product (**64/ 65,** 12 mmol) was dissolved in dry THF (20 mL), then sodium thiophenolate (1.53 g, 12 mmol) was added and thiophenol was dropweise until pH=7. The mixture was stirred under argon at room temperature for 30 min. The TLC showed the starting UV inactive compounds to be transformed in a 2:1 mixture of two strongly UV active spots, but almost with the same Rf value. The mixture was then dilute with CH_2Cl_2 (100 mL) and was washed with NaOH 0.1M solution (20 mL), and saturated NaCl (30 mL), dried with $MgSO_4$, concentrated and well dried in high vacuum. The residue was purified by column chromatography using toluene:acetone 4:1 as eluant, to give pure compounds **66/67**

66 (48%):$[\alpha]_D$ =+ 83 (c = 1.4, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$): δ 1.81-2.12 (4s, each 3H, 3AcO, AcHN), 3.62 (s, 3H, OMe), 3.68 (d, $J_{3,4}$= 9.8 Hz, 3-H), 3.90-4.10 (m, 3H, 9a, 5-H), 4.30-4.40 (m, 2H, 9b, 6-H), 4.65 (d, 1H, *CH*HPh), 4.85 (b, 1H, OH), 4.95 (d, 1H, *CH*HPh), 5.17 (m, 1H, 8-H), 5.31 (dd, 1H, 7-H), 5.21 (d, 1H, NH), 7.08-7.48 (m, 10H, Ph).

67 (25%): $[\alpha]_D$ =+ 21 (c = 1.1, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$): δ 2.00-2.12 (4s, each 3H, 3AcO, AcHN), 3.65 (s, 3H, OMe), 3.80 (d, $J_{3,4}$= 2.4, Hz, 3-H), 3.90-4.10 (dd, 2H, 5, 9a-H), 4.40 (dd, 1H, 4-H), 4.50 (dd, 1H, 6-H), 4.60-4.76 (dd, 3H, CH_2Ph, 9b-H), 4.80 (b, 1H, OH), 5.00 (d, 1H, NH), 5.30 (m, 1H, 8-H), 5.60 (dd, 1H, 7-H), 7.08-7.48 (m, 10H, Ph).

$C_{34}H_{34}O_{11}NS$ (667.71) MS (MALDI, positive mode, Matrix:DHB): m/z = 690 $[M+Na]^+$

Isomerization 67→66:

The syrupy residue **67** was dissolved in dry toluene (20 mL) and DBU (15 μL, 0.1 mmol) at 0°C with stirring under argon. The solution was let slowly to reach room temperature and stirred overnight. TLC: toluene: acetone 1:1. The slower moving spot was transformed in the faster moving one, trough no quantitatively. The residue was purified by column chromatography using toluene: acetone 4:1 as eluant, to give pure compound **66.**

Methyl 5-Acetamido-7,8,9-tri-O-acetyl-4-O-benzyl-3-phenylthio-3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl)onate diethylphosphite (68).-

Compound **66** (2 g, 3.0 mmol) was dissolved in acetonitril p.a (8 mL), diisopropylethylamine (0.76 mL, 4.4 mmol) and diethylclorophosphite (0.56 mL, 3.6 mmol) were successively added. TLC toluene:acetone 2:1. After 15 min the reaction was complete. The solvent was evaporated and the residue purified by column chromatography toluene:acetone 3:1 containing 0.1% Et_3N to afford donor **68** as a white foam (2.0 g, 91%): ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.35 (m, 6H, $2CH_3$), 1.85-2.11 (4s, each 3H, AcO, AcHN), 3.59 (s, 3H, OMe), 3.61 (d, 1H, 5-H), 3.70 (dd, 1H, 3-H), 4.00-4.30 (m, 4H, $2CH_2$), 4.40 (dd, 1H, 6-H), 4.50 (dd, 1H, 4-H), 4.70 (d, 1H, *CH*HPh), 4.90 (d, 1H, *CH*HPh), 5.10 (dd, 1H, 8-H), 5.35 (dd, 1H, 7-H), 5.56 (d, 1H, NH), 7.08-7.48 (m, 10H, Ph).

Methyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-O-benzyl -3-thiophenyl-3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl)onate-(2→8)-5-acetamido-7-O-acetyl-4,9-di-O-benzyl- 2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonate (69).-

A solution of compounds **62** (1 g, 2.08 mmol) and **68** (2.15 g, 2.8 mmol) in dry acetonitril (10 mL) was cooled to –25°C and then treated with trimethylsilyltrifluoromethanesulfonate (100 μL, 0.56 mmol). The solution was let to warm up slowly to room temperature. TLC $CHCl_3$:MeOH 15:1. The solution was neutralized with Et_3N, concentrated and purified by chromatography column using toluene:acetone 5:1 as eluate, to afford **69** (761 mg, 31%) as a white foam.$[\alpha]_D$ = +128 (c = 1.1, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.80-2.07 (5s, each 3H, AcO, AcHN), 3.46 (dd, 1H, $J_{8a,9'a}$ = 2.1 Hz, 9a-H), 3.64 (m, 1H, 5b-H), 3.72 (d, 1H, $J_{3b,4b}$ = 9.8 Hz, 3b-H), 3.73 (s, 3H, OMe), 3.77 (dd, 1H, $J_{9a,8a} < 1$, $J_{9a,9'a}$ = 10.1 Hz, 9a-H), 3.83 (s, 3H, OMe), 3.89 (d, 1H, 7a-H), 4.07 (d, 1H, 9b-H), 4.08 (d, 1H, 4a-H), 4.21 (dd, 1H, 6b-H), 4.27 (d, 1H, 5a-H), 4.31 (d, 1H, 9b-H), 4.32 (d, 1H, 4b-H), 4.47 (d, 1H, $J_{5a,6a}$= 6.7 Hz, 6a-H), 4.53 (m, 1H, 8a- H), 4.61-4.69 (dd, 2H, CH_2 Ph), 4.56-4.89 (dd, 2H, CH_2Ph), 4.18-4.33 (dd, 2H, CH_2Ph), 5.22 (d, 1H, 7b-H), 5.25(d, J= 8.3 Hz, NH-b), 5.40 (d, 1H, 8b-H), 5.99 (d, 1H, $J_{3a,4a}$ = 3.6 Hz, 3a-H), 6.18 (d, J=5.8 Hz, NH), 7.13-7.33 (m, 20H, Ph).
^{13}C-NMR (600 MHz, $CDCl_3$): δ 48.2 (C-5a), 52.3 (C-5b, OMe), 52.7 (OMe), 57.6 (C-3b), 62.4 (C-9b), 67.6 (C-7b), 69.2 (C-8b), 69.6 (C-7a), 69.9 (C-9a), 70.3 (CH_2Ph) 71.0 (C-6b), 71.1 (C-4a), 72.8 (CH_2Ph), 73.4 (C-8a), 75.0 (CH_2Ph), 75.7 (C-6a), 79.2 (C-4b), 101.5 (C-2b), 107.1 (C-3a), 145.6 (C-2a), 162.1 (C-1a), 167.7 (C-1b).

$C_{93}H_{57}O_{18}N_2S$ (1522.5) MS (MALDI, positive mode, Matrix:DHB): m/z = 1545 $[M+Na]^+$

Methyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-O-benzyl -3-phenylthio-3,5-dideoxy-α-D-galacto-nonulopyranosyl)onate-(2→8)-5-acetamido-7-O-acetyl-4,9-di-O-benzyl-3-phenyl-thio - 3,5-trideoxy-D-glycero-D-galacto-nonulopyranosonate (70).-

Compound **69** (860 mg, 0.73 mmol) was acetylated overnight (5 mL Pyridine, 5 mL acetic anhydride, 30 mg DMAP per gram), and stirred at room temperature for 12h. All volatiles were evaporate and the residue was dissolved in a 4:1 acetonitril:water mixture (5 mL) and heated at 80°C, then N-bromosuccinimide (144 mg, 0.80 mmol) was added and the solution was refluxed with stirring at this temperature for 20 min. TLC toluene:acetone 1:1. The starting material was transformed in a 3:1 mixture of diasteromeric trans-bromohydrines, being the slower moving spot the major one (diaxial). The solvents were removed under vacuum, and the syrup so obtained was used in the next step without further purification.
The crude product was dissolved in dry THF (10 mL), then sodium thiophenolate (115 mg, 87 mmol) was added and thiophenol was dropweise until pH=7. The mixture was stirred under argon at room temperature for 30 min. The TLC showed the starting UV inactive compounds to be transformed in a 1:1 mixture of two strongly UV active spots, but almost with the same Rf value. The mixture was then dilute with CH_2Cl_2 (100 mL) and was washed with NaOH 0.1M solution (20 mL), and saturated NaCl (30 mL), dried with $MgSO_4$, concentrated and well dried in high vacuum. The residue was purified by column chromatography using toluene:acetone 4:1-3:1 as eluant, to give pure compound **70** (362 mg, 40%): $[\alpha]_D$= + 71 (c = 1.6, $CHCl_3$); ^{1}H-NMR (600 MHz,$CDCl_3$) δ 1.86-2.21 (6s, each 3H, AcO, AcHN), 3.34 (dd,1H, $J_{9'a,,8a}$= 5.4 Hz, 9'a-H), 3.39 (d, 1H, $J_{3b,4b}$=10 Hz, 3b-H), 3.45 (m, 1H, 5b-H), 3.55 (s, 3H, OMe), 3.67 (dd, 1H, 9b-H), 3.72 (dd, 1H, 5a-H), 3.69 (d, 1H, 3a-H), 3.92 (s, 3H, OMe), 3.95 (dd, 1H, 9a-H), 4.18 (dd, 1H, $J_{3a,4a}$= 10.5 Hz, $J_{4a,\ 5a}$= 8.1 Hz, 4a-H), 4.23 (dd, 1H, J_{gem}= 12.6, $J_{9'b,8b}$= 4.9 Hz, 9'b-H), 4.24 (d, 1H, 6b-H), 4.24-4.35 (dd, 2H, CH_2Ph), 4.34 (d, 1H, 4b-H), 4.57 (m, 1H, 8a-H), 4.60 (d, 1H, 6a-H), 4.60-4.65 (dd, 2H, CH_2Ph), 4.90-5.04 (dd, 2H, CH_2Ph), 5.19 (dd, 1H, $J_{6b,7b}$<1 Hz, $J_{6b,7b}$<1 Hz, $J_{7b,8b}$= 9.1 Hz, 7b-H), 5.24 (dd, 1H, $J_{6a,7a}$<1 Hz, $J_{6b,7b}$<1 Hz, $J_{7a,8a}$= 4.0 Hz, 7a-H), 5.30 (dd, 1H, 8b-H), 5.42 (brd.s, 1H, OH), 4.85 (brd.s, 1H, OH), 5.53 (m, 2H, 2×NH), 7.08-7.54 (m, 25H, Ph).
^{13}C-NMR (600 MHz, $CDCl_3$): δ 52.6 (OMe), 52.8 (C-5a), 52.9 (C-5b), 56.3 (C-3a), 60.1(C-3b), 62.0 (C-9b), 67.3 (C-7b), 68.5 (C-8b), 69.9 (C-6b), 70.4 (7a, 6a), 70.9 (C-9a), 73.1 (C-

8a), 73.3 (CH_2Ph), 74.9-75.0 ($2CH_2Ph$), 78.4 (C-4b), 79.4 (C-4a), 97.3 (C-2b), 100.8 (C-2a), 168.2, 168.8 (C-1a, 1b).

Methyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-O-benzyl -3-phenylthio-3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl)onate-(2→8)-(5-acetamido-7-O-acetyl-4,9-di-O-benzyl-3-phenylthio- 3,5-trideoxy-D-glycero-D-galacto-nonulopyranosyl)onate diethyl-phosphite (71).-

Compound **70** (300 mg, 0.2 mmol) was dissolved in acetonitril p.a (5 mL), diisopropylethylamine (75 μL, 0.38 mmol), and diethylclorophosphite (56 μL, 0.30 mmol) were successively added. TLC toluene:acetone 2:1. After 40 min the reaction was complete. The solvent was evaporated and the residue purified by MPLC toluene:acetone 3:1 containing 0.1% Et_3N to afford donor **71** as colorless syrup (205 mg, 74 %):^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.35 (2t, 6H, CH_3), 1.85-2.11 (6s, each 3H, AcO, AcHN), 3.00-3.40 (m, 2H, 3b, 9a-H), 3.49 (s, 3H, OMe), 3.50-3.89 (m, 4H,3a, 5a, 5b, 9a-H), 3.90 (s, 3H, OMe), 4.00-4.30 (m, 7H, $2CH_2$ 6a, 6b, 9b-H), 4.30-4.70 (m, 5H, 9b, 4a, 4b-H, CH_2Ph), 4.82 (m, 1H, 8a-H), 4.90-5.20 (m, 4H, CH_2Ph, 7b, 7a-H), 5.30-5.40 (m, 2H, NH, 8b-H), 5.60 (d, 1H, NH), 7.00-7.50 (m, 25H, Ph).

Benzyl O-(2,6-Di-O-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-O-pivaloyl-β-D-glycopyranoside (72).-

Compounds **72** was synthesized following a procedure from Lassaletta *et al*. The analytical data are identical with the published values.[90]

Benzyl O-{Methyl (5-acetamido-7,8,9-tri-O-acetyl-4-O-benzyl -3-phenylthio-3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl)onate-(2→8)[methyl (5-acetamido-7-O-acetyl-4,9-di-O-benzyl-3-phenylthio- 3,5-trideoxy-D-glycero-D-galacto-nonulopyranosyl)onate] -(2→3)-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (73).-

A solution of compound **71** (94 mg, 0.11 mmol) and **72** (100 mg, 0.07 mmol) in dry acetonitril (5 mL) containing activated 0.3 nm molecular sieves (0.4 g) was cooled to –20^{o}C and treated with trimethylsilyl trifluormethanesulfonate (3 μL, 0.01 mmol). The solution was

stirred until it reach room temperature .TLC toluene:acetone 3:2. When all the starting donor was transformed, the solution was neutralized with Et_3N, filtered and concentrated. Purification of the residue by column chromatography using toluene:acetone 5:1 as eluant gave pure tetrasacharide **73** (58 mg, 31%): ^{1}H-NMR (600 MHz, $CDCl_3$) δ=1.10 (s, 9H, OPiv), 1.67-2.09 (6s, 3H each, AcO, AcHN), 3.13 (m, 1H, H-5b), 3.24 (d, 1H, $J_{3c,4c}$=9.8 Hz, 3c-H), 3.26 (d, 1H, 6b-H), 3.30 (d,1H, 5d-H), 3.32 (d, 1H, $J_{3d,4d}$= 9.9 Hz, 3d-H), 3.37 (d, 1H, 5a-H), 3.39 (dd, 1H, 2b-H), 3.48 (m, 2H, 5c-H, 6b-H), 3.56 (t, 1H, J= 9.9 Hz, 3a-H), 3.58 (m, 2H, 9c, 6a-H), 3.71 (m, 2H, 4b-H, 6'a-H), 3.83 (m, 4H, OMe, 4a-H), 3.86 (s, 3H, OMe), 3.93 (dd, 1H, J_{gem}=12.2 Hz, $J_{9c,8c}$= 2.5 Hz, 9c-H), 3.96 (dd, 1H, $J_{2b,3b}$=9.7 Hz, $J_{3b,4b}$=3.2 Hz, 3b-H), 4.08 (dd, 1H, J_{gem}= 12.7 Hz, $J_{9'd,8d}$=5 Hz, 9'd-H), 4.13-4.26 (dd, 2H, CH_2Ph), 4.17 (d, 1H, $J_{1b,\ 2b}$= 7.7 Hz, 1b-H), 4.20 (dd, 1H, $J_{5d,6d}$=10.0 Hz, 6d-H), 4.35 (d, 1H, 9d-H), 4.34 (d, 1H, 6c-H), 4.38-4.47 (dd, 2H, CH_2Ph), 4.39 (d, 1H, $J_{1a,2a}$=7.8 Hz, 1a-H),4.39-4.82 (dd, 2H, CH_2Ph), 4.43 (d, 1H, 4c-H), 4.51-4.92 (dd, 2H, CH_2Ph), 4.54-4.96 (dd, 2H, CH_2Ph), 4.72 (m, 1H, 8c-H), 5.06 (dd, 1H, $J_{1a,2a}$= 8.6 Hz, 2a-H), 5.20 (dd, 1H, $J_{6d,7d}$ =1.1, $J_{7d,8d}$= 9.1 Hz, 7d-H), 5.32 (m, 2H, NH, H-7c), 5.36 (m, 2H, NH, 8d-H), 7.26-7.04 (m, 50H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 52.6, 52.8 (2 OMe), 53.4 (C-3d), 53.6 (C-5c), 59.1(C-3c), 60.3(C-5d), 62.0 (C-9d), 67.0 (C-4b), 67.4 (C-7d), 68.4 (C-8d, 6a), 68.8 (C-6b), 69.5 (C-6d), 69.9 (CH_2Ph), 70.7 (C-9c), 71.2 (C-7c), 72.2 (C-6c), 72.7 (C-5b, CH_2Ph), 73.2 (C-3a, CH_2Ph), 73.7 (CH_2Ph), 74.4 (C-8c), 75.2-75.5 (2 CH_2Ph), 75.7 (C-5a), 76.7 (C-4a), 76.8 (C-3b), 78.0 (C-2b), 78.2 (C-4d), 78.7 (C-4c), 80.9 (C-3a), 99.5 (C-1a), 100.8 (C-2d), 101.1 (C-2c), 102.6 (C-1b), 167.9, 168.1 (C-1c, 1d).

$C_{117}H_{133}O_{32}N_2S_2$ (2143.0) MS (MALDI, positive mode, Matrix:DHB): m/z = 2167 $[M+Na]^+$

Benzyl O-(2,6-Di-O-benzyl-3-O-p-methoxybenzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-O-pivaloyl-β-D-glycopyranoside (74).-

A suspension of **72** (3 g, 3.3 mmol) and dibutyltin oxide (1.26 g, 4.95mmol) in toluene (100 mL) was refluxed (Dean-Stark apparatus) over night under azeotropic removal of water. The reaction mixture was cooled and treated with tetrabutylammonium bromide (0.5 g, 1.65 mmol) and p-methoxybenzyl chloride (0.92 mL, 6.6 mmol) and then gently refluxed for 3 h. The solution was cooled to room temperature, the precipitate filtered and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography (toluene .Acetone 20:1) to afford **74** (3.13 g, 93%): $[\alpha]_D$ 119° (c = 1.2, $CHCl_3$)^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.10 (s, 9H, OPiv), 3.03-3.70 (m, 4H, 5b, 5a, 2b, 3b, 2b, 6b-H), 3.80 (s, 3H, OMe), 3.90 (d,

1H, 4a-H), 4.05 (dd, 1H, 3a-H), 4.30 (d, 1H, $J_{1,2}$ =8.3 Hz, 1b-H), 4.45 (d, 1H, $J_{1,2}$ =8.3 Hz, 1a-H), 4.40-5.00 (m, 12H, $6CH_2Ph$), 5.12 (dd, 1H, 2a-H), 6.90 (d, 1H, MPM), 7.10-7.40 (m, 17H, Ph).

Texyldimethylsilyl O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-O-acetyl-2-dimethylmaleimido -β-D-glucopyranoside (75).-

Compound **75** was synthesized following a published procedure[102]

Texyldimethylsilyl O-(6-O-benzyl-β-D-galactopyranosyl)-(1→3)-2-dimethylmaleimido -β-D-glucopyranoside (76).-

A solution of **75** (1 g, 1.02 mmol)in dry methanol (15 mL) was treated with catalytic amounts of 0.1 M sodium methanolate (800 µl) an stirred at room temp. After 3h the solution was neutralized with ion-exchange resin (Amberlite IR-120 H^+), the resin filteres off and the filtrate concentrated *in vacuo*. Flash chromatography (toluene:Acetone: 2:1) of the residue gave compound **76** (0.68 g, 98%)as a white powder: ^{1}H-NMR (250 MHz, $CDCl_3$) δ 0.03 (2s, 6H, $Si(CH_3)_2$), 0.65 (m, 12H, 4 Me-TDS), 1.44 (m, 1H; CH-TDS), 1.90 (s, 6H, DMM), 3.40 (m , 2H, 5b, 5a), 3.60-3.80 (m, 9H, CH_2Ph, 6b, 6a, 3b, 4a, 4b-H), 3.90 (dd, 1H, $J_{2,3}$ = 8.5 Hz, 2a-H), 4.30 (d, 1H, 3a-H), 4.50 (d, 1H, 1b-H), 5.10 (d, 1H, $J_{1,2}$ =10 Hz, 1a-H), 7.30 (m, 5H, Ph).

Methyl (5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-O-diethylphosphite-D-glycero-β-D-galacto-nonulopyranosyl)onate (77).-

Compound **77** was synthesized following a published procedure.
The analytical data are identical with the published values.[39]

Texyldimethylsilyl -O[(Methyl [5-acetamido-4,7,8,9-tetra-O-acetyl -3,5-dideoxy-D-glycero -α-D-galacto-nonulopyranosyl]onate)]-(2→3)-(2,4-di-O-acetyl-6-O-benzyl-β-D-galacto pyranosyl)-(1→3)-[(methyl [5-acetamido-4,7,8,9-tetra-O-acetyl -3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl]onate)]-(2→6)-(4-O-acetyl-2-deoxy-2-[3,4-dimethy lmaleimido]-β-D-glucopyranoside (78).-

Texyldimethylsilyl -O(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)- [(methyl [5-acetamido-4,7,8,9-tetra-O-acetyl -3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl]onate)] –(2→6)-(4-O-acetyl-2-deoxy-2-[3,4-dimethylmaleimido] -β-D-glucopyranoside (79).-

Disialylation I

A solution of the acceptor **76** (160 mg, 0.23mmol) and neuraminic acid phosphite **77** (300 mg, 0.46 mmol) in dry acetonitril pa was cooled to –40^{o}C. Then TfOH (8 μL) was added and the reaction was stirred 10 min. To this mixture was added dropweise under a nitrogen atmosphere a solution of the neuraminic acid phosphite **77** (300 mg, 0.46 mmol) in dry acetonitril pa. After 1h, the reaction mixture was neutralized with Et_3N and evaporated *in vacuo*. The residue was dissolved in pyridine (5 mL) and was added acetic anhydride (5 mL) and the mixture was stirred a room temperature 2h. The solvents were evaporated and coevaporated with toluene. The residue was purified by MPLC (toluene: acetone 4:1) to gave a a lower migrating spot corresponding to disialyl compound (**78**), and fast migrating spot corresponding to monosialyl compound (**79**).

(78)- $[\alpha]_D$ = - 72 (c = 0.8, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 0.04 (2s, 6H; 2Me-Si), 0.67-0.73 (2s, 12H, 4Me-TDS), 1.45 (m, 1H, CH-TDS), 1.64 (dd, 1H, J_{gem}12.4 Hz, 3d-H_{ax}), 1.83-2.33 (m, 46H, AcO, NDMM, 3c-H_{ax}), 2.52 (d, 1H, 3d-H_{eq}), 2.56 (dd, 1H, 3e-H_{eq}), 3.32 (dd, 1H, 6b-H), 3.40 (d, 1H, 6a-H), 3.51 (d, 1H, 6b-H), 3.54-3.56 (m, 2H, 5a, 6d -H), 3.63 (d, 1H, 5b-H), 3.75 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.82 (s, 1H, 6a-H), 3.87 (dd, 1H, 9d-H), 3.93 (dd 1H, 2a-H), 3.95 (dd, 1H, 5d-H), 3.99 (m, 2H, 6c, 5c-H,), 4.09 (d, 1H, 9c-H), 4.25 (d, 1H, 9'd-H), 4.28 (d,1H, 9'c-H), 4.33 (d, 1H, 3b-H), 4.39 (d, 1H, 1b-H), 4.40 (d, 1H, *CH*HPh), 4.52 (m, 2H, 3a-H, CH_2Ph), 4.73 (dd, 1H, 2b-H), 4.85 (d, 1H, 4c-H), 4.87 (d, 1H, 4d-H), 4.96-4.98 (m, 2H, 4a, 4b-H), 5.08 (d, 1H, 1a-H), 5.29-5.32 (m, 3H, 7c, 8c, 8d-H), 5.40 (dd, 1H, 7d-H), 7.13-7.31(m, 5H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 33.8 (CH-TDS), 37.4 (C-3c, 3d), 49.1, 49.4 (C-5c, 5d), 52.7, 53.1 (2 OMe), 57.4 (C-2a), 61.4, 62.2 (C-9c, 9d), 63.3 (C-6a), 66.6 (C-7d), 67.4 (C-6b), 67.5 (C-7c, 8d, 4b), 68.6 (C-8c), 69.0, 69.3 (C-4c, 4d), 70.1 (C-4a), 70.6 (C-2b), 71.1 (C-5b), 71.7 (C-6d, 3b), 72.4 (C-6c), 72.5 (C-5a), 74.4 (C-3a), 93.2 (C-1a), 99.7 (C-1b), 96.7, 98.3 (C-2c, 2d), 167.8 (C-1c, 1d).
$C_{80}H_{113}O_{39}N_3Si$ (1768.7) MS (MALDI, positive mode, Matrix:DHB): m/z = 1791 $[M+Na]^+$

(79)-$[\alpha]_D$ = - 240 (c = 1, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 0.05(2s, 6H; 2Me-Si), 0.67-0.74 (4s, 12H, 4Me-TDS), 1.46 (m, 1H, CH-TDS), 1.86-2.12 (m, 34H, AcO, NDMM, 3c-H_{ax}), 2.58 (dd, 1H; J_{gem}=13.0, $J_{3,4}$=4.6 Hz, 3c-H_{eq}), 3.40 (m, 2H, 6b, 6a-H), 3.54-3.57 (m, 2H; H-6'b, 5a), 3.68 (t, 1H, $J_{5b,6b}$=6.1 Hz, 5b-H), 3.76 (s, 3 H; OMe), 3.85 (dd, 1H, J_{gem}=10.9, $J_{6a,5a}$=5.2 Hz, 6a-H), 3.93 (dd, 1 H, $J_{2a,1a}$=8.2, $J_{2a,3a}$=10.7 Hz, 2a-H), 4.00 (m, 2H, 6c, 5c-H), 4.09 (dd, 1H, J_{gem} =12.4 Hz, $J_{9c,8c}$=5.77 Hz, 9c-H), 4.29 (dd 1H, H-9'c), 4.41 (d, 1H, *CH*HPh), 4.49 (dd, 1H, $J_{3a,4a}$=9.4 Hz, 3a-H), 4.54 (d, 1H, *CH*HPh,), 4.85-4.86 (m, 2H, 4c, 2b-H), 4.91 (dd, 1H, $J_{2b,3b}$=10.2, $J_{2b,1b}$=7.8 Hz, 2b-H), 4.96 (dd, 1H, 4a-H), 5.05 (d, 1H, 1a-H), 5.14 (d, 1H, NH), 5.29 (d, 1H, $J_{7c,8c}$=8.1 Hz, H-7c), 5.34 (m, 1H, 8c-H), 5.37(d, 1H, 4b-H).
^{13}C-NMR (600 MHz, $CDCl_3$): δ 37.6 (C-3c), 49.6 (C-5c), 52.8 (OMe), 57.6 (C-2a), 62.4 (C-9c), 63.5 (C-6a), 67.1 (C-6b), 67.4 (C-4b), 67.6 (C-7c), 68.7 (C-8c), 69.3 (C-4c), 69.4 (C-2b), 69.6 (C-4a), 71.3 (C-3b), 71.8 (C-5b), 72.6 (C-5a, 6c),75.2 (C-3a), 93.4 (C-1a), 98.3 (C-2c), 100.4 (C-1b), 167.9 (C-1c).

Methyl [5-Acetamido-4,7,8,9-tetra-O-acetyl -3,5-dideoxy-D-glycero-α-D-galacto-nonulo-pyranosyl]onate)]-(2→3)-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)- [(methyl [5-acetamido-4,7,8,9-tetra-O-acetyl -3,5-dideoxy-D-glycero-α-D-galacto-nonulo-pyranosyl]onate)] -(2→6)-(4-O-acetyl-2-deoxy-2-[3,4-dimethylmaleimido]- -β-D-gluco-pyranosyl) Trichloroacetimidate (80).-

To a solution of compound **78** (122 mg, 0.080 mmol) in dry THF (10 mL) was added tetrabutylammonium fluoride (0.3 mL of a 1M solution in THF) and acetic acid (30 μl) at –10°C under nitrogen. The mixture was stirred for 6h at –10°C, then was poured in ice water (20 mL) and extracted with diethylether (3x20 mL). The organic extracts were dried ($MgSO_4$), filtered and concentrated. A solution of the residue, trichloracetonitrile (0.09 mL) and DBU (0.1 μL, mmol) in dichloromethane (5 mL) was stirred for 45 min at room temperature, then concentrated. The residue was eluted from a column of silica gel (toluene-acetone: 4:1

containing 0.1% of Et_3N to afford **80** (74 mg, 65%) as a white foam: ^{1}H-NMR (250 MHz, $CDCl_3$) : δ 1.60 (dd, 1H, $3c_{ax}$-H), 1.70-2.10 (m, 46H, OAc, NHAc, NDMM,, 3c-H_{ax}), 2.50 (m, 2H, 3d-H_{eq}, $3c_{eq}$ -H), 3.30-3.70 (m, 8H, 6c, 5a, 6a, 5b, 6b-H,), 3.70 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.80-4.40 (m, 8H, 5d, 5c, 9c, 9d, 3b, 2a-H), 4.40-4.50 (dd, 2H, CH_2Ph), 4.50-5.50 (m, 12H, 2b, 4d, 4c, 4a, 4b, 7d, 8c, 8d, 7c, 3a-H, 2NH), 6.10 (d, 1H, J=10.4 Hz, 1a-H), 7.30 (m, 5H, Ph), 8.50 (s, 1H, NH).

O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-O-acetyl-2-dimethyl-maleimido -β-D-glucopyranosyl Trichloroacetimidate (81).-

Compound **81** was synthesized following a published procedure.[102]

Benzyl O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)-O-(4,6-di-O-acetyl-2-N-[3,4-dimethylmaleimido]-β-D-glucopyranosyl)-(1→3)-(2,6-di-O- benzyl -β-D-galacto-pyranosyl)-(1→4)- 3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (82).-

Benzyl O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)-O-(4,6-di-O-acetyl-2-N-[3,4-dimethylmaleimido]-β-D-glucopyranosyl)-(1→4)-(2,6-di-O- benzyl -β-D-galacto-pyranosyl)-(1→4)- 3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (83).-

A mixture of imidate **81** (1.1 g, 1.2 mmol) and lactose acceptor **74** (0.736 g, 0.7 mmol) in anhydrous dichloromethane (20 mL) was stirred 30 min at room temperature. Tin (II) triflate (0.05 g, 0.07 mmol) was added and the mixture was stirred for 1h, neutralized with Et_3N and concentrated. Column chromatography of the residue (PE:EtOAc 5:1) afforded a mixture of two compounds **82** β(1→ 3) (486 mg, 41%) and **83** β(1→ 4) (462 mg, 39%).

(82)- $[\alpha]_D$ –210^o(c=1,$CHCl_3$).^{1}H-NMR (600 MHz, $CDCl_3$) δ=1.14 (s, 9H, OPiv), 1.90-2.34 (m, 21H, AcO, NDMM), 2.66 (b, 1H, OH), 3.13 (d, 1H, $J_{5a,6a}$=7.5 Hz, 5a-H), 3.36-3.43 (m, 5H, 3b, 5b, 6b, 6d, 6a-H,), 3.47 (dd, 1H, 2b-H), 3.53 (m, 2H, 3a, 6d-H), 3.58-3.63 (m, 3H, 6b', 5d ,6a-H), 3.72 (m, 1H, 5c-H), 3.97 (d, 1H, 4b-H), 3.99 (d, 1H, 4a-H), 4.05 (d, 1H, $J_{1d,2d}$=7.7 Hz ,1d-H), 4.09 (dd, 1H, 2c-H), 4.10-4.18 (dd, 2H, 6c-H), 4.18-4.93 (6dd, 12H, 6CH_2Ph), 4.33 (d, 1H, $J_{1a,2a}$=8.3 Hz, 1a-H), 4.38 (d, 1H, $J_{1b,2b}$=7.3 Hz, 1b-H), 4.48 (dd, 1H, 3c-H), 4.79 (d, 1 H, 3d-H), 4.88 (dd, 1H, $J_{2d,3d}$= 10.2 Hz, 2d-H), 4.93 (d, 1H, $J_{4c,3c}$=3.2 Hz, 4c-H), 5.06 (dd, 1H, $J_{2a,3a}$= 8.5 Hz, 2a-H), 5.13 (d, 1H, $J_{1c,2c}$= 8.5 Hz, 1c-H), 5.36 (d, 1H, $J_{4d,3d}$= 3.0 Hz, 4d-H), 7.09-7.30 (m, 30H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 55.1(C-2c), 62.2 (C-6c), 66.6 (C-6d), 67.1 (C-4d), 67.3 (C-4b), 67.7 (C-6a), 68.5 (C-6b), 69.0 (C-4c), 69.1 (C-2d), 70.9 (C-3d), 71.8 (C-5c, 5d), 72.1 (C-2a), 74.2 (C-3c), 75.1 (C-5a), 75.9 (C-4a), 77.8 (C-2b), 80.1 (C-3a), 83.9 (C-3b), 98.6 (C-1c), 99.6 (C-1a), 100.0 (C-1d), 102.1 (C-1b).

(83)- $[\alpha]_D$ −162°(c=1.1,$CHCl_3$).^{1}H-NMR (600 MHz, $CDCl_3$) δ =1.17 (s, 9H, OPiv), 1.84-2.01 (m, 21H, AcO, NDMM), 2.24 (d, 1H, OH), 3.03 (dd, 1H, 2b-H), 3.21 (dd, 1H, 6b-H), 3.33 (dd, 1H, 5b-H), 3.38 (dd, 1H, 6d-H), 3.43 (m, 2H, 3b, 5a-H), 3.53 (m, 2H, 6'b, 6'd-H), 3.61 (d, 1H, 5c-H), 3.63 (d, 1H, 3a-H), 3.69 (d, 1H, 5d-H), 3.71 (m, 2H, 6a-H), 3.87 (d, 1H, $J_{4b,3b}$=2.1 Hz, 4b-H), 3.99 (d, 1H, 4a-H), 4.01 (d, 1H, 6c-H), 4.05 (dd, 1H, 2c-H), 4.17 (d, 1H, 6c-H), 4.17-5.00 (6dd, 12H, CH_2Ph), 4.20 (d, 1H, $J_{1d,2d}$=7.8 Hz, 1d-H), 4.32 (d, 1H, $J_{1b,2b}$= 7.6 Hz, 1b-H), 4.46 (d, 1 H, $J_{1a,2a}$= 8.0 Hz, 1a-H), 4.65 (dd, 1H, 3a-H), 4.85 (dd, 1H, 3d-H), 4.91 (dd, 1H, 2d-H), 5.00 (d, 1H, 4c-H), 5.08 (d, 1H, $J_{1c,2c}$= 8.3 Hz, 1c-H), 5.11 (dd, 1H, 2a-H), 5.37 (d, 1H, $J_{4d,3d}$ =3.0 Hz, 4d-H), 7.19-7.33 (m, 30H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 55.2 (OMe), 55.7 (C-2c), 62.1(C-6c), 66.8 (C-6d), 67.2(C-4d), 68.1 (C-6a, 4c), 68.9 (C-6b), 69.3 (C-2d), 71.0 (C-3d), 71.4 (C-5), 71.1 (C-5d), 72.2 (C-2a), 73.6 (C-5b), 74.8(C-3c), 75.5 (C-5a, 3b), 75.7 (C-4b), 76.8 (C-4a), 80.8 (C-3a), 81.3 (C-2b), 99.2 (C-1c), 99.6 (C-1a), 100.3 (C-1d),102.1 (C-1b).

Benzyl O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)-O-(4,6-di-O-acetyl-2-N-[3,4-dimethylmaleimido]-β-D-glucopyranosyl)-(1→4)-(2,6-di-O- benzyl–3-O-p-methoxy-benzyl-β-D-galactopyranosyl)-(1→4)- 3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (84).-

A solution of **81** (2.01 g, 3.9 mmol) and lactose acceptor **74** (1.5 g, 2.6 mmol) was stirred 30 min at room temperature, then the reaction mixture was cooled to 0°C and a solution of TMSOTf (0.01 mL, 0.039 mmol) was added. After 2h the reaction was neutralized with Et_3N and concentrated. Column chromatography of the residue afforded **84** (1.5 g, 61 %).$[\alpha]_D$ − 116°(c=1,$CHCl_3$).^{1}H-NMR (600 MHz, $CDCl_3$) δ=1.15 (s, 9H, OPiv), 1.81-2.04 (m, 21H, AcO, NDMM), 3.02 (dd, 1H, 2b-H), 3.10 (dd, 1H, 6b-H), 3.15 (dd, 1H, 5b-H), 3.21 (dd, 1H, 2b-H), 3.39 (m, 3H, 6d, 6b', 5a-H), 3.56-3.61 (m, 4H, 6d', 4b, 5c, 3a-H), 3.68-3.71 (m, 3H, 5d, 6a-H,), 3.78 (s, 3H, OMe), 3.92 (dd, 1H, 4a-H), 3.98 (dd, 1H, 6c-H), 4.02 (dd, 1H, 2c-H), 4.19 (dd, 1H, 6c-H), 4.19 (d, 1H, $J_{1d,2d}$=7.6 Hz, 1d-H), 4.27 (dd, 1H, $J_{1b,2b}$= 7.6 Hz, 1b-H), 4.44 (d, 1H, $J_{1a,2a}$=8.1 Hz, 1a-H), 4.71 (dd, 1H, 3c-H), 4.19-5.07 (7dd, 14H, CH_2Ph), 4.85 (dd, 1H, $J_{1c,2c}$=8.3 Hz, 3d-H), 4.92 (dd, 1H, 2d-H), 4.98 (d, 1H, $J_{1c,2c}$ = 8.3 Hz, 1c-H), 4.99 (d, 1H,

4c-H), 5.08 (d, 1H, 2a-H), 5.38 (d, 1H, $J_{4c,3c}$= 3.0 Hz, 4d-H), 6.80 (d, 2H, MPM), 7.16-7.34 (m, 32H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 55.3 (OMe), 55.8 (C-2c), 61.9 (C-6c), 66.9 (C-3c), 67.2(C-4d), 68.1 (C-6a), 68.8 (C-6b), 69.0 (C-4c), 69.2 (C-2d), 71.1 (C-3d), 71.4 (C-5c), 71.7 (C-5d), 72.3 (C-2a), 73.0 (C-5b), 74.0 (C-4b), 74.7 (C-3c), 75.6 (C-5a), 76.9 (C-4a), 80.3 (C-2b), 80.4 (C-3b), 81.0 (C-3b), 98.9 (C-1c), 99.6 (C-1a), 100.4 (C-1d), 102.2 (C-1), 100.8 (C-2d), 101.1 (C-2c), 102.6 (C-1b), 167.9, 168.1 (C-1c, 1d).

Anal.Calcd for $C_{95}H_{110}O_{29}N$ (1729.9): C 65.96, H 6.41, N 0.81; Found . C 65.87, H 6.53, N 0.63.

Benzyl O-(6-O-benzyl-β-D-galactopyranosyl)-(1→3)-O-(2-N-[3,4-dimethylmaleimido]-β-D-glucopyranosyl)-(1→4)-(2,6-di-O- benzyl-3-O-p-methoxybenzyl -β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (85).-

A solution of the tetrasaccharide **84** (140 mg, 0.081 mmol) in dry MeOH (10 mL) was added DBU (0.1 mL) and the mixture was stirred 3h at room temperature, then neutralized and concentrated. Column chromatography of the residue (toluene:acetone 3:1) afforded **85** (118 mg, 96%): ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.15 (s, 9H, OPiv), 1.70-1.90 (2b, 6H, NDMM), 3.02 (d, 1H, 5b, 6b-H), 3.07-3.11 (m, 2H, 3b, 2b-H), 3.36 (d, 1H, 5a-H), 3.43-3.50 (m, 4H, 4c, 5d, 6b, 3d-H), 3.50-3.78 (m, 7H, 4b, 3a, 6d, 6a, 5c, 6c, 2d-H), 3.78 (s, 3H, OMe), 3.89 (m, 3H, 6c, 4d-H), 3.92 (d, 1H, 4a-H), 4.01 (dd, 1H, 2c-H), 4.04 (d, 1H, 1d-H), 4.21 (d, 1H, $J_{1b,2b}$=7.7 Hz, 1b-H), 4.22-5.00 (dd, 14H, 7CH_2Ph), 4.40 (dd, 1H, $J_{1b,2b}$= 7.6 Hz, 1b-H), 4.44 (d, 1H, $J_{1a,2a}$=8.1 Hz, 1a-H), 4.85 (dd, 1H, $J_{1c,2c}$=8.3 Hz, 3d-H), 4.92 (dd, 1H, 3c-H), 4.40 (d, 1 H, 1a-H), 5.01 (d, 1H, 1c-H), 5.06 (dd, 1H, 2a-H), 6.80 (d, 2H, MPM), 7.20-7.30 (m, 32H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 55.0 (C-2c), 55.2 (OMe), 63.2 (C-6c), 67.5 (C-6b), 67.9(C-6d), 68.7 (C-4d), 69.0 (C-6a), 71.0 (C-4c), 71.1 (C-2d, 5b), 72.2 (C-2a), 72.9 (C-3d), 73.5 (C-5c), 74.2 (C-4b), 74.9 (C-4b), 75.4 (C-5a), 76.6 (C-4a), 80.2 (C-3b, 2b), 80.8 (C-3a), 82.8 (C-3c), 99.1 (C-1c), 99.7 (C-1a), 102 (C-1b), 103.7 (C-1d).

$C_{85}H_{100}O_{24}N_1$ (1519) ; MS (MALDI,positive mode, Matrix:DHB): m/z = 1542 $[M+Na]^+$

Benzyl O-(6-O-benzyl-β-D-galactopyranosyl)-(1→3)-O-[methyl (5-acetamido-7,8,9-tri-O-acetyl-4-O-benzyl -3-phenylthio-3,5-dideoxy-D-glycero-α-D-galactononulopyranosyl) onate]

-(2→6)- (2-N-[3,4-dimethylmaleimido]-β-D-glucopyranosyl)-(1→4)-(2,6-di-O- benzyl -β-D-galactopyranosyl)-(1→4)- 3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (86).-

A solution of the tetrasaccharide **85** (98 mg, 0.06 mmol) and neuraminic acid phosphite **68** (100 mg, 0.12 mmol) in dry acetonitril pa was cooled to –40°C. Then TfOH (5 μL) was added and the mixture was stirred 10 min. To this mixture was added dropweise under a nitrogen atmosphere a solution of the neuraminic acid phosphite **68** (100 mg, 0.12 mmol)) in dry acetonitril pa. After 1h, the reaction mixture was neutralized with Et_3N and evaporated *in vacuo*. The residue was purified by MPLC (toluene:acetone 3:1 v/v) afforded **86** (49 mg, 36% yield); $[\alpha]_D$ +8°(c=1,$CHCl_3$), $[\alpha]_D$ +8°(c=1,$CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$) δ1.12 (s, 9H, OPiv), 1.67-2.06 (6s, 18H, Ac_2O, NDMM), 2.97-2.99 (m, 2H, 6b, 5b-H,), 3.09 (m, 1H, 2b-H), 3.31 (d, 1H, $J_{3e,4e}$=9.8 Hz, 3e-H), 3.35-3.37 (m, 2H, 2d, 5a-H,), 3.45-3.51 (m, 3H, 5c, 4c, 6b-H), 3.58-3.69 (m, 8H, 3a, 6a, 3b, 4b, 6c, 5d, 6d-H), 3.78 (s, 3H, OMe), 3.88-3.94 (m, 6H, OMe, 2c, 6c', 4a-H,), 3.97-3.99 (m, 2H, 3d, 9e-H,), 4.15 (d, 1H, 1d-H), 4.19 (d, 1H, $J_{1d,2d}$= 9.4 Hz, 1b-H), 4.20-4.26 (m, 2H, 4e, 9e'-H,), 4.32-5.04 (7dd, 14H, CH_2Ph), 4.42 (d, 1H, 6e-H), 4.45 (d, 1H, 1a-H), 4.49 (dd, 1H, 3c-H), 4.95 (d, 1H, 1c-H), 5.07 (dd, 1H, $J_{2a,3a}$= 9.9 Hz, 2a-H), 5.19 (m, 2H, 8e, 7e-H), 5.31 (d, 1H, $J_{NH,\ 4c}$=8.2 Hz, NH), 6.80 (d, 2H, MPM), 7.16-7.34 (m, 40H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 52.9 (C-5c), 54.9 (C-2c), 53.3 (OMe), 55.6 (OMe-MPM), 54.9 (C-2c), 60.5 (C-3e), 62.5 (C-9e), 63.8 (C-6c), 67.6 (C-8e), 68.0 (C-6b), 68.1 (C-4d), 68.5 (C-6c), 68.6 (C-2d), 68.7 (C-7e), 71.6 (C-6e, 4c), 71.7 (C-5b), 72.6(C-2a), 73.3-74.4 (C-3), , 4b, 5d, 6d), 75.3 (C-5c), 75.9 (C-5a), 77.1 (C-4a), 77.1 (C-4a), 77.7 (C-3d), 78.2 (C-4e), 80.7 (C-2b), 81.3 (C-3a), 81.4 (C-3c), 99.8 (C-1c), 100.1 (C-1a), 102.5 (C-1b, 1d).

$C_{116}H_{135}O_{35}N_2S$ (2151) ; MALDI MS (MALDI,positive mode, Matrix:DHB): m/z = 2174 $[M+Na]^+$

Benzyl O-(6-O-benzyl-β-D-galactopyranosyl)-(1→3)-O-(2-N-[3,4-dimethylmaleimido]-β-D-glucopyranosyl)-(1→4)-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)- 3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (87).-

A solution of tetrasaccharide **83** (100 mg, 0.062 mmol) in dry MeOH (14 mL) was added DBU (0.14ml) and the mixture was stirred 3h at room temperature, then neutralized and concentrated. Column chromatography of the residue (toluene:acetone 3:1) afforded **87** (75 mg, 92% yield): ^{1}H-NMR (600 MHz, $CDCl_3$) δ=1.15(s, 9H, OPiv), 1.87 (2s, 6H, NDMM), 3.07 (d, 1H, 6b-H), 3.08 (dd, 1H, 2b-H), 3.18 (dd, 1H, 5b-H), 3.18 (dd, 1H, 5b-H), 3.35 (dd, 1H, $J_{3b,2b}$= 9.7, $J_{3b,4b}$=2.2 Hz, 3b-H), 3.39 (d, 1H, $J_{5a,6a}$= 9.4 Hz, 5a-H), 3.45-3.48 (m, 3H, 3d, 4c, 5c-H), 3.58 (dd, 1H, 2d-H), 3.60 (dd, 1H, 3a-H), 3.62-3.74 (m, 6H, 6a, 6b', 6c, 5d, 6d-H), 3.87 (m, 2H, 4b, 6c'-H), 3.92 (dd, 1H, $J_{4d,3d}$=2.3 Hz, 4d-H), 3.98 (dd, 1H, 4a-H), 4.03.(m, 2H, 1d, 2c-H), 4.27-4.28 (m, 2H, 1b, 3c-H), 4.45 (d, 1H, $J_{1a,2a}$=7.9 Hz, 1a-H), 4.30-4.98 (6dd, 12H, CH_2Ph), 5.09 (dd, 1H, $J_{2a,3a}$= 8.8 Hz, 2a-H), 5.12 (d, 1H, $J_{1c,2c}$ = 8.5 Hz, 1c-H), 7.20-7.33 (m, 30H, Ph).
^{13}C-NMR (600 MHz, $CDCl_3$): δ 55.2 (C-2c), 63.1(C-6c), 67.6 (C-6b), 68.1 (C-6a), 68.8 (C-4d), 69.1 (C-6d), 71.1 (C-4c), 71.3 (C-2d), 71.8 (C-5b), 72.2 (C-2a), 72.9 (C-3d), 73.0 (C-3b), 73.5 (C-5d), 75.1 (C-5c), 75.4 (C-5a), 76.4 (C-4b), 76.7 (C-4a), 80.7 (C-3a), 81.3 (C-2b), 83.4 (C-3c), 99.6 (C-1c), 99.7 (C-1a), 102,4 (C-1b), 103.8 (C-1d).

Benzyl{ (Methyl [5-acetamido-7,8,9-tetra-O-acetyl-4-O-benzyl-3-O-phenylthio-3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl]onate)} -(2→3)-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)- { (methyl [5-acetamido-7,8,9-tetra-O-acetyl -4-O-benzyl-3-O-phenylthio-3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl]onate)}-(2→6)-(4-O-acetyl -2-deoxy-2-[3,4-dimethylmaleimido]- -β-D-glucopyranosyl}-(1→4)-(3-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (88).-

Benzyl -(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)-{ (methyl [5-acetamido-7,8,9-tetra-O-acetyl-4-O-benzyl-3-O-phenylthio-3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl]onate)} –(2→6)-(4-O-acetyl-2-deoxy-2-[3,4-dimethylmaleimido] -β-D-glucopyranoside-(1→4)-(3-O-acetyl-2,6-di-O-benzyl-β-D-galacto pyranosyl)-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (89).-
Disialylation II

A solution of the acceptor **87** (60 mg, 0.037 mmol) and neuraminic acid phosphite **68** (57 mg, 0.074 mmol) in dry acetonitril pa was cooled to –40°C. Then TfOH (1 μL) was added and the mixture was stirred 10 min. To this mixture was added dropweise under a nitrogen atmosphere a solution of the neuraminic acid phosphite **68** (104 mg, 0.148 mmol) in dry acetonitril pa. After 1h, the reaction mixture was neutralized with Et_3N and evaporated *in vacuo*. The residue was dissolved in pyridine (5 mL) and was added acetic anhydride (5 mL) and the mixture was stirred a room temperature 2h. The solvents were evaporated and coevaporated with toluene. The residue was purified by MPLC (toluene: acetone 4:1) to gave a disialyl compound (**88**), and a monosialyl compound (**89**).

(88)- ^{1}H-NMR (600 MHz, $CDCl_3$) δ=1.15 (s, 9H, OPiv), 1.86-2.16 (m, 42H, AcO, NHAc, NDMM) 3.02 (dd, 1H, 3e-H), 3.06 (dd, 1H, 2b-H), 3.17 (dd, 1H, 3f-H), 3.19 (d, 1H, 6d-H), 3.34 (d, 1H, 5b-H), 3.38-3.55 (m, 5H, 5d, 5a, 6b-H), 3.62-3.70 (m, 8H, 3a, 5f, 5c, 6a-H, OMe,), 3.70-3.90 (m, 6H, 6c, 4b, 5e-H, OMe,), 3.90-4.18 (m, 7H, 9e, 9f, 4a, 2c, 3d-H), 4.23 (d, 1H, 1d-H), 4.31 (d, 1H, 1b-H), 4.30-4.33 (m, 2H, 4e, 4f-H), 4.46 (d, 1H, 1a-H), 4.72 (dd, 1H, 3c-H), 4.93 (d, 1H, 4c-H), 5.01-5.04 (m, 4H, 3b, 2d-H), 5.10 (dd, 1H, 2a-H), 5.14 (d, 1H, 1c-H), 5.25-5.30 (m, 4H, 8e, 8f, 7e, 7f-H), 5.41 (d, 1H, 4d-H), 7.17-7.34 (m, 50H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 52.8 (C-5e), 53.3, (OMe), 53.4(C-5f), 56.2 (C-2c), 61.4-60.9 (C-3e, 3f), 62.9-62.5 (C-9e, 9f), 64.6(C-6e), 67.4 (C-4d), 67.6(C-8e), 67.9 (C-8f), 68.3-69.9 (C-6a, 6b, 6c) 70.0 (C-4c), 72.0 (C-5d), 72.7(C-2a), 73.6 (C-5c), 74.1 (C-4b), 74.9 (C-2d), 75.9 (C-5a), 76.2 (C-3c), 77.4 (C-4a), 79.8-79.9 (C-4e,ef) 81.0 (C-3a), 81.7 (C-2b), 99.7 (C-1c), 99.6 (C-1a), 102.7 (C-1a), 103.1 (C-1d).

(89)- ^{1}H-NMR (600 MHz, $CDCl_3$): 1.10 (s, 9H, OPiv), 1.74-2.18 (m, 33H, AcO, NHAc, NDMM), 3.00 (dd, 1H, 2b-H), 3.22 (d, 1H, 5b-H), 3.30-3.42 (m, 4H, 6b 5a, 3e, 6d-H), 3.57-3.70 (m, 5H, 5e, 4a, 6a, 3a-H), 3.70 (s, 3H, OMe), 3.70-4.10 (d, 4b, 6c 2c-H), 4.10-4.35 (m, 5H, 9e, 4e, 3c-H), 4.20 (d, 1H, 1d-H), 4.35-5.00 (dd, 14H, CH_2Ph), 4.42 (d, 1H, 1b-H), 4.74 (d, 1H, 1a-H), 4.85-4.99 (m, 4H, 3d, 4c, 2b, 2d-H), 5.01 (d, 1H, 1c-H), 5.16 (d, 1H, 2a-H), 5.27-5.29 (m, 2H, 7e ,8e-H), 5.40 (d, 1H, 4d-H), 7.16-7.31 (m, 40H, Ph).

O-[3,4,6-Tri-O-acetyl-2-deoxy-2-dimethylmaleimido-β-galactopyranosyl] Trichloroacetimidate (90).-

Compound **90** was synthesized following a published procedure.[91]

Benzyl O-(3,4,6-Tri-O-acetyl-2-deoxy-2-N-[3,4-dimethylmaleimido]- β-D-galactopyranosyl)-(1→4)- (2,6-di-O-benzyl-3-O-(4-methoxy)benzyl-β-D-galactopyranosyl)-(1→4)- 3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (91).-

A mixture of imidate **90** (410 mg, 0.73 mmol) and lactose acceptor **74** (370 mg, 0.37 mmol) in dry acetonitril (2 mL) was stirred for 30 min at room temperature. Tin (II) triflate (1.7 mg, 0.042 mmol) was added, and the mixture was stirred for 2 h. The solution was neutralized with Et_3N and evaporated *in vacuo*. Column chromatography of the residue (toluene:acetone 20:1-15:1) afforded **91** (380 mg, 75%): $[\alpha]_D$ -115°(c=1,$CHCl_3$), $[\alpha]_D$ -115°(c=1,$CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$) δ=1.22 (s, 9H, OPiv), 1.76-2.34 (5s, 15H, AcO, DMM), 3.08-3.12 (m, 2H, 6b, 2b-H), 3.16 (dd, 1H, 5b-H), 3.23 (dd, 1H, 3b-H), 3.41-3.42 (m, 2H, 6b, 5a-H), 3.59 (d, 1H, 4b-H), 3.62 (dd, 1H, 3a-H), 3.69 (m, 2H, 6a-H), 3.79 (s, 3H, OMe), 3.89 (dd, 1H, 4a-H), 3.99 (d, 1H, 5c-H), 4.04 (d, 1H, 6c-H), 4.05-5.02 (6dd, 12H, CH_2Ph), 4.21 (dd, 1H, 6c-H), 4.26 (d, 1H, 1b-H), 4.38 (dd, 1H, 2c-H), 4.46 (d, 1H, 1a-H), 5.07 (dd, 1H, 2a-H), 5.17 (d, 1H, 1c-H), 5.50 (d, 1H, 4c-H), 5.95 (dd, 1H, 3c-H), 6.81 (d, 2H, MPM), 7.16-7.31 (m, 32H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 51.7 (C-2c) , 55.7 (OMe), 61.7 (C-6c), 67.1 (C-4c), 68.0 (C-3c), 68.5 (C-6a), 69.4 (C-6b), 70.7 (C-5c), 73.0 (C-2a), 73.8 (C-5b), 75.3 (C-4b), 76.2 (C-5a), 77.4 (C-4a), 80.9 (C-3b), 81.0 (C-2b), 81.4 (C-3a), 99.9 (C-1c), 100.2 (C-1a), 102.7 (C- 1b).

C_{78} $H_{89}O_{22}N$ (1392) MS (MALDI, positive mode, Matrix:DHB): m/z = 1415 $[M+Na]^+$

Texyldimethylsilyl 2-Deoxy-2-dimethylmaleimido-β-galactopyranoside (92).-

Compound **92** was synthesized following a published procedure.[91]

Texyldimethylsilyl 2-Deoxy-2-N-(3,4-dimethylmaleimido)-3,4-O-isopropylidene-β-D-galactopyranoside (93).-

To a solution of compound **92** (5 g, 12.4 mmol), in acetone (100 mL) was added dimethoypropane (5 mL) and camphorsulphonic acid (50 mg). The mixture was stirred at room temperature until TLC (petroleum ether:ethyl acetate 1:1) showed complete conversion of the starting material into a faster moving spot (~30min). The solution was neutralized with Et_3N and concentrated in vacuum. Column chromatography of the residue in petrol ether:ethylacetate 2:1 afforded pure compound (5.06 g, 92%).$[\alpha]_D$ -87° (c=1.2,$CHCl_3$), ^{1}H-

NMR (250 MHz, $CDCl_3$) δ=0.01-0.19 (2s, 6H, $2CH_3Si$), 0.60-0.80 (m, 12H, $4CH_3$-TDS), 1.25 (s, 3H, CH_3-isop), 1.50 (m, 1H, CH-TDS), 1.55 (s, 3H, CH_3-isop), 1.80 (s, 6H, NDMM), 3.80 (dd, 1H, $J_{2,1}$=7.8 Hz, $J_{2,3}$= 8.5 Hz, 2-H), 3.90-4.05 (m, 2H, 6, 5-H), 4.15 (d, 1H, H-4), 4.70 (dd, 1H, 3-H), 5.02 (d, 1H, $J_{1a,2a}$=7.4 Hz, 1-H).

Texyldimethylsilyl O-(Methyl [5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl]onate)-(2→6)-(2-deoxy-2-[3,4-dimethylmaleimido]-3,4-O-isopropylidene-β-D-galactopyranoside (94).-

To a solution of compounds **93** (1.0 g, 2.26 mmol) and **77** (2.0 g, 3.40 mmol) in acetonitrile was added trimethylsilyl trifluoromethanesulfonate (0.12 mL, 0.67 mmol) at –40°C under argon atmosphere. After stirring for 3 h the solution was neutralized with Et_3N, let to reach room temperature and concentrated in vacuum. The residue was purified by column chromatography in toluene:acetone 5:1-3:1 to afford compound **94** (0.5 g, 65%): ^{1}H-NMR (600 MHz, $CDCl_3$) δ=0.01-0.17 (2s, 6H, $2CH_3Si$), 0.63-0.69 (m, 12H, $4CH_3$-TDS), 1.26 (s, 3H, CH_3-isop), 1.35 (m, 1H, CH-TDS), 1.53 (s, 3H, CH_3-isop), 1.82-2.08 (m, 27H, AcNH, NDMM, $3b_{ax}$-H), 2.57 (dd, 1H, J_{gem}=12.3 Hz, $J_{3,4}$= 2.6 Hz, $3b_{eq}$-H), 3.65 (d, 1H, 6a-H), 3.76 (s, 3H, OCH_3), 3.91 (d, 1H, 2a-H), 3.94 (m, 2H, 5a, 6'a-H), 4.02 (m, 2H, 5b, 6b-H), 4.10-4.12 (m, 2H, 4a, 9b-H), 4.31 (d, 1H, J_{gem}=12.3 Hz, $J_{9',8}$= 2.6 Hz, 9'b-H), 4.60 (dd, 1H, $J_{3a,4a}$=4.9 Hz, $J_{3a,2a}$=9.3 Hz, 3a-H), 4.84 (d, 1H, 4b-H), 5.04 (d, 1H, $J_{1a,2a}$=7.4 Hz, 1a-H), 5.27 (d, 1H, $J_{7b,8b}$= 7.0 Hz, 7b-H), 5.34 (m, 1H, 8b-H), 5.68 (d, 1H, $J_{NH,4b}$=4.5 Hz, NH).
$C_{43}H_{66}O_{19}N_2Si$ (943.1) MS (MALDI, positive mode, Matrix:DHB): m/z = 966 $[M+Na]^+$

Texyldimethylsilyl O-(Methyl [5-acetamido-4,7,8,9-tetra-O-acetyl -3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl]onate-(2→6)-(3,4-di-O-acetyl-(2-deoxy-2-[3,4-dimethyl-maleimido] - β-D-galactopyranoside (95).-

Compound **94** (0.4g, 0.4mmol) was dissolved in CH_2Cl_2 (4 mL) and treated with a 50% aqueous trifluracetic acid solution (0.4 mL)and stirred for 2 h at room temperature. TLC toluene:acetone 1:1. The organic phase was collected and washed with water (5mL), saturated $NaHCO_3$ (5mL) and brine (2 mL), dried and concentrated. The residue was dissolved in pyridine(10 mL) and acetic anhydride (5mL). the mixture was stirred at room temperature 2h. Then, the solvents were evaporated an coevaporated with toluene. Chromatography column of the residue afforded **95** (0.32 g, 92%).

^{1}H-NMR (600MHz, $CDCl_3$) δ=0.01-0.17 (2s, 6H, $2CH_3$-Si), 0.70-0.75 (m, 12H, CH_3-TDS), 1.47-1.50 (t, 1H, CH-TDS), 1.83-2.18 (m, 27H, AcNH, NDMM, $3b_{ax}$-H), 2.55 (dd, 1H, J_{gem}=12.8 Hz, $J_{3b,4b}$=4.5 Hz, $3b_{eq}$-H), 3.29 (dd, 1H, 6a-H), 3.79 (s, 3H, OMe), 3.88 (dd, 1H, 6b-H), 3.95 (d, 1H, 5a-H), 4.01 (d, 1H, 5b-H), 4.08 (d, 1H, 6b-H), 4.12 (dd, 1H, 9b-H), 4.21 (dd, 1H, $J_{1a,2a}$=8.0, $J_{2a,3a}$=11.4 Hz, 2a-H), 4.28 (dd, 1H, 9b'-H), 4.87(m, 1H, 4b-H), 5.31 (d, 1H, 1a-H), 5.32 (dd, 1H, 7b-H), 5.36 (m, 1H, 8b-H), 5.40 (d, 1H, $J_{4a,3a}$=3.0 Hz, 4a-H), 5.64 (dd, 1H, $J_{3a,4a}$=3.0 Hz, $J_{3a,2a}$=9.1 Hz, 3a-H).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 49.4 (C-5b), 52.8 (OMe), 53.3 (C-2a), 62.2 (C-9b), 63.1 (C-6a), 67.0 (C-4a, 7b), 67.8 (C-8b), 68.1 (C-3a), 68.9 (C-4b), 71.9 (C-5a), 72.4 (C-6b), 71.9 (C-5a), 93.6 (C-1a).

$C_{44}H_{74}O_{16}N_2Si$ (915.1)MS (MALDI, positive mode, Matrix:DHB): m/z = 939 $[M+Na]^+$

O-(Methyl [5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl]onate)-(2→6)-(3,4-di-O-acetyl-(2-deoxy-2-[3,4-dimethylmaleimido]- β-D-galactopyranosyl) Trichloroacetimidate (96).-

To a solution of **95** (630 mg, 0.07 mmol) in THF (10 mL) was added at –10°C acetic acid (59μL) and TBAF (1M in THF, 1mL). The mixture was stirred 6h, then was poured in ice water and extracted with diethylether (3x mL). The organic extracts were dried $MgSO_4$ filtered and concentrated. A solution of the residue, trichloroacetonitrile and DBU in dichloromethane was stirred for 45 min at room temperature, then concentrated. The residue was eluated from a column of silica gel toluene acetone containing 0.1% of Et_3N. to afford **96** (554 mg, 65%)

^{1}H-NMR (250 MHz, $CDCl_3$) δ= 1.80-2.30 (m, 28H, NDMM, 6AcO, AcHN, $3b_{ax}$-H), 2.51 (dd, 1H, J_{gem}=12.8 Hz, $J_{3b,4b}$=4.5 Hz, $3b_{eq}$-H), 3.42 (dd, 1H, 6a-H), 3.51(dd, 1H, 5b-H), 3.72 (s, 3H, OMe), 4.01-4.87 (m, 5H ,6b, 9b, 2a, 4b-H), 5.03-5.90 (m, 5H, 7b, 8b, 4a, 3a-H, NH), 6.42 (2d, 2H, 1a-H), 8.60 (s, 1H, NH).

Benzyl O-(Methyl [5-acetamido-4,7,8,9-tetra-O-acetyl -3,5-dideoxy-D-glycero-α-D-galacto-nonulopyranosyl]onate)-(2→6)-(2-deoxy-2-[3,4-dimethylmaleimido]-3,4-O-di-O-acetyl-β-D-galactopyranosyl)-(1→4)- (2,6-di-O-benzyl-3-O-(4-methoxy)benzyl -β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (97).-

A solution of imidate **96** (233 mg, 0.26 mmol) and lactose acceptor **74** (218 mg, 0.17 mmol) in dry acetonitril was stirred 30 min at room temperature . Tin (II) triflate (0.7 mg, 0.0017 mmol) was added and stirred under argon atmosphere for 1h, then neutralized with Et_3N and concentrated. Column chromatography of the residue (toluene:acetone 3:1) afforded **97** (252 mg, 68%), $[\alpha]_D$ –8°(c=1,$CHCl_3$).^{1}H-NMR (600MHz, $CDCl_3$) δ=1.19 (s, 9H, OPiv), 1.70-2.35 (m, 21H, AcO, AcHN, NDMM, $3c_{ax}$-H), 2.56 (dd, 1H, $3c_{eq}$-H), 3.09 (dd, 1H, 2b-H), 3.15-3.17 (m, 2H, 5b, 6b-H), 3.22 (dd, 1H, 3b-H), 3.38 (d, 1H, 5a-H), 3.50 (dd, 1H, 6b-H), 3.56 (dd, 1H, 6c-H), 3.61 (dd, 1H, 3a-H), 3.6 (m, 2H, 6a-H), 3.77 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.82-3.84 (m, 2H, 6b', 4a-H), 3.93 (d, 1H, 4b-H), 3.99-4.04 (m, 2H, 5d, 9d-H), 4.12 4.13 (m, 2H, 6d, 5c-H), 4.27 (d, 1H, 9'd-H), 4.23 (d, 1H, 1b-H), 4.38 (dd, 1H, 2c-H), 4.45 (d, 1H, 1a-H), 4.89 (d, 1H, 4d-H), 4.14-5.05 (6dd, 12H, CH_2Ph), 5.05 (dd, 1H, 2a-H), 5.19 (d, 1H, NH), 5.27 (d, 1H, 1c-H), 5.33 (dd, 1H, 3c-H), 6.77 (d, 2H, MPM), 7.18-7.30 (m, 32H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 38.0 (C-3d), 49.5 (C-5d), 51.6 (C-2a), 53.0 (OMe), 55.3 (MeO), 62.2 (C-9d), 63.2 (C-6c), 66.9 (C-4c), 67.0 (C-7d), 67.9 (C-3c), 68.1 (C-6a), 68.3 (C-8d), 68.8 (C-4d), 68.9 (C-6b), 71.3 (C-5c), 72.6 (C-2a), 72.8 (C-6d), 73.5 (C-5b), 74.1 (C-4b), 75.7 (C-5a), 76.8 (C-4a), 80.3 (C-2b), 80.5 (C-3b), 81.0 (C-3a), 99.5 (C-1c), 99.8 (C-1a), 102.2 (C-1b).

Anal. Calcd for $C_{96}H_{115}O_{32}N_2$ (1808.9): C 63.74, H 6.41, N 1.55; found . C 63.79, H 6.47, N 1.57.

O-(2,3,4,6-tetra-O-benzoyl-α-D-galactopyranosyl) Trichloacetimidate (98).-

Compound **98** was synthesized following a published procedure.[103]

Texyldimethylsilyl O-(4,6-O- benzylidene-2-deoxy-2-(3,4-dimethylmaleimido)-β-D-galacto-pyranoside (99).-

Compound **99** was synthesized following a published procedure.[91]

Texyldimethylsilyl O(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-(3,4-dimethylmaleimido)-β-D-galactopyranoside (100).-

A solution of imidate **98** (500 mg, 0.68 mmol) and acceptor **99** (244 mg, 0.45 mmol) in dichloromethane (10 mL) was stirred 30 min at room temperature , then a solution of TMSOTf (0.01M, 60µl) was added under nitrogen atmosphere. After 3h, the solution was neutralized with Et_3N and evaporated *in vacuo*. The residue was purified by column chromatography (toluene:acetone 10:1) to afford **100 (**435 mg**,** 87%) ^{1}H-NMR (600MHz, $CDCl_3$) δ=0.0-0.12 (2s, 6H, 2Me-TDS), 0.66-0.72 (m, 12H, TDS), 1.41-1.46 (2s, 6H, NDMM), 3.40 (s, 1H, 5a-H), 3.62 (d, 2H, $J_{6b,5b}$=6.0 Hz, 6b-H), 3.78 (d, 1H, $J_{6a,6a'}$=11.6 Hz, 6a-H), 4.15 (d, 1H, $J_{5b,4b}$=6.0Hz, 5b-H), 4.18 (d, 1H, 6a'-H), 4.40 (m, 1H, 2a-H), 4.46 (d, 1H, $J_{4a,3a}$=2.9 Hz, 4a-H), 4.65 (dd, 1H, $J_{3a,2a}$=11.2, $J_{3a,4a}$=3.3 Hz, 3a-H), 4.95 (d, 1H, $J_{1b,2b}$=7.8 Hz, 1b-H), 5.14 (d, 1H, $J_{1a,2a}$=8.0 Hz, 1a-H), 5.43 (s, 1H, PhH), 5.49 (dd, 1H, $J_{3b,4b}$=3.2 Hz, $J_{3b,2b}$=10.2 Hz, 3b-H), 5.71 (dd, 1H, 2b-H), 5.86 (d, 1H, 4b-H), 7.19-8.04 (m, 25H, Ph).
Calcd for $C_{62}H_{69}O_{15}N$ (1096.3):calcd C 67.93, H 6.34, N 1.28; found . C 67.19, H 6.21, N 1.32.

O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1→3)-4,6-O- benzylidene-2-deoxy-2-(3,4-dimethylmaleimido)-β-D-galactopyranosyl) Trichloacetimidate (101).-

To a solution of **100** (890 mg, 0.9 mmol) in THF (13 mL) was added at –10°C acetic acid (45µL) and TBAF (1M in THF, 2 mL). The mixture was stirred 6h , then was poured in ice water and extracted with diethylether (3x mL). The organic extracts were dried $MgSO_4$ filtered and concentrated. The residue was dissolved in dry dichloromethane (8 mL) and treated with trichloroacetonitrile (350 µl) and DBU (1.1 µl) under nitrogen at room temperature. After stirring for 1h, the reaction mixture was concentrated *in vacuo* and the residue was purified by flash chromatography (toluene acetone, 4.1 containing 0.1% of Et_3N) to give **101** (790 mg, 89%) as white foam. ^{1}H-NMR (250 MHz, $CDCl_3$) δ= 1.48-1.52 (2s, 6H, NDMM), 3.72 (s, 1H, 6a-H), 4.20 (d, 1H, 6a'-H), 4.43 (m, 2H, 6b, 5b-H), 4.50 (d, 1H, 4a-H), 4.70-4.90 (m, 4H, 3a, 2a, 6b, 5a-H), 5.05 (d, 1H, $J_{1b,2b}$= 7.9 Hz, 1b-H), 5.49 (s, 1H, PhCH), 5.55 (dd, 1H, $J_{3b,4b}$=3.6 Hz, $J_{3b,2b}$=9.8 Hz, 3b-H), 5.75 (dd, 1H, $J_{2b,3b}$=11.2 Hz, 2b-H), 5.95 (d, 1H, 4b-H), 6.10 (d, 1H, $J_{1a,2a}$=7.7 Hz, 1a-H), 7.15-8.00 (m, 25H, Ph), 8.50 (s, 1H, NH).

Benzyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1→3)-(4,6-O- benzylidene-2-deoxy-2-(3,4-dimethylmaleimido)-β-D-galactopyranosyl)-(1→4)-(2,6-di-O- benzyl-3-O-(4-methoxy)benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (102).-

A solution of imidate **100** (920 mg, 1.2 mmol) and lactose acceptor **74** (450 mg, 1 mmol) in dry acetonitril was stirred 30 min at room temperature. Tin (II) triflate (49 mg, 0.012 mmol) was added and stirred under argon atmosphere for 1h, then neutralized with Et_3N and concentrated. Column chromatography of the residue (toluene:acetone 3:1) afforded **102** (252 mg, 62%) ^{1}H-NMR (600 MHz, $CDCl_3$) δ=1.14 (s, 9H, OPiv), 1.40-1.42 (2s, 6H, NDMM), 2.89 (dd, 1H, 6b-H), 2.93 (dd, 1H, 2b-H), 3.08 (dd, 1H, 5b-H), 3.17 (dd, 1H, $J_{3b,4b}$=2.5 Hz, $J_{3b,2b}$= 9.5 Hz, 3b-H), 3.29-3.34 (m, 3H, 6b, 5c, 5a-H), 3.52 (dd, 1H, 3a-H), 3.63 (d, 2H, 6a-H), 3.68 (m, 2H, 6c, 4b-H), 3.78 (s, 3H, CH_3O), 3.79 (d, 1H, 4a-H), 4.03 (d, 1H, 6c-H), 4.15 (d, 1H, $J_{1b,1a}$= 7.0 Hz, 1b-H), 4.20-5.00 (6dd, 12H, CH_2Ph), 4.30 (d, 1H, 5d-H), 4.35 (d, 1H, 6d-H), 4.42 (d, 1H, $J_{1a,2a}$= 9.6 Hz, 1a-H), 4.54 (dd, 1H, 2c-H), 4.65 (dd, 1H, 6d-H), 4.95 (dd, 1H, 3c-H), 4.99 (d, 1H, $J_{1c,2c}$ = 8.4 Hz, 1c-H), 5.03 (dd, 1H, 2a-H), 5.12 (d, 1H, 1d-H), 5.52 (s, 1H, PhH), 5.57 (dd, 1H, $J_{3d,2d}$= 10.3 Hz, $J_{3d,4d}$= 3.1 Hz, 3d-H), 5.77 (dd, 1H, $J_{1d,2d}$=8.0, $J_{2d,3d}$=10.0 Hz, 2d-H), 5.92 (d, 1H, 4d-H), 6.77 (d, 2H, MPM), 7.03-7.43 (m, 25H, Ph).
MS (MALDI,positive mode, Matrix:DHB): m/z = 1958 $[M+Na]^+$
Anal. Calcd for $C_{113}H_{114}O_{28}N$ (1934.1): C 70.17, H 5.94, N 0.72; found . C 65.58, H 5.88, N 0.82.

Benzyl O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→3)-O-(4,6-di-O-acetyl-2-N-[3,4-dimethylmaleimido]-β-D-glucopyranosyl)-(1→3)-(2,6-di-O- benzyl -β-D-galactopyranosyl)-(1→4)- 3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (103).-

A mixture of **82** (180 mg, 0.10 mmol) and sodium hydroxide in dioxane-water (4:1, 18 mL) was stirred over night at room temp. A solution of 1M HCL was slowly added dropwise until pH= 4.5 was reached; the reaction mixture was stirred and the pH constantly checked by a Ph-meter. After 3d the mixture was neutralized with a solution of 1M NaOH, ethanolamine was added and the solvent evaporated *in vacuo*. The residue was lyophilized from dioxane-water and used for the next step without further purification.
The residue was dissolved in pyridine (10 mL), treated with acetic anhydride (5 mL) and the mixture stirred at room temperature for 16 h. The solvent was evaporated *in vacuo* by co

distillation with toluene and the residue purified by flash chromatography to afford **103** (78%) as white powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 1.13 (s, 9H, OPiv), 1.63 (s, 3H, AcHN), 1.94-2.10 (6s, 18H, AcO), 3.18 (d, 1H, 6b-H), 3.23 (d, 1H, 6'b-H), 3.37-3.38 (m, 2H, 5a, 2c-H), 3.41-3.77 (m, 11H, 2b, 6d, 5c, 3b, 3a, 6a, 5d, 5b, 5a-H), 4.09-4.10 (m, 2H, 3c, 4a-H), 4.14 (m, 2H, 6c-H), 4.14-4.90 (6dd, 12H, CH_2Ph), 4.34 (d, 1H, 1d-H), 4.43 (d, 1H, 1a-H), 4.46 (d, 1H, 1b-H), 4.86 (m, 2H, 4c, 1c-H), 4.89 (dd, 1H, 3d-H), 4.96 (dd, 1H, 2d-H), 5.13 (dd, 1H, $J_{2a,3a}$= 8.7 Hz, 2a-H), 5.32 (d, 1H, $J_{3b,4b}$= 2.9 Hz, 4b-H), 5.39 (d, 1H, $J_{3d,4d}$= 2.8 Hz, 4d-H), 7.18- 7.44 (m, 30H, Ph).

^{13}C-NMR (600 MHz, $CDCl_3$): δ 57.0 (C-2c), 62.3 (C-6c), 67.5 (C-6d. 4d), 68.0 (C-6a), 68.5 (C-6b), 69.0 (C-4c), 69.5 (C-2d, 4b), 71.2 (C-3d), 71.9 (C-5c), 72.0 (C-2a, 5d), 72.9 (C-5b), 75.5 (C-5a), 75.7 (C-4a), 76.7 (C-3c), 78.8 (C-3b), 80.1 (C-2b), 99.6 (C-1a), 99.9 (C-1c), 100.6 (C-1d), 102.2 (C-1b), 125.2-128.9 (C-Ph

$C_{85}H_{101}O_{28}N$ (1584) ; MS (MALDI,positive mode, Matrix:DHB): m/z = 1607 $[M+Na]^+$

Benzyl O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-(2,6-di-O-benzyl-3-O-(4-methoxy)benzyl -β-D-galactopyranosyl)-(1→4)- 3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (104).-

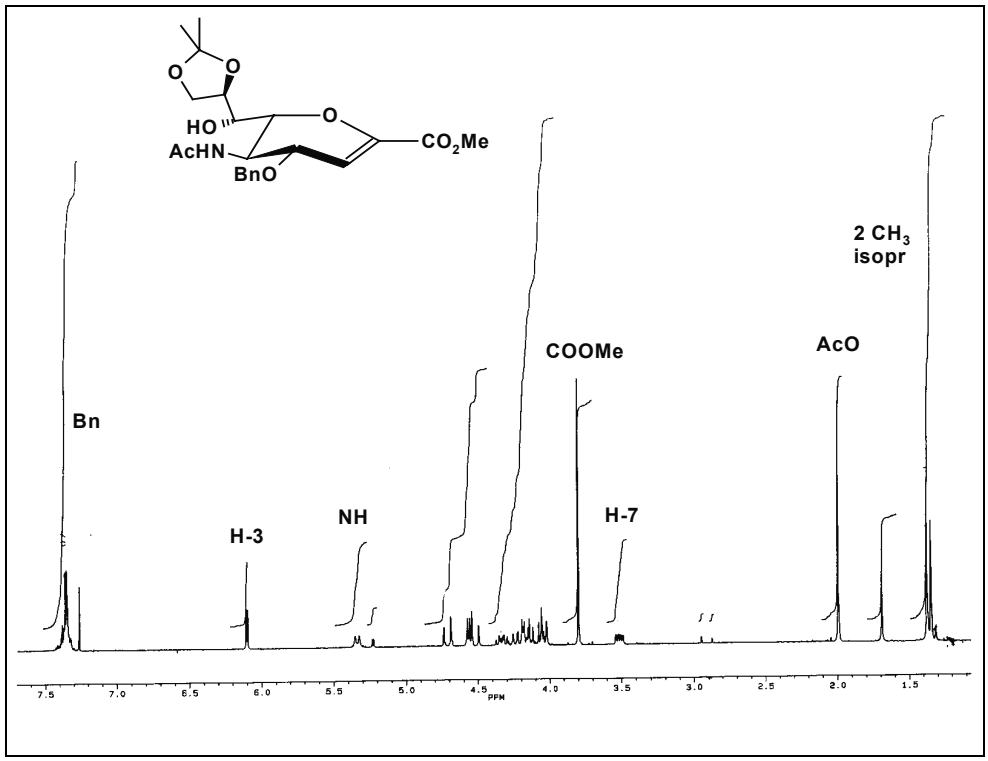
A mixture of **91** and sodium hydroxide in dioxane-water (4:1, 10 mL) was stirred over night at room temp. A solution of 1M HCL (4.5 mL) was slowly added dropwise until pH= 4.5 was reached; the reaction mixture was stirred and the pH constantly checked by a Ph-meter. After 3d the mixture was neutralized with a solution of 1M NaOH, ethanolamine was added and the solvent evaporated *in vacuo*. The residue was lyophilized from dioxane-water and used for the next step without further purification.

The residue was dissolved in pyridine (10 mL), treated with acetic anhydride (5 mL) and the mixture stirred at room temperature for 16 h. The solvent was evaporate *in vacuo* by codistillation with toluene and the residue purified by flash chromatography to afford **104** (75%) as white powder. ^{1}H-NMR (600 MHz, $CDCl_3$):δ1.15 (s, 9H, OPiv), 1.83 (s, 3H, AcHN), 1.99-2.18 (4s, 12H, AcO), 3.28 (d, 1H, 6b-H), 3.42-3.44 (m, 2H, 5b 5a-H), 3.51 (d, 1H, 6'b-H), 3.56 (dd, 1H, 2b-H), 3.66 (dd, 1H, 3a-H), 3.71-3.73 (m, 3H, 6a 2c-H), 3.84-3.85 (m, 2H, 6c ,5c-H), 4.03-4.07 (m, 2H, 4a , 4b-H), 4.15 (d, 1H, 6c-H), 4.07-5.00 (5dd, 10H, CH_2Ph), 4.47 (m, 2H, 1b, 1a-H), 4.76 (d, 1H, 1c-H), 4.86 (dd, 1H, 3b-H), 5.11 (dd, 1H, $J_{2a,3a}$= 8.8 Hz, 2a-H), 5.36 (d, 1H, 4c-H), 5.47 (d, 1H, $J_{3c,2c}$ = 11.1 Hz, $J_{3c,4c}$= 3.1 Hz, 3c-H), 7.16-7.31 (m, 25H, Ph).

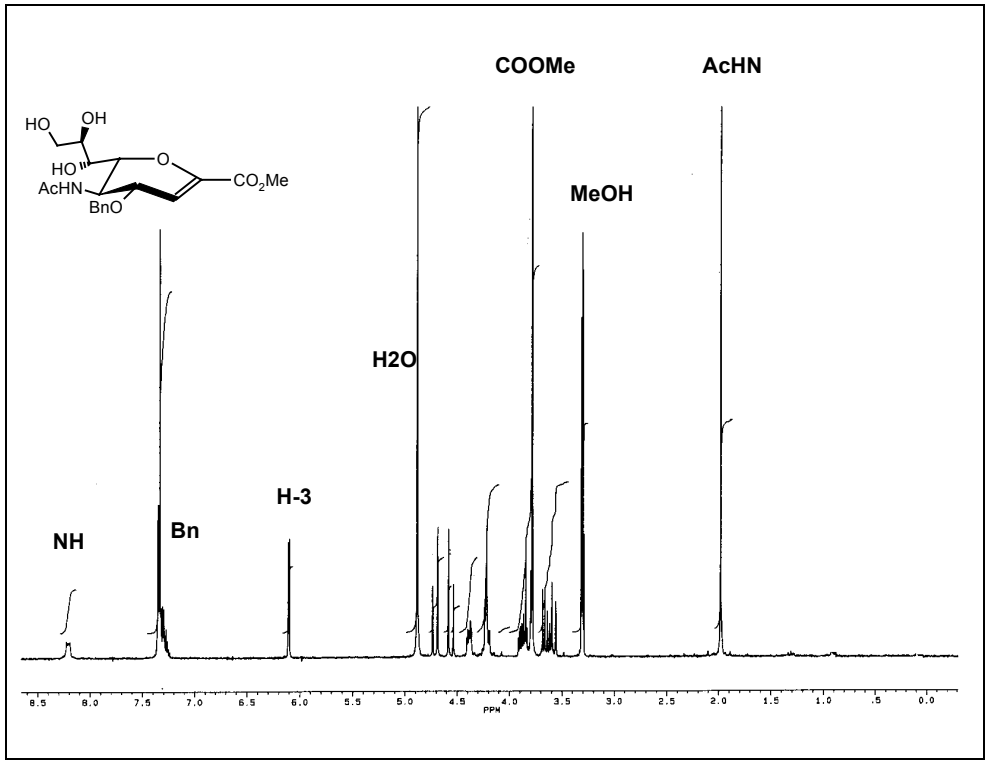
^{13}C-NMR (600 MHz, $CDCl_3$): δ 52.7 (C-2c), 61.2 (C-6c), 66.8 (C-4c), 67.9 (C-6a), 68.8 (C-6b), 69.1 (C-3c), 70.4 (C-5c), 71.8 (C-2a), 73.4 (C-5b), 74.4 (C-4b), 74.7 (C-3b), 75.6 (C-5a), 76.0 (C-4a), 78.2 (C-2b), 80.7 (C-3a), 99.7 (C-1a), 100.8 (C-1c), 102.5 (C-1b), 127.1-128.6 (C-Ph).

$C_{68}H_{81}O_{21}N$ (1248) ; MS (MALDI,positive mode, Matrix:DHB): m/z = 1271 $[M+Na]^+$

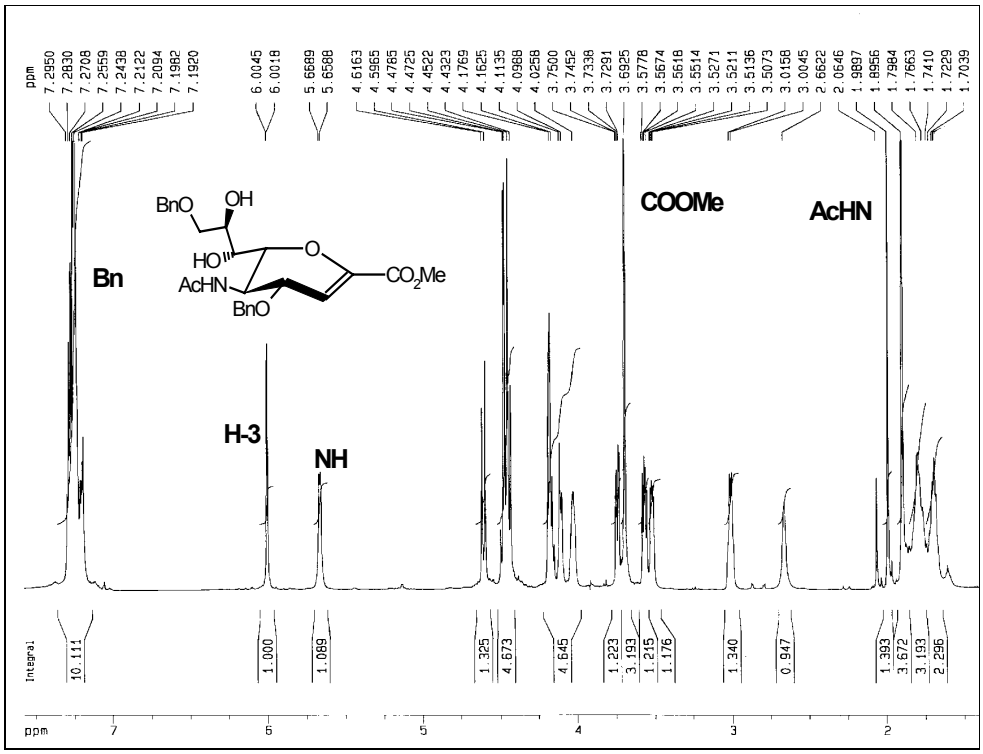
3.3 ^{1}H-NMR and HMQC Spectra



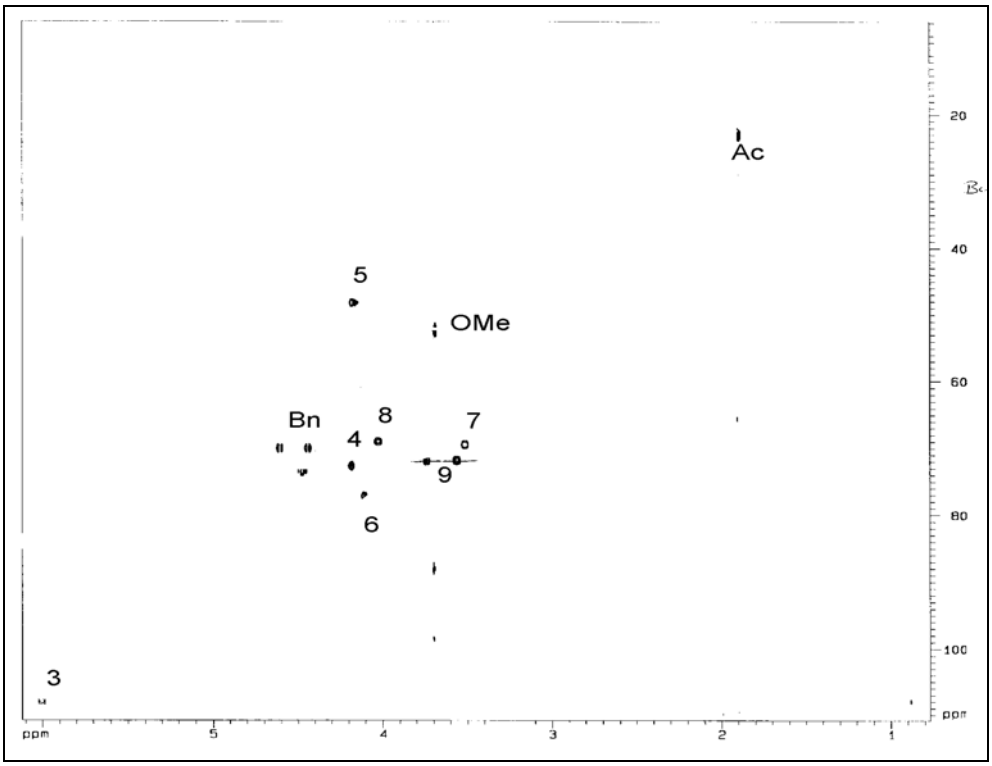
^{1}H-NMR Spectrum of compound **60** (250 MHz, $CDCl_3$)



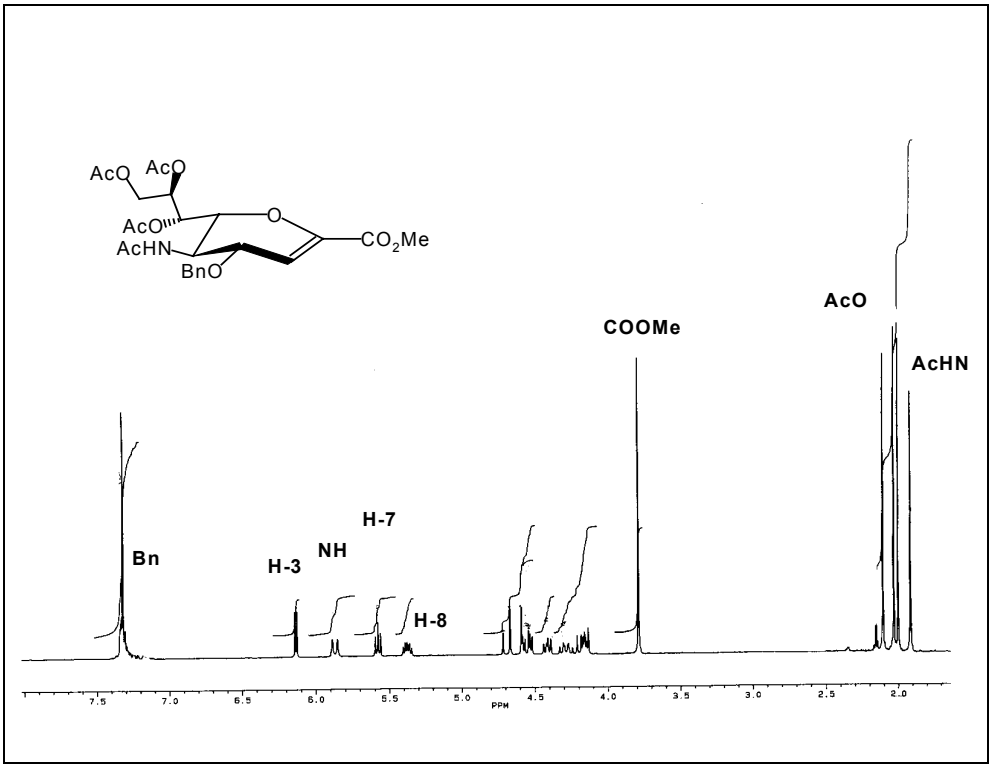
^{1}H-NMR Spectrum of compound **61** (250 MHz, MeOD)



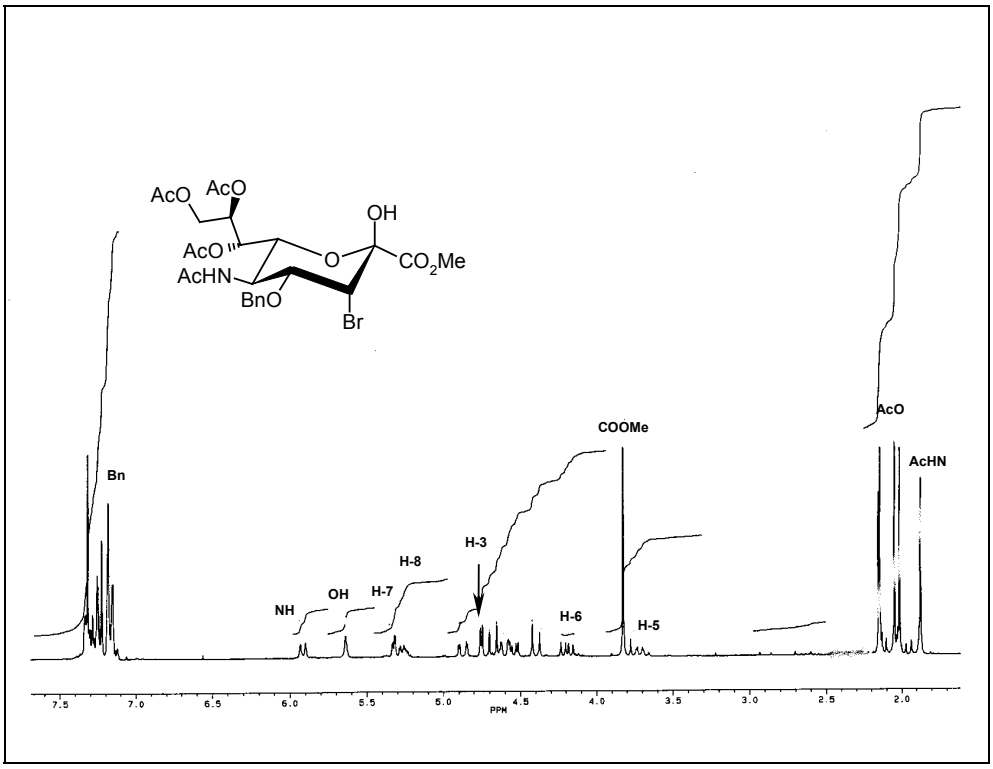
^{1}H-NMR Spectrum of compound **62** (600 MHz, $CDCl_3$)



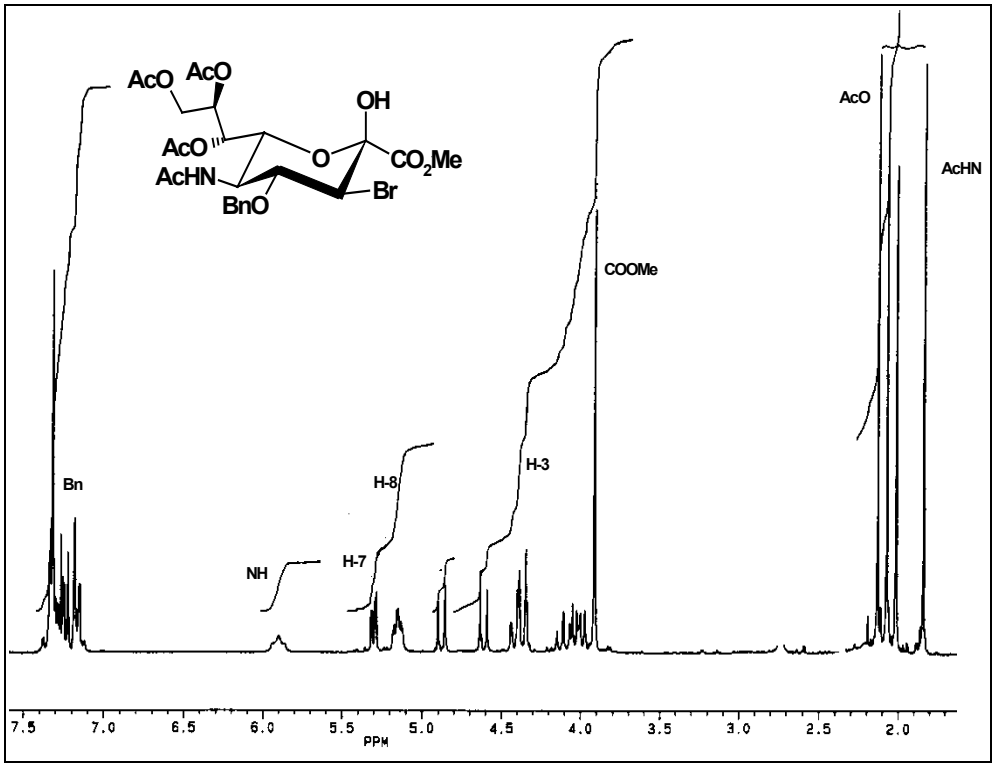
HMQC Spectrum of compound **62**



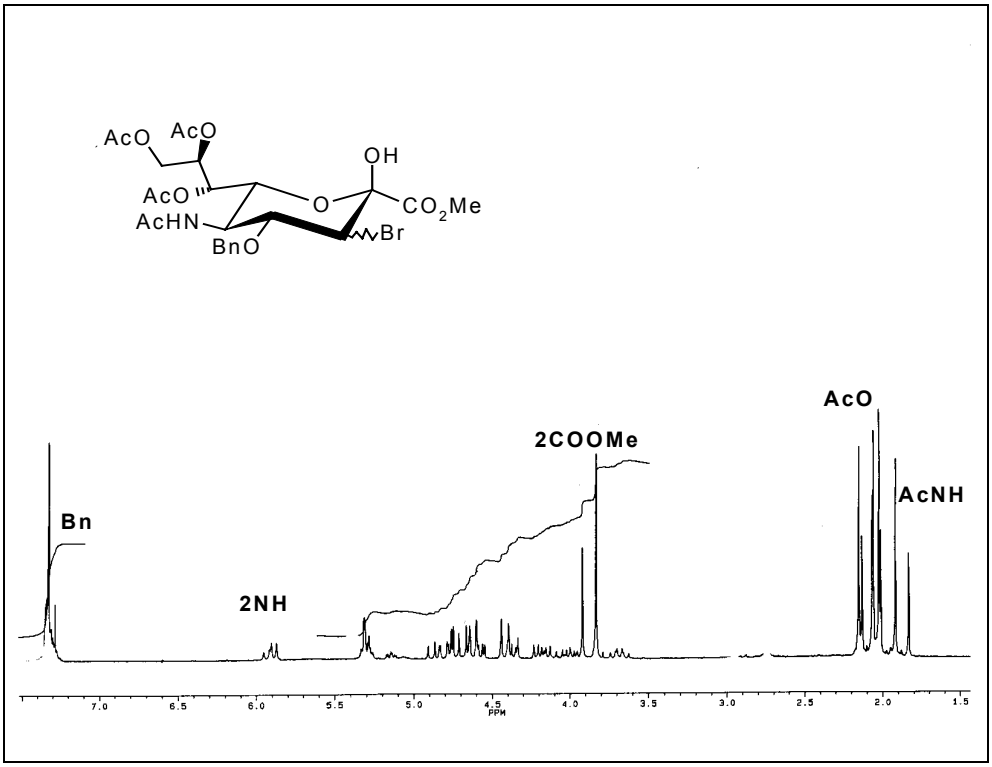
^{1}H-NMR Spectrum of compound **63** (250 MHz, $CDCl_3$)



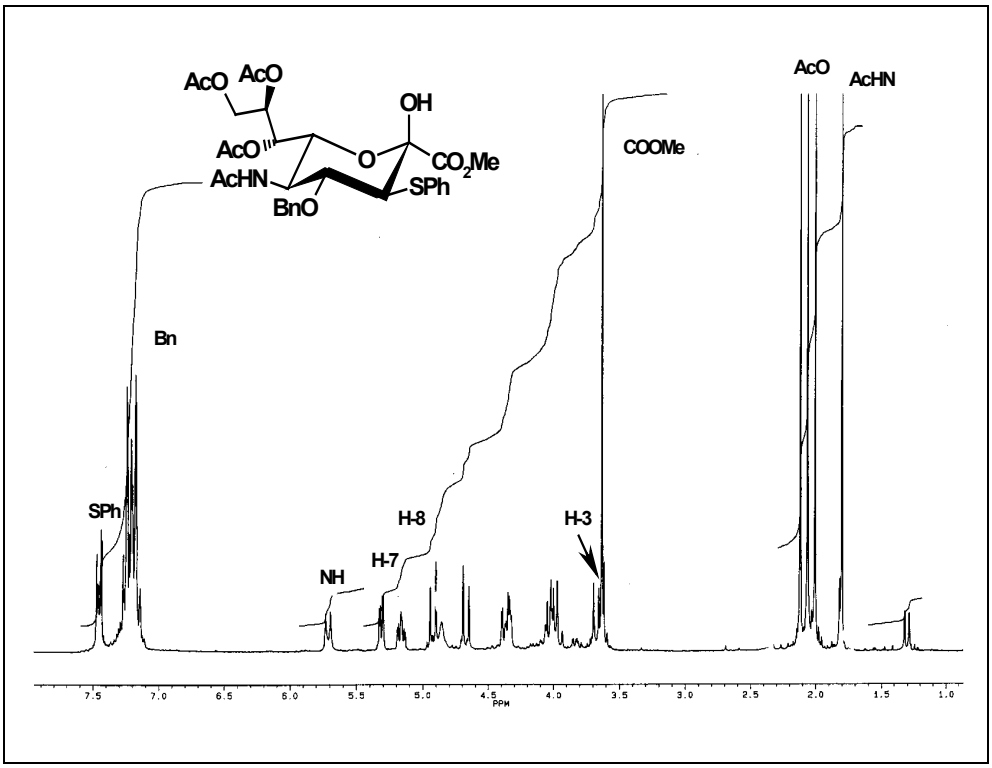
^{1}H-NMR Spectrum of compound **64** (250 MHz, $CDCl_3$)



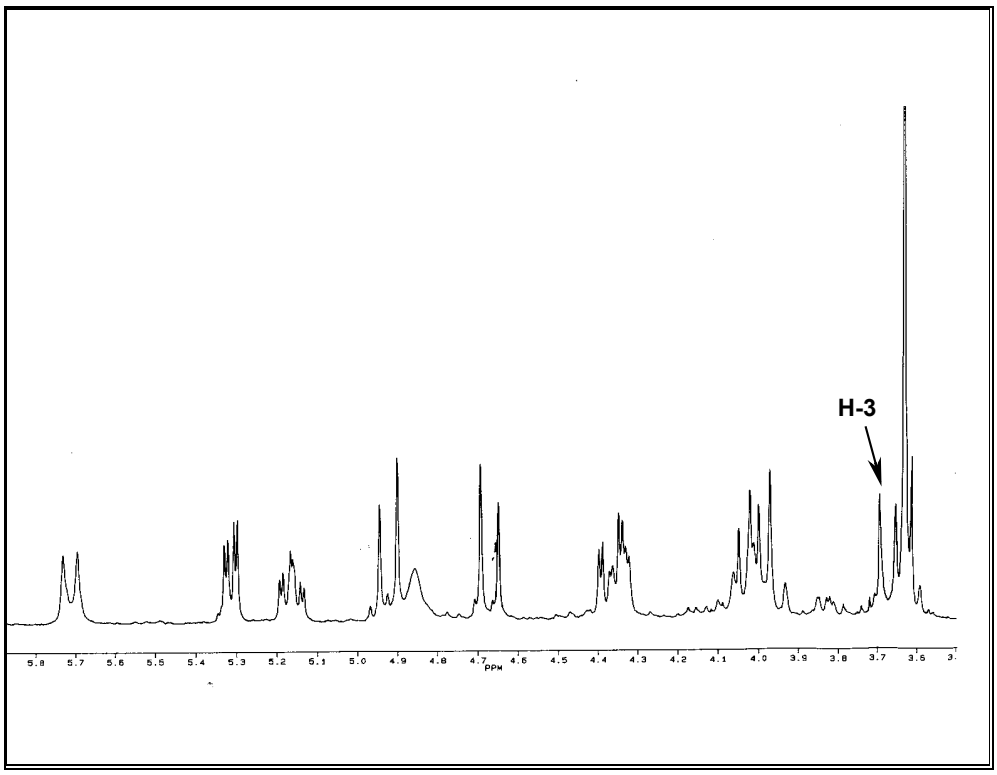
^{1}H-NMR Spectrum of compound **65** (250 MHz, $CDCl_3$)



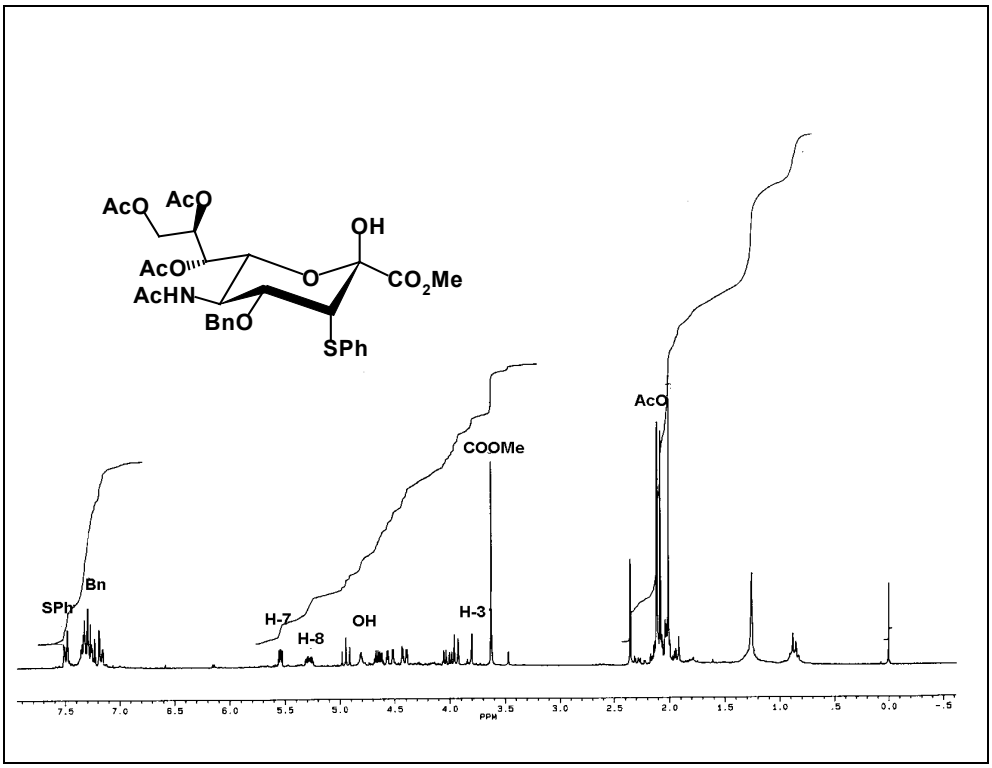
^{1}H-NMR Spectrum of compounds **64/65** (250 MHz, $CDCl_3$)



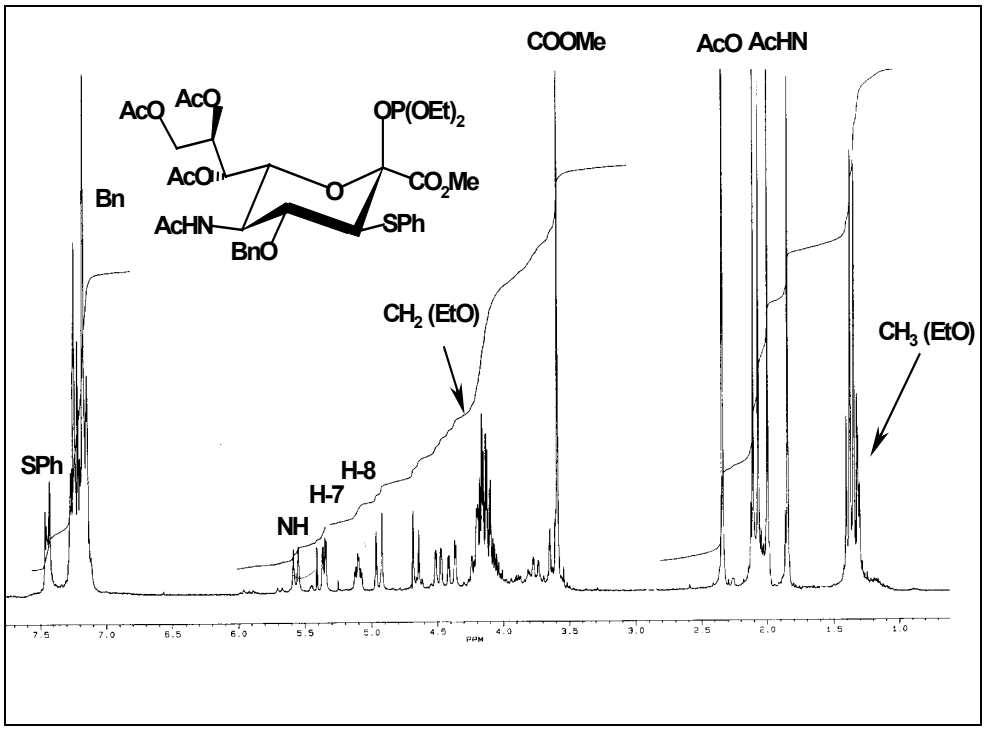
^{1}H-NMR Spectrum of compound **66** (250 MHz, $CDCl_3$)



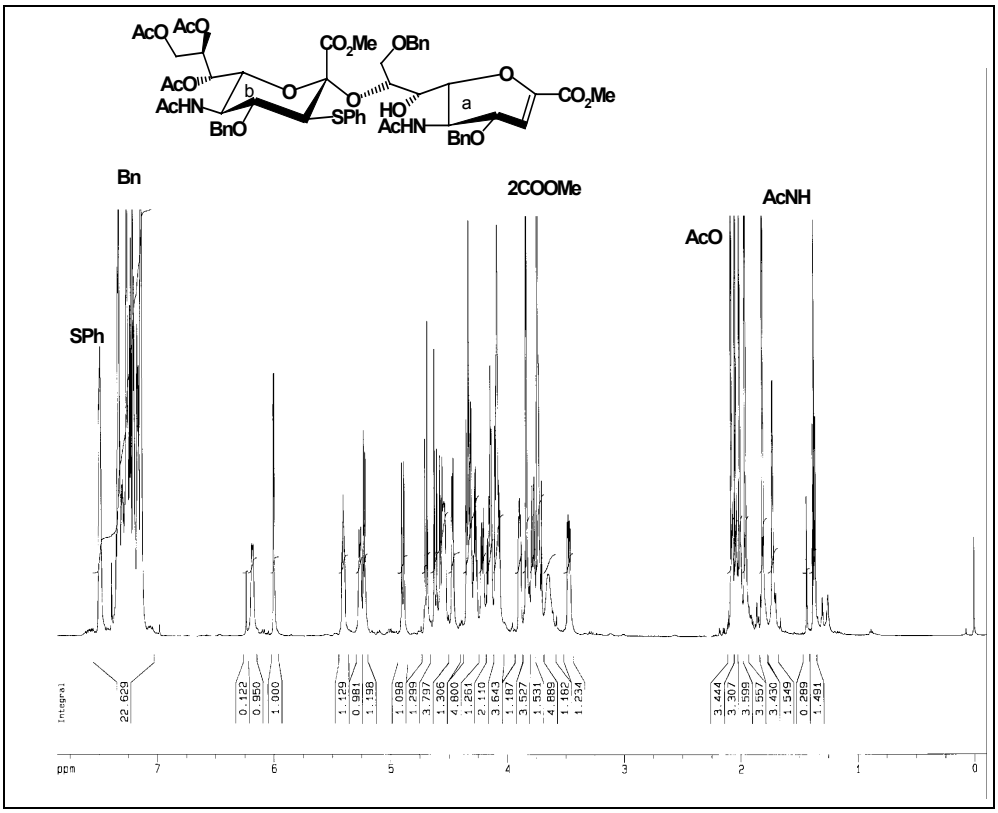
^{1}H-NMR Spectrum of compound **66** (range: 3.60-5.80 ppm)



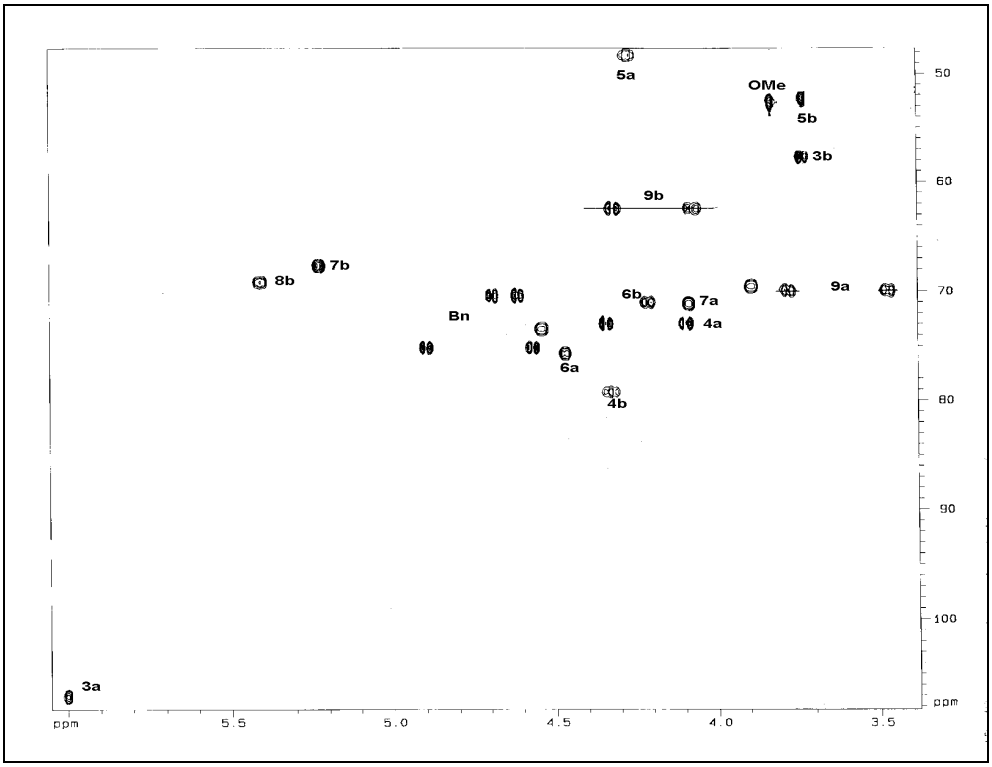
^{1}H-NMR Spectrum of compound **67** (250 MHz, $CDCl_3$)



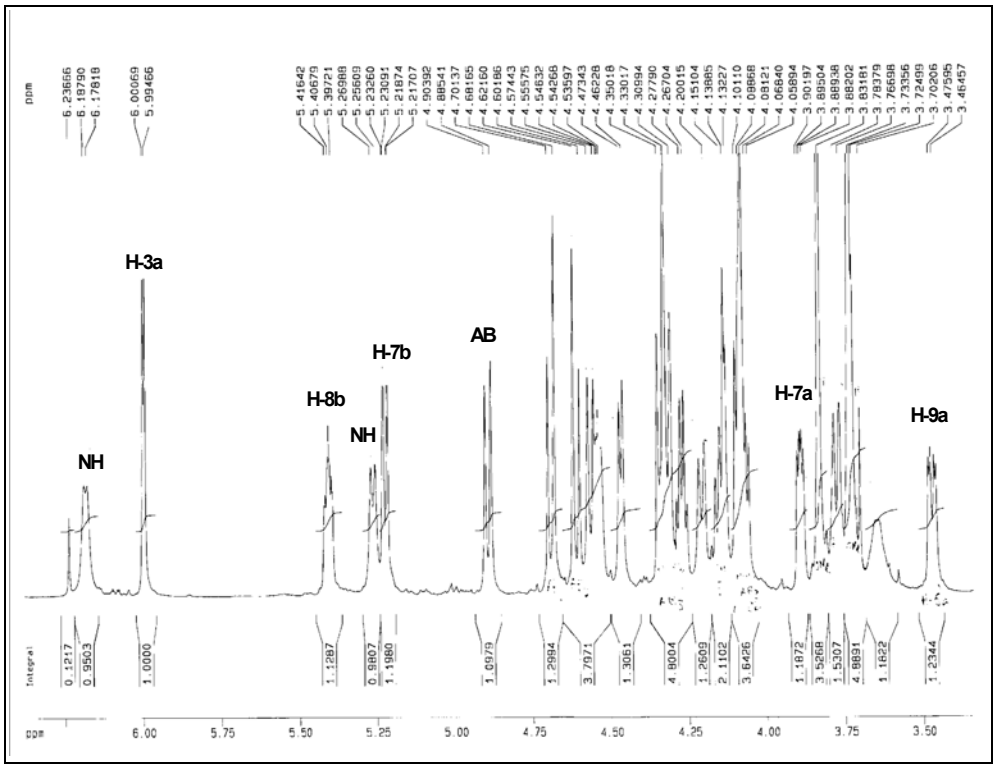
^{1}H-NMR Spectrum of compound **68** (250 MHz, $CDCl_3$)



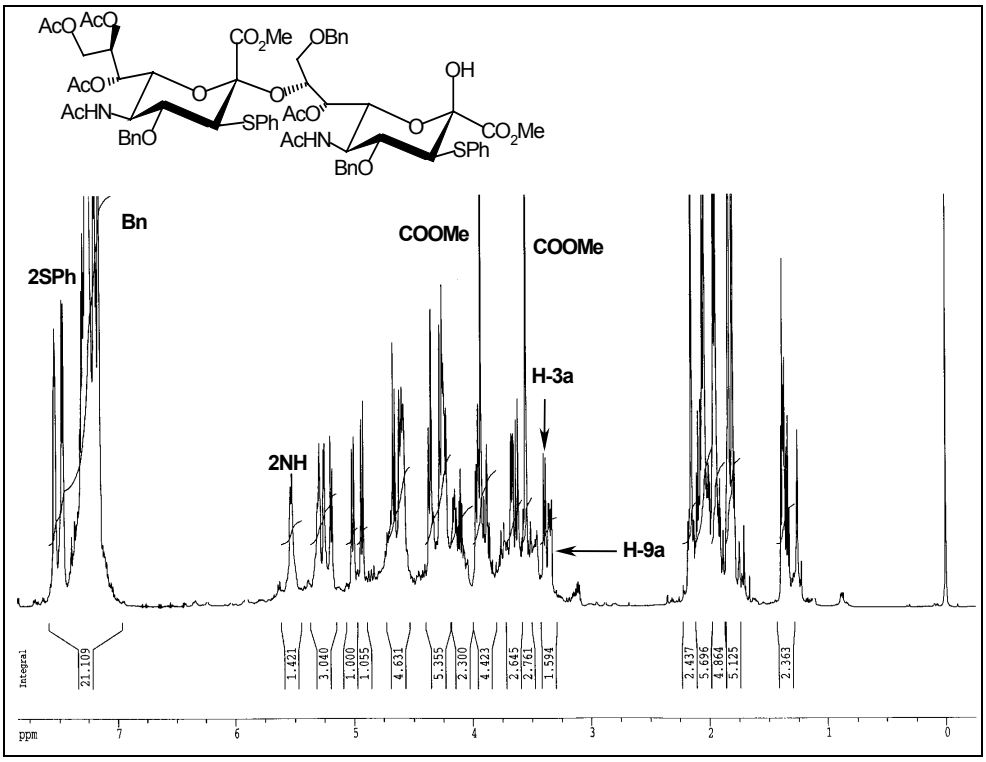
^{1}H-NMR Spectrum of compound **69** (600 MHz, $CDCl_3$)



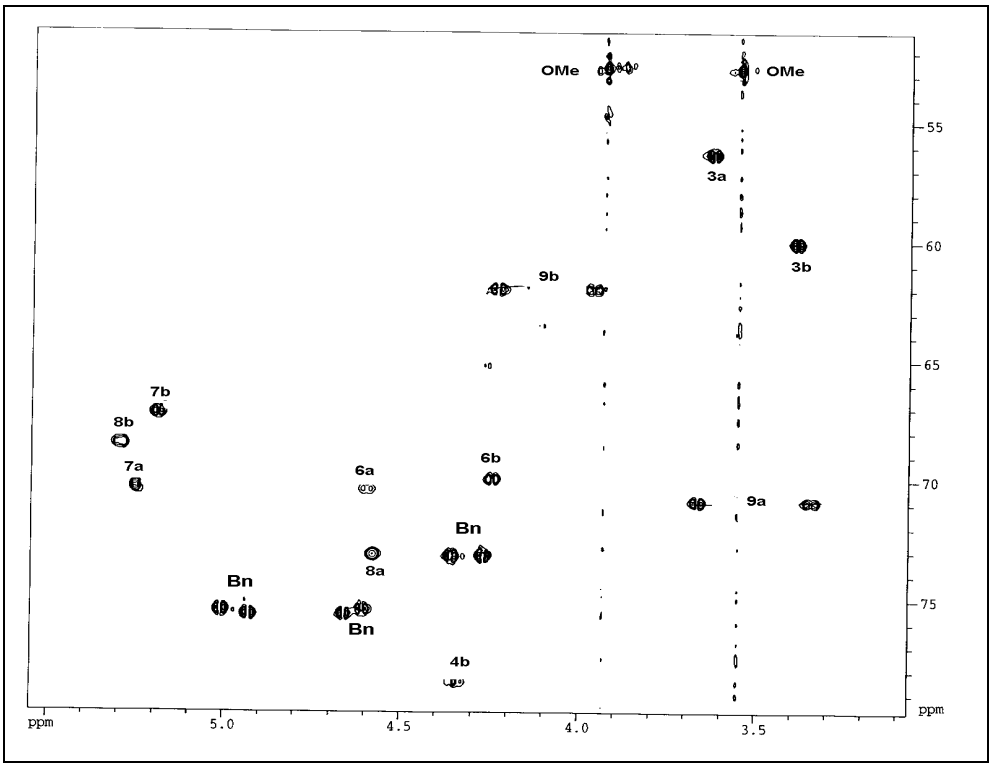
HMQC Spectrum of compound **69**



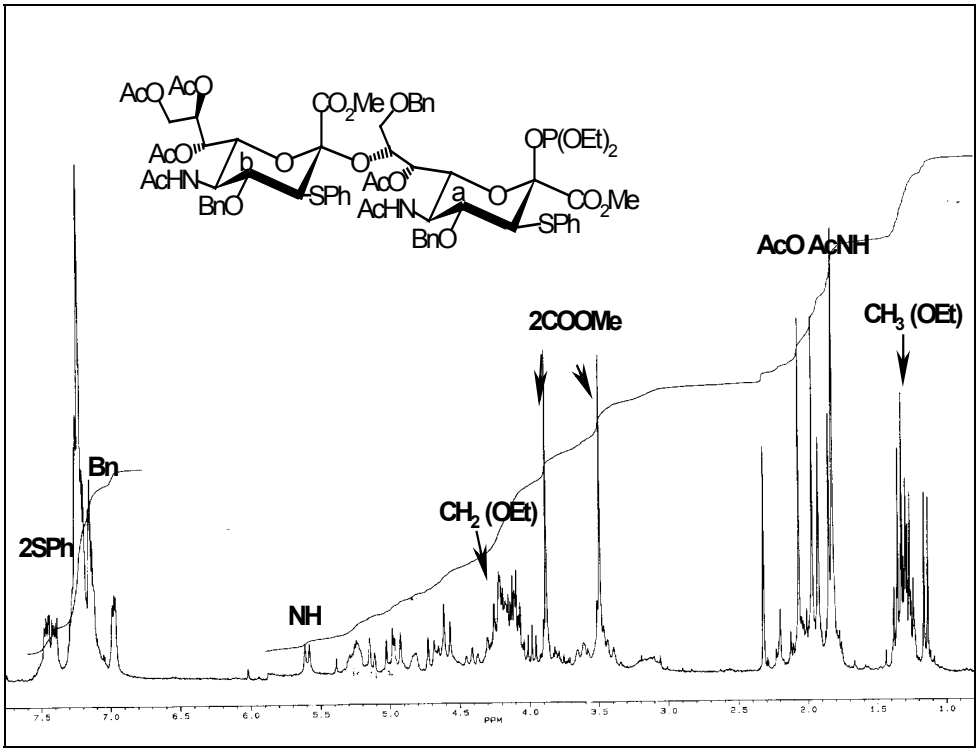
^{1}H-NMR Spectrum of compound **69** (range: 3.60-6.50)



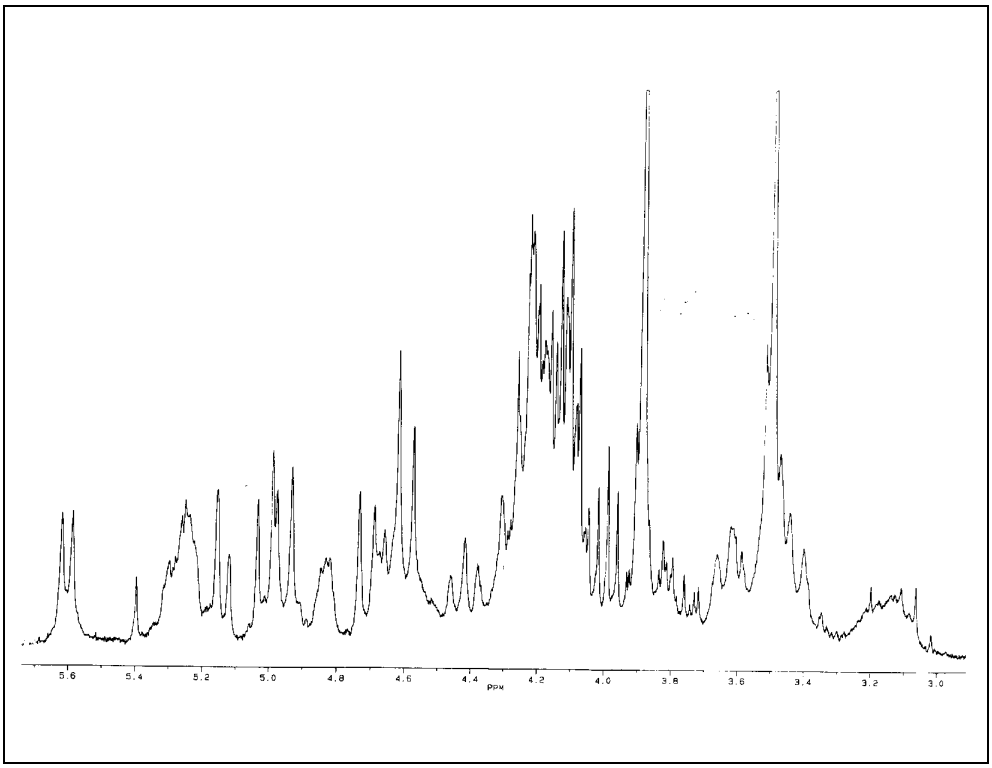
^{1}H-NMR Spectrum of compound **70** (600 MHz, $CDCl_3$)



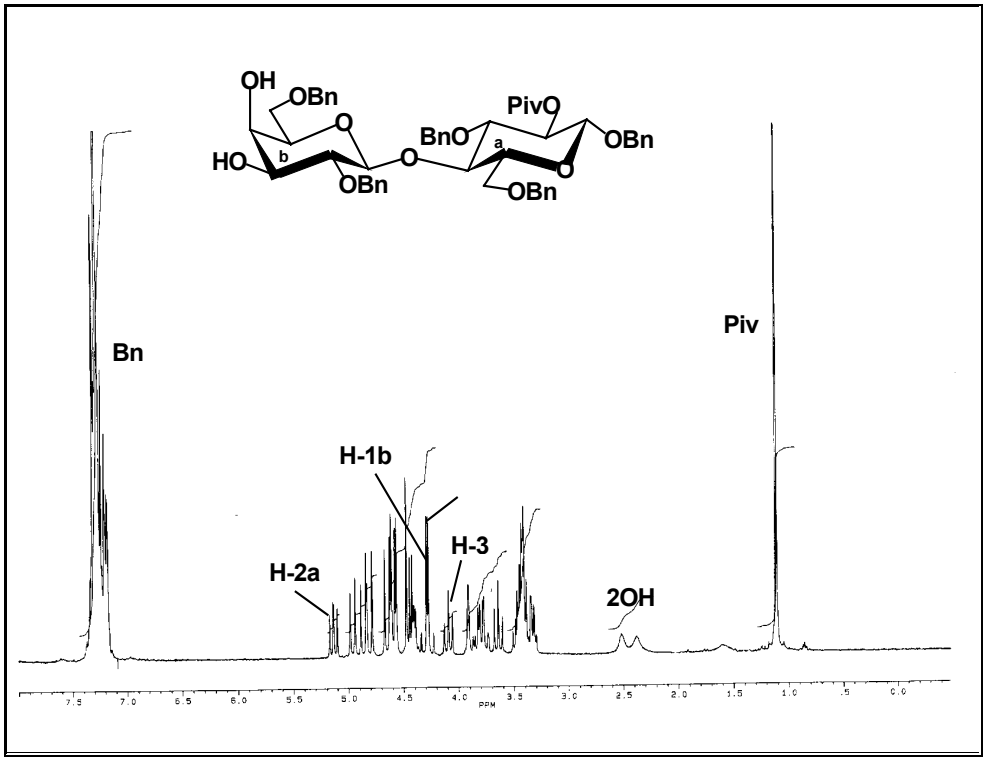
HMQC Spectrum of compound **70**



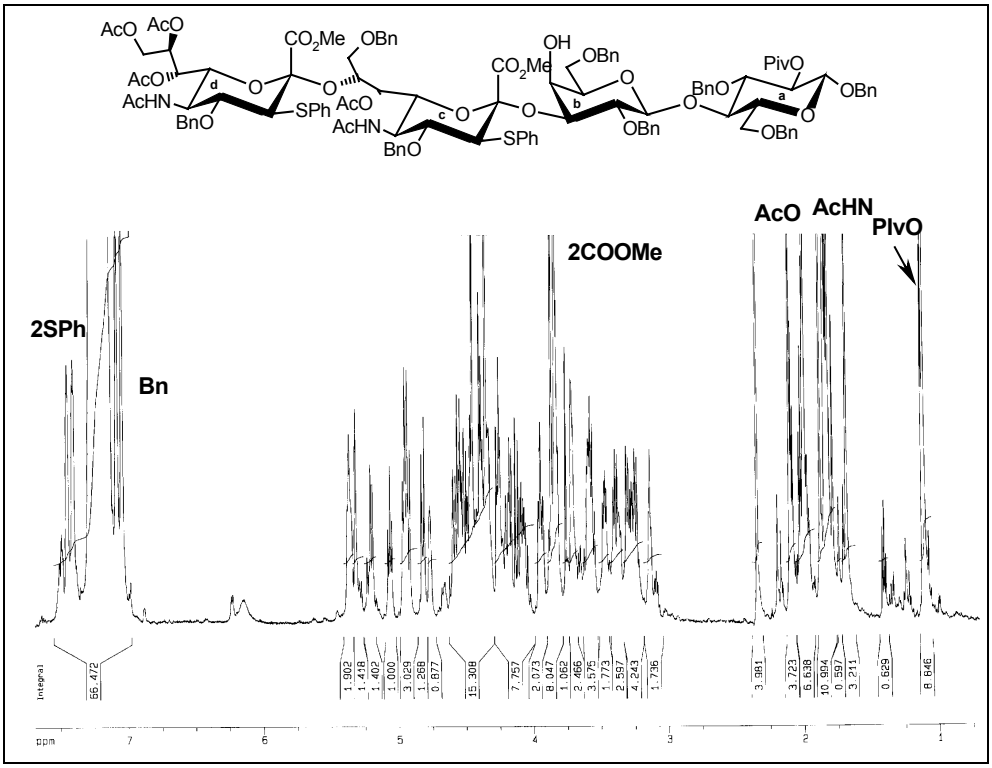
^{1}H-NMR Spectrum of compound **71** (250 MHz, $CDCl_3$)



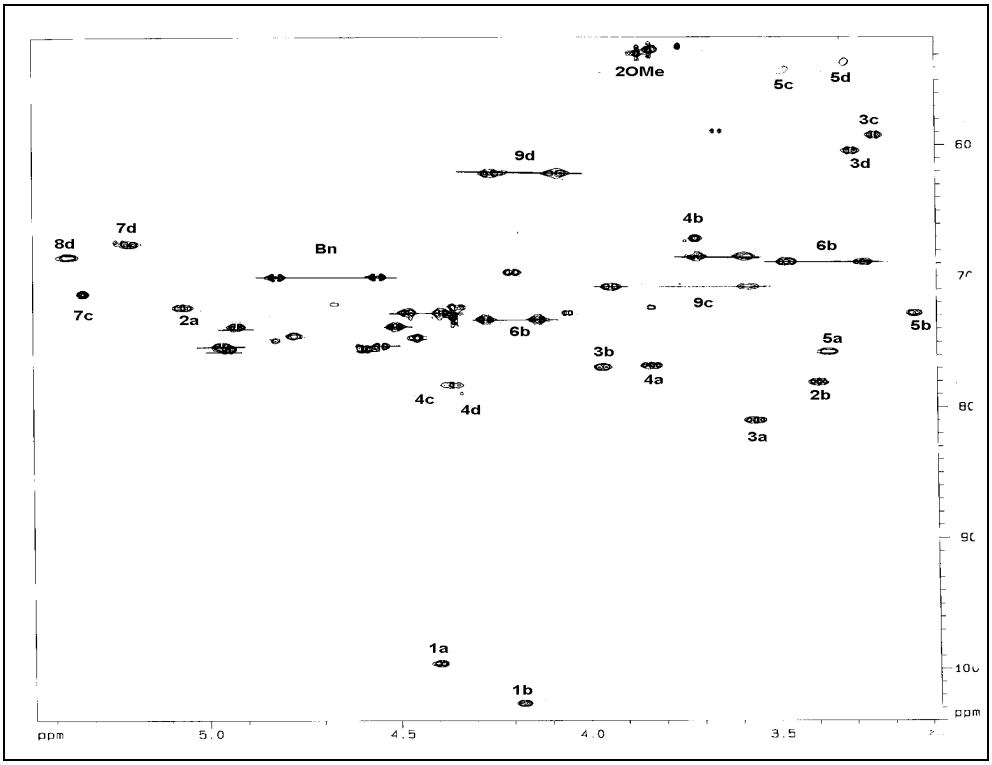
^{1}H-NMR Spectrum of compound **71** (range: 3.00-5.60 ppm)



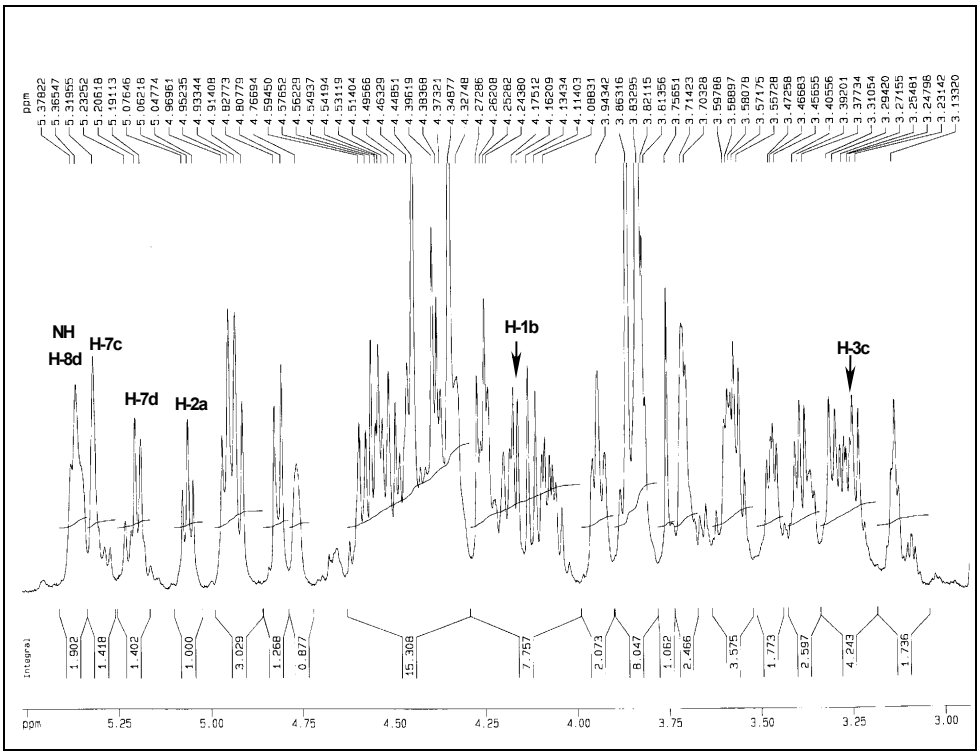
^{1}H-NMR Spectrum of compound **72** (250 MHz, $CDCl_3$)



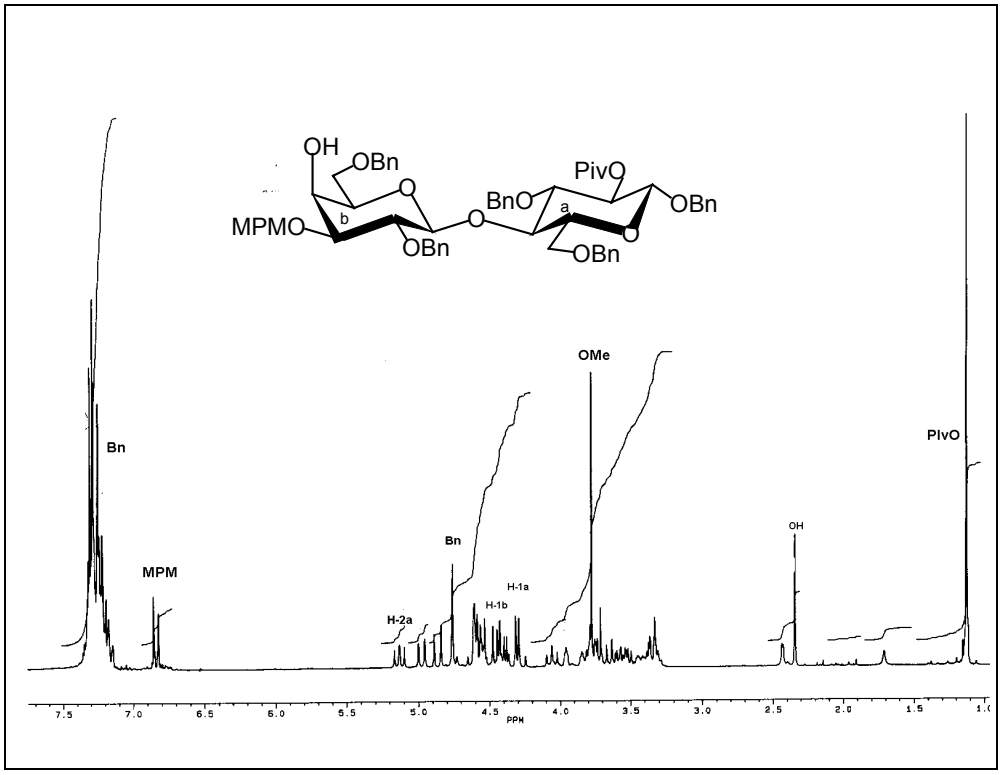
^{1}H-NMR Spectrum of Compound **73** (600 MHz, $CDCl_3$)



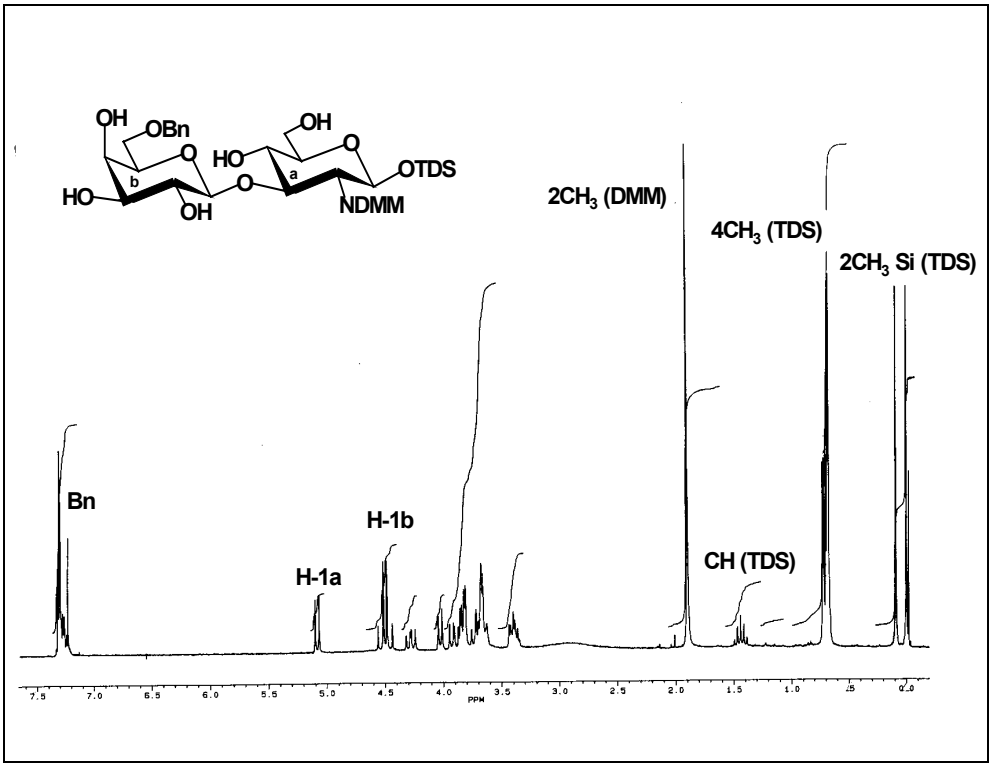
HMQC Spectrum of compound **73**



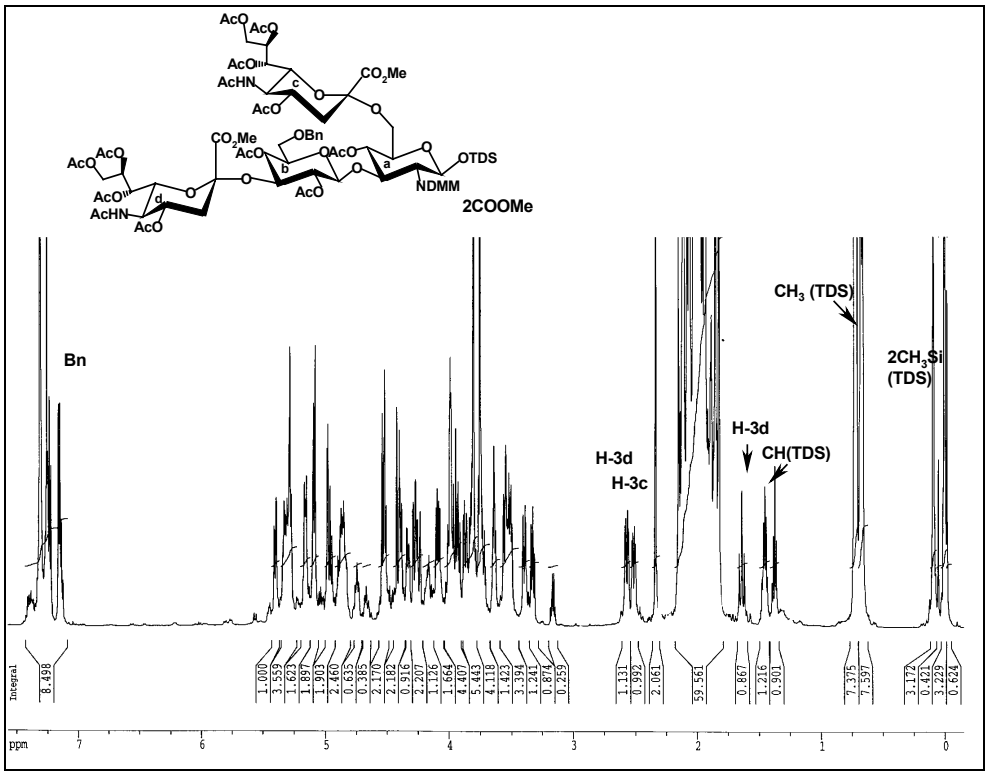
^{1}H-NMR Spectrum of Compound **73** (range: 3.00-5.30)



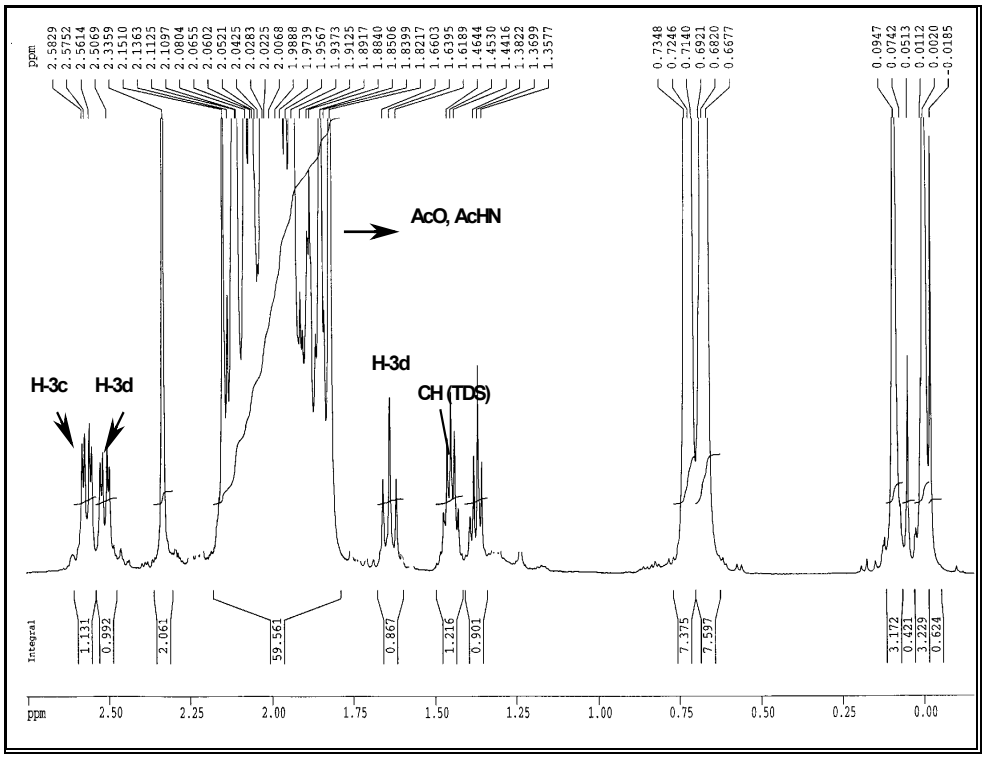
^{1}H-NMR Spectrum of Compound **74** (250 MHz, $CDCl_3$)



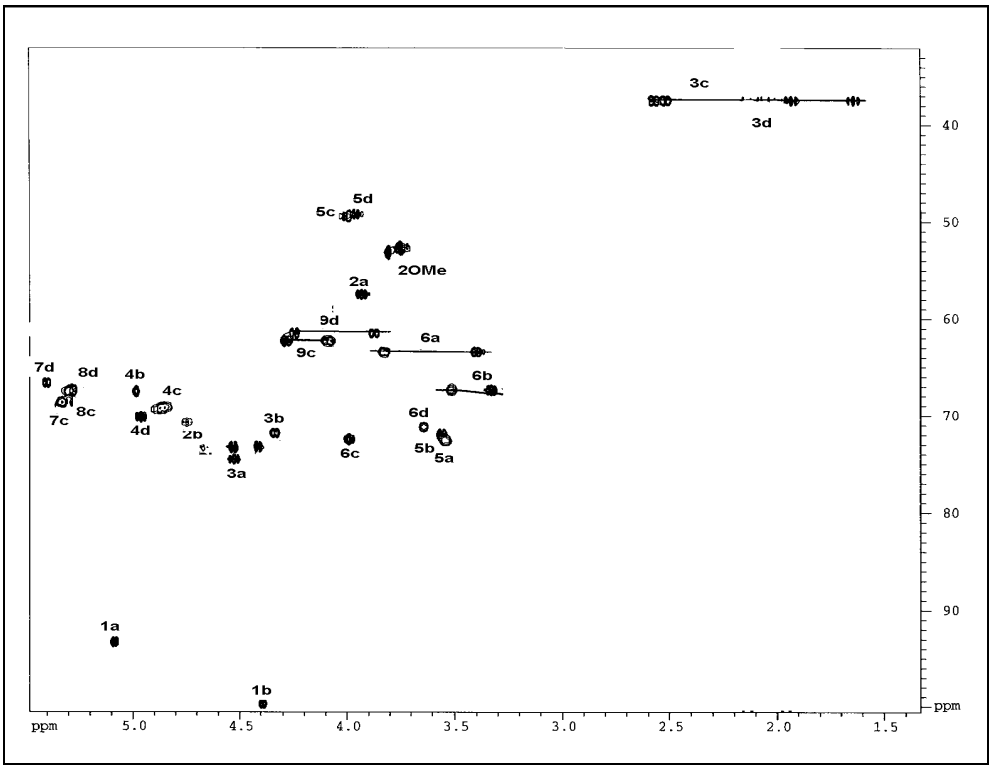
^{1}H-NMR Spectrum of compound **76** (250 MHz, $CDCl_3$)



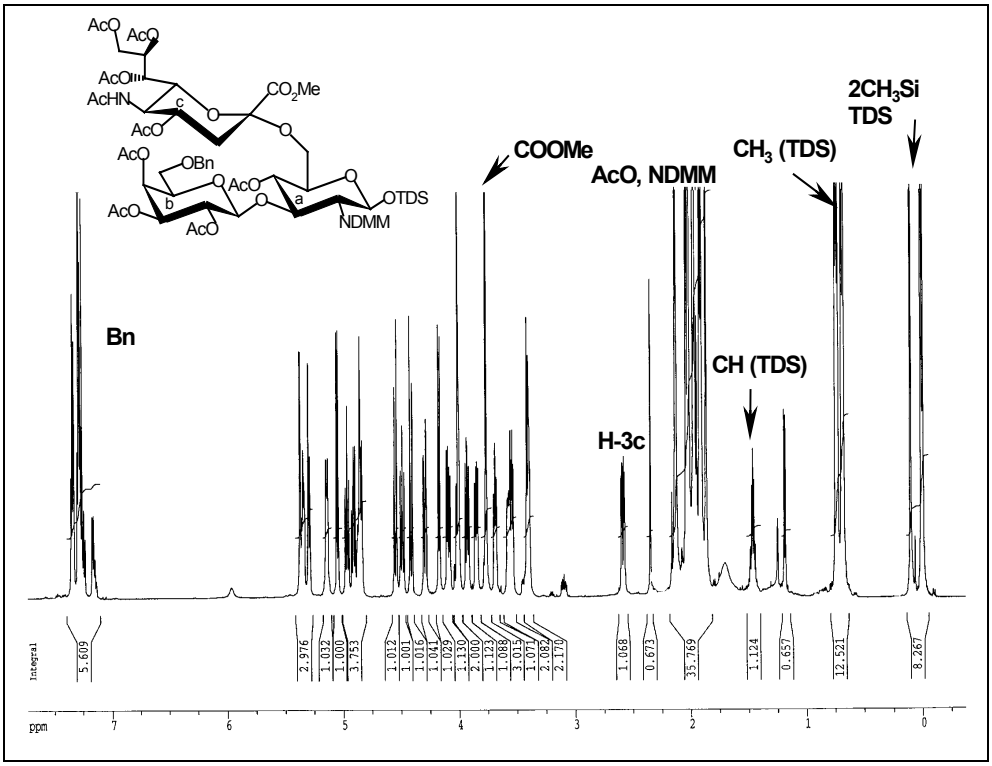
^{1}H-NMR Spectrum of Compound **78** (600 MHz, $CDCl_3$)



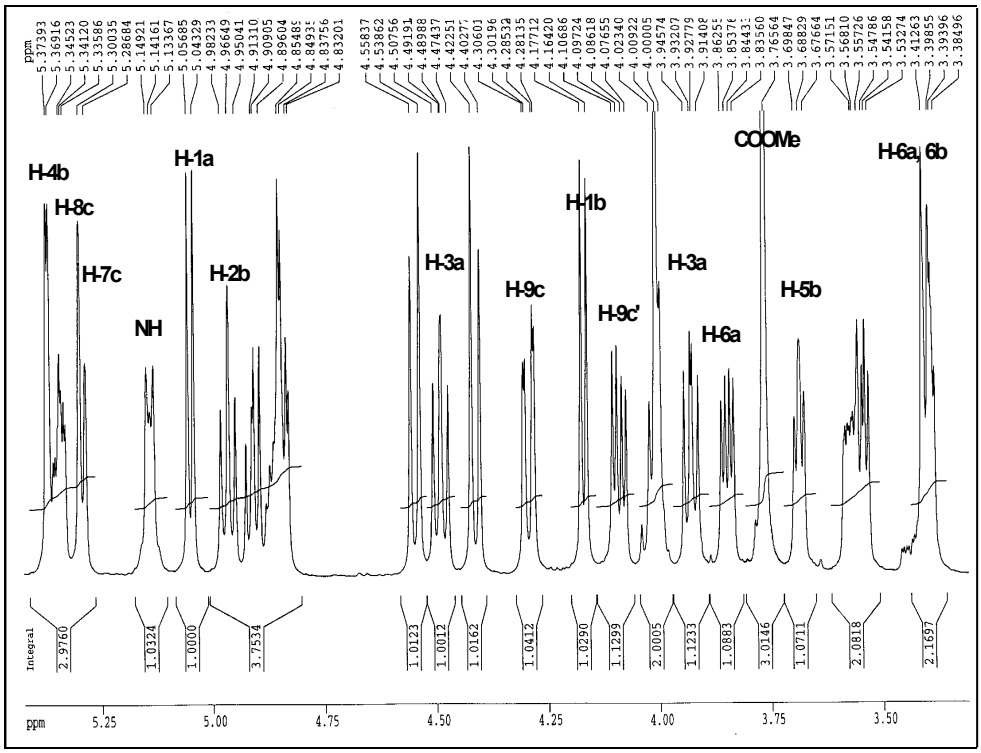
^{1}H-NMR Spectrum of Compound **78** (range: 0.00-2.30 ppm)



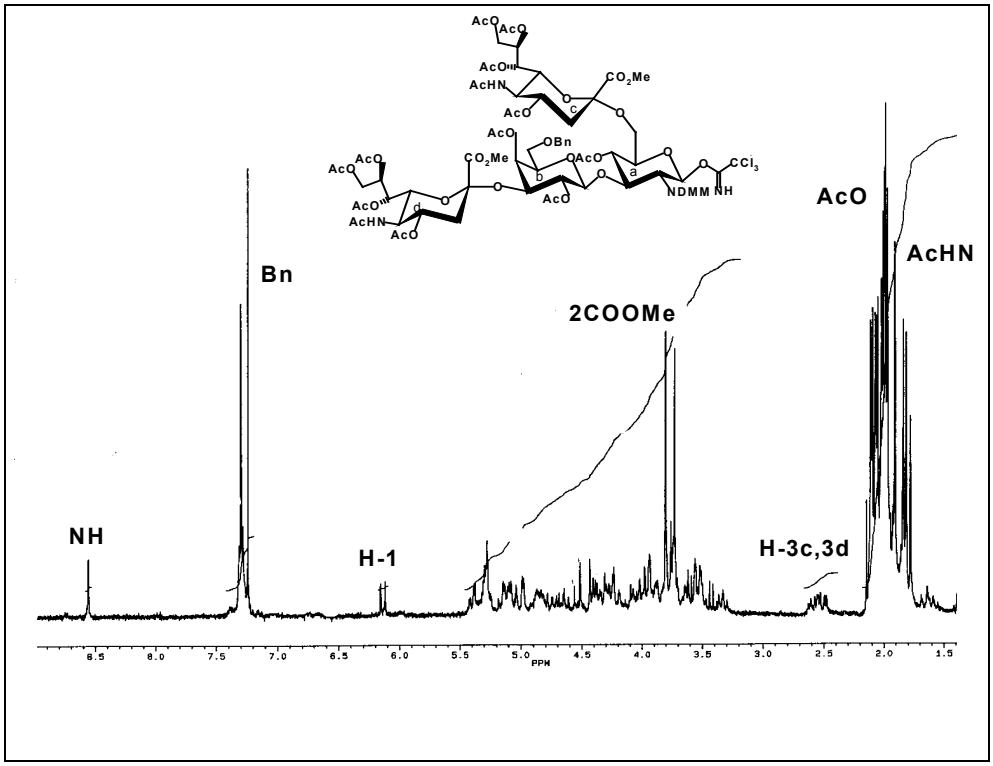
HMQC Spectrum of compound **78**



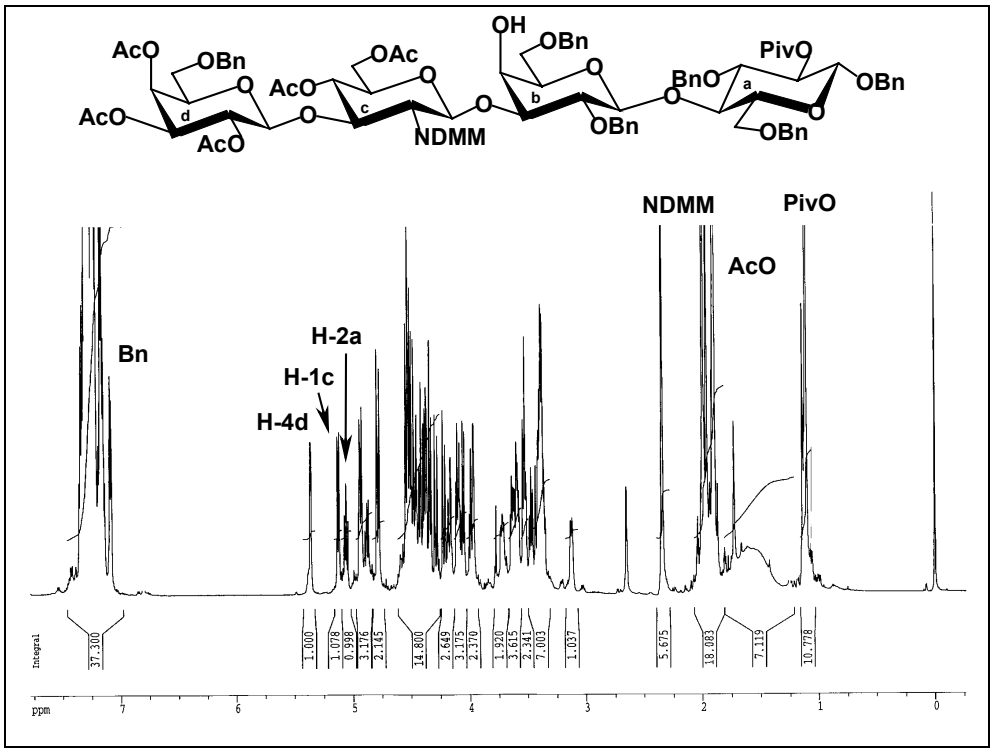
^{1}H-NMR Spectrum of Compound **79** (600 MHz, $CDCl_3$)



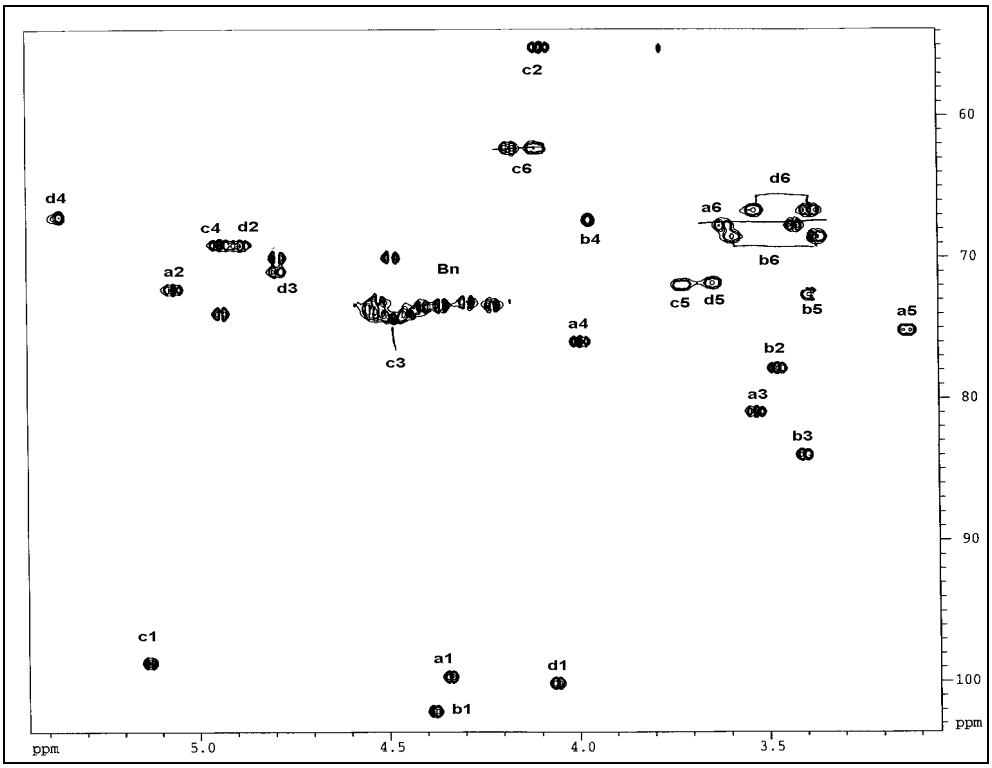
^{1}H-NMR Spectrum of Compound **79** (range: 3.38-5.40 ppm)



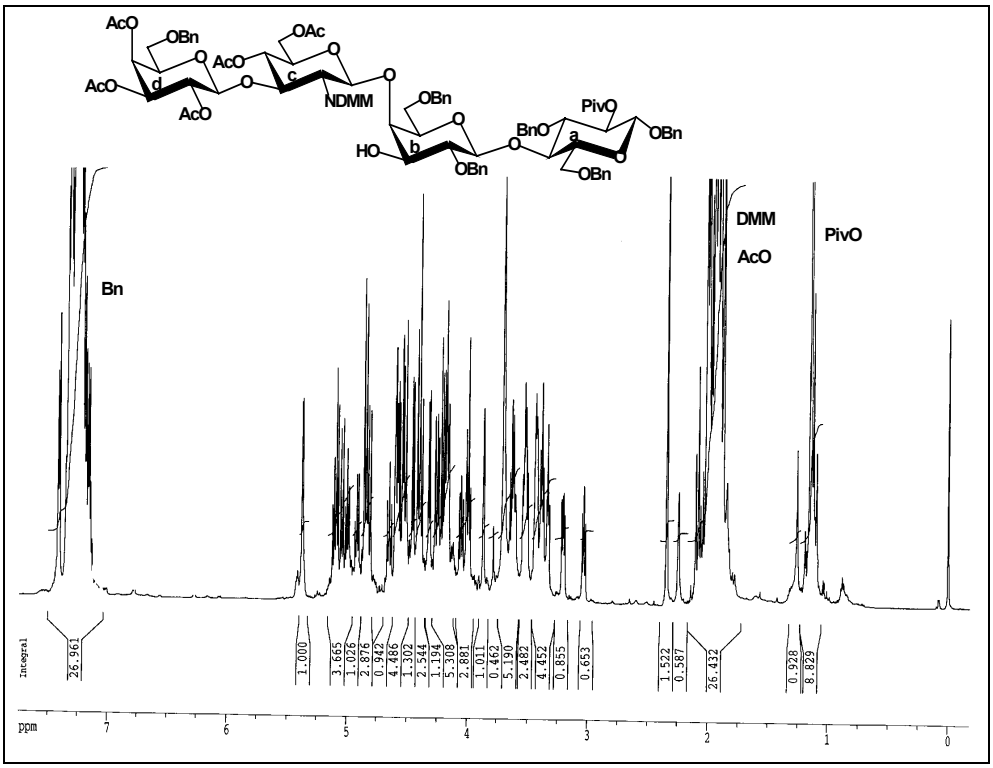
^{1}H-NMR Spectrum of Compound **80** (250 MHz, $CDCl_3$)



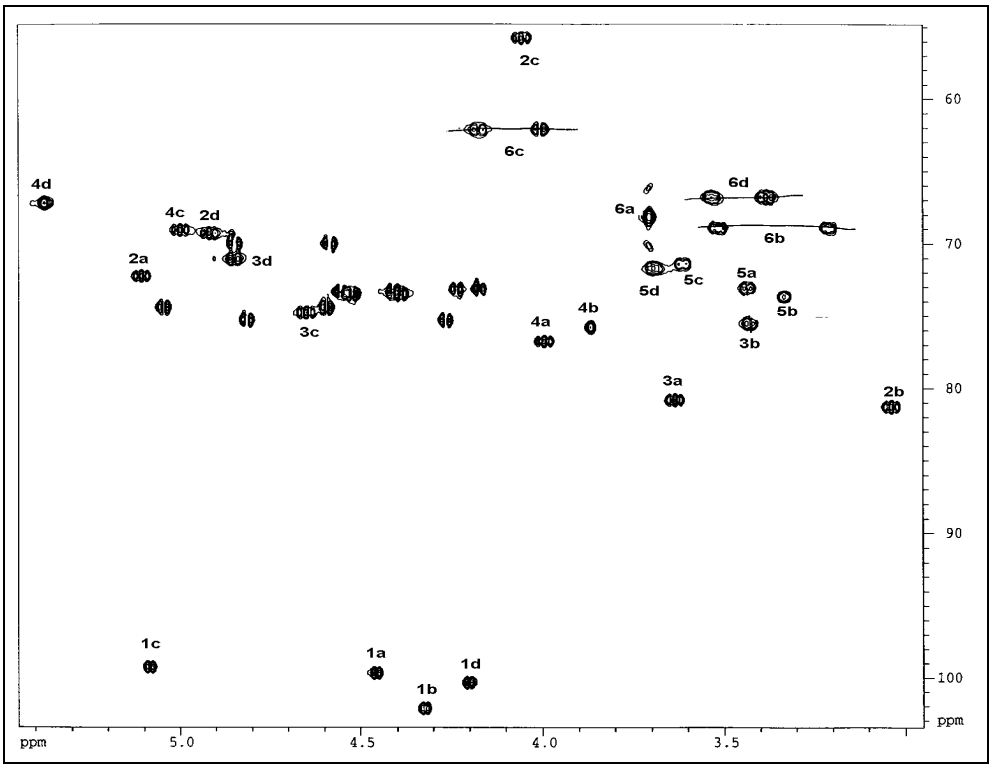
^{1}H-NMR Spectrum of compound **82** (600 MHz, $CDCl_3$)



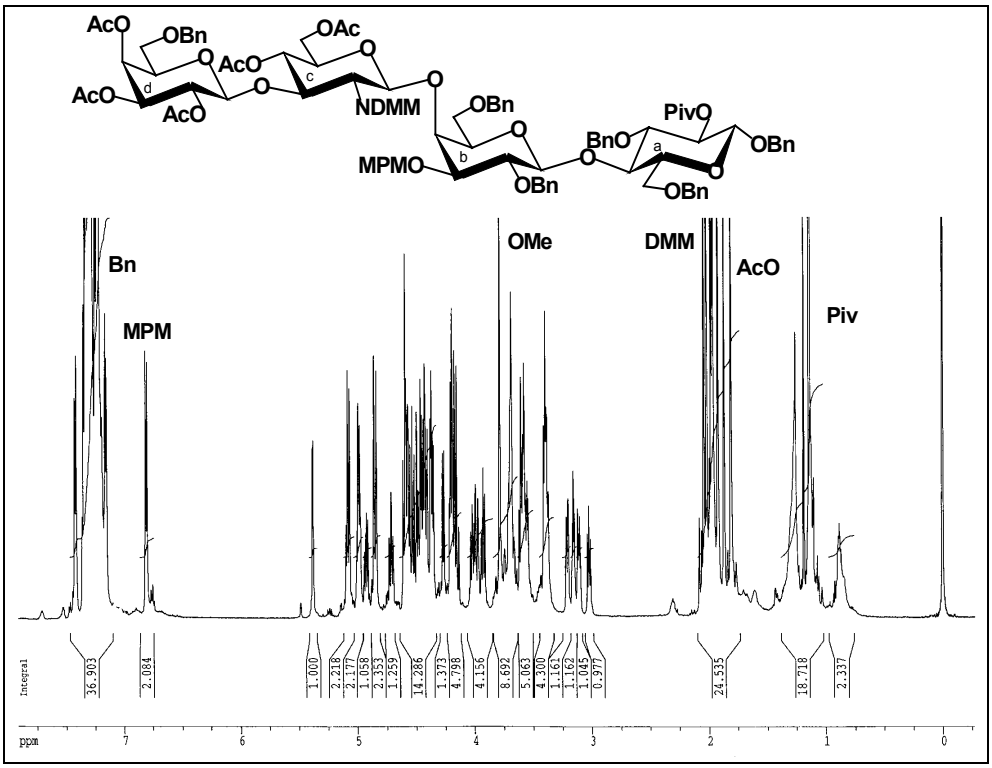
HMQC Spectrum of compound **82**



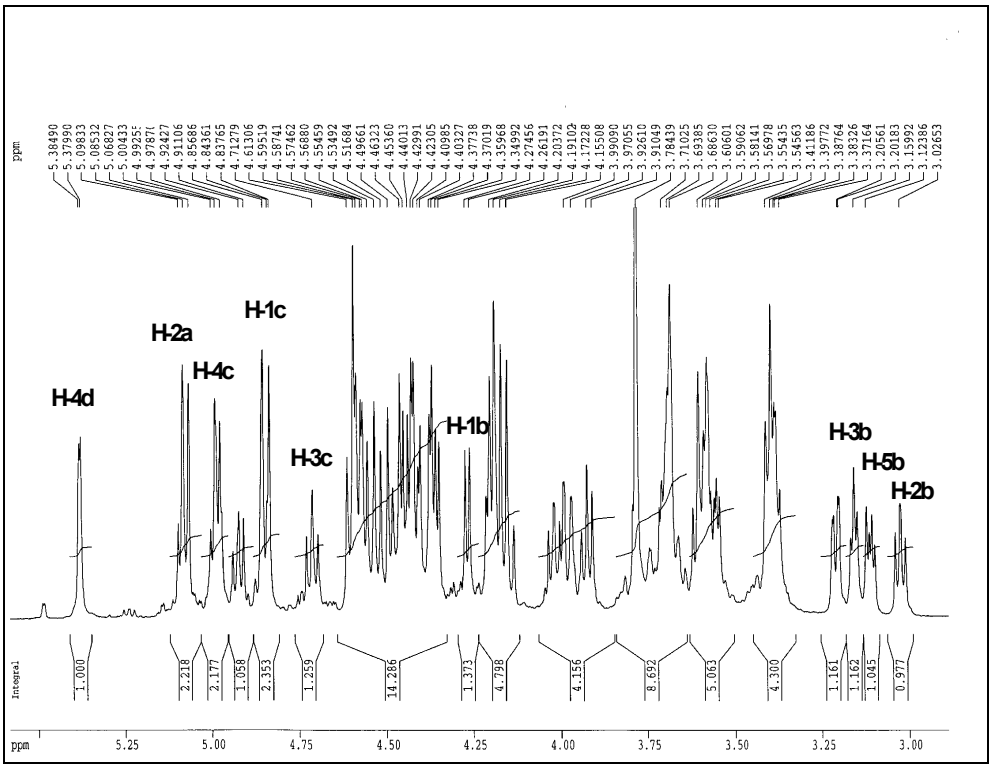
^{1}H-NMR Spectrum of compound **83** (600 MHz, $CDCl_3$)



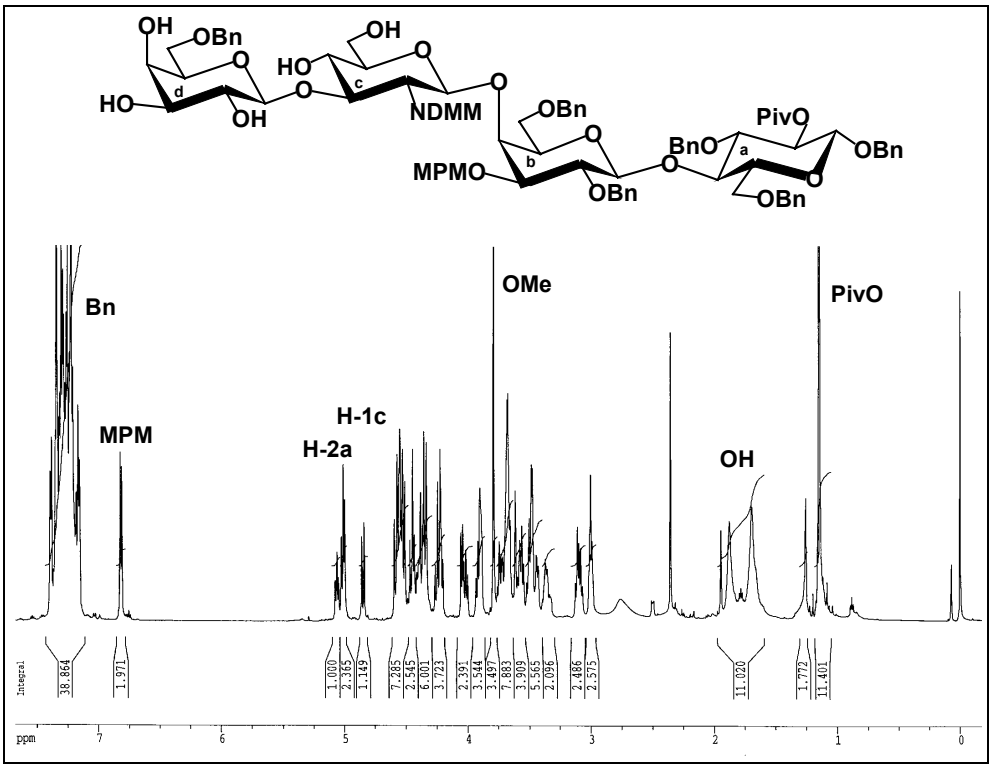
HMQC Spectrum of compound **83**



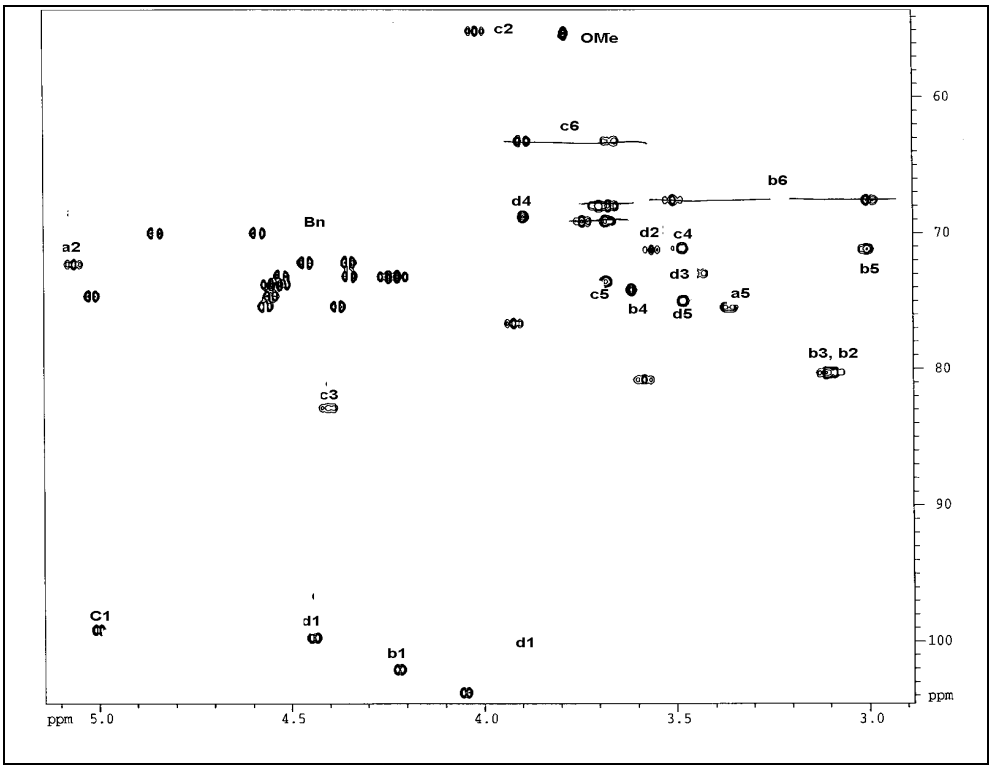
^{1}H-NMR Spectrum of compound **84** (600 MHz, $CDCl_3$)



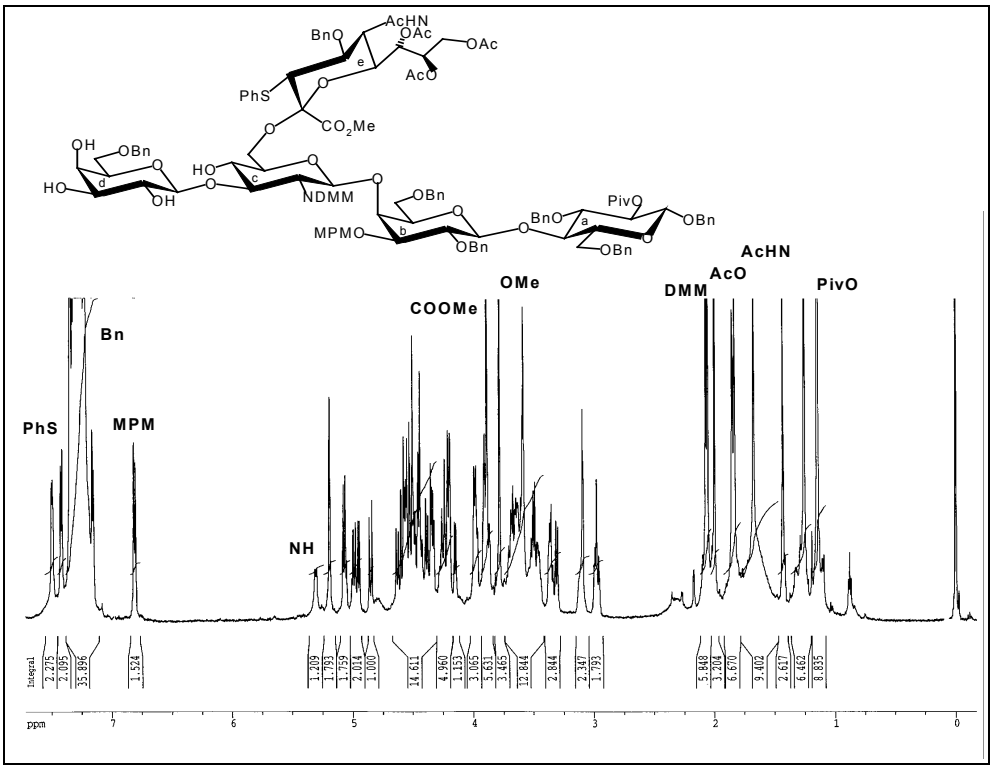
^{1}H-NMR Spectrum of compound **84** (range: 3.00-5.30 ppm)



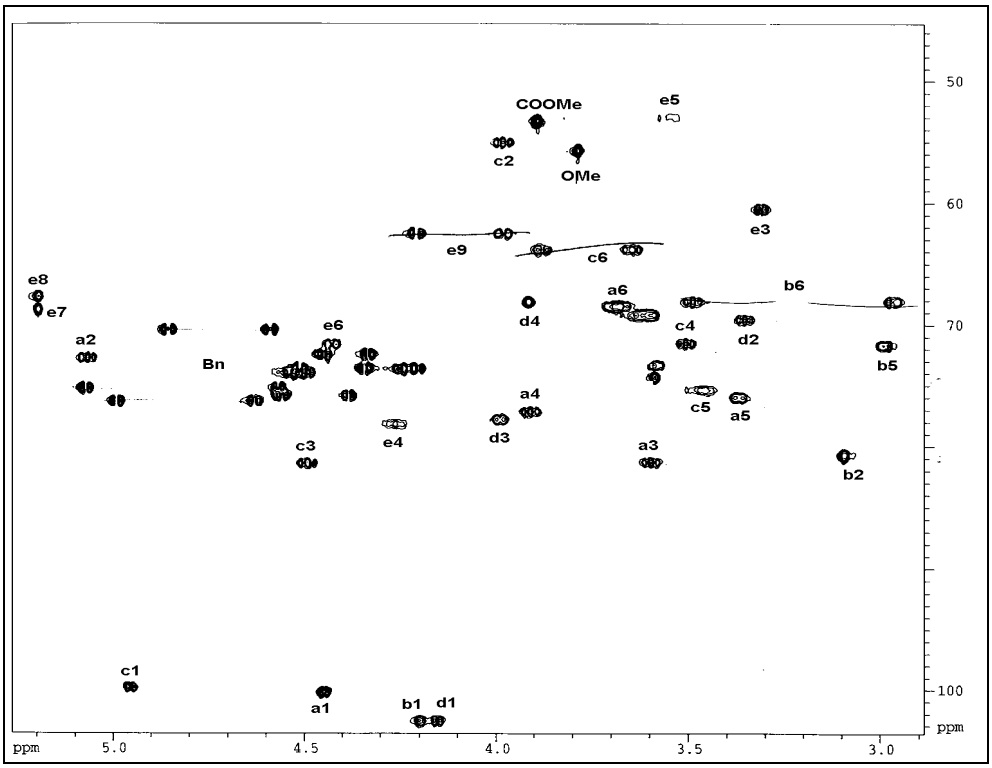
^{1}H-NMR Spectrum of compound **85** (600 MHz, $CDCl_3$)



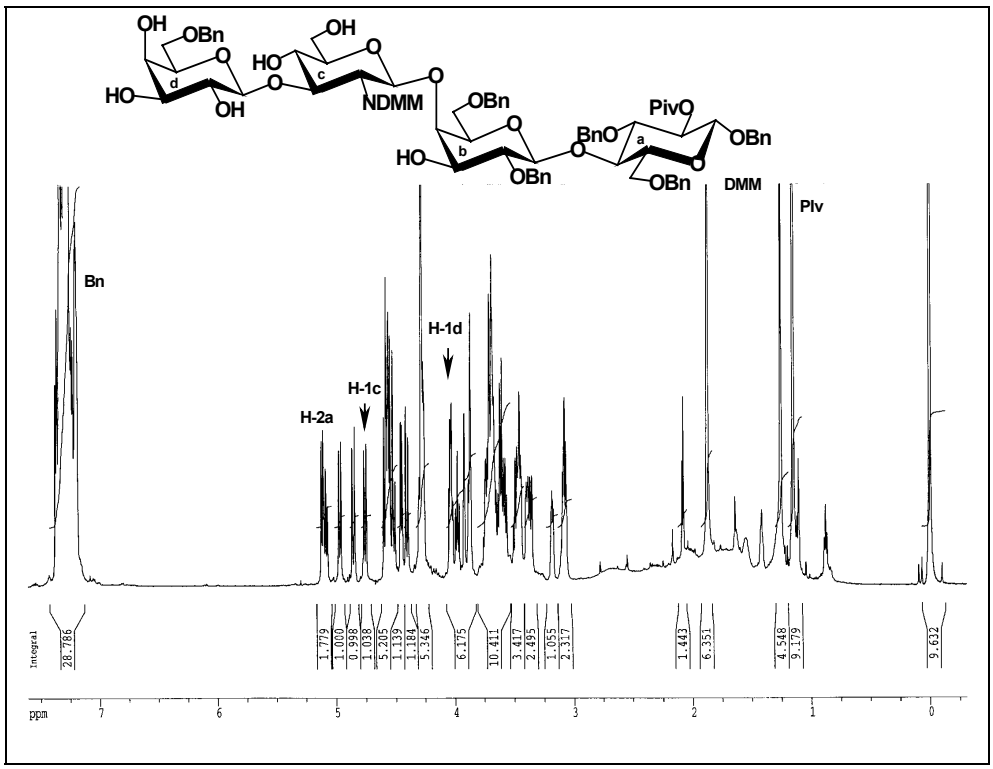
HMQC Spectrum of compound **85**



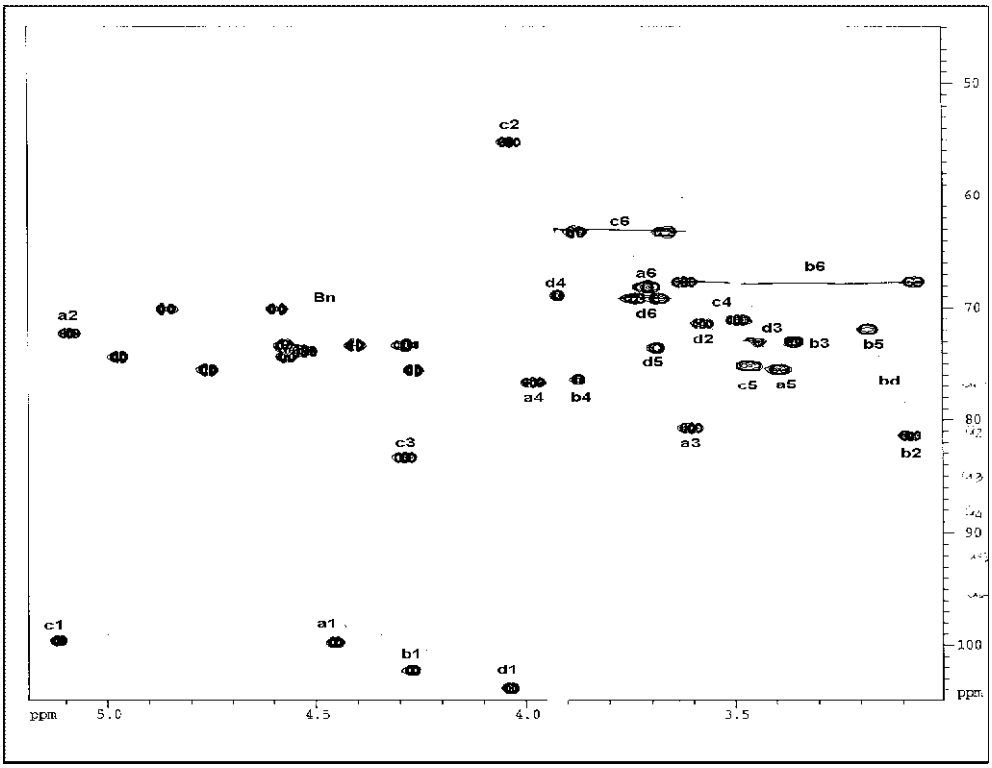
^{1}H-NMR Spectrum of compound **86** (600 MHz, $CDCl_3$)



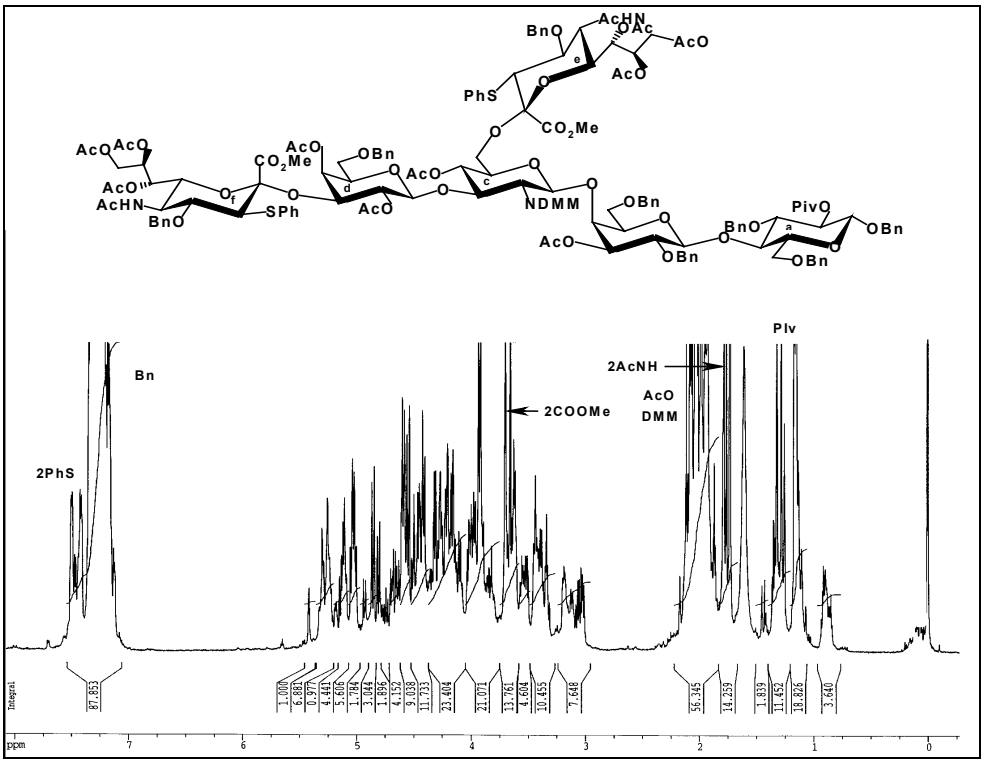
HMQC Spectrum of compound **86**



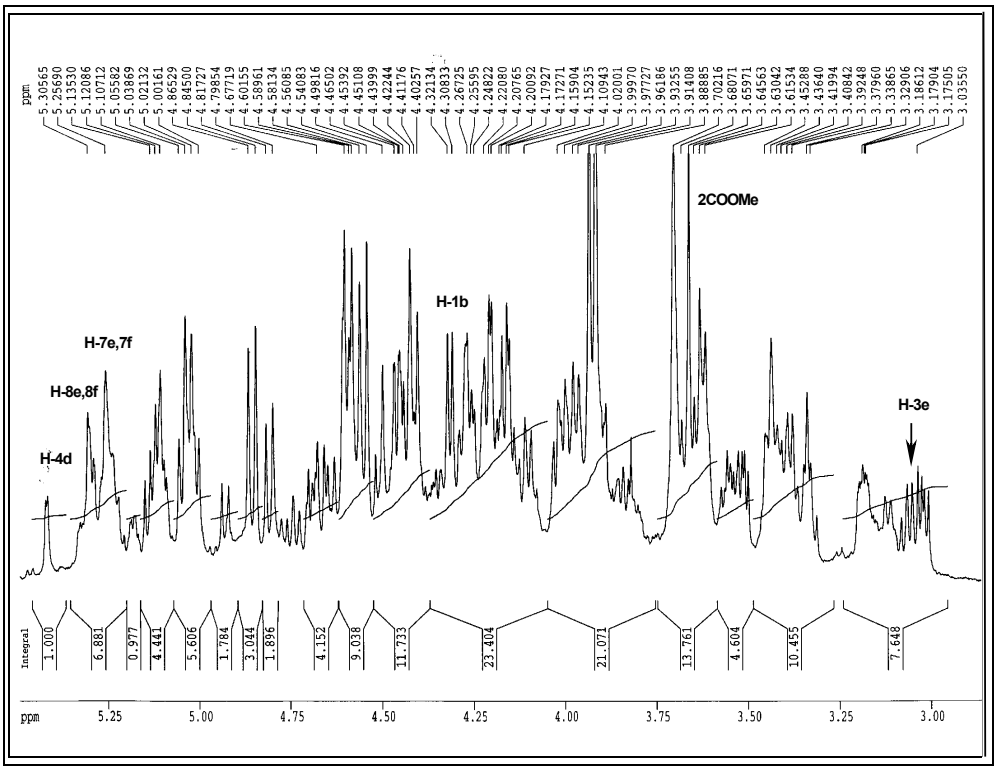
^{1}H-NMR Spectrum of compound **87** (600 MHz, $CDCl_3$)



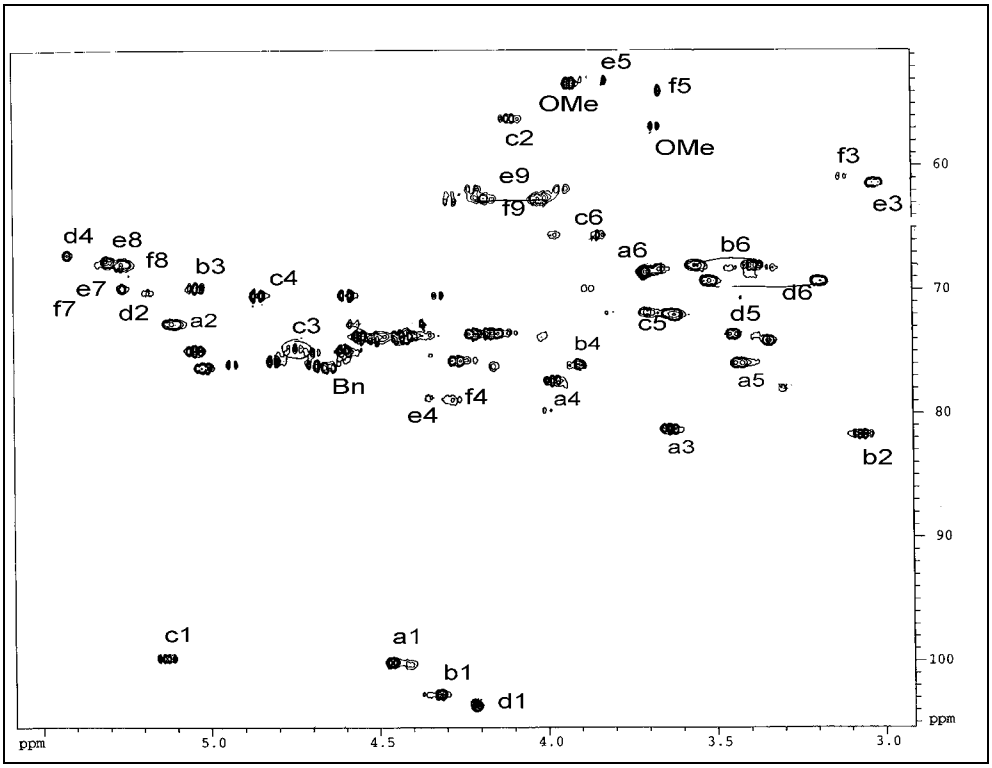
HMQC Spectrum of compound **87**



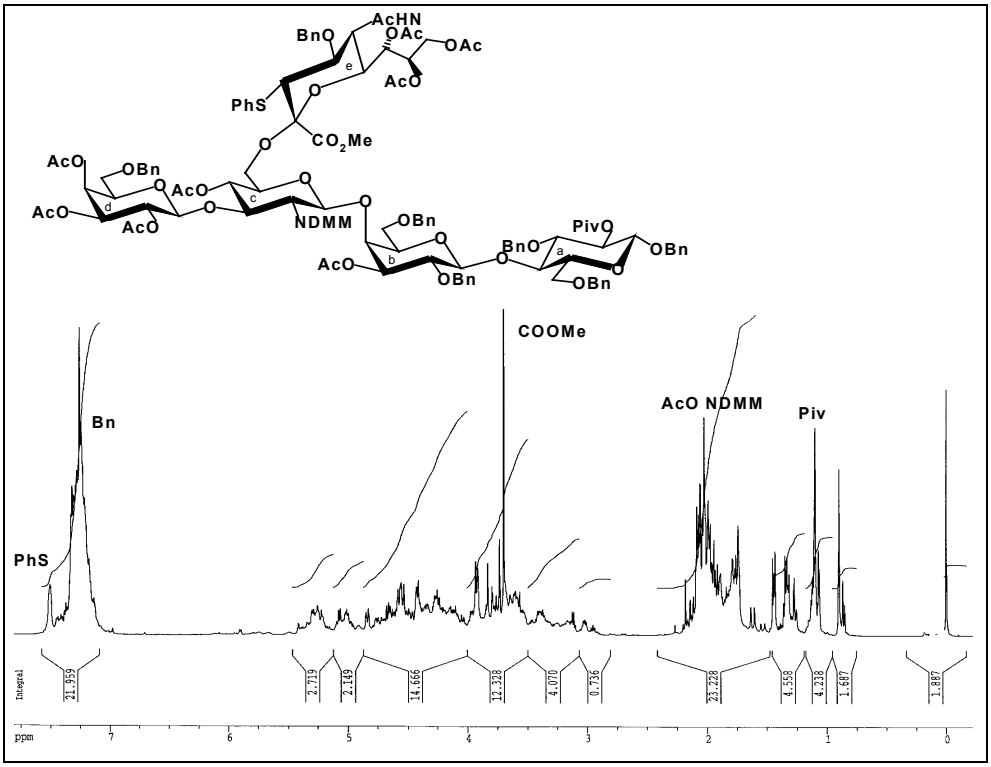
^{1}H-NMR Spectrum of compound **88** (600 MHz, $CDCl_3$)



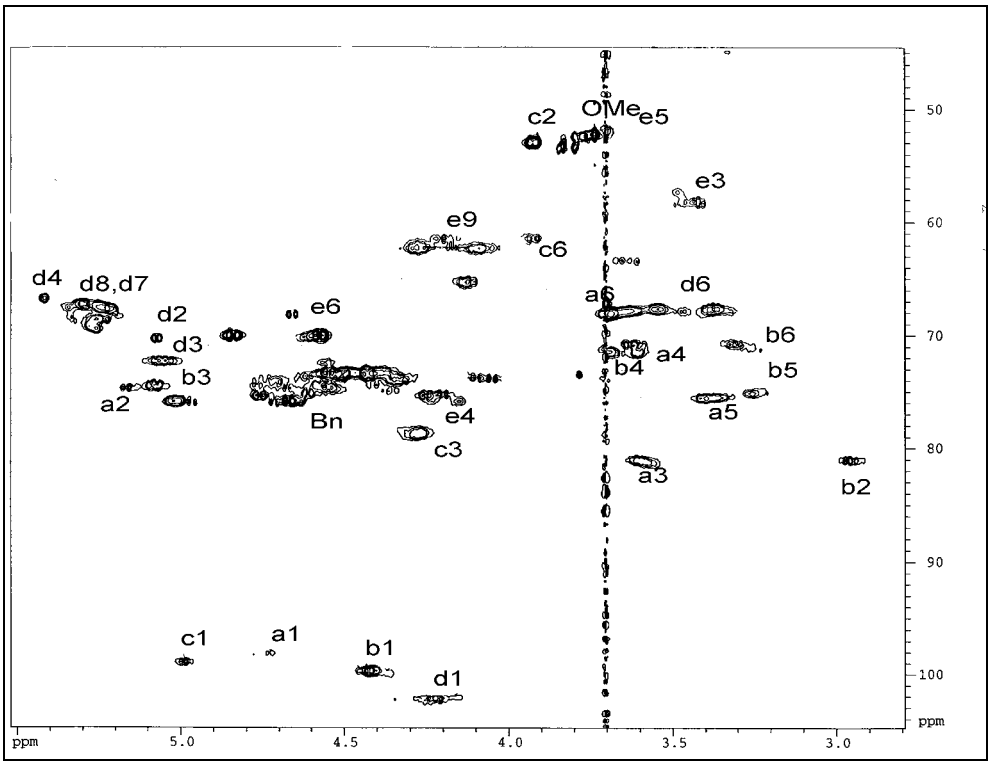
^{1}H-NMR Spectrum of compound **88** (range: 3.00-5.30 ppm)



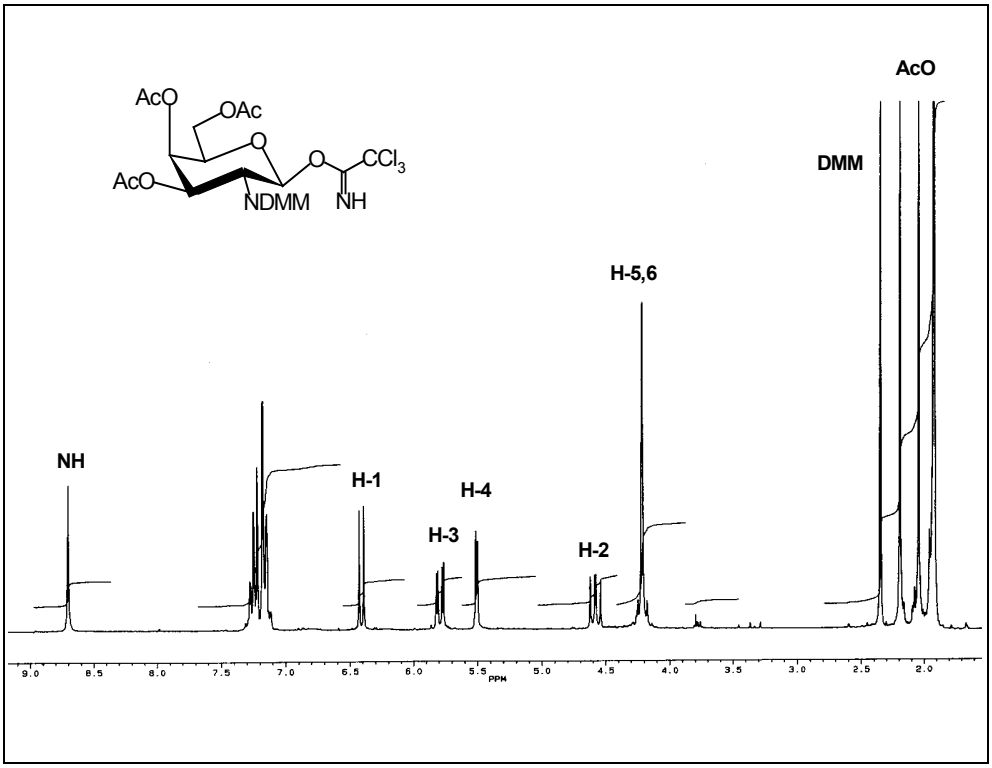
HMQC Spectrum of compound **88**



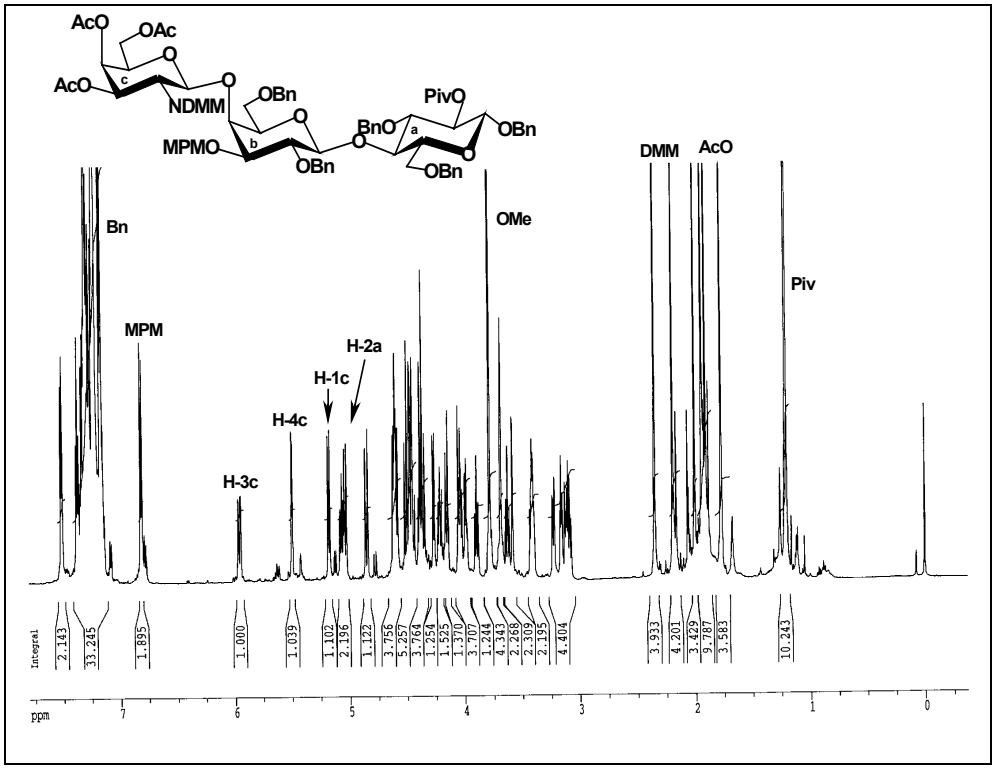
^{1}H-NMR Spectrum of compound **89** (600 MHz, $CDCl_3$)



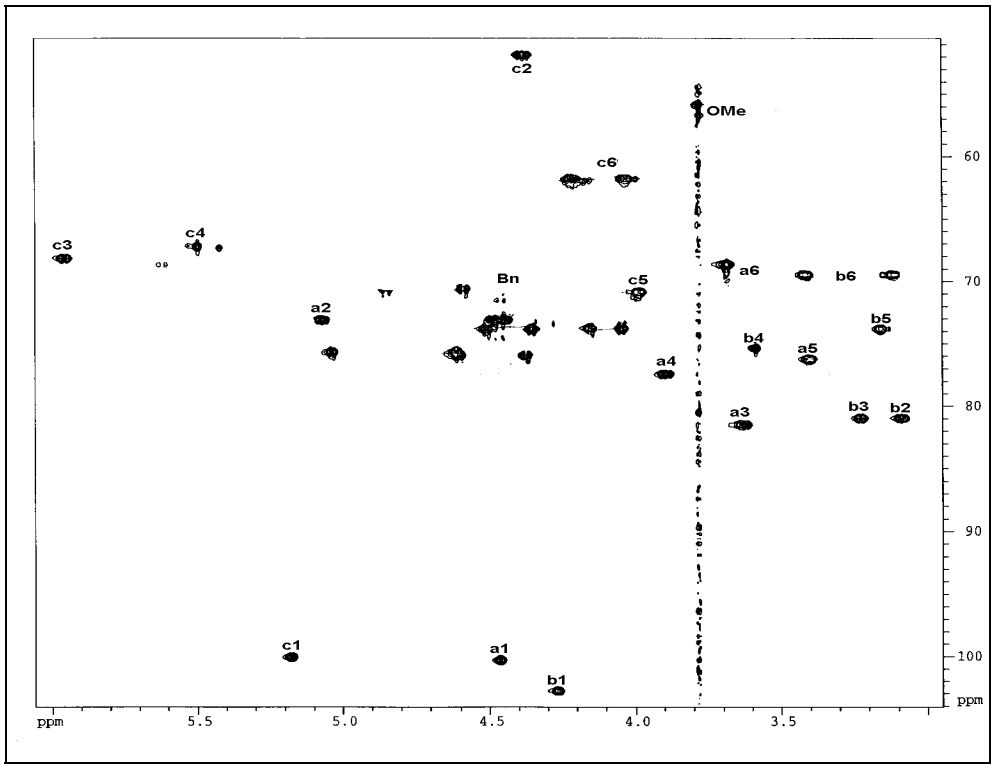
HMQC Spectrum of compound **89**



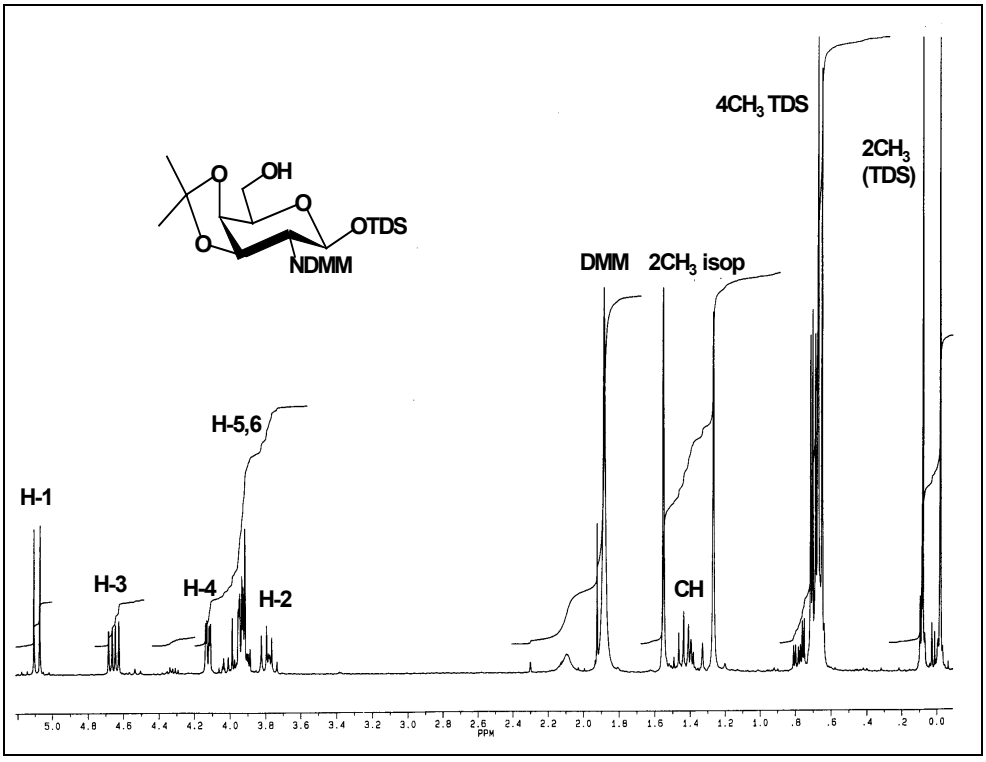
^{1}H-NMR Spectrum of compound **90** (250 MHz, $CDCl_3$)



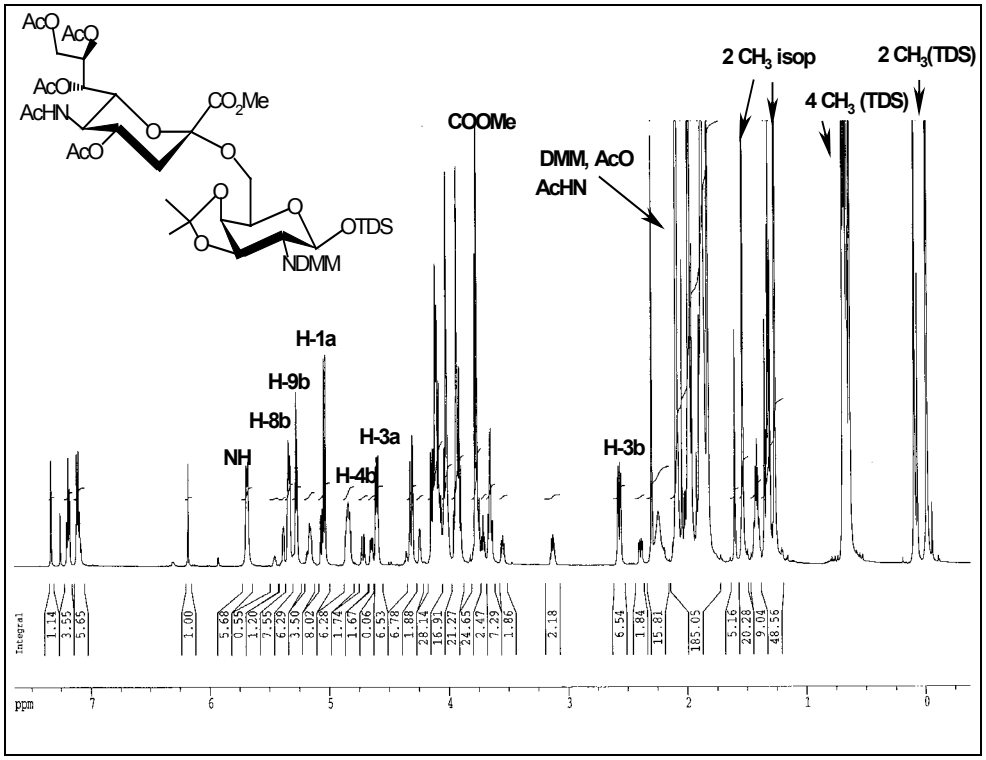
^{1}H-NMR Spectrum of compound **91** (600 MHz, $CDCl_3$)



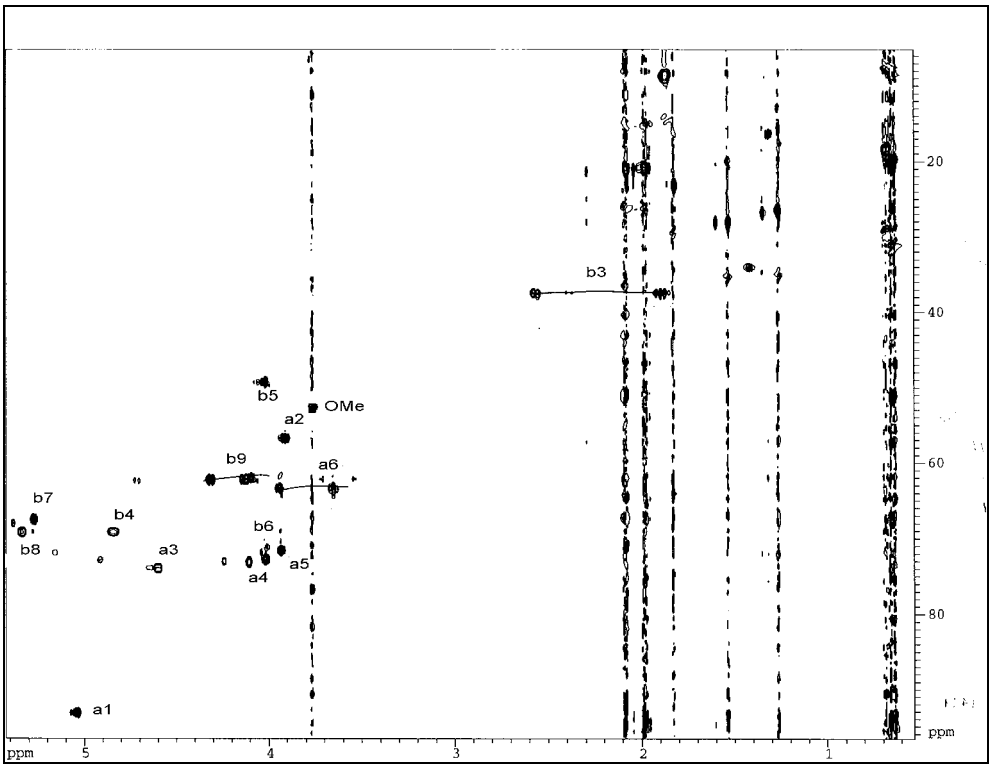
HMQC Spectrum of compound **91**



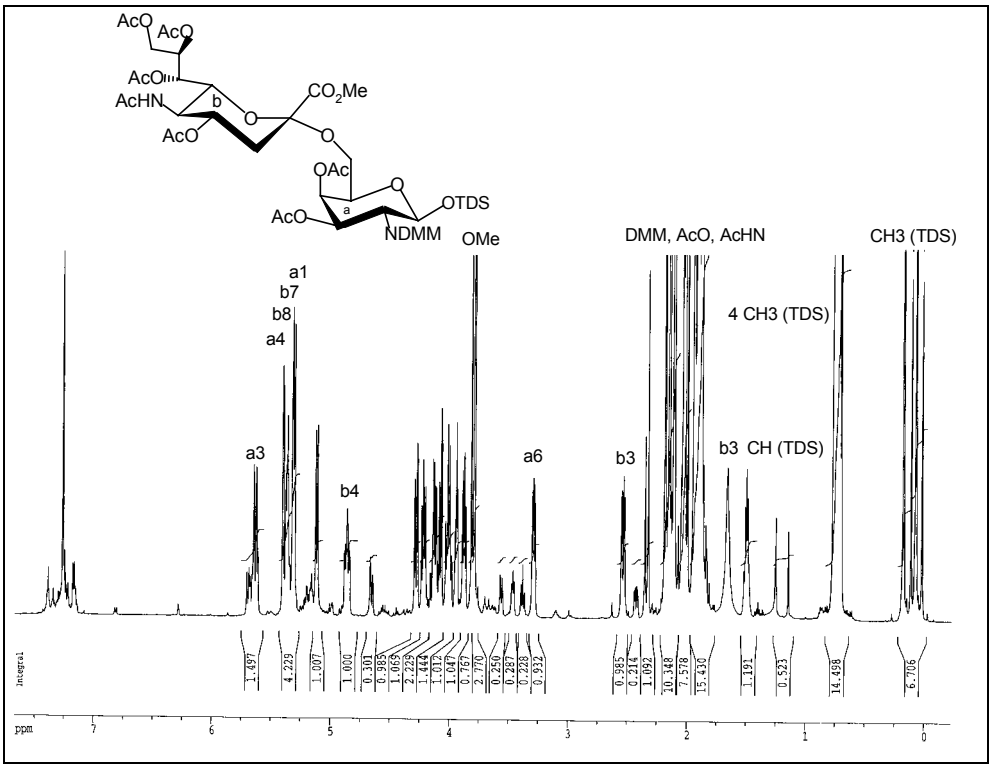
^{1}H-NMR Spectrum of compound **93** (250 MHz, $CDCl_3$)



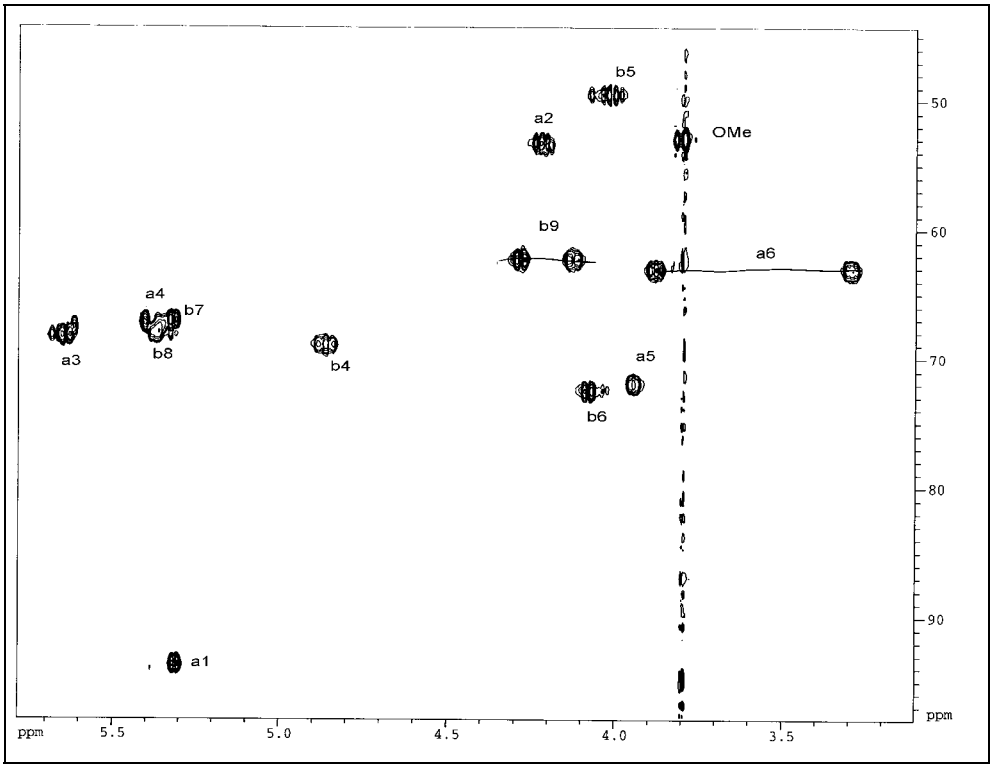
^{1}H-NMR Spectrum of compound **94** (600 MHz, $CDCl_3$)



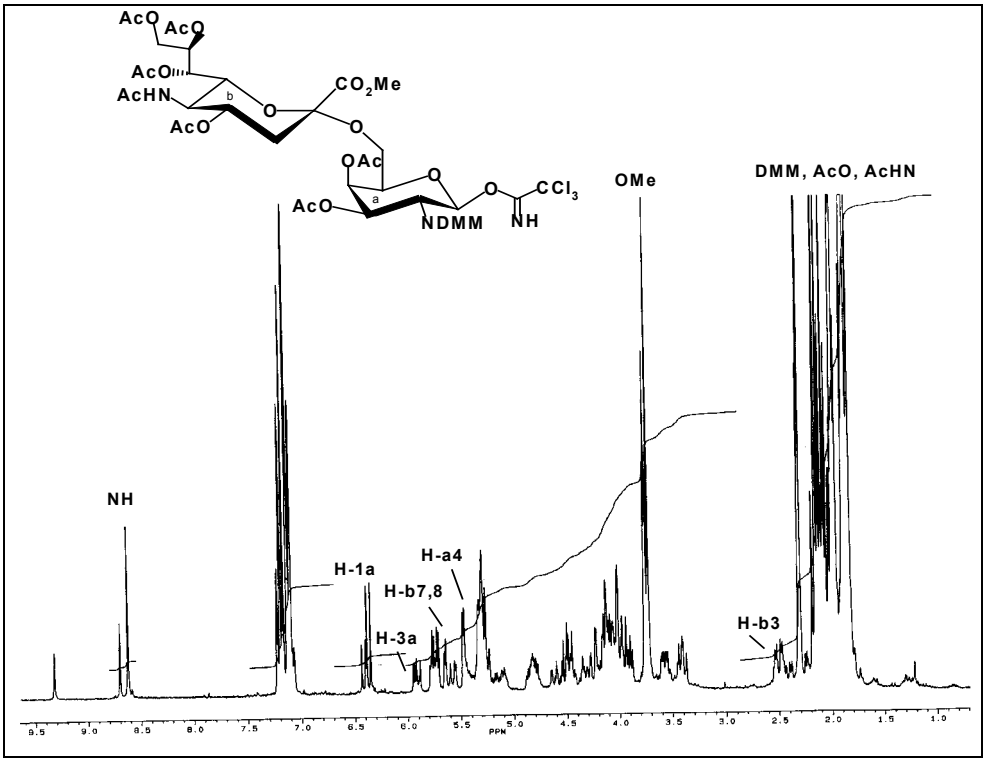
HMQC Spectrum of compound **94**



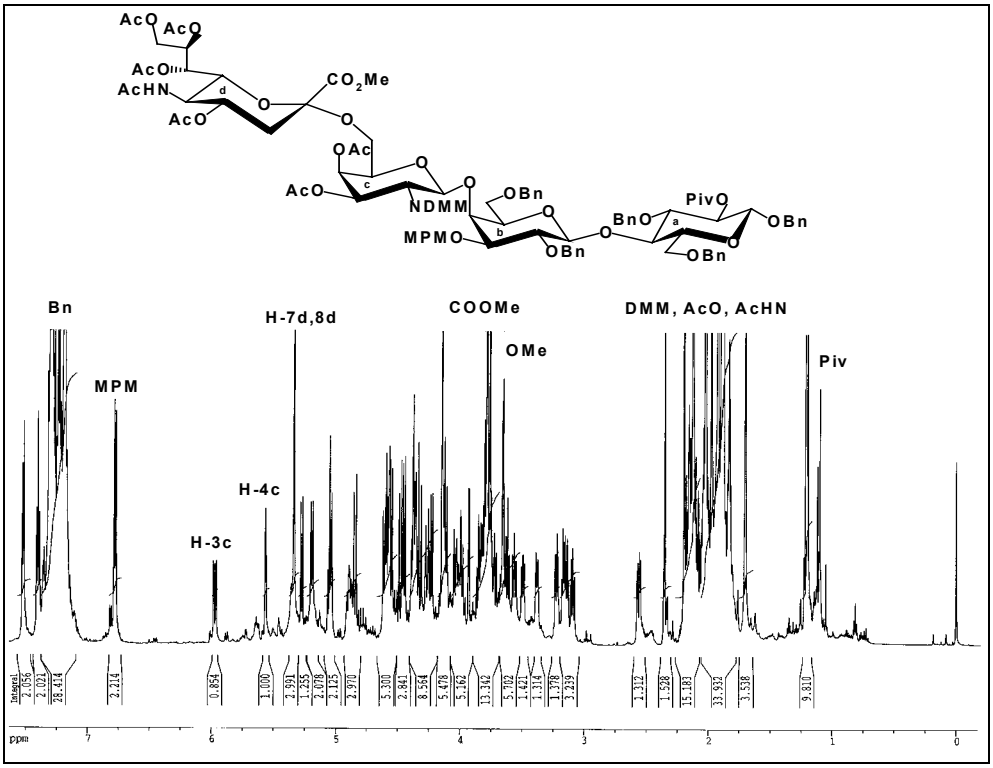
^{1}H-NMR Spectrum of compound **95** (600 MHz, $CDCl_3$)



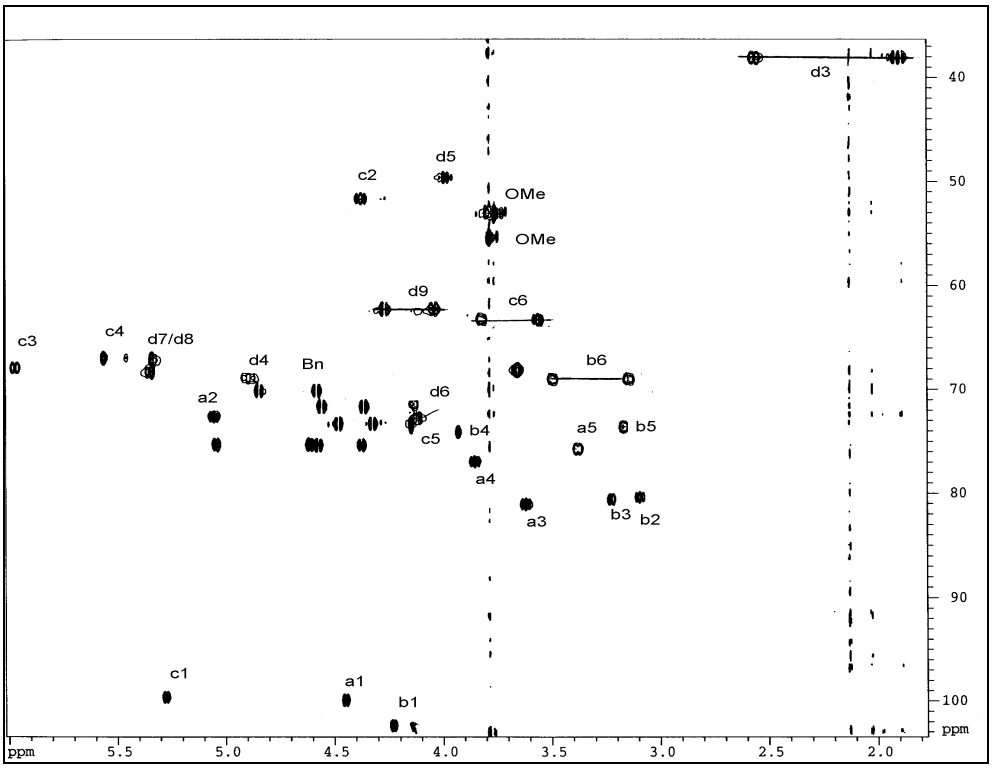
HMQC Spectrum of compound **95**



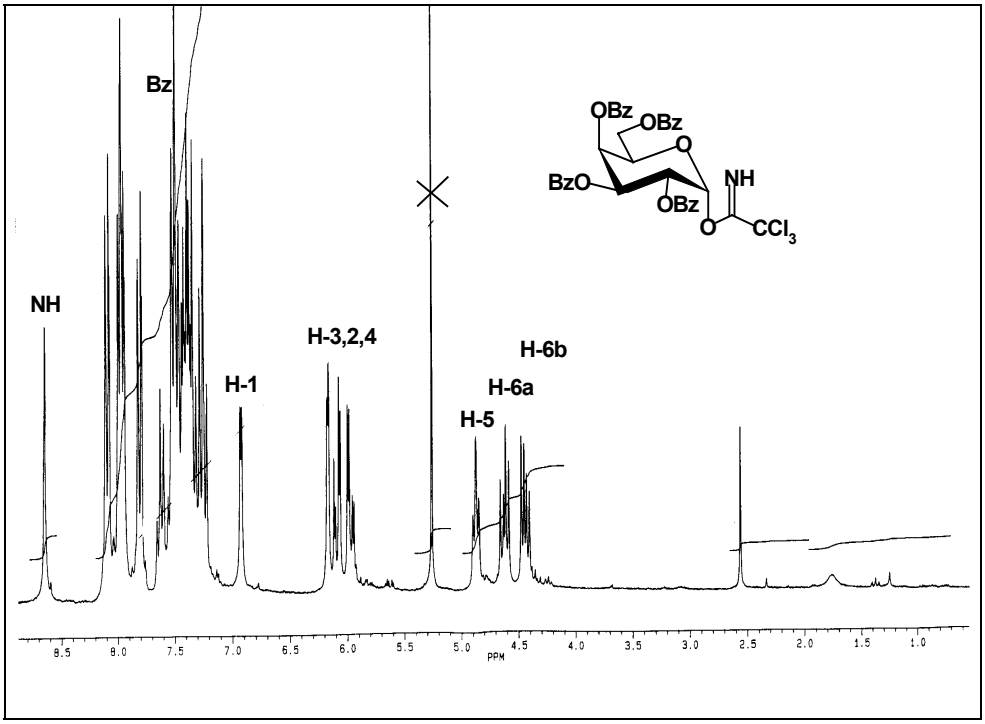
^{1}H-NMR Spectrum of compound **96** (250 MHz, $CDCl_3$)



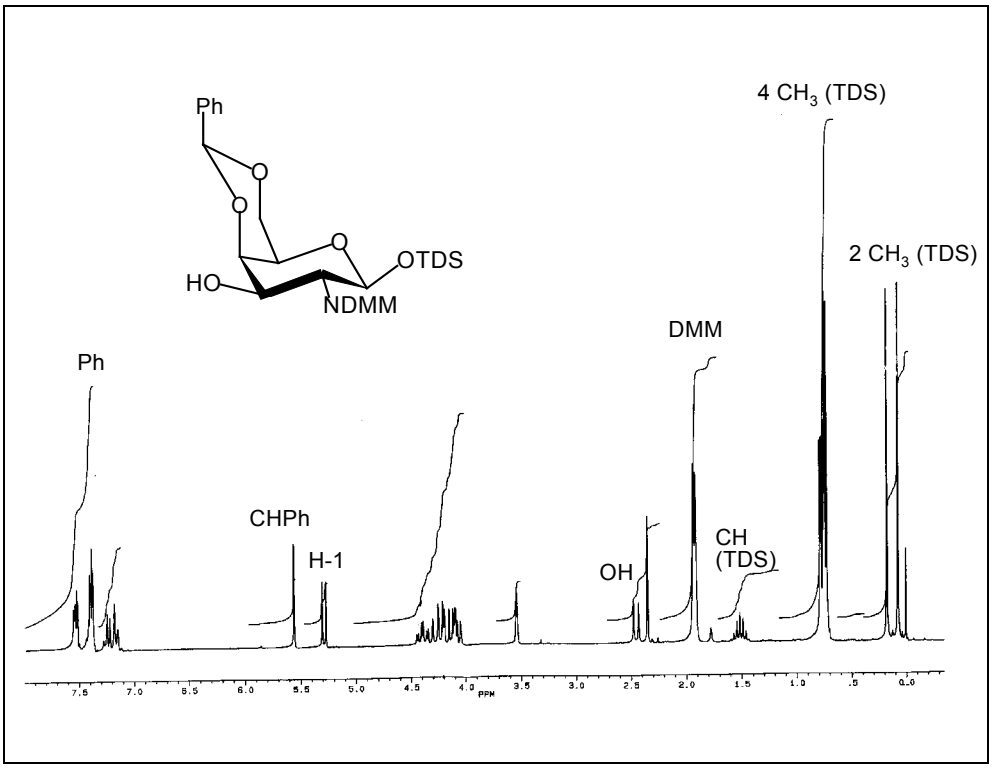
^{1}H-NMR Spectrum of compound **97** (600 MHz, $CDCl_3$)



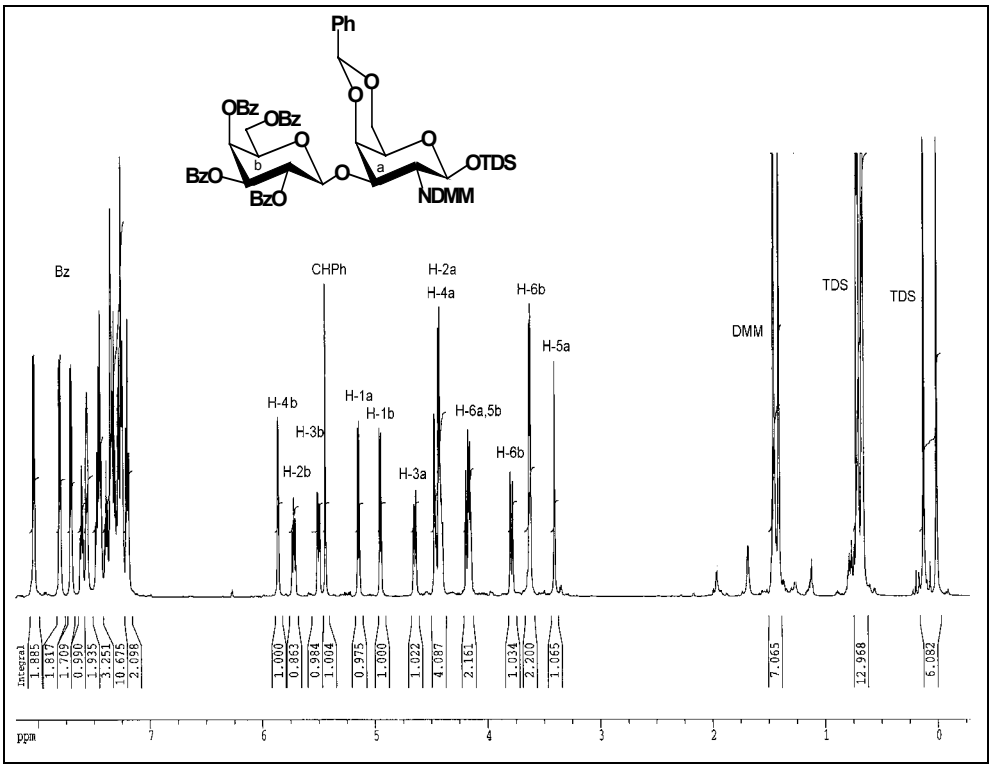
HMQC Spectrum of compound **97**



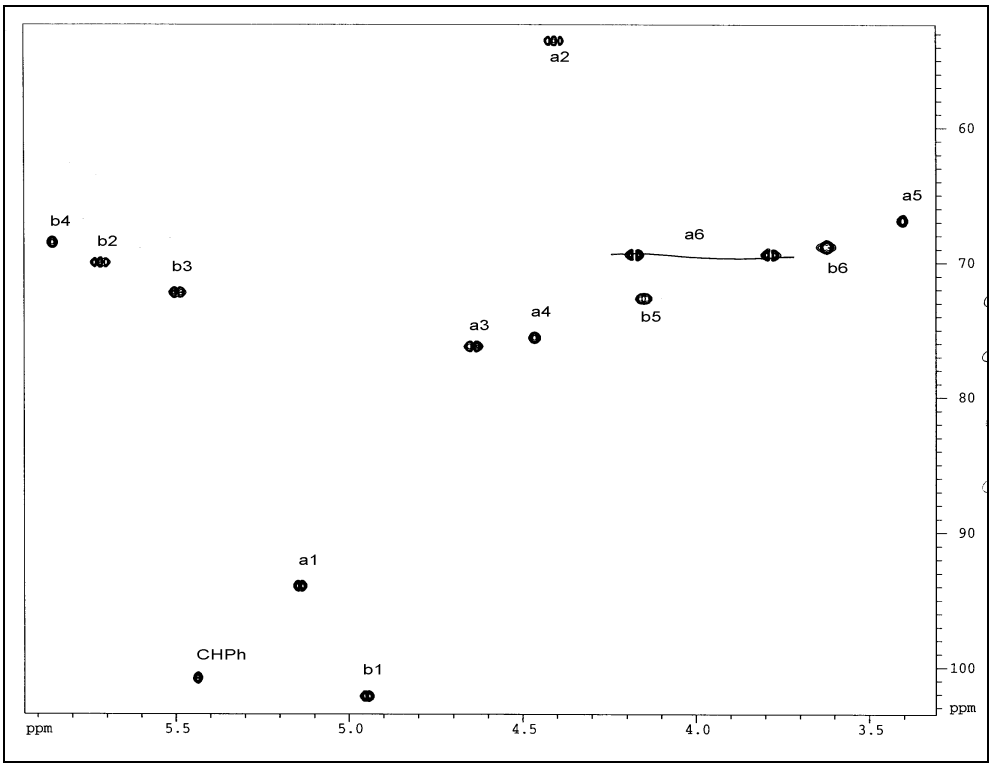
^{1}H-NMR Spectrum of compound **98** (250 MHz, $CDCl_3$)



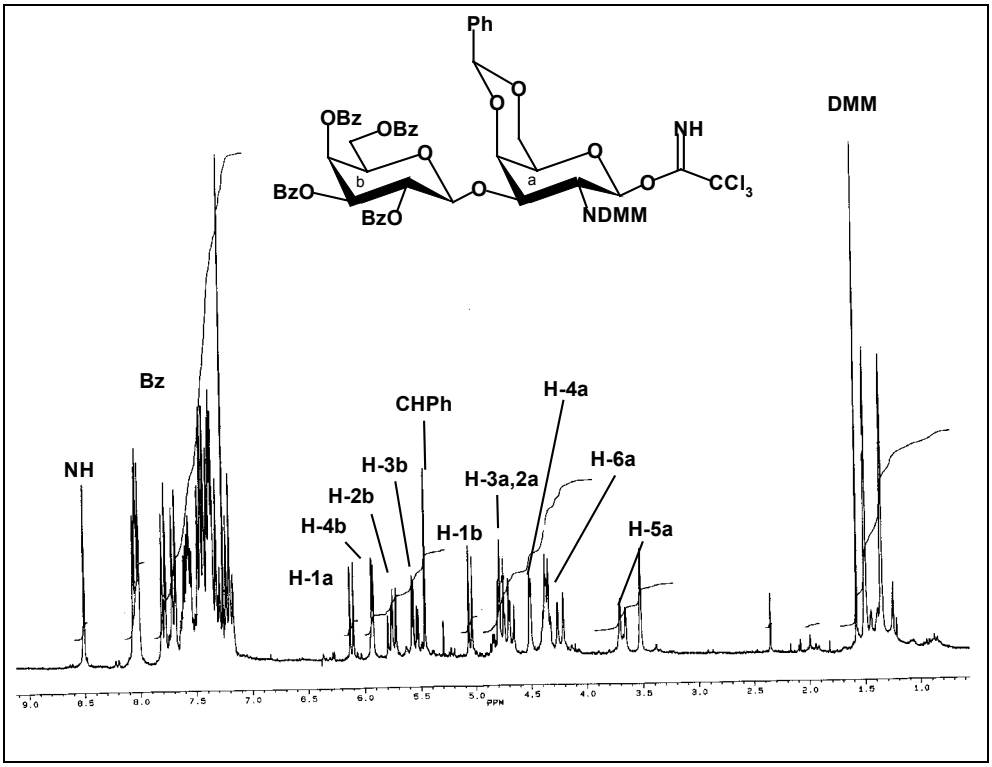
^{1}H-NMR Spectrum of compound **99** (250 MHz, $CDCl_3$)



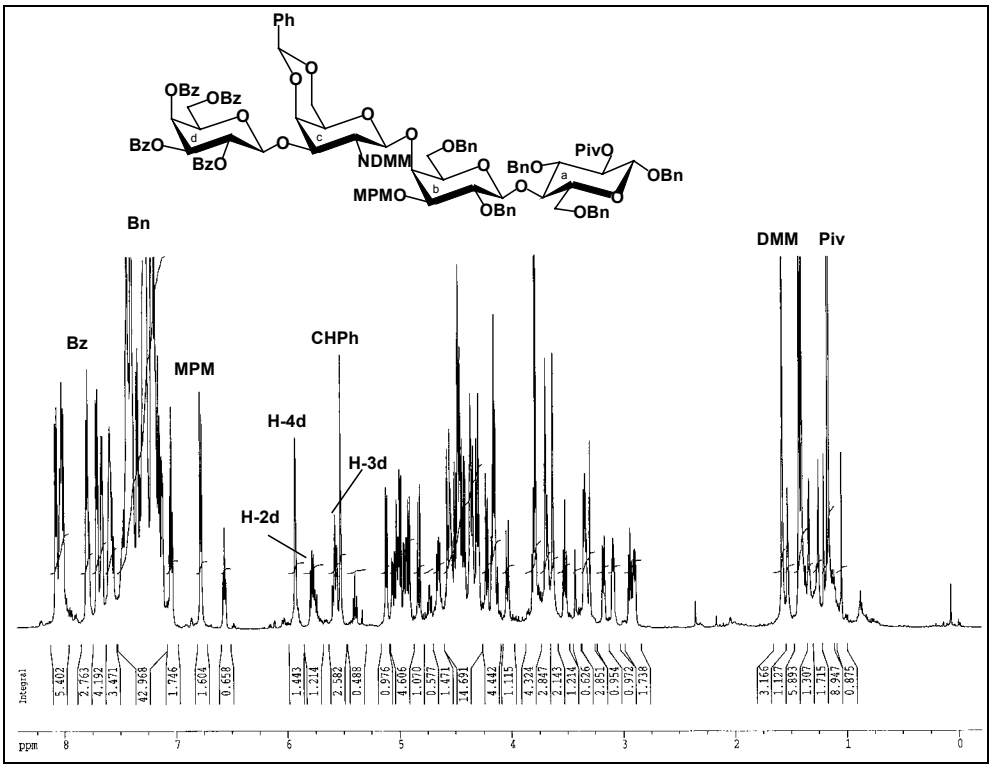
^{1}H-NMR Spectrum of compound **100** (600 MHz, $CDCl_3$)



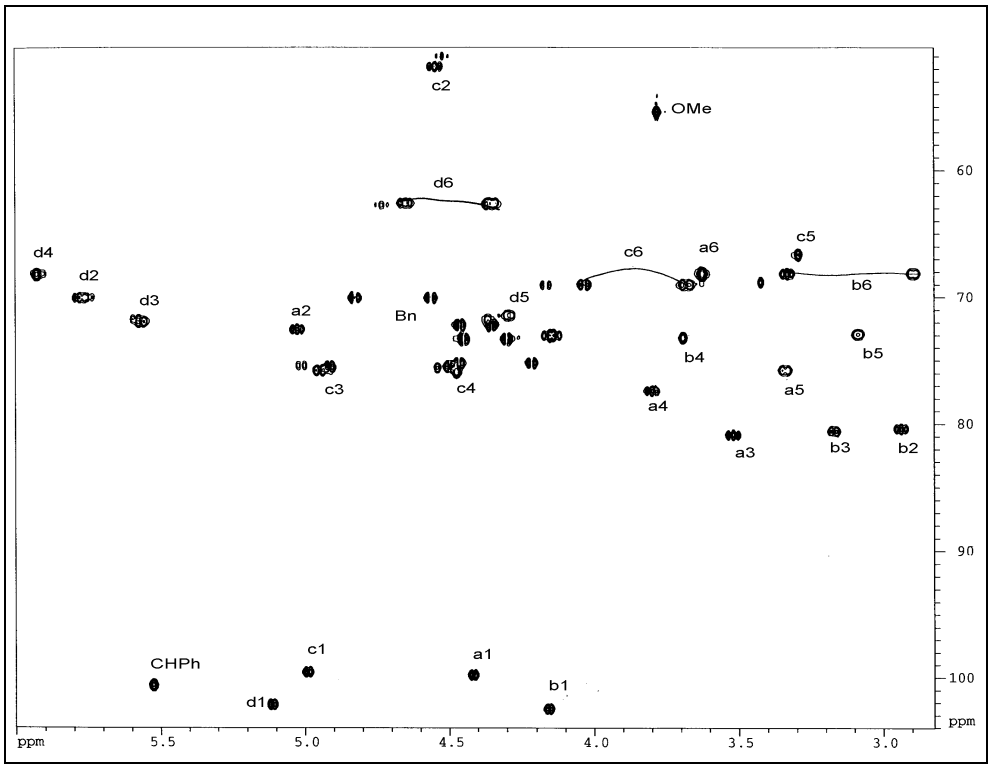
HMQC Spectrum of compound **100**



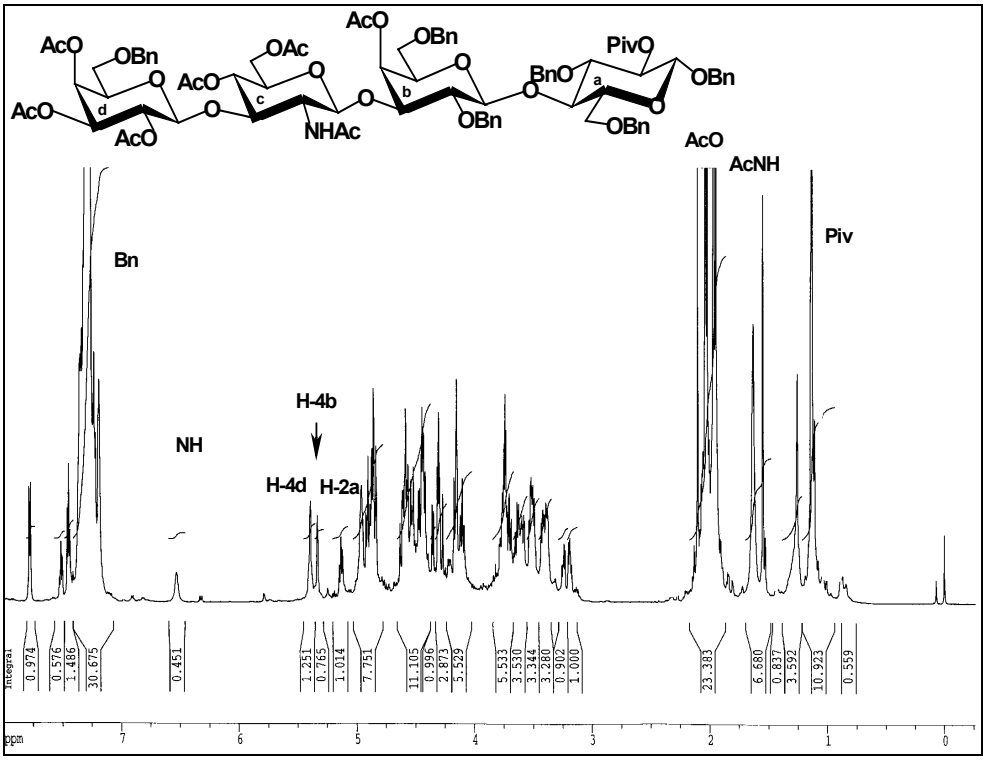
^{1}H-NMR Spectrum of compound **101** (250 MHz, $CDCl_3$)



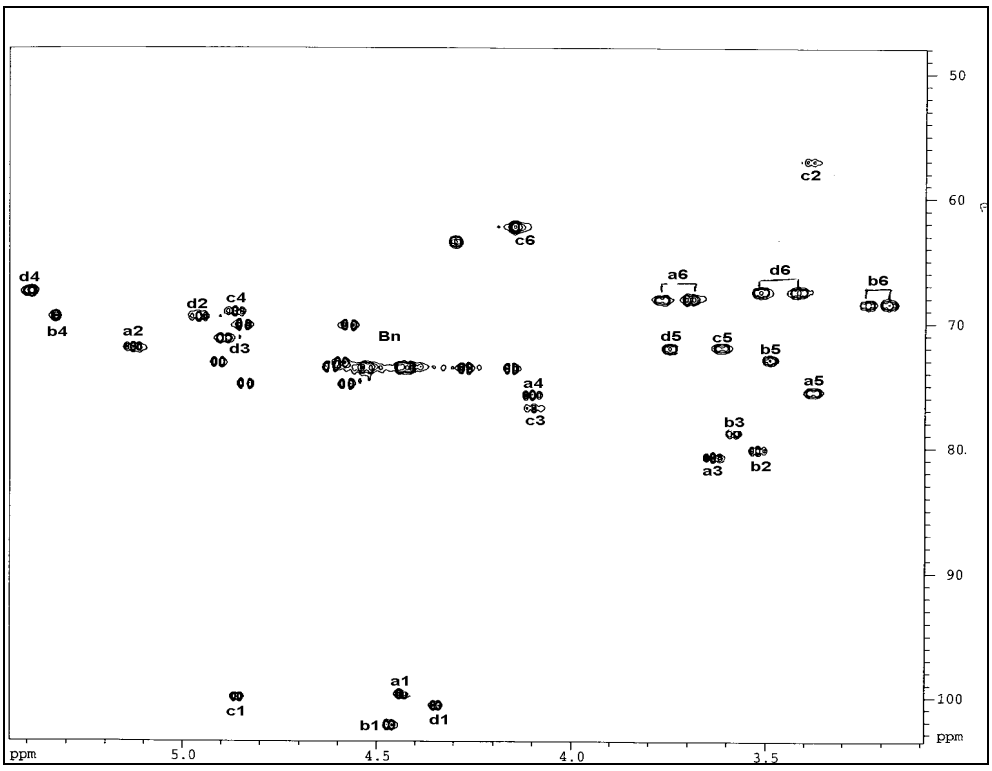
^{1}H-NMR Spectrum of compound **102** (600 MHz, $CDCl_3$)



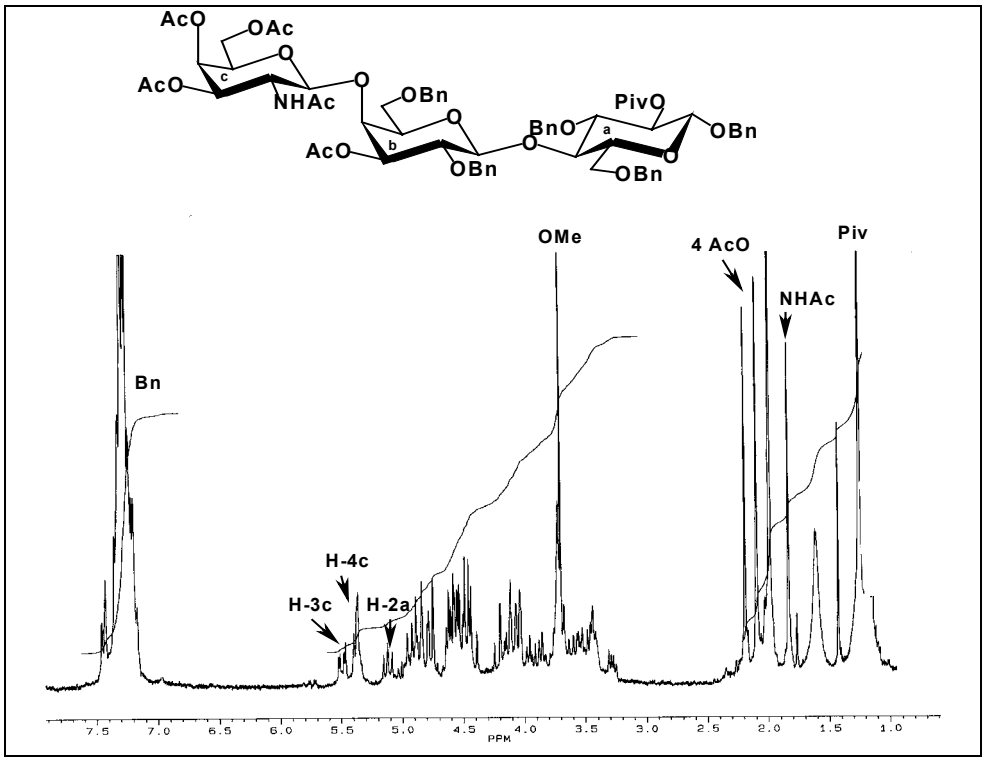
HMQC Spectrum of compound **102**



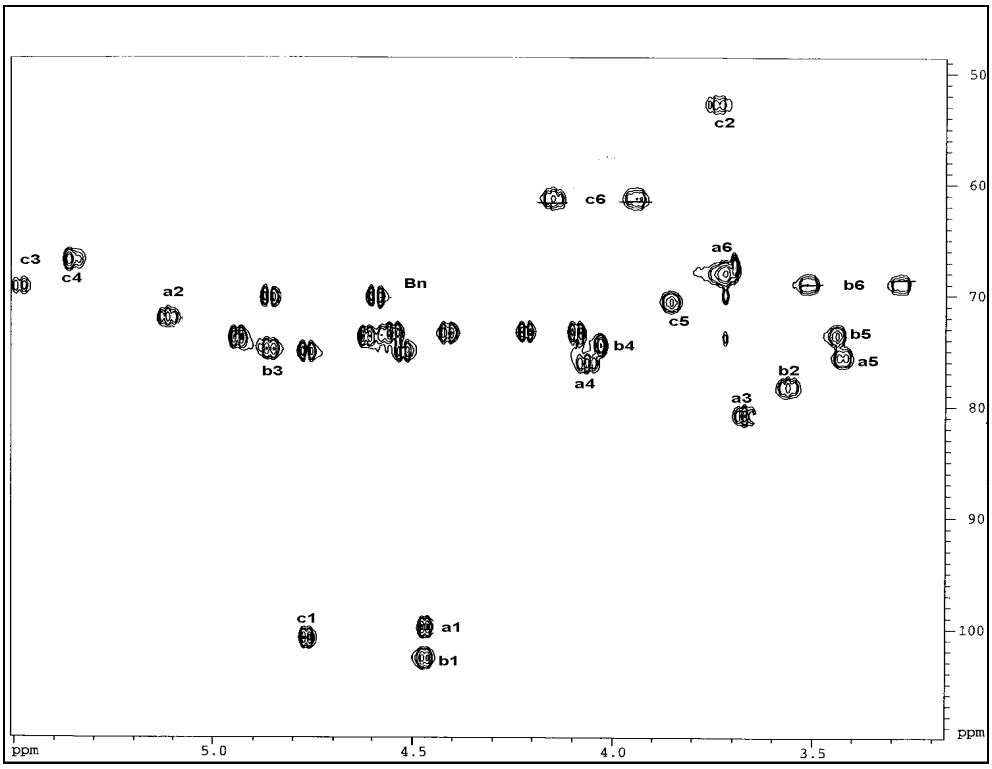
^{1}H-NMR Spectrum of compound **103** (600 MHz, $CDCl_3$)



HMQC Spectrum of compound **103**



^{1}H-NMR Spectrum of compound **104** (600 MHz, $CDCl_3$)



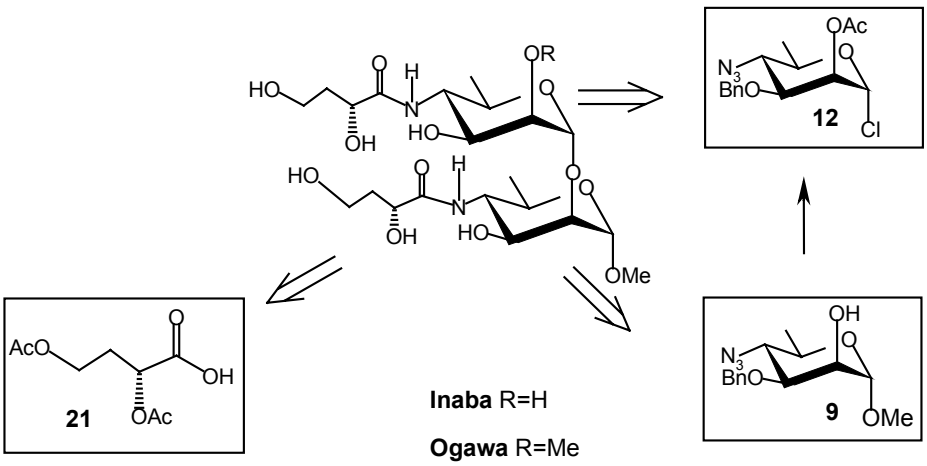
HMQC Spectrum of compound **104**

4. Summary

4.1 Synthesis of methyl α-glycoside-linked mono- and disaccharides mimicking the OPS of *V.cholerae* O1

Systematic prevention of cholera by immunization has not been achieved so far, because of the lack of a protective vaccine. Optimized syntheses of oligosaccharides in combination with efficient strategies for covalent attachment of these structures to protein surfaces permits the use of well defined synthetic compounds as substitutes for polysaccharides of bacterial origin in serological tests and possibly even as vaccines.

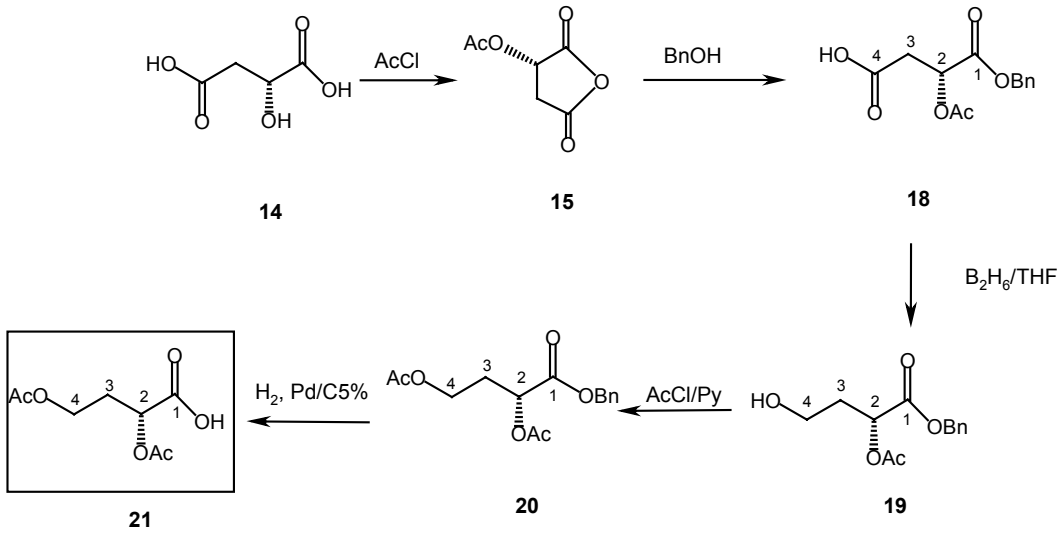
The synthesis of such substances related to the O-polysaccharide of *V. Cholerae O1* was the first objective of this work. In the initial stage, an efficient synthesis of the methyl α-glycoside of the monomeric intracatenary repeating unit of the O-PS was elaborated. Based on the general structure of the OPS of *V.Cholerae* O1 serotypes Inaba and Ogawa, two different constituents were synthesized, i.e. the D-perosamine derivatives **9** and **12**, and the 3-deoxy-L-*glycero*-tetronic acid derivative **21** (Scheme 64).



Scheme 64. Retrosynthetic analysis of the disaccharides

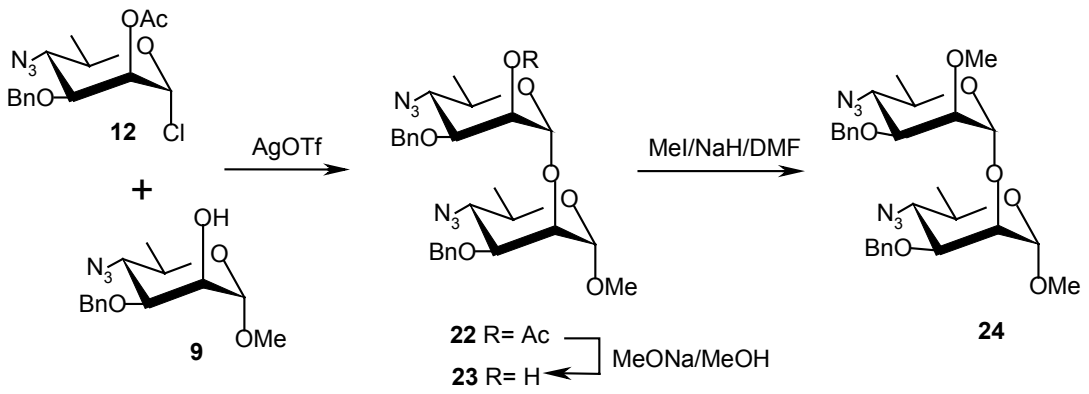
Since 4-amino-4,6-dideoxy-D-mannose (perosamine), the sole monosaccharide component of the *Vibrio cholerae* O1 OPS, is a rare sugar, its synthesis in acceptable yields was the first problem to resolve for the synthesis of oligosaccharides related to the polysaccharide antigens. A strategy was developed to provide this saccharide in larger scale and its subsequent use in a unified approach to develop immunologically active di- and trisaccharides.

To achieve this goal, methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**9**) was envisioned as a common precursor for a selectively protected acceptor and glycosyl halides (**12**) that serve as donor in silver trifluoromethanesulfonate (triflate)-promoted oligosaccharide synthesis (Chapter 2.2.2). On the other hand, *the new N-acylating reagent* **21** was developed from commercially available L-malic acid **14** (Scheme 65, Chapter 2.2.3).



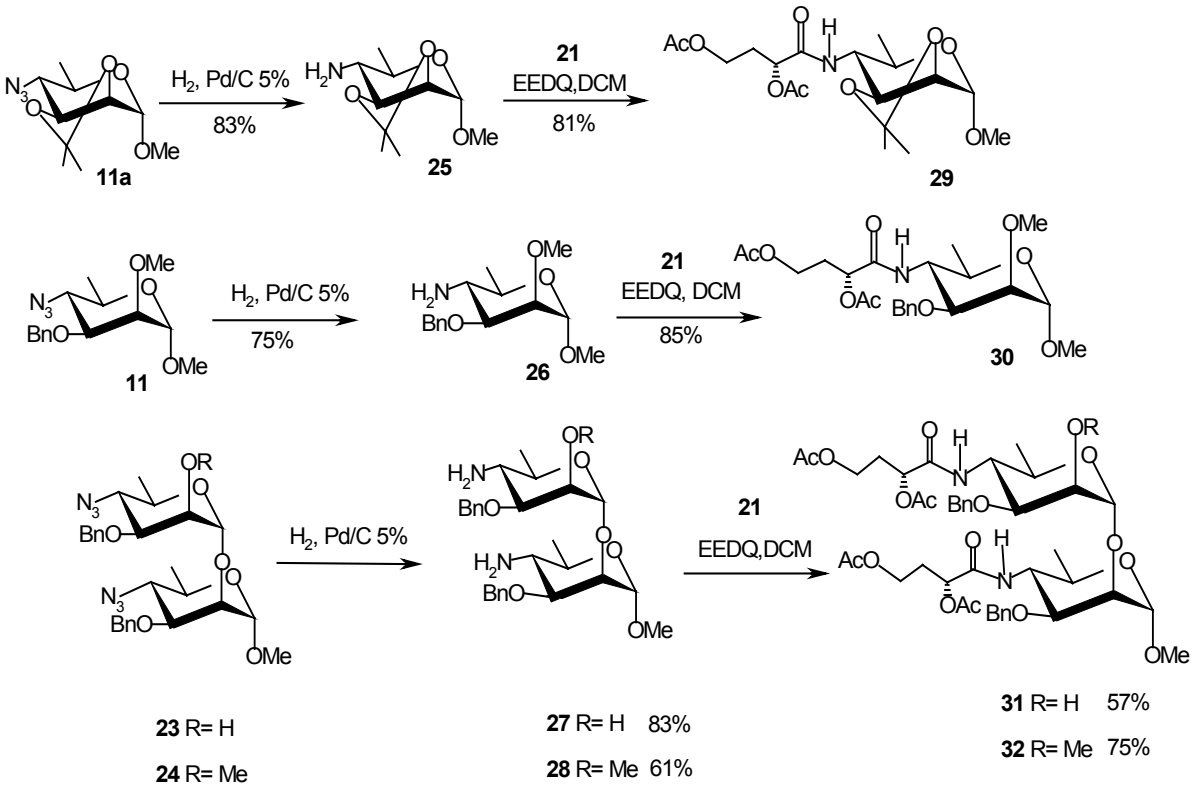
Scheme 65. Synthesis of a new precursor of the 3-deoxy-L-glycero tetronic acid

The synthesis of the disaccharide was based on the use of the *O*-acetyl group as temporary and the benzyl group as permanent protecting group. Formation of the α-mannoside was achieved using silver triflate as promoter of the glycosylation reaction with neighboring group participation (Scheme 66). Subsequent deacetylation of **22** and methylation gave **24** which mimicks the terminal portion of the serotype Ogawa (Chapter 2.2.4).



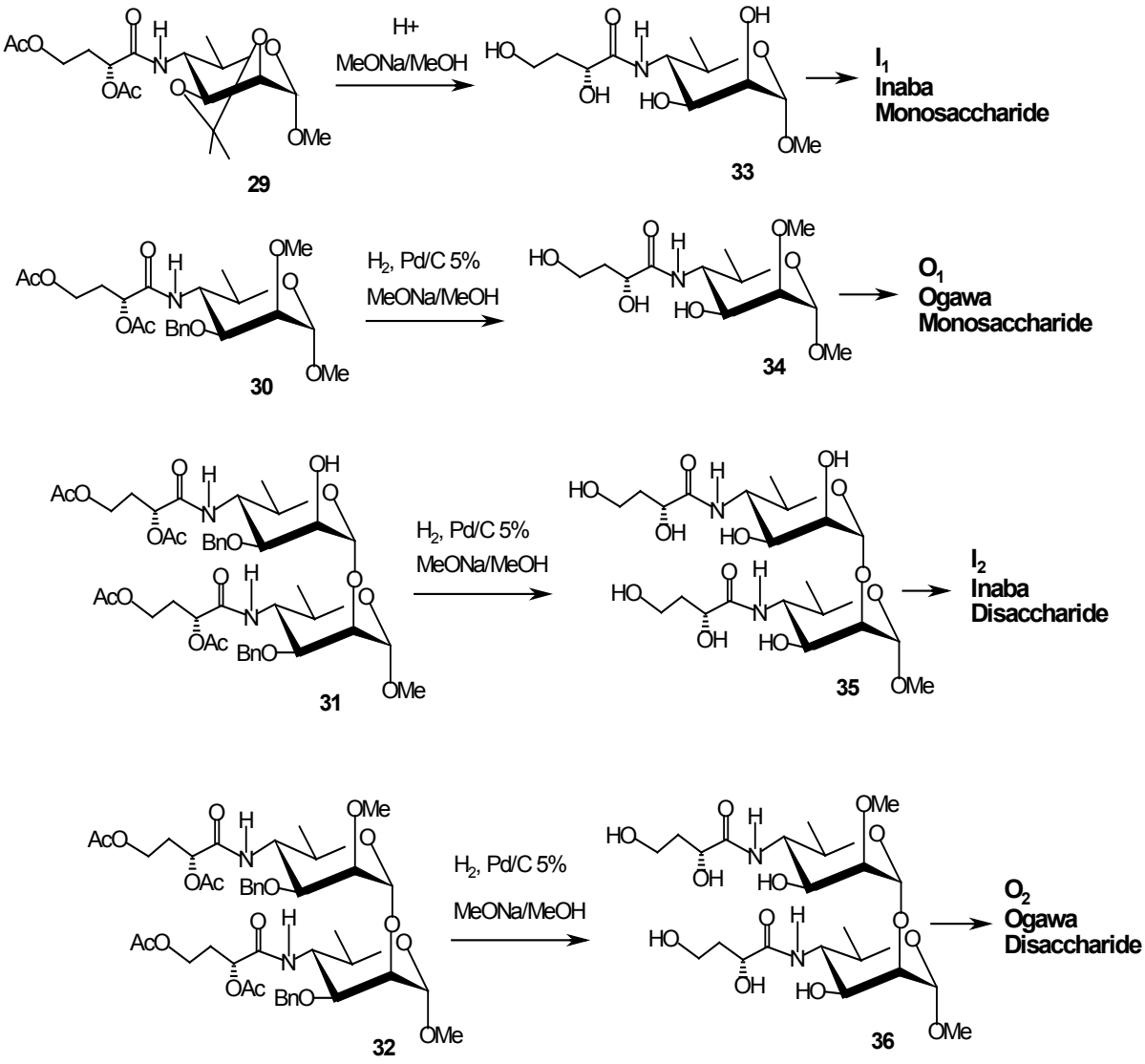
Scheme 66. Synthesis of the disaccharides

The azido oligosaccharides of the required size (→ **11a, 11, 23 and 24**) were subsequently converted via the corresponding 4-amino derivatives (→ **25, 26, 27** and **28**) into oligosaccharides containing the required 4-acylamino group (→ **29, 30, 31, 32**). These analogs were obtained by *N*-acylation with 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid (**21**) and EEDQ as coupling reagent in good yields (→ **29**, 81%, →**30**, 85%, →**31**, 57%, →**32**, 75%) (Scheme 67, Chapter 2.2.5, 2.2.6.2).



Scheme 67. Reduction of the azido group and N-acylation

Deprotection of the oligosaccharides was achieved by acid hydrolysis of the isopropylidene group (→ **33**) or hydrogenolysis of the benzyl groups (→ **34, 35, 36**), followed by Zemplén deacetylation (Scheme 68, Chapter 2.2.6.3).

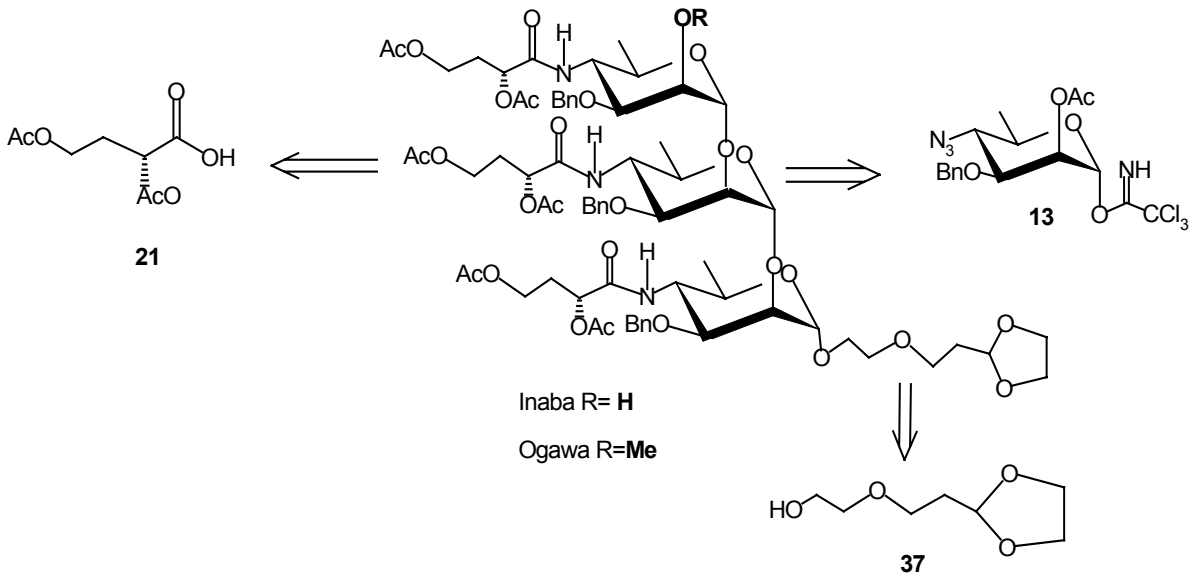


Scheme 68. Deprotection

The ability of synthetic model compounds (**I_1, I_2, O_1** and **O_2**) to inhibit the reaction of mouse poylclonal antibodies was studied by ELISA. From this preliminary study it was concluded that the 2-*O*-methylated terminal monosaccharide (**O_1**) seems to be the dominant serotype-specific determinant of the Ogawa strain (Chapter 2.2.7).

4.2. Synthesis of mono-, di- and trisaccharide fragments representing the terminus of the O-polysaccharide of *V.Cholerae* O1, serotype Inaba and Ogawa, bearing a dioxolane spacer arm

To further produce useful intermediates for the synthesis of immunogens the mono-, di- and trisaccharide fragments of the O-specific polysaccharide (O-PS) equipped with functionalized spacer as aglycon were synthesized to allow their conjugation to suitable carrier proteins (Scheme 69).

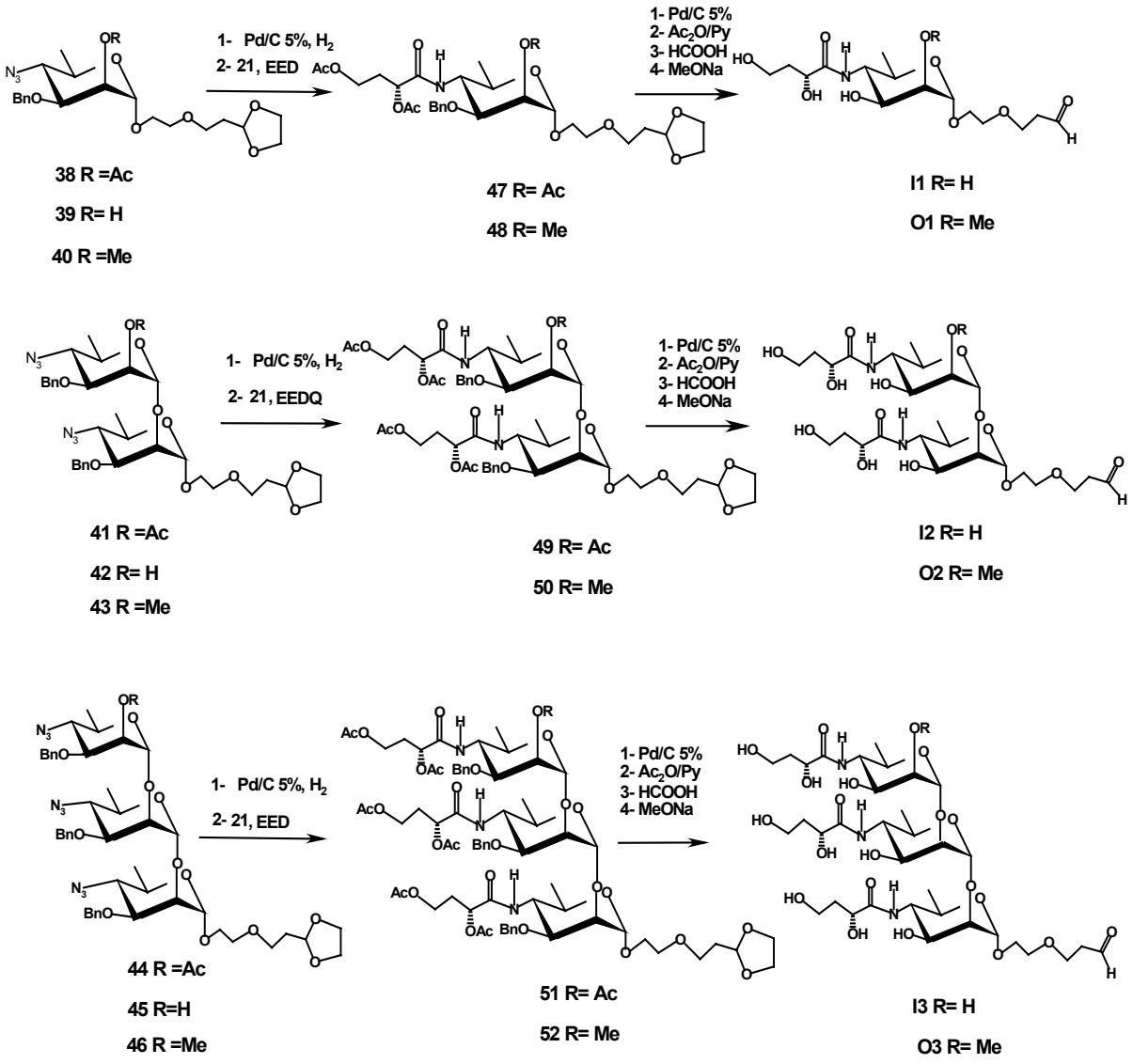


Scheme 69. Retrosynthetic pathway for synthesis of linker-equipped haptens

The stepwise construction of the haptens was based on the use of the glycosyl imidate **13** as the key glycosyl donor. Due to its stability during many chemical manipulation, the dioxolane spacer arm **37** was introduced at the first synthetic step. Accordingly, the glycosyl donor **13** and the linker acceptor **37** were condensed to give the fully protected linker-equipped monosaccharide **38**. To extend the monosaccharide into the desired oligosaccharide chain, it was deacetylated (→ **39**) and the resulting product was reacted with the same donor **13**. This sequence of reactions (→ **39**→ **41**→ **42**→ **44**) was repeated up to the trisaccharide. By this approach useful intermediates for the synthesis of fragments of *V.cholerae* O1 in both the Ogawa and Inaba series as well as for synthesis of other members were obtained, since the approach allows for chemical manipulations such as methylation (→ **40, 43, 46**) or chain extension at the position O-2 in the upstream terminal moiety (Chapter 2.3.4).

The azides (→ **38, 40, 41, 43, 44** and **46**) were converted to the corresponding amines which in turn were reacted with 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*tetronic acid (**21**) in the presence

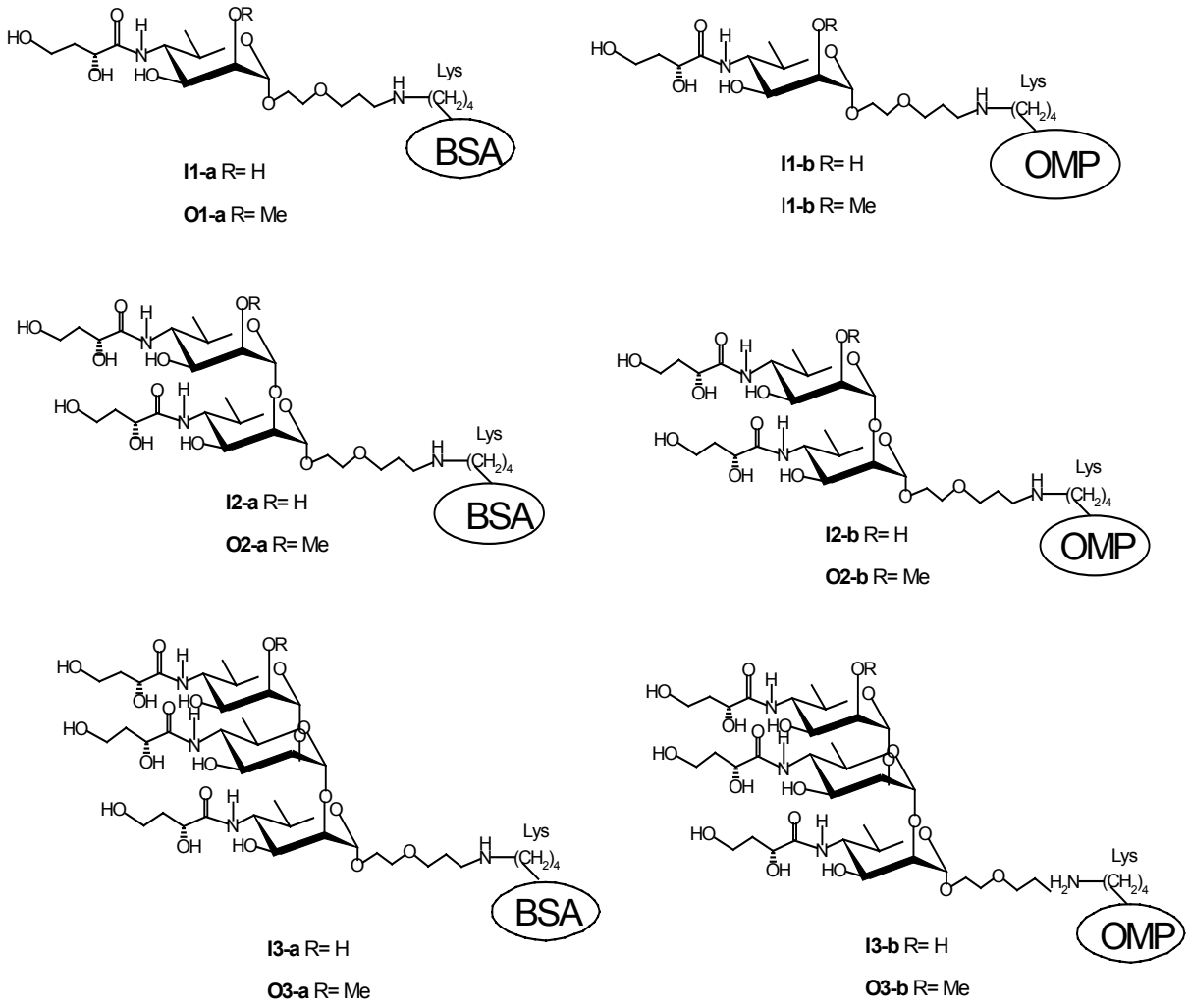
of EEDQ to produce the desired 4-(3-deoxy-tetronamido) derivatives (→ **47-52**) (Chapter 2.3.5).



Scheme 70. Reduction, coupling and deprotection steps

To convert the assembled oligosaccharides (→ **47-52**) into targets suitable for coupling to proteins, additional four steps were required: catalytic hydrogenolysis of benzyl protecting groups and acetylation, followed by hydrolysis of the dioxolane ring and deacetylation under basic conditions to yield the desired aldehydes → **I1, O1, I2, O2, I3, O3** (Chapter 2.3.6).

Finally, the linker-equipped haptens (I1-O3) were grated onto BSA and OMP via reductive amination. The resulting neoglycoproteins carrying different incorporation levels of haptens (Table 2 of Chapter 2.3.7.2) were analyzed for their immunological properties.



Scheme 71. Neoglycoconjugates using synthetic oligosaccharides

Conclusions:

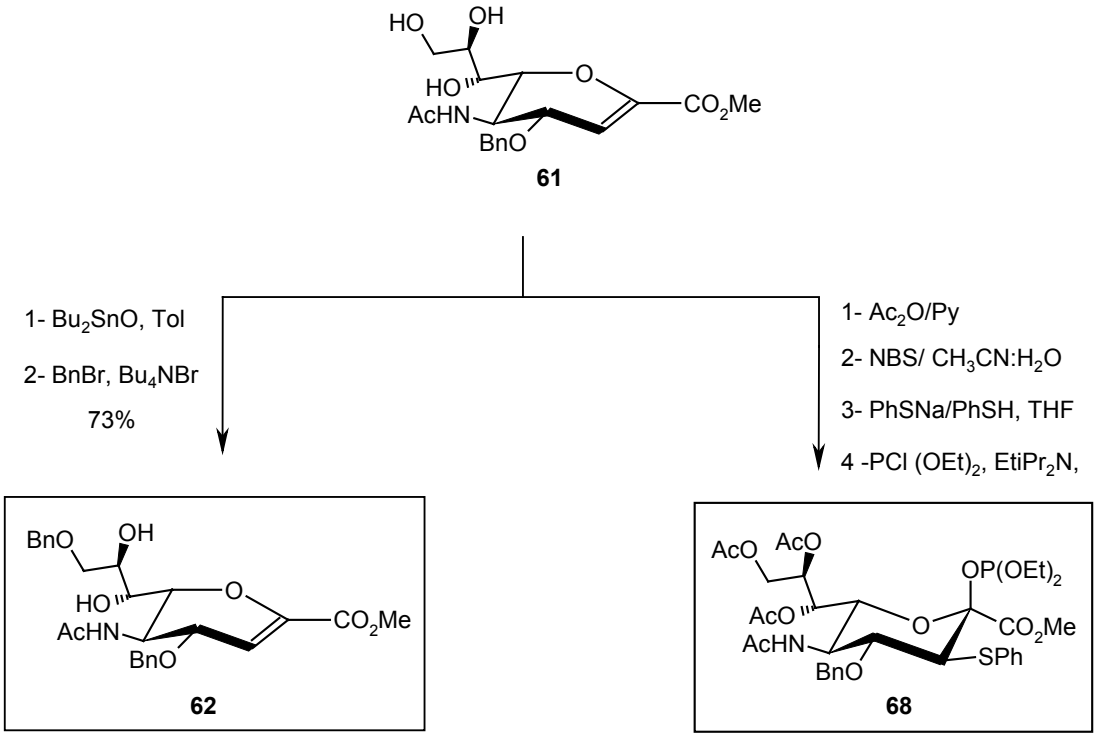
- Synthetic routes to the preparation of oligosaccharides corresponding to the OPS of *V.cholerae* O1 serotypes Inaba and Ogawa were elaborated which allowed the production of the antigens in quantities sufficient for detailed studies of their immunological and biological properties.
- Consistently higher yields were obtained when the trisaccharides were assembled from monosaccharide precursors (**13**, **39**) in a stepwise fashion using Schmidt's glycosylation technique that proved superior to other methods comparatively examined.
- Selective attachment of the saccharides to BSA and OMP using the dioxolane spacer **37** via reductive amination afforded a range of neoglycoproteins for use in immunological experiments.

4.3 Approaches to the synthesis of GQ1bα

Aim of this part was the development of new synthetic routes for the synthesis of the complex GQ1bα ganglioside. For this purpose new tools for the formation of different sialyl glycosides as central structural cores were elaborated.

4.3.1 Synthesis of Neu5Acα(2→8)Neu5Ac donor

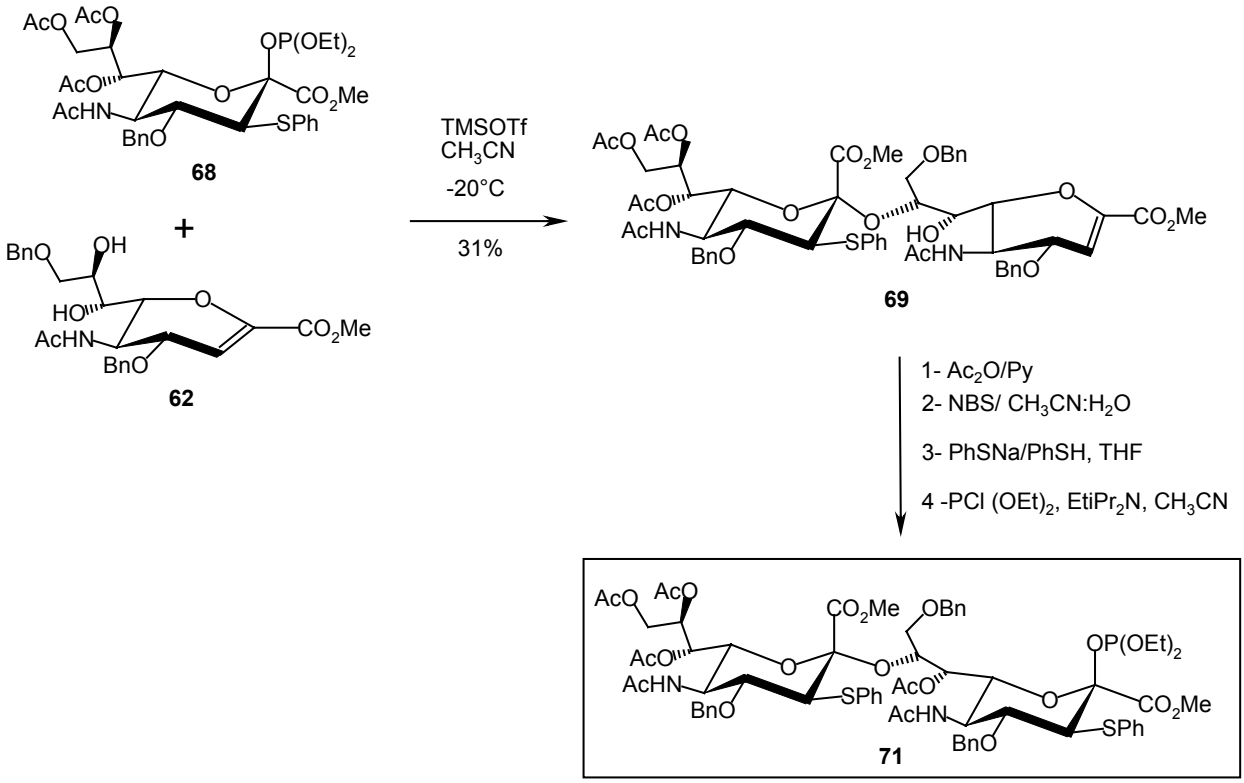
At first, *a new and totally synthetic Neu5Acα(2→8)Neu5Ac donor* was prepared and analyzed for its usefulness in sialylation reactions. The key step in the synthesis of the disaccharide **69** was the glycosylation of the sialyl acceptor **62** with the sialyl donor **68**. Both intermediates were derived from the readily available glycal **61** as common precursor (Scheme 72).



*Scheme 72. Synthesis of the sialyl acceptor **62** and donor **68** from a common precursor **61***

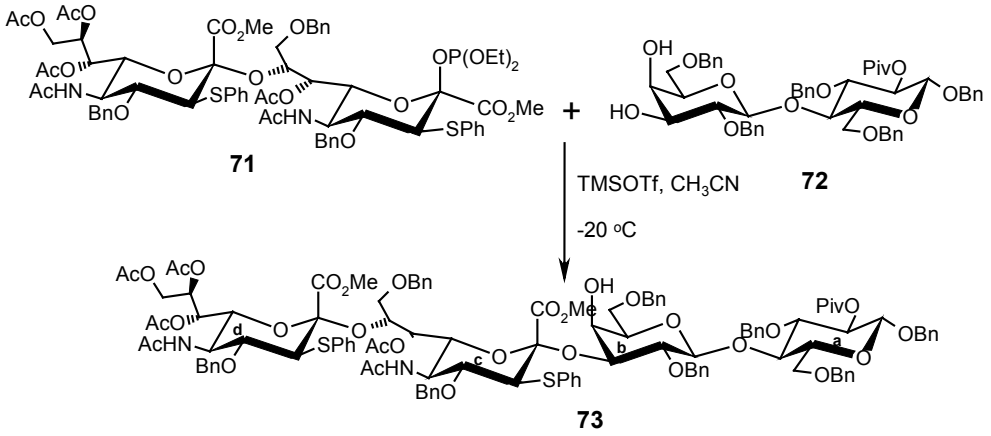
In order to avoid losses of donor (via side reactions) and also to improve the α,β-ratio in sialylations, donors of the type **68** were introduced that carry a thiophenyl substituent at C-3. Our method of generating the auxiliary phenylthio group (PhSNa/PhSH, THF) provides a higher reaction rate than the original method (PhSNa, THF), and may be used in new synthetic targets. Since the reactivity order of the hydroxy groups of sialic acid is HO-9>> HO-4> HO-8>>HO-7 the diol **62** could be 8-O sialylated in a regioselective mode with the

donor **68** to generate the disaccharide **69**. Once the α (2→8) linkage was formed, the disaccharide **69** could be converted by the same procedure into the desired donor **71** (Scheme 73, Chapter 2.5.3).



*Scheme 73. Synthesis of the disialyl donor **71***

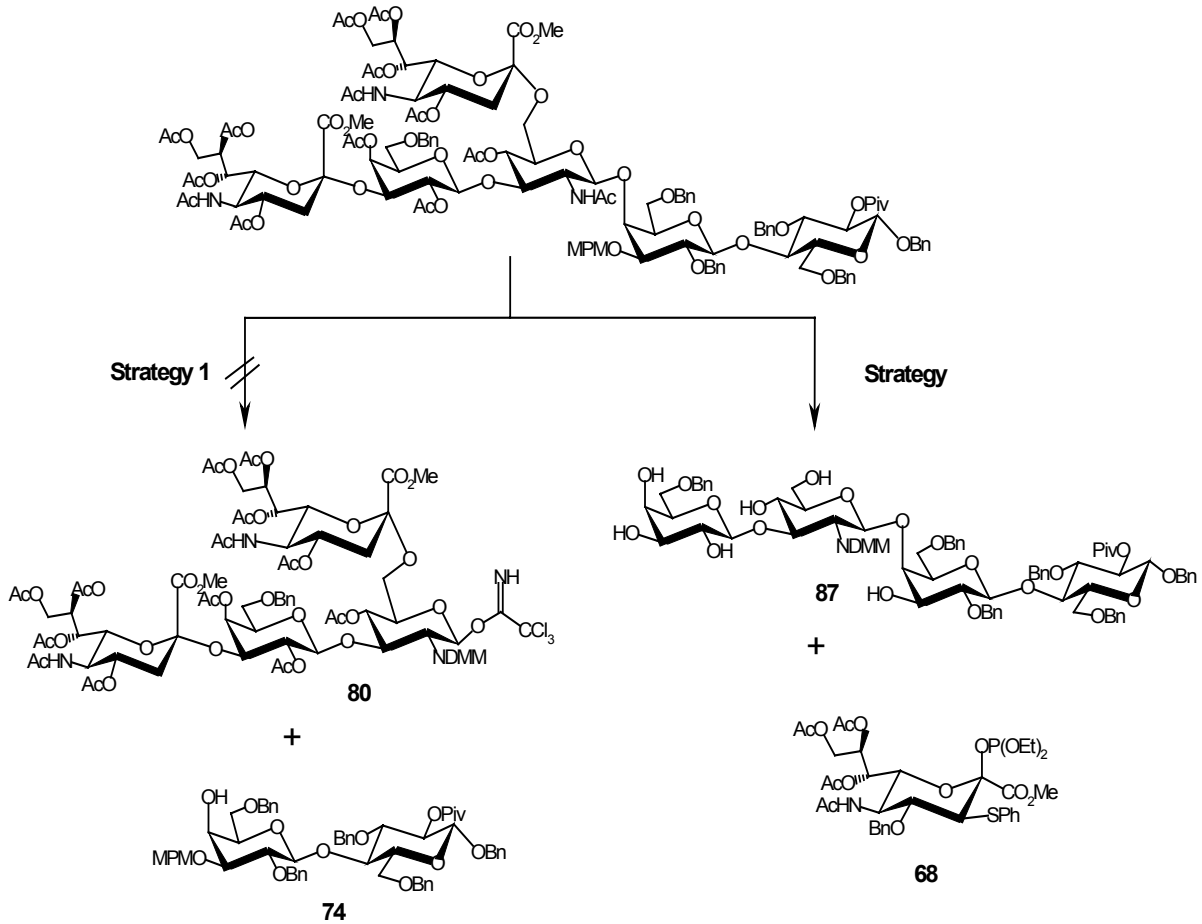
The efficiency of **71** as a sialyl donor was investigated by reaction with the lactose acceptor **72**. Activation of **71** with TMSOTf in the presence of the acceptor **72** and acetonitrile as the solvent led to the desired α-sialylated compound **73** in 31 % yield after chromatographic purification . Formation of the related β-glycoside was not observed (Chapter 2.5.4).



*Scheme 74. Synthesis of GD3 derivative using a new donor **73***

4.3.2 Synthesis of the protected target hexasaccharide

Based on the structure of the hexasaccharide shown in Scheme 68, two different synthetic strategies were developed.



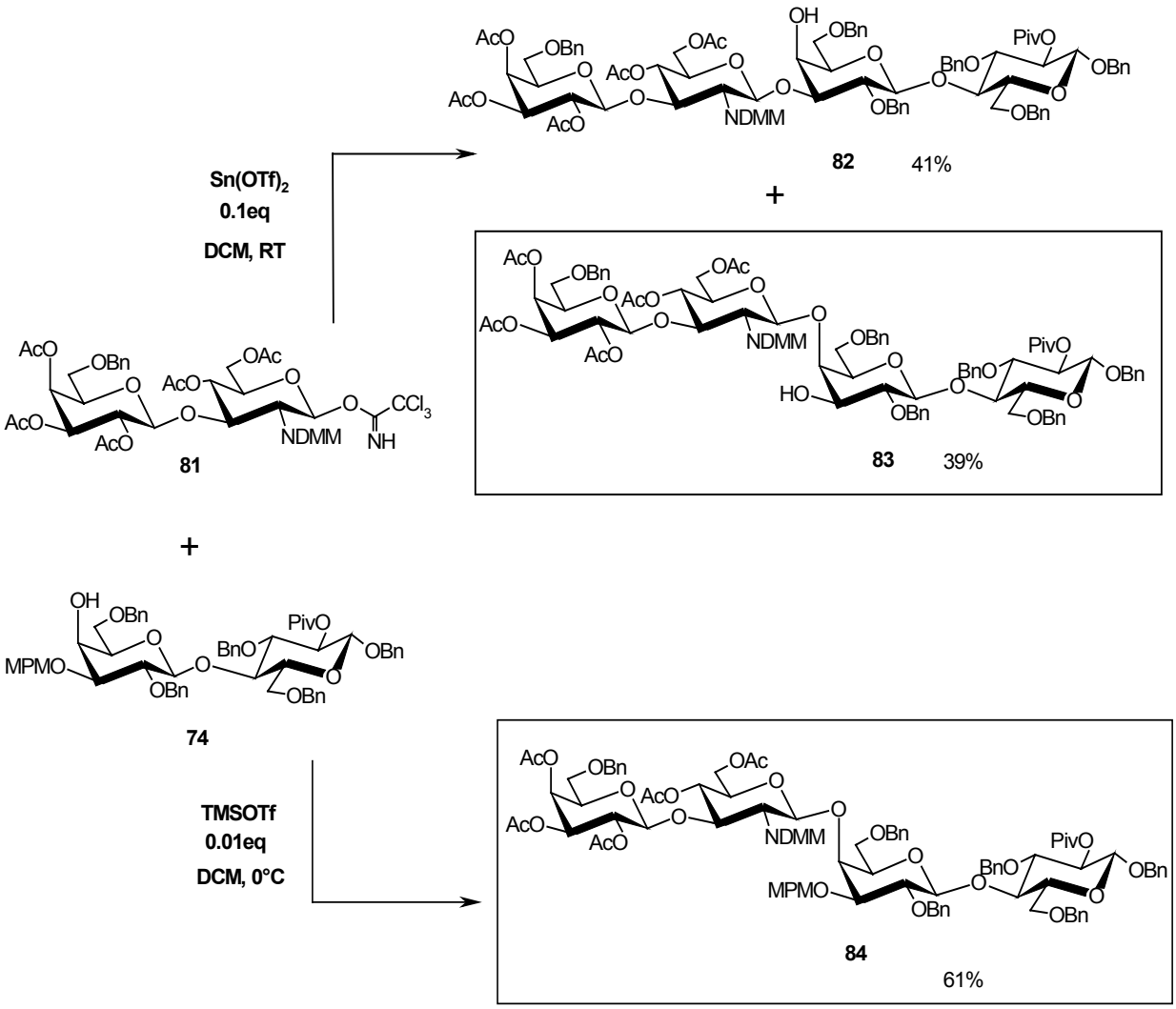
Scheme 75. Synthetic strategies

According to the first synthetic strategy, the disconnection led to compounds **74** and **80** as the required building blocks (Scheme 75). Compound **74** was readily synthesized from the know lactose **72** (Chapter 2.5.5.1), while the required tetrasaccharide donor **80** was synthesized over five steps from the known disaccharide **75**. The structure of the tetrasaccharide **78** was unequivocally assigned by NMR analysis (Chapter 2.5.5.2).

For coupling of the tetrasaccharide donor **80** and the lactose acceptor **74** different methods were examined. As described in chapter 2.5.5.3, the hexasaccharide could not be obtained by strategy 1, probably because of some sterical hinderance in donor **80** and the rather unreactive 4b-position of the lactose acceptor **74**. Therefore, the attention was focussed on the synthesis of this important building block using a new procedure (Strategy 2).

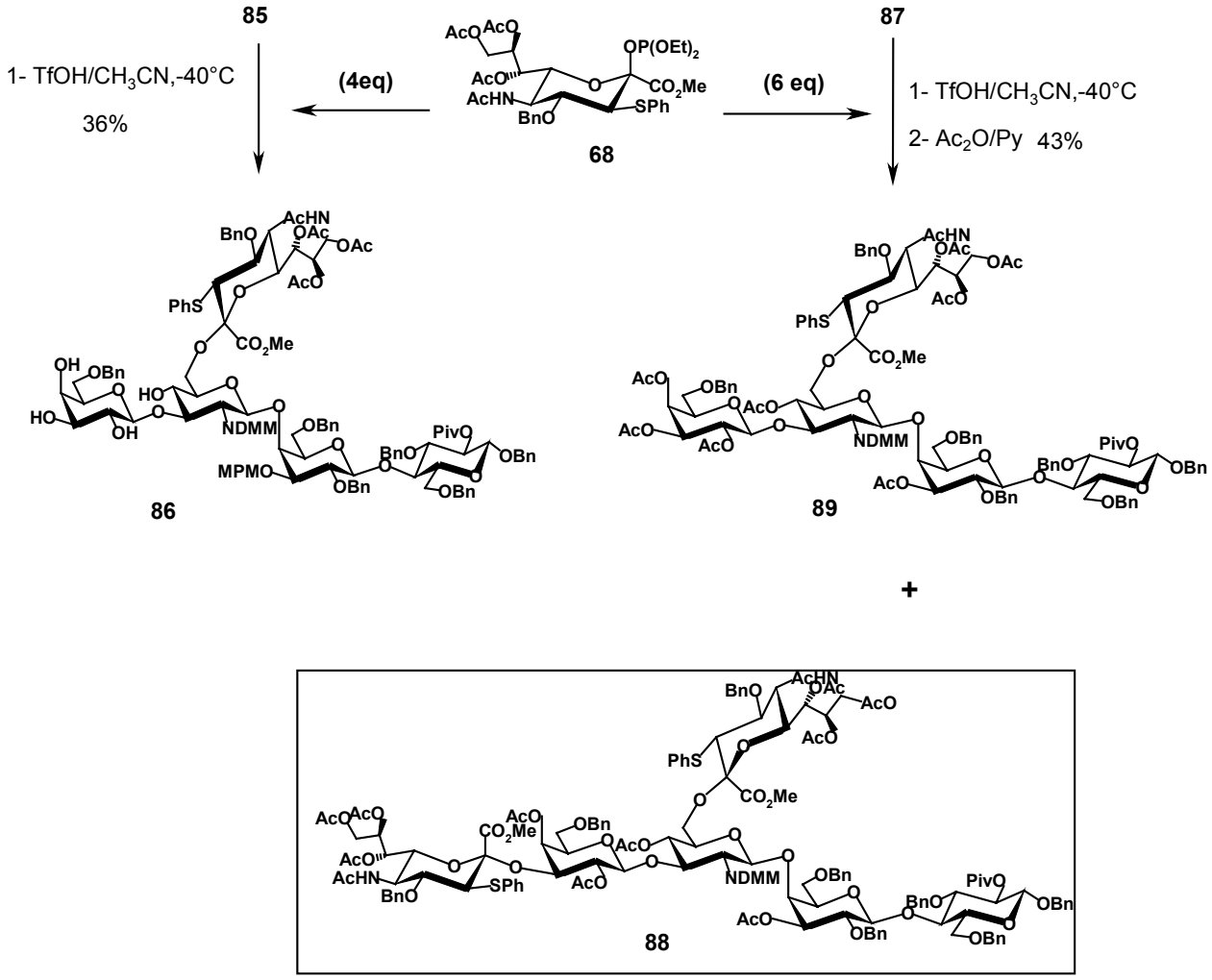
Here, the bis-sialylation as the last synthetic step was the most important variation. Correspondingly, two main building blocks had to be assembled, i.e. the tetrasaccharide acceptor **87** and the already synthesized donor **68** (Scheme 75).

For the synthesis of the tetrasaccharide **87** the lactose **74** was chosen as acceptor and the known disaccharide **81** as donor. When the glycosylation reaction was performed in the presence of $Sn(OTf)_2$ (0.1eq), the NMR analysis revealed formation of **82** (41 %) and **83** (39 %) as main compounds (Scheme 68). From this experience we knew that the concentration of catalyst had to be lowered (<0.1M) in order to avoid uncontrollable formation of **82**. A second attempt using TMSOTf (0.01 eq) at 0°C gave the protected tetrasaccharide **84** in 61 % yield (Scheme 76, Chapter 2.5.6.1).



*Scheme 76. Glycosylation reaction using the donor **81** and the acceptor **74***

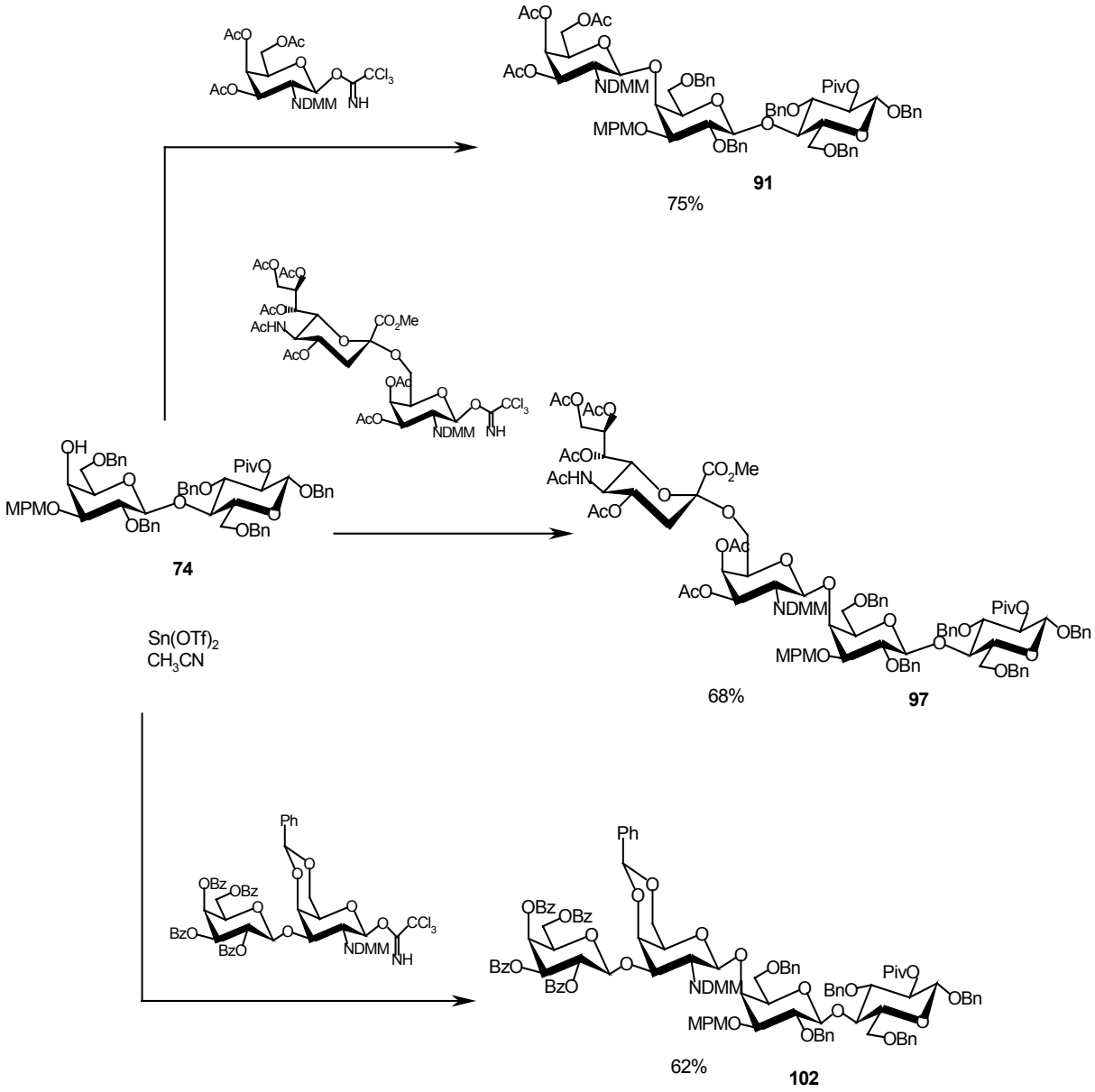
Careful attempts to regio- and stereoselectively disialylate compound **85** (4eq) in the presence of catalytic amounts of TfOH in acetonitrile at -40°C gave mostly the pentasaccharide **86** (Chapter 2.5.6.2). Attention was then turned to the acceptor **87**, which could be potentially sialylated at the three positions 3b, 6c and 3d. The versatility of the synthetic strategy developed in this work could be demonstrated once more in the preparation of **88**. Coupling of the donor **68** (6 eq) with the glycosyl acceptor **87** under identical reaction conditions followed by *O*-acetylation afforded the pentasaccharide **89** and the desired hexasaccharide **88** (Chapter 2.5.6.3).



Scheme 77. Synthesis of target oligosaccharides

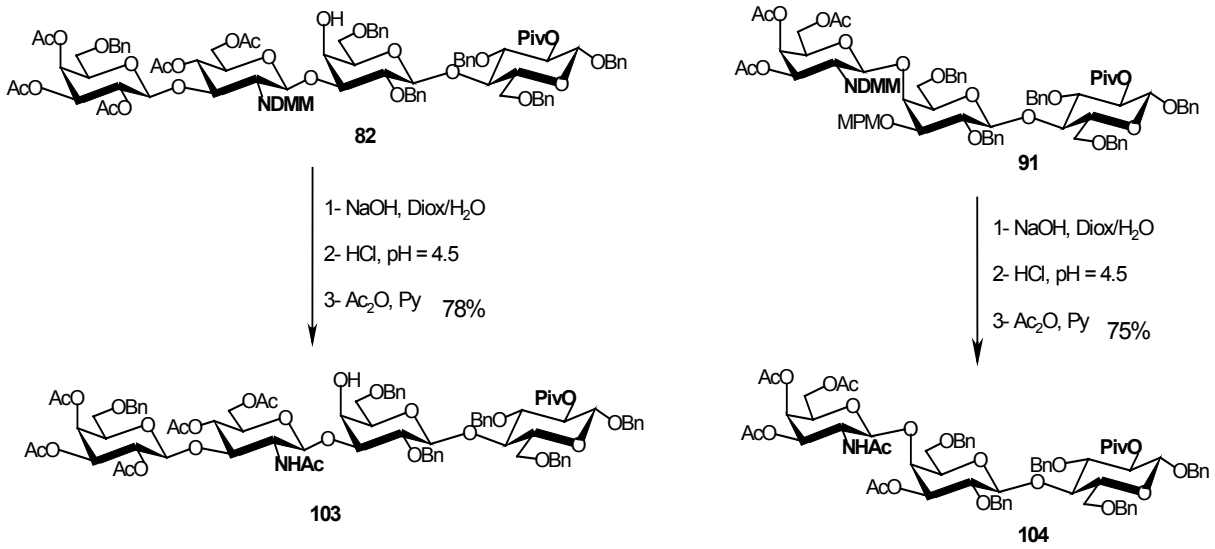
4.3.3 Synthesis of structures containing GalNAcβ(1→4)Lac linkage

In the final part of this thesis work a versatile and efficient strategy for the synthesis of compounds containing the ubiquitous GalNAc moieties was established. For this purpose three key intermediaries (→ **91, 97, 102)** in the synthesis of gangliosides were prepared. As model acceptor compound the lactose derivative **74** was chosen, and the results are illustrated in Scheme 78.



Scheme 78. Synthesis of core structures containing GalNDMMβ(1-4)Lac linkage

In order to confirm the versatility of our synthetic strategy, methods compatible with the concurrent presence of both the pivaloyl and dimethylmaleoyl protecting group were required. Thus, the successful cleavage of the dimethylmaleoyl (DMM) group in the presence of pivaloyl (Piv) was studied using the model compounds **82** and **91** and the reaction sequence applied is outlined in chapter 2.6.4 (Scheme 79).



Scheme 79. DMM Cleavage in the presence of Piv group

Conclusions

The conclusions that can be drawn from the experimental work are the following:

- a novel Neu5Acα(2→8)Neu5Ac donor was synthesized in reasonable yield and used on the synthesis of an GD2 ganglioside derivative;
- two entirely different synthetic strategies and protecting group schemes for the synthesis of the hexasaccharide were investigated;
- an efficient procedure was developed for regio- and stereoselective bis-sialylation of oligosaccharides; this may be useful for the synthesis of highly complex gangliosides;
- a number of core structures bearing the ubiquitous GalNAcβ(1→4)Lac sequence were successfully prepared and the methodology was shown to be compatible with a number of different protecting groups.

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