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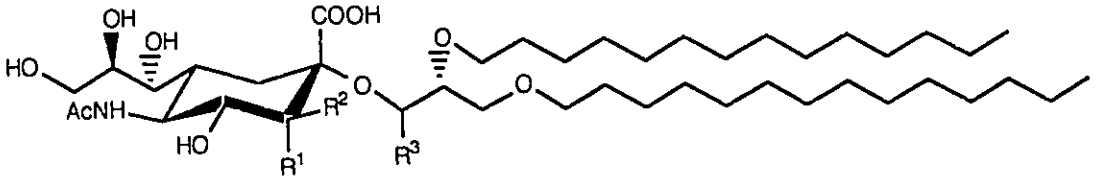
To my husband, Frederick
and my family, Charles,
Claire, Catherine and François

ABSTRACT

Sialic acid has been sought as an excellent candidate for ^2H NMR studies because of its ubiquitous presence at the penultimate non-reducing ends of membrane glycoproteins and glycolipids and because it has been associated with a number of biological and immunological phenomena. Regio- and stereoselectively deuteriated sialyl glycerolipids **1a**, **1b** and **1c** were required for these studies and were synthesized.



1a $R^1 = D, R^2 = R^3 = H$
1b $R^1 = R^2 = D, R^3 = H$
1c $R^1 = R^2 = H, R^3 = D$

The first step of the synthetic route developed, involved the synthesis of unlabeled and labeled glycerolipids. The regio- and stereoselective deuterium incorporations in sialic acid and subsequent transformation of the deuteriated sialic acids into their respective glycosyl donors followed. The glycosylation of the glycerolipids with the appropriate glycosyl donors completed the synthesis of the model sialylglycerolipids and prompted further investigations of glycosidation methods for the synthesis of sialyl glycosides. A phase transfer catalysis procedure, particularly useful in the synthesis of thiosialosyl donors, was developed and the applicability of the procedure to produce a wide range of S- and O-sialosyl derivatives was explored. An extensive study of thioglycosylation reactions using a series of thiosialosyl donors, the glycerolipid and various thiophilic promoters resulted in the introduction of the concept of active and latent thiosialosyl donors which concluded the synthetic aspect of this research.

ACKNOWLEDGMENTS

I would like to extend my deepest appreciation and profound gratitude to my supervisor professor René Roy for his insight, precious comments and unequivocal support over the course of this research project.

I would also like to thank Dr. Harold Jarrell and Dr. David Fenske. Without their interest in ^{2}H NMR spectroscopy of regio- and stereo-selectively deuteriated sialyl glycerolipids I would not have had the opportunity of working on this challenging research project. I was honored that they requested me to perform the difficult synthesis of the sialylglycerolipids they required for their study.

I wish to thank my fellow colleagues, especially Alan Rey and Chantal Grand-Maître who shared the laboratory with me along with helpful discussions and memorable moments.

Many thanks go out to Raj Capoor for his expert NMR spectral data contributions and to Dr. Clem Kazakoff for his prompt mass spectral services.

I would like to single out the immense contribution of my family in the preparation of this document. I commend my parents Claire and Charles for their utmost commitment, determination, support and encouragement in the production of this manuscript. I would also like to thank my sister Catherine and my parents for typing countless pages and my cousin David Lamarche for his revision work. Without them, the successful completion of this thesis would surely not have been possible.

Finally, I am greatly appreciative of the support, encouragement and love given to me by my husband Frederick throughout the past years.

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List of Abbreviations

Ac	Acetyl
ADEPT	Auto DEPT
Anal.	Analytical
arom	Aromatic
Å	Angstrom
bd	Broad Doublet
bn	Benzyl
bs	Broad Singlet
BSA	Bovine Serum Albumin
bz	Benzoyl
C	Celsius
c	Concentration
C.I.	Chemical Ionization
calcd	Calculated
cat.	Catalytic
^{13}C NMR	Carbon Nuclear Magnetic Resonance.
d	Doublet
δ	Chemical Shift
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
dd	Doublet of Doublets
ddd	Doublet of Doublets of Doublets
DEPT	Distortionless Enhanced Polarization Transfer
DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulfoxide
DMTST	Dimethyl(methylthio)sulfonium Triflate
DTSL	1,2-Di-O-tetradecyl-3-O-α-D-sialyl-*sn* glycerol
E.I.	Electron Impact
EDG	Electron Donating Group
eq	Equivalent
Et	Ethyl
ether	Diethyl Ether
EWG	Electron Withdrawing Group

FAB	Fast Atom Bombardment
g	Grams
gem	Geminal
h	Hour
HA	Human Influenza Virus Hemagglutinin
^{1}H NMR	Proton Nuclear Magnetic Resonance
[$^2H_1$-3G] DTSL	1,2-Di-O-tetradecyl-3-O-α-D-sialyl-*sn*-[3–$^2H_1$] glycerol
[$^2H_1$-NeuAc] DTSL	1,2-Di-O-tetradecyl-3-O-α-D-[3ax-$^2H_1$] sialyl-*sn*-glycerol
[$^2H_2$-NeuAc] DTSL	1,2-Di-O-tetradecyl-3-O-α-D-[3ax,3eq-$^2H_2$] sialyl-*sn*-glycerol
^{2}H NMR	Deuterium Nuclear Magnetic Resonance.
Hz	Hertz
IDCP	Iodonium Dicollidine Perchlorate
lit.	Literature
M	Molar
M	Molecular Ion
m	Multiplet
m.p.	Melting Point
M.S.	Mass Spectrum
m/z	Mass to Charge Ratio
Me	Methyl
MeIm	1-Methyl Imidazolin-2-yl
MHz	Megahertz
mg	Milligrams
ml	Milliliters
mmol	Millimoles
mol	Moles
µl	Microliters
NBS	N-Bromosuccinimide
NIS	N-Iodosuccinimide
Ph	Phenyl
ppm	Parts per Million
Py	Pyridin-2-yl
q	Quartet
R.T.	Room Temperature
rf	Rate of Flow
rpm	Rotation per Minute

s	Second
s	Singlet
sat.	Saturated
sint'd	Sintered
t	Triplet
TBAHS	Tetrabutylammonium Hydrogen Sulfate
Tf	Triflate
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMSiOTf	Trimethylsilyl Trifluoromethanesulfonate
TMU	1,1,3,3- Tetramethylurea
U.V	Ultraviolet
V/V	Volume per Volume
W/V	Weight per Volume
W/W	Weight per Weight

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INTRODUCTION

Carbohydrates play important structural and functional roles at the surface of biological membranes. Attached to lipid and proteins, carbohydrates are a major element of the cell surface, where, anchored in the membrane, they modulate interactions with the outside world. Glycolipids are intimately involved with immune functions[1], interactions with toxins and biological pathogens[2], cell recognition and differentiation as well as growth control in both normal and diseased cells[3] (Figure I). They also have the potential to modulate membrane physical properties[4].

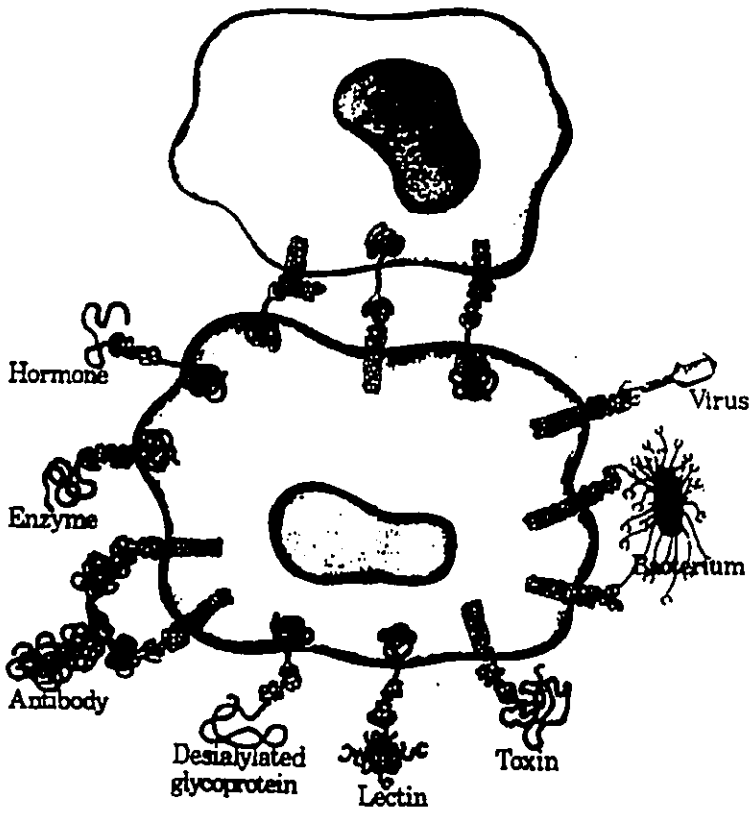

Figure I. Schematic Illustration of Membrane Glycolipids Interactions with their Environment.

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Glycolipids are most frequently composed of a carbohydrate head group glycosidically linked to a diacyl- (or dialkyl-) glycerol or a sphingosine residue. The carbohydrate headgroup can be relatively simple (single sugar residue) or very complex (oligosaccharide) and may be neutral or charged. The involvement of the headgroup in such biologically important functions is not only dependent on the structure of the surface carbohydrate but also on its accessibility to external factors. Thus, the spacial organization of the membrane surface which will be determined by such factors as the orientation, ordering and dynamics of the carbohydrate residues, is expected to be of critical importance in understanding cell-surface phenomena.

An attractive possibility for studying the ordering, conformation and dynamics of molecules in an anisotropic environment, such as the molecular environment of the biological membrane, is the use of Deuterium (^{2}H) Nuclear Magnetic Resonance. ^{2}H NMR affords an excellent tool with which to probe membrane surfaces and to observe surface components directly. It is a technique that has been applied extensively to the study of glycolipid headgroups in cerebrosides[5-7] in glycoglycerolipids[8-12] and in model glycolipid systems[13]. It has proved to be particularly valuable because of the ease of obtaining information about specific molecular sites on carbohydrate headgroups by isotopic labeling. Detailed information on the orientation, conformation and motion of monosaccharide headgroups[8-10], disaccharide headgroups[11,12] and the glycerol backbone of glycoglycerolipids[9,10,14-17] has been obtained.

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6 R. Skarjune and E. Oldfield, Biochemistry, **21** (1982) 3154.
7 D.B. Fenske, K. Hamilton, H.C. Jarrell, E. Florio, K.R. Barber and C.W.M. Grant, Biochemistry, **30** (1991) 4503.
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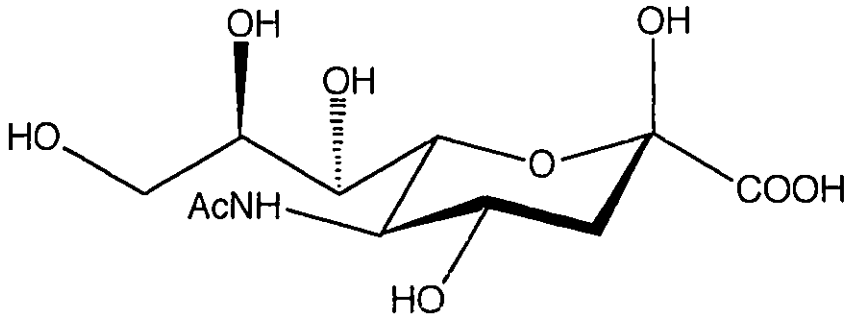

Figure II. A Sialic Acid, N-Acetylneuraminic Acid.

Sialic acid (Figure II) has been sought as an excellent candidate for ^2H NMR studies because its ubiquitous presence at the penultimate non-reducing ends of glycoproteins and glycolipids has been associated with a number of biological and immunological phenomena[18]. Among the great variety of biological functions of sialic acids there are those functions attributed to the endowment of glycoconjugates and cellular membranes with a negative charge. For instance it has been determined that the charge associated to the membrane of erythrocytes is primarily due to the presence of sialic acid residues[19]. Such charged membranes covering cells can both prevent cell aggregation by electrostatic repulsion or facilitate aggregation, through calcium bridging[20]. The negative charge also affects the overall rigidity of cell surfaces and aids in the transportation of ions through the cellular membrane[21]. Sialic acids are also involved in the regulation of the viscosity of many biofluids and mucins[22]. The presence or absence of sialic acid residues on the oligosaccharide portion of the glycoproteins elicits conformational changes in the system. The extent of syalylation has been considered to be the controlling factor in the viscosity of the mucous substances. Sialic acids also play an important role as biological receptors. It has been demonstrated that sialic acid groups on cell surfaces interact with biomolecules such as hormones,

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enzymes, viruses and toxins[23]. For instance, sialic acids have been shown to be the cell surface receptors for the influenza virus haemagglutinins which were known to be responsible for binding the virus to receptors during infection[24]. Accumulated evidence also indicates that gangliosides, sphingoglycolipids characterized by the presence of ceramide, sialic acids and other sugars, play an important role as receptors for different toxins and hormones. For example, cholera toxin has been found to exhibit a high degree of specificity and affinity for the ganglioside GM_1[1,25]. Finally, sialic acids also play a role that has come to be known as the anti-recognition effect[26,27], the protection of glycoconjugates and cells from recognition and degradation. Glycosidically linked to the terminal sugar residues of glycoconjugates, sialic acids effectively block important antigenic sites and recognition markers on cell surfaces, protecting them from identification and degradation by the surrounding immune system. It is a known phenomena that younger cells have a higher sialic acid content then older ones[28,29]. Moreover, cancer cells have also been found to contain more sialic acid residues than normal cells. However, specific sialylated conjugates occur in much higher concentration in tumor cells than in normal cells[30-32] . In fact, sialic acid containing tumor-associated antigens have been identified in various tumor cells; typical examples are listed in Table 1. Novel diagnostics and anti-tumor agents are being developed based on these important findings.

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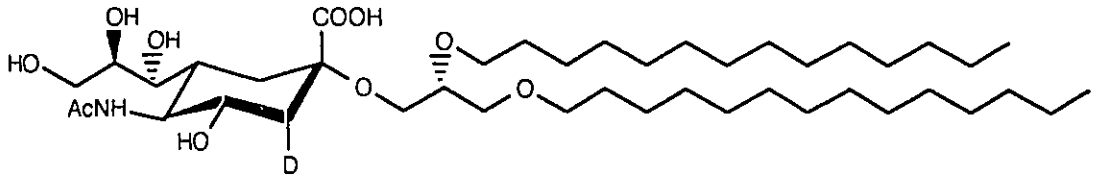
31 K. Furukawa, H. Yamagachi, H.F. Oettgen, L.J. Old and K.O. Lloyd, Cancer Res., **49** (1989) 191.
32 C. Wargalla, D.J. Sanders,R.A. Reisfeld, K. Mujos, T.J. Kipps, H.M. Yang and D.A. Cheresh, Cancer Res., **49** (1989) 2857.

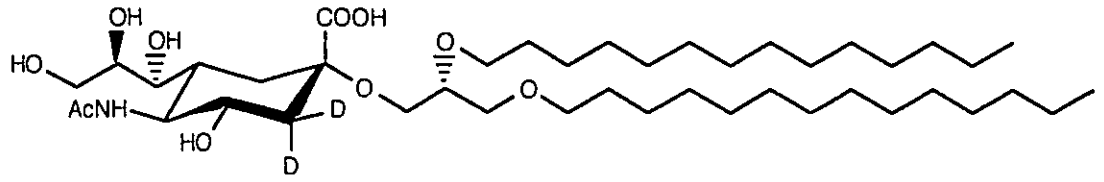
Table 1. Tumor-Associated Sialic Acid (NeuAc) Glycolipid Antigen.[1,25]

ANTIGEN	STRUCTURE	CANCER
GD_3 ganglioside	NeuAcα2→8NeuAcα2→3Galß1→ 4Glcß1→1Cer	Human Melanoma
GD_2 ganglioside	GalNAcß1→4Galß1→4Glcß1→1Cer 3 ↑ NeuAcα2→8NeuAcα2	Human Melanoma Human Neuroectoderm
GM_2 ganglioside	GalNAcß1→4Galß1→4Glcß1→1Cer 3 ↑ NeuAcα2	Breast Cancer Brain Tumor Human Melanoma
Sialyl Le^a	Galß1→3GlcNAcß1→3Galß1→4Glcß1 3 4 1 ↑ ↑ ↓ NeuAcα2 Fucα1 1Cer	Human Colorecto- adenocarcinoma
Sialyl Le^x	Galß1→4GlcNAcß1→3Galß1→4Glcß1 3 4 1 ↑ ↑ ↓ NeuAcα2 Fucα1 1Cer	Human Colorecto- adenocarcinoma

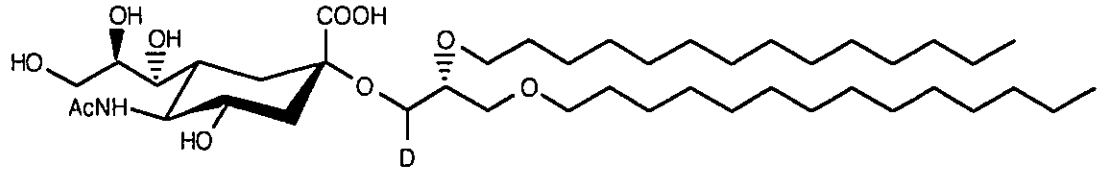
The ultimate objectives of ^2H NMR studies of membrane surfaces is to relate structural and motional properties of glycolipids in membranes to their biological roles. It seems that a fundamental prerequisite to such studies is to reach some understanding of the properties of these glycolipids in simpler systems. With this motivation, the deuteriated 1,2-di-O-tetradecyl-3-O-α-D-sialyl-*sn*-glycerol (DTSL) **1a**, **1b** and **1c** (Figure III) have been selected as deuteriated probes for the ^2H NMR study of sialic acid containing glycoglycerolipids in model membranes.



1a



1b



1c

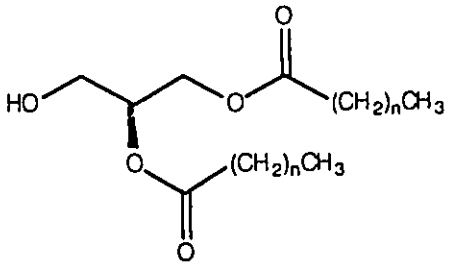
Figure III. Deuteriated Sialylglycerolipids. 1,2-Di-O-tetradecyl-3-O-[3ax-deutero-α-D-sialyl]-*sn*-glycerol, [^2H-NeuAc] DTSL, (**1a**); 1,2-di-O-tetradecyl-3-O-[3ax,3eq-dideutero-α-D-sialyl]-*sn*-glycerol, [$^2H_2$-NeuAc] DTSL (**1b**); 3-deutero-1,2-di-O-tetradecyl-3-O-α-D-sialyl-*sn*-glycerol [^2H-3G] DTSL (**1c**).

The main objective of this thesis was the chemical synthesis of the deuteriated sialylglycerolipids described above **1a-c**. The first step towards attaining that goal was the synthesis of the labeled and unlabeled glycerolipid moiety. The regio- and stereoselective deuterium incorporations in sialic acid and the subsequent transformation of the deuteriated sialic acids into their respective glycosyl donors followed. The glycosylation of the glycerolipids with the appropriate glycosyl donors completed the synthesis of the model sialylglycerolipids and prompted further investigations of glycosylation methods for the synthesis of sialyl glycosides. A phase transfer catalysis procedure particularly useful in the synthesis of thioglycosyl donors was developed and the applicability of the procedure to produce a wide range of S- and O-sialoside derivatives was explored. An extensive study of thioglycosylation reactions using a series of thiosialosyl donors, the glycerolipid and various thiophilic promoters resulted in the introduction of the concept of active and latent thiosialosyl donors which concluded this research.

CHAPTER 1 Synthesis of the Glycerolipid

1.1 Introduction

Glycolipids are a structurally diverse group of membrane components. They may be divided into two major classes, distinguished by the nature of their lipophilic anchor. The glycolipids of bacteria and plants generally consist of a mono- or oligosaccharide glycosidically linked to the 3-position of 1,2-di-O-acylglycerol **2** (Figure IV). Diacylglycerol-based glycolipids also occur in animal membranes, but only in very small quantities. The major glycolipids of animals consist of mono- or oligosaccharides whose reducing end is glycosidically linked to ceramide **3** a sphingosine base whose amino function is amide-linked to a fatty acid (Figure IV). The naturally occurring structures of membrane lipids were considered when the selection of a lipid moiety, required as glycosyl acceptor for the synthesis of the model deuteriated glycolipids, was made. The lipid chosen was a dialkyl glycerol derivative: 1,2-di-O-tetradecyl-*sn*-glycerol **4** (Figure IV).



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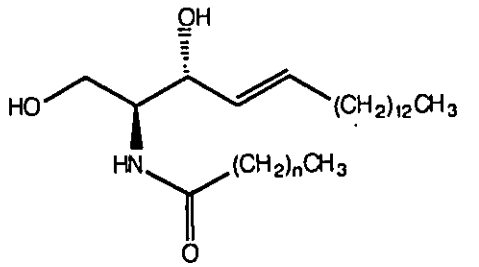

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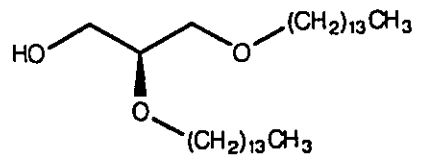

4

Figure IV. Comparison of Naturally Occurring and Model Lipids. Chemical Structures of a 1,2-Diacylglycerol **2**, a Ceramide **3** and the Glycerolipid 1,2-Di-O-tetradecyl-*sn*-glycerol **4**.

The glycerolipid **4** combines the glycerol backbone of the 1,2-di-O-acylglycerol, the stereocenter exhibited on the sphingosine and on the glycerol residue and the two long lipophilic chains. A dialkylglycerol lipid was selected rather than a diacylglycerol lipid for several reasons.

First, the attachment of the lipophilic chains to glycerol by an ether linkage is stable compared to the usual ester bond, and thus, further elaboration of the head group oligosaccharides is facilitated. The dialkylglycerol moiety would therefore serve as a stable lipophilic group used to anchor the monosaccharide head group to the membrane.

Second, the glycerolipid provides a model of the dialkylglycerolipid which have been isolated from bacteria such as *Halobacterium cutirubrum*[33] and *Thermoplasma acidophilum*[34].

Third, although the replacement of fatty acid chains with the corresponding alkyl chains in phospholipids does lead to some differences in the physical properties (i.e. gel to liquid-crystal phase transition temperature) of the lipid, these changes do not appear to be dramatic[35-37].

Finally, the selection of the glycerolipid 1,2-di-O-tetradecyl-*sn*-glycerol and its C-3 deuteriated derivative is further motivated by the fact that several studies have used that specific glycerolipid in the study of cell surface glycans[8-12], thus allowing for the possibility of comparison between glycoglycerolipids bearing various headgroups.

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35 D.L. Dorset and W.A. Pangborn, Chem. Phys. Lipids, **30** (1982) 1.
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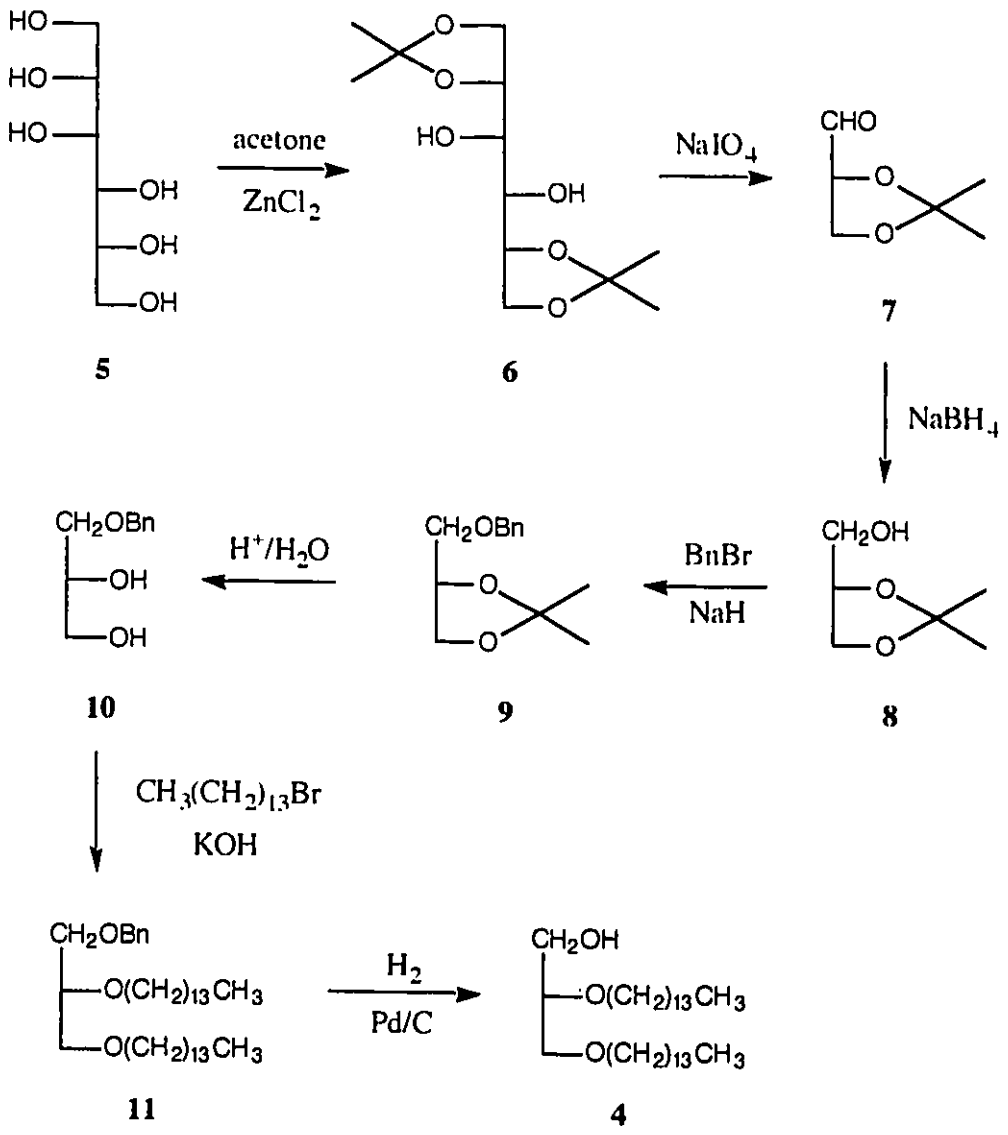
37 H. Eibl and A. Blume, Biochem. Biophys. Acta, **553** (1979) 476.

The glycerolipid 1,2-di-O-tetradecyl-*sn*-glycerol **4** has previously[38] been synthesized from D-mannitol **5** according to the procedure depicted in Scheme 1.



Scheme 1. Classical Procedure used in the Synthesis of 1,2-Di-O-tetradecyl-*sn*-glycerol **4**.

38 M. Kates, T.H. Chan and N.Z. Stanacev, Biochemistry, **2** (1963) 394.

This seven-step procedure involved the periodate cleavage of the C-C bond at the 3,4-position of 1,2;5,6-di-O-isopropylidene-D-mannitol **6** followed by the reduction of the aldehyde **7** to (R)-2,3-O-isopropylidene-*sn*-glycerol **8**. Hydrolysis of the benzyl protected (R)-2,3-O-isopropylidene-*sn*-glycerol **9** furnished the diol **10**. Finally, alkylation and deprotection of the diol **10**, yielded the desired glycerolipid **4**.

Although the previously used synthetic route yields the desired glycerolipid in an acceptable overall yield (20 to 30%), several inconveniences hinder the procedure. The kinetic acetalation of the D-mannitol **5** has to be carefully monitored to avoid the production of the triacetal, 1,2;3,4;5,6-tri-O-isopropylidene-D-mannitol as a side product. The synthetic conditions for the preparation of aldehyde **7** and alcohol **8** must be safe against decomposition, polymerization or oxidation. Furthermore, racemization of the alcohol **8** occurs at acidic pH-values resulting in a notable loss of optical rotation of the product[39]. The possibility of racemization is of great concern since retention of the chiral center at the 2-position of the glycerol moiety is necessary to obtain a glycerolipid that mimics the naturally occurring lipid portion of glycolipids. Moreover, the extremely high water solubility of the aldehyde **7** and the alcohol **8** also complicates the synthetic route by limiting the selection of available purification methods. Finally, since a C-3 labeled glycerolipid is also needed, the previously used synthesis lacks the necessary flexibility to accomplish the deuteriation. Although the introduction of the deuterium atom could be performed during the reduction of aldehyde **7**, the following four steps would have to be duplicated to yield both the glycerolipid **4** and its C-3 labeled derivative.

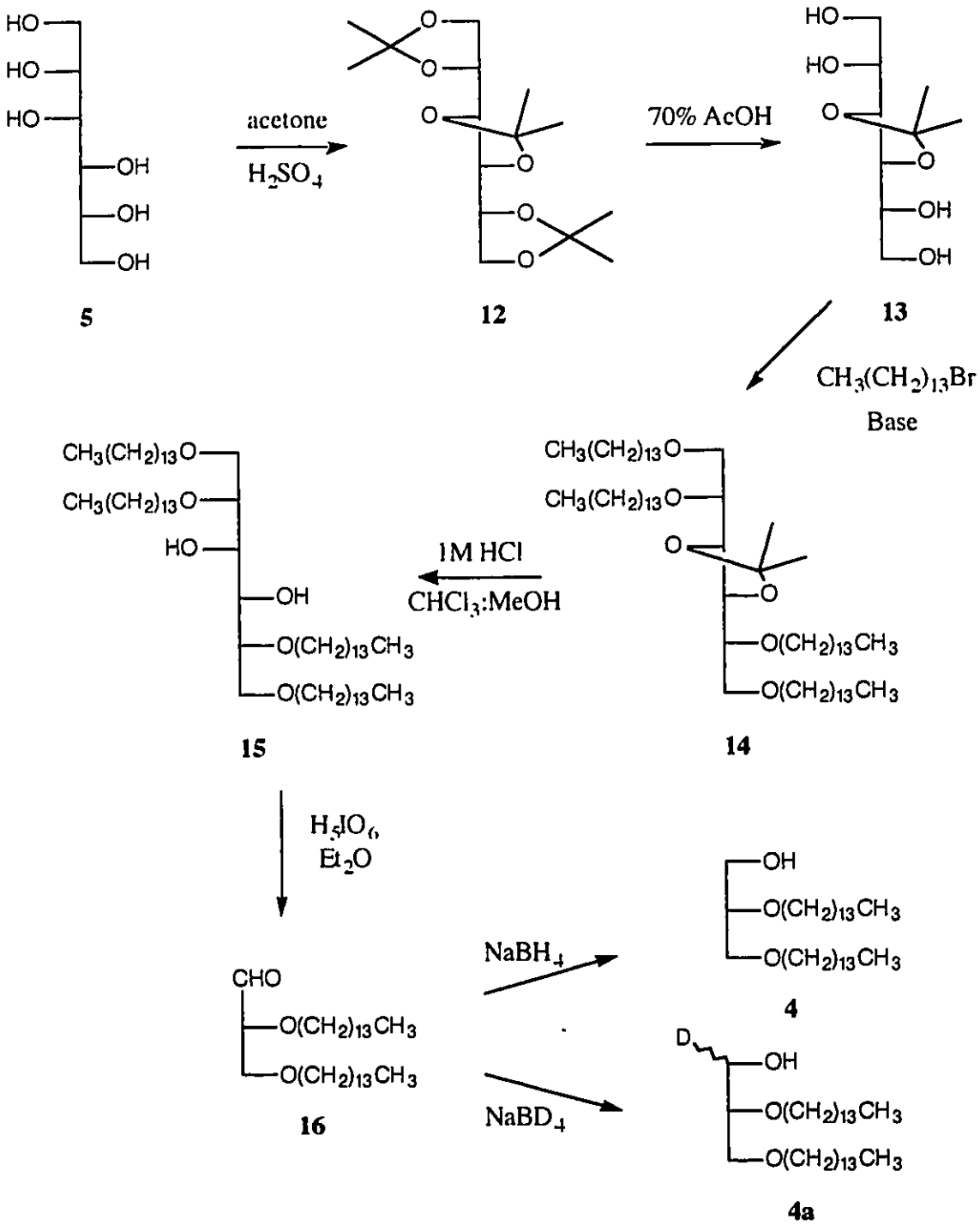
It was therefore apparent that improvements to the existing synthetic route were desirable. The development of a strategy involving less synthetic steps, avoiding the use of unstable intermediates having inconvenient properties to handle and which can lead to the loss of optical activity of the desired chiral product and allowing for the introduction of the deuterium label in the last step of the synthesis is presented in the following discussion.

39 H. Eibl, Chem. Phys. Lipids, **28** (1981) 113.

1.2 Discussion

1.2.1 Development of a Strategy for the Synthesis of the Glycerolipid

After consideration of the various problems pertaining to the previous synthesis of the glycerolipid, 3,4-O-isopropylidene-D-mannitol **13** was selected as key intermediate instead of the more usual 2,3-O-isopropylidene-D-glyceraldehyde **7**. The hexitol D-mannitol **5** was chosen as the starting material for the synthesis as it furnished a chiral center having the desired stereochemical requirements. Because of the C_2 axis of symmetry of D-mannitol, oxidative cleavage of the C_3-C_4 bond renders the desired chiral center accessible. The synthetic route proposed uses one less step then the previous synthesis[38] and allows for the introduction of the deuterium in the last step of the procedure. The synthetic route is depicted in Scheme 2.

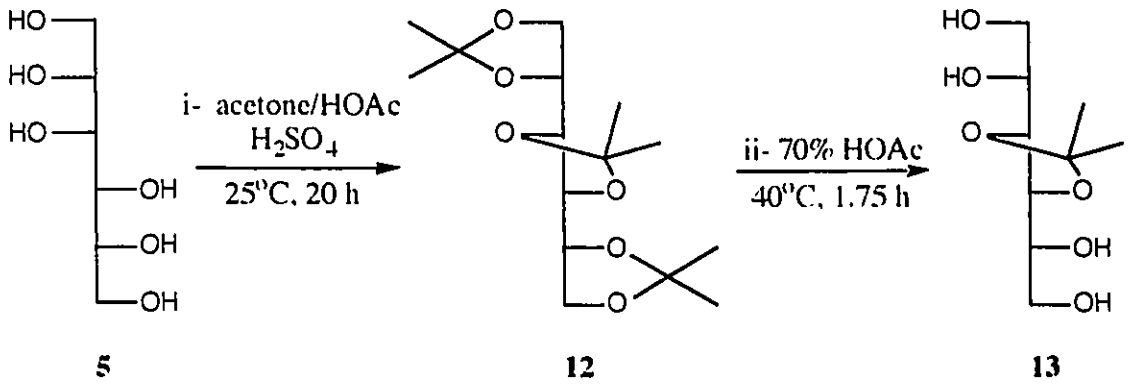


Scheme 2. Procedure used in the Synthesis of 1,2-Di-O-tetradecyl-*sn*-glycerol **4** and C-3 Deuteriated Derivative **4a**

1.2.2 Synthesis of Glycerolipid 4

- **Tris-acetonation/Kinetic De-acetonation**

The first stage of the procedure involves the tris-acetonation of D-mannitol (step i) followed by the kinetically controlled hydrolysis of the two primary isopropylidene groups (step ii) (Scheme 3).



5 **12** **13**

Scheme 3. Tris-acetonation/Kinetic De-acetonation.

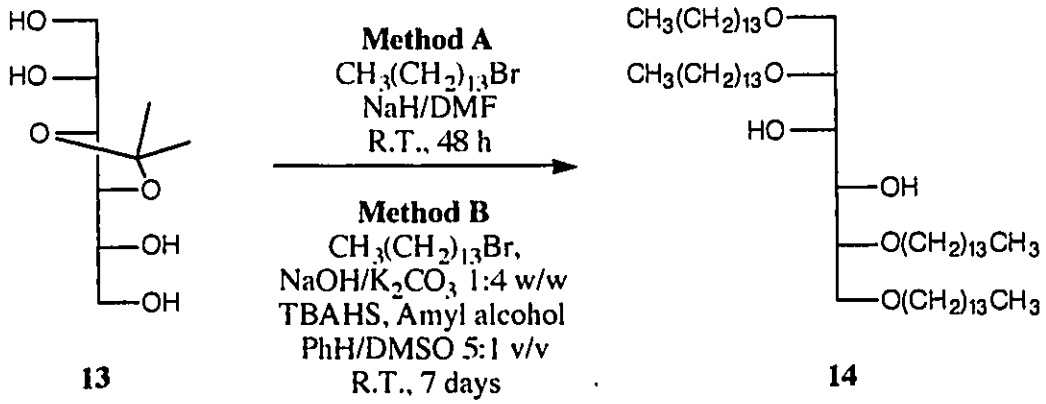
The conversion of the D-mannitol **5** to the 1,2;3,4;5,6-tri-O-isopropylidene-D-mannitol **12** was accomplished by a slight modification of a procedure developed by Mannock et al.[40]. The acetalation was performed under acid catalysis (concentrated H_2SO_4) using acetone as solvent. Acetic acid was also added to the reaction mixture to improve the solubility of the D-mannitol in acetone. It was found that preparing the concentrated H_2SO_4:acetic acid:acetone solution (2:1:40) at 0°C was necessary in order to avoid the polymerization of acetone. The reaction proceeded at room temperature. Although TLC indicated that even after 20 hours of stirring the reaction was not complete, the reaction mixture was neutralized because it appeared that a state of equilibrium had been reached between the various acetalated derivatives of D-mannitol. Addition of molecular sieves to the reaction mixture in an attempt to push the reaction to completion reduced the reaction time but failed to improve the yield of the reaction. Typically, yields of 65 to 67% were obtained. Usually, the tri-O-isopropylidene derivative was purified solely by crystallization[40,41] but the addition of a simple extraction step to remove the water soluble partially acetalated D-mannitol derivatives prior to crystallization was found to slightly improve the purity of 1,2;3,4;5,6-tri-O-isopropylidene-D-mannitol **12**. The melting point and optical rotation were in accord with

40 D.A. Mannock, R.N.A.H. Lewis and R.N. McElhaney, Chem. Phys. Lipids, **43** (1987) 113.
41 J. Kuszmann and E. Tomori, Carbohydr. Res., **137** (1985) 276.

literature[40,41]. The ^1H NMR spectrum (200 MHz, $CDCl_3$) confirmed the triple acetalation of D-mannitol; the six methyl groups appeared at δ (ppm) 1.39, 1.35, 1.31 as three singlets, each integrating for six protons.

The kinetic de-acetonation of the tri-O-isopropylidene **12** was first reported by Wiggins[42]. A modification of that procedure, described by Mannock et al.[40] was used. Hydrolysis of the primary isopropylidene groups of tri-O-isopropylidene **12** was effected in a 70% aqueous solution of acetic acid at 40°C over a period of 1.75 hours. Tetrol **13** was crystallized from acetone/benzene in good yields (86%) after complete neutralization of the acetic acid and evaporation of the solvent. A small amount of D-mannitol, obtained from complete hydrolysis of tri-O-isopropylidene **12** was easily separated from the final product before the crystallization by filtration of an acetone solution of the evaporated crude product. The melting point and optical rotation of pure tetrol **13** is comparable to those obtained by Mannock et al.[40]. The presence of a unique singlet (δ (ppm) 1.42, 6H) in the ^1H NMR (200 MHz, D_2O) spectrum of tetrol **13** indicated the complete hydrolysis of the two primary isopropylidene groups.

- **Alkylation of 3,4-O-Isopropylidene-D-mannitol 13**



Scheme 4. Alkylation of Tetrol **13**

Alkylation of the tetrol **13** to form 1,2;5,6-tetrakis-O-tetradecyl-3,4-O-isopropylidene-D-mannitol **14** was carried out under fairly mild conditions, at room temperature in the presence of sodium hydride and 1-bromotetradecane (Method A). These conditions avoided the use of high temperatures which had been found to increase the formation of side products. The reaction was complete in two days and provided the tetraalkylated derivative **14** as a clear oil (76% yield) after removal of the excess 1-bromotetradecane by silica gel column chromatography.

42 J.F. Wiggins, J. Chem. Soc., (1946) 13.

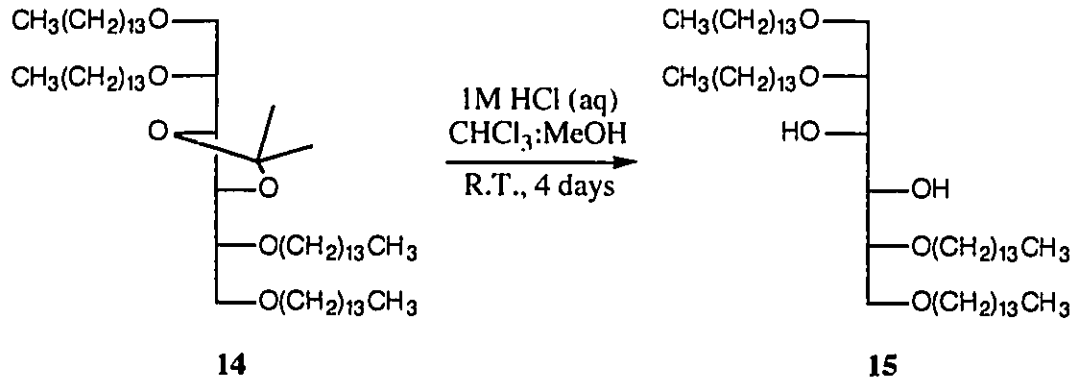
The alkylation was also attempted under phase transfer catalysis as previously described by Szeja et al.[43] for the benzylation of sugar polyols (Method B). The solid-liquid reaction mixture was composed of powdered NaOH/K_2CO_3 (1:4 w/w), benzene and DMSO as co-solvents (5:1 v/v), amyl alcohol, tetrabutylammonium hydrogen sulfate (TBAHS) as phase transfer catalyst, 1-bromotetradecane (1.2 eq per OH group) and the tetrol **13**. The role of the tertiary alcohol, according to Szeja et al.[43] is to assist in bringing measurable amounts of the hydroxide ions in the non polar phase without forming the corresponding alkylated ether. Efficient stirring of the reaction mixture was necessary to maximize the surface area of the solid phase exposed to the organic reaction mixture. The reaction, performed at room temperature, was very slow. After a week, the reaction was quenched and a 33% yield of the tetraalkylated product **14** was recovered. Since the only difference between the published results of Szeja et al.[43] who obtained a 80% yield of 1,2,5,6-tetra-O-benzyl-3,4-O-isopropylidene-D-mannitol in 12 hours lies in the selection of the alkylating agent, it is presumed that the difference of reactivity between the benzyl bromide and the alkylbromide explain the longer reaction time observed with the 1-bromotetradecane.

The ^1H NMR (200 MHz, $CDCl_3$) spectrum of the 1,2;5,6-tetrakis-O-tetradecyl-3,4-O-isopropylidene-D-mannitol **14** clearly showed the presence of the tetradecyl groups since it displayed characteristic intense methylene signals centered at δ = 1.22 ppm integrating for 88 methylene protons and a triplet appearing at 0.85 ppm representing 12 methyl protons.

43 W. Szeja, I. Fokt and G. Grynkiewicz, Recl. Trav. Chim. Pays-Bas, **108** (1989) 224.

- **Synthesis of 1,2,5,6-Tetrakis-O-tetradecyl-D-mannitol 15**

The next step of the synthetic route involves the hydrolysis of the oily tetraalkylated isopropylidene D-mannitol derivative **14**.



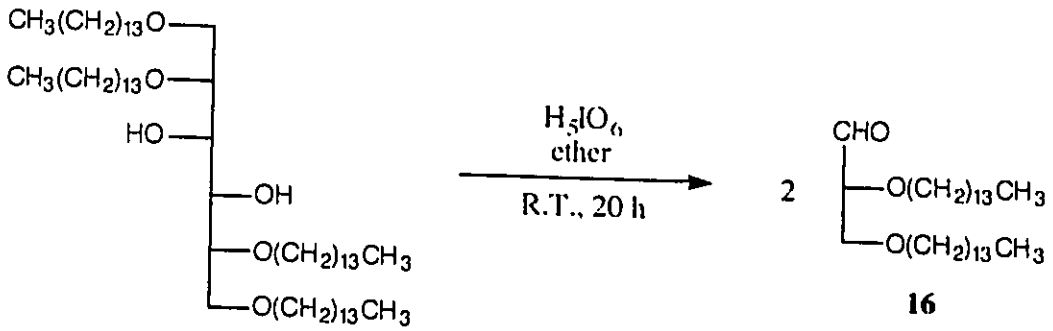
14 **15**

Scheme 5. Hydrolysis of 1,2,5,6-Tetrakis-O-tetradecyl-3,4-O-isopropylidene-D-mannitol **14**

The hydrolysis of the tetraalkylated D-mannitol derivative **14** is hindered by an obvious solubility problem lying in the hydrophobicity of the derivative **14.** In order to overcome the problem, a reaction medium wherein both the derivative **14** and an aqueous acid solution are soluble had to be designed. To achieve such a medium, a biphasic mixture of a solution of the tetraalkylated D-mannitol derivative **14** in chloroform and of a 1M HCl solution was diluted with methanol until the phases became miscible and formed an homogeneous solution. The reaction proceeded at reflux and was complete within 4 days. The oil obtained was crystallized from ethanol to give the diol **15** (m.p. 41.3-42.2°C, $[\alpha]_D$ -8.1° (c1.0, chloroform)) in 91% yield. The completion of the hydrolysis was confirmed by ^1H NMR spectroscopy (200 MHz, $CDCl_3$) since the singlet found at 1.35 ppm in the ^1H NMR spectrum of the tetraalkylated D-mannitol derivative **14**, which indicated the presence of the acetonide group was no longer present in the spectrum of diol **15**.

- **Oxidative Cleavage of 1,2,5,6-Tetrakis-O-tetradecyl-D-mannitol 15**

The oxidative cleavage of the vicinal hydroxyl groups of diol **15** was performed in the presence of the periodate anion.



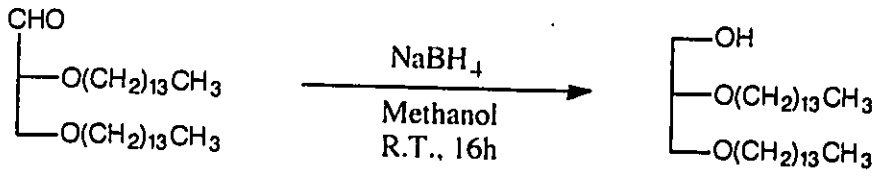
15 **16**

Scheme 6. Oxidative Cleavage of 1,2,5,6-Tetrakis-O-tetradecyl-D-mannitol **15**.

In order to perform the oxidative cleavage, the commonly used sodium periodate could not be utilized because of the great differences in solubility of diol **15** and of the periodate salt. Therefore, instead of using that salt, the acid form of the periodate salt was selected to accomplish the desired reaction in dry ether. Under such conditions, the reaction progressed smoothly and produced a high yield (88%) of aldehyde **16** in 20 hours. The aldehyde obtained solidified spontaneously (m.p. 28.0-29.6°C, $[\alpha]_D$ +9.4° (c1.0, chloroform)) and was sufficiently pure to be used in the next step without further purification. The formation of the aldehyde was confirmed by ^{1}H NMR. The characteristic aldehyde signal was found at δ = 9.69 ppm. Furthermore, the ^{13}C NMR spectrum of aldehyde **16** displayed a signal at δ = 203.4 ppm indicating the presence of the carbonyl function.

- **Reduction of 2,3-Di-O-tetradecyl-D-glyceraldehyde 16**

The last step of the synthesis involves the reduction of aldehyde **16**.



16 **4**

Scheme 7. Reduction of 2,3-Di-O-tetradecyl-D-glyceraldehyde **16**.

Sodium borohydride was used as reducing agent to produce the glycerolipid **4**. The reaction was performed at room temperature and yielded, after 6 hours, the alcohol **4** in 93% yield.

Purification was accomplished by crystallization from methanol/ether and gave the pure alcohol as white flaky crystals, m.p. 42.0-42.6°C. The melting point measured was identical to that found in the literature[44].

The optical rotation of the final product ($[\alpha]_D$ -9.3° (c1.0, chloroform)) is higher than that obtained for the same product when prepared by the previously used procedure (lit.[44] $[\alpha]_D$ -7.79° (c0.95, chloroform)). The improvement in optical rotation is a reflection on the stability of the reaction intermediates which were carefully selected in order to avoid any possibility of racemization or loss of chirality during the synthesis.

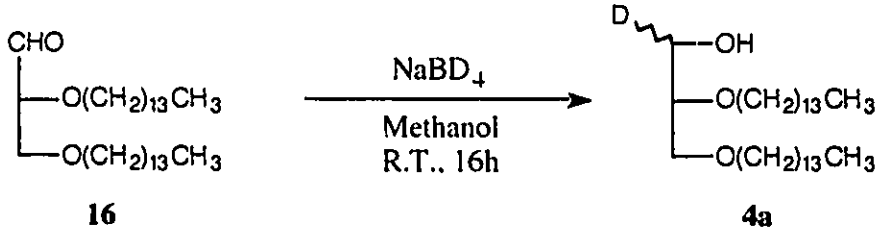
The disappearance of the aldehyde proton signal on the ^1H NMR spectrum of the crude alcohol **4** confirmed the completion of the reduction. The ^1H NMR spectrum of the pure alcohol **4** displayed multiplets at δ = 3.64-3.37 ppm representing the glycerol and the first methylene protons of the alkyl chains and multiplets appearing at 1.52, 1.22 and 0.84 ppm depicted the other protons of the lipid chains ($OCH_2(C\underline{H_2})_{13}C\underline{H_3}$).

The ^{13}C NMR spectrum also confirmed the formation of the alcohol as the signal indicating the presence of the carbonyl function, which appeared on the ^{13}C NMR spectrum of aldehyde **16** is not found on the ^{13}C NMR spectrum of alcohol **4**.

Mass spectrometry and chemical analysis were further used to fully characterize alcohol **4** and confirmed the successful synthesis of the desired glycerolipid **4**.

1.2.3 Synthesis of Labeled Glycerolipid 4a

The synthesis of the labeled glycerolipid **4a** was also accomplished through reduction of aldehyde **16**.



16 **4a**

Scheme 8. Deuteride Reduction of 2,3-Di-O-tetradecyl-D-glyceraldehyde **16**.

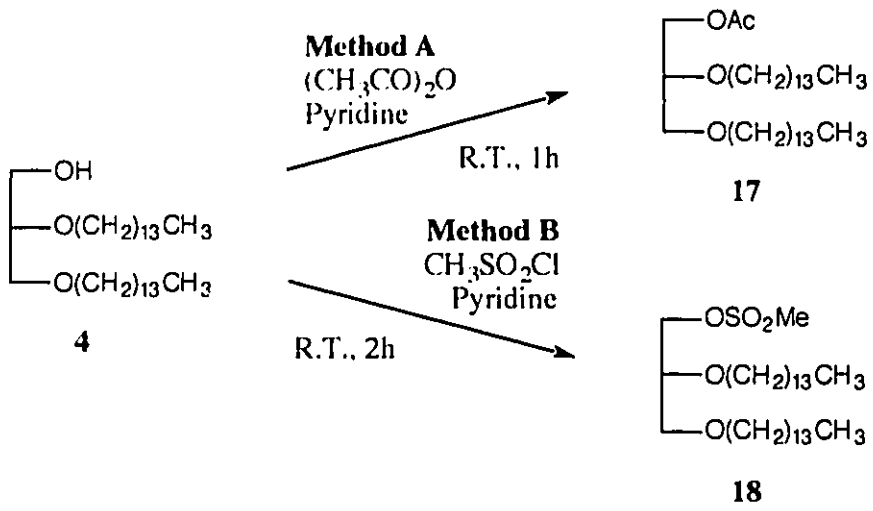
The use of sodium borodeuteride as reducing agent in the same reaction conditions as those used for the synthesis of the unlabeled glycerolipid, yielded the deuteriated alcohol **4a** as white crystals (m.p. 42.0-42.6°C, $[\alpha]_D$ -9.3° (c1.0, chloroform)) in 91% yield.

The absence of an aldehyde signal, which appeared at δ = 9.69 ppm in the ^{1}H NMR spectrum of aldehyde **16**, used as starting material, in the ^{1}H NMR spectrum of the crude labeled glycerolipid **4a** indicated the completion of the reduction reaction. The proton nuclear magnetic resonance spectrum of 3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol **4a** displayed a multiplet at δ = 3.61-3.37 ppm representing the glycerol and first methylene protons (integrating for 8 protons) and multiplets centered at δ = 1.54, 1.22 and 0.84 ppm confirming the presence of the remaining tetradecyl chain protons. The mass spectrum (C.I./ether) of labeled glycerolipid **4a** displayed the molecular ion peak at 486 rather than at 485 as it appeared in the mass spectrum of unlabeled glycerolipid **4**. Although the integration of the ^{1}H NMR spectrum and the molecular ion of the mass spectrum indicated clearly that a deuterium atom was introduced in the glycerolipid molecule it was impossible to determine whether any stereoselection had taken place during the reduction. Such stereochemical information which is usually readily available from ^{1}H NMR spectroscopy, was not accessible in the present situation because the glycerol and first methylene protons of the glycerolipid highly overlapped to form a complex multiplet. In order to verify the possibility that some diastereofacial stereoselectivity had occurred during the deuteride reduction of aldehyde **16**, a method to simplify the complex multiplet or rather to separate the H-3 glycerol proton from the multiplet was necessary.

1.2.4 Assignment of the H-3 Glycerol Protons of Glycerolipids 4 and 4a

- Methods for the Characterization of the H-3 Glycerol Protons

The simplest method to accomplish a simplification of the ^{1}H NMR spectrum of the glycerolipid was to derivatize the glycerolipid alcoholic function with a group that would cause a downfield shift of the H-3 signal in the ^{1}H NMR spectrum of the substituted glycerolipid. The groups selected to be used as substituents were the acetyl (Method A) and the methylsulfonyl (Method B) groups. To verify and compare the effect of each of these groups, the acetylation and sulfonation were first attempted on the unlabeled glycerolipid **4** (Scheme 9).



Scheme 9. Substitution of Glycerolipid **4**.

The acetylation reaction (Method A) was performed with acetic anhydride in pyridine. After one hour of stirring at room temperature, the acetate **17** was obtained as a clear oil ($[\alpha]_D$ +0.4° (c1.0, chloroform)) in quantitative yield. Acetylation of glycerolipid **4** was confirmed by the ^1H NMR spectrum which showed a singlet at δ = 2.02 ppm characteristic of an acetyl group and by the ^{13}C NMR spectrum of acetate **17** where the carbonyl carbon signal appeared at δ = 171.1 ppm.

Sulfonylation of glycerolipid **4** was carried out with methylsulfonyl chloride in the presence of a base, pyridine. The reaction proceeded at room temperature and yielded, after two hours, the methylsulfonate **18** (85% yield) which was recrystallized from ether/methanol to give white crystals (m.p. 141.1°C, sint'd 106.3-107.7°C, $[\alpha]_D$ -0.9° (c1.0, chloroform)). In the ^1H NMR spectrum of methylsulfonate **18**, a singlet indicating the presence of the methyl group of the methylsulfonate moiety was found at δ = 3.01 ppm. Evidence for the sulfonylation of glycerolipid **4** was also found from the ^{13}C NMR spectrum of methylsulfonate **18** which showed its methyl signal at δ = 37.0 ppm.

As expected, the H-3 protons of both acetate **17** and methylsulfonate **18** exhibited a downfield shift in their respective ^1H NMR spectra. The ^1H NMR spectra of the H-3 protons of the unsubstituted, acetyl and methylsulfonyl substituted glycerolipids are shown in Figure V.

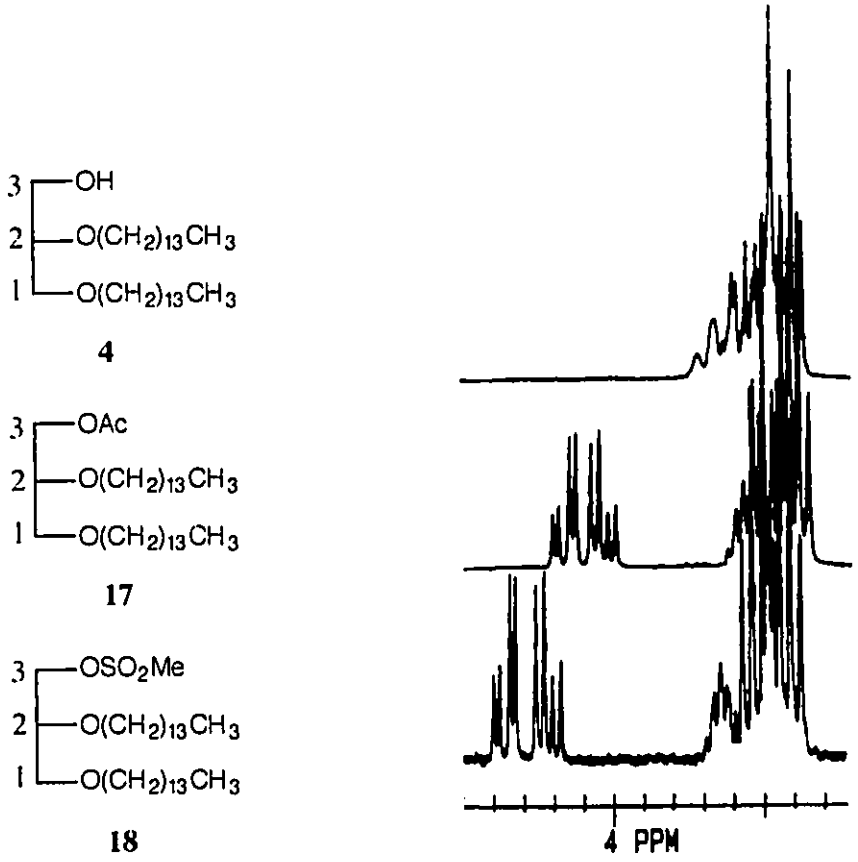
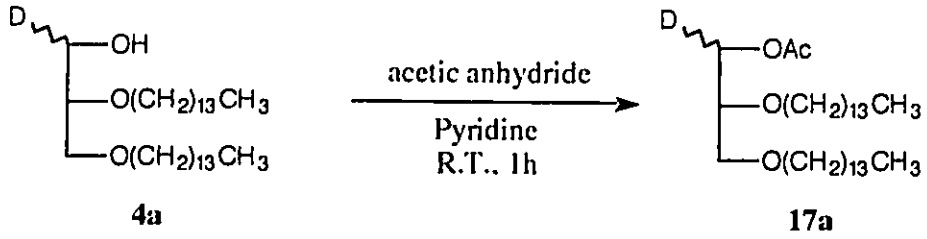


Figure V. ^{1}H NMR Spectra of the Unsubstituted, Acetyl and Methylsulfonyl Substituted Glycerolipids showing the H-3 Region

In the ^{1}H NMR spectrum of the acetylated glycerolipid **17**, the H-3 protons appeared downfield to 4.18 ppm and 4.04 ppm relative to the complex multiplet of glycerolipid **4**. Each of the H-3 protons clearly appeared as a doublet of doublets at 4.18 ppm (J_{gem} = 11.6 Hz, $J_{2,3}$ = 4.1 Hz) and at 4.04 ppm ($J_{2,3}$ = 5.6 Hz). In the ^{1}H NMR spectrum of methylsulfonate **18**, the shift exhibited by each H-3 protons was approximately 0.7 ppm downfield. The protons also formed two doublets of doublets, centered at 4.36 ppm (J_{gem} = 10.9 Hz, $J_{2,3}$ = 3.6 Hz) and at 4.22 ppm ($J_{2,3}$ = 5.6 Hz).

As the two methods proved very efficient at accomplishing the desired downfield shift of the H-3 protons from the complex multiplet which hindered the interpretation of the ^{1}H NMR spectrum of glycerolipid **4**, the acetylation method was selected to facilitate the assignment of the H-3 glycerol protons of the labeled glycerolipid because of its simplicity of manipulation and of purification.

- **Acetylation of Deuteriated Glycerolipid 4a**



4a **17a**

Scheme 10. Acetylation of Deuteriated Glycerolipid **4a**

The acetylation of labeled glycerolipid **4a** was performed under the same conditions as the acetylation of glycerolipid **4**. The product was obtained in quantitative yield. The H-3 protons exhibited a shift of approximately 0.6 ppm. The H-3 signals converged to two doublets of almost identical intensity (integration 41:39) at $\delta = 4.17$ ppm ($J_{2,3} = 4.1$ Hz) and at $\delta = 4.05$ ppm ($J_{2,3} = 5.7$ Hz). The partial ^{1}H NMR spectra of the unsubstituted and acetyl substituted deuteriated glycerolipids **4a** and **17a** are shown in Figure VI.

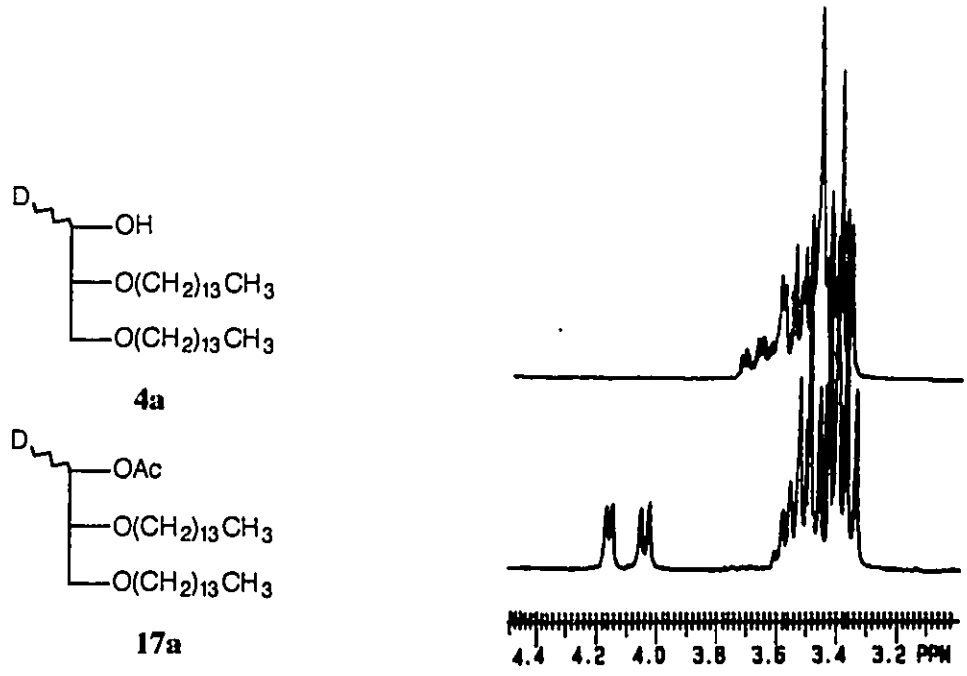


Figure VI. ^{1}H NMR Spectra of Unsubstituted and Acetyl Substituted Deuteriated Glycerolipids **4a** and **17a** showing the H-3 Region

Theses results are indicative of no strong preferential stereochemical induction.

In order to see whether any diastereofacial stereoselectivity can be induced during the reduction, a study of different chelating agents and reaction conditions was made.

1.2.5 Study of Stereochemical Induction in the Borodeuteride Reduction

1.2.5.1 Temperature and Chelation Study

The deuteriation reactions were performed at various temperatures and in the presence of different chelating agents in order to induce some diastereofacial stereoselectivity in the labeled glycerolipid. The results of the various stereochemical induction attempts are summarized in Table 2.

Table 2. Temperature and Induction Study of Deuteride Reduction

	Room Temperature		**-78°C**	
Reducing Agent	Ratio of peaks at δ = 4.17 vs 4.04 ppm	Stereoselectivity Pro-R: Pro-S	Ratio of peaks at δ = 4.17 vs 4.04 ppm	Stereoselectivity Pro-R: Pro-S
$NaBD_4$	1:1	50%:50%	-	-
$NaBD_4/MgBr_2$	-	-	2:3	60%:40%
$Zn(BD_4)_2$	2:3	60%:40%	1:2	67%:33%

- **Low Temperature Reduction using Sodium Borodeuteride**

The original reduction, which was performed at room temperature, in the presence of sodium borodeuteride in methanol was repeated at a lower temperature, -78°C (CO_2/acetone bath) in an attempt to reduce molecular motion and thereby observe some stereoselectivity. As the initial solution of aldehyde **16** was cooled, the insolubility of the aldehyde became obvious. In spite of that problem, the reducing agent was introduced into the reaction flask and the reaction was followed by TLC. It soon became apparent that no reaction was occurring, therefore, after 2 days,

the reaction was quenched and the reaction products were isolated. A ^{1}H NMR spectrum of the resulting oil showed the presence of aldehyde **16**. The reaction product was acetylated in order to render any trace of deuteriated product detectable by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum revealed no trace of acetate signal. The reaction with sodium borodeuteride was also tried at 0°C in an attempt to increase the solubility of the aldehyde in the solvent but it also resulted in a failure.

- Borodeuteride Reduction in the Presence of a Chelating Agent

In order to favor induction of diastereoselectivity (chelation control) during the reduction of aldehyde **16**, the reduction was performed in the presence of various chelating agents. The chelating agents selected were magnesium and zinc salts.

- a - Magnesium Salt as Chelating Agent

The reduction of aldehyde **16** using a magnesium salt as chelating agent was performed in the presence of magnesium bromide etherate in anhydrous tetrahydrofuran at -78°C. The reaction was followed by TLC and was deemed complete after a period of 10 hours. The labeled glycerolipid was isolated from the reaction mixture and acetylated according to Method A. The acetylated product was obtained in 90% yield. The ^{1}H NMR spectrum of the diastereomeric mixture of acetates exhibited two doublets at δ = 4.17 (0.4 H, $J_{2,3}$ = 4.1 Hz) and at 4.05 ppm (0.6 H, $J_{2,3}$ = 5.7 Hz), integrating for a total of one proton in a ratio of 2:3 respectively. The difference of intensity of the proton signals confirmed that some diastereofacial stereoselectivity has taken place during the reduction. The stereoselectivity of the reduction was 60% in favor of the deuteron located at the same position on the glycerol moiety of the acetate as the proton appearing at δ = 4.17 ppm (pro-R) and the level of induction for the deuteron located at the same position as the proton displaying a peak at δ = 4.05 ppm (pro-S) was of 40%.

- b - Zinc Salt as Chelating Agent

Zinc borodeuteride was selected as both reducing and chelating agent for the stereoselective deuteriation of aldehyde **16**. The zinc borodeuteride was prepared according to a slight modification of a procedure developed by Gensler et al.[45]. The publication of Gensler et al.

45 W.J. Gensler, F. Johnson and A.D.B. Sloan, J. Am. Chem. Soc., **82** (1960) 6074.

describes the preparation of zinc borohydride using as starting materials zinc chloride and sodium borohydride. The addition of an etherial solution of the borohydride to a refluxing solution of zinc chloride produced, after 10 hours, a solution of zinc borohydride. The insoluble sodium chloride formed was left to settle and the zinc borohydride was used as a clear solution. The use of sodium borodeuteride instead of sodium borohydride yielded the corresponding deuteriated zinc salt according to the following equation:

$$ZnCl_2 + 2\ Na(BD_4) \rightarrow Zn(BD_4)_2 + 2\ NaCl$$

The zinc borodeuteride was used to reduce aldehyde **16**. The reaction was performed either in diethyl ether or tetrahydrofuran, at -78°C. Within 3 days, the reaction was deemed complete and the isolated labeled glycerolipid was acetylated according to Method A. The acetate was obtained in yields varying from 70 to 80%. The ^1H NMR spectrum of the diastereomeric mixture of acetates displayed two doublets characteristic of the H-3 protons of the glycerol moiety at $\delta = 4.15$ (0.33 H, $J_{2,3} = 4.1$ Hz) and 4.04 ppm (0.67 H, $J_{2,3} = 5.7$ Hz), integrating for a total of one proton in a ratio of 1:3 respectively. The occurrence of stereoselectivity in the reduction was also confirmed by the difference in the intensity of the two doublets. The integration ratio indicated that the deuteriation occurred preferentially for the signal appearing at $\delta = 4.15$ ppm since that proton signal only integrates for 0.33 proton. The stereoselectivity of the reduction was therefore 67% in favor of the pro-R deuteron and the level of induction for the pro-S deuteron was of 33%. The use of tetrahydrofuran as solvent instead of diethyl ether did not improve the stereoselectivity of the reaction. Figure VII illustrates the difference of stereoselectivity observed in the ^1H NMR spectra of the deuteriated derivatives as obtained from the room temperature sodium borodeuteride experiment, where no stereoselectivity took place, and the -78°C zinc borodeuteride reaction were the highest induction was observed.

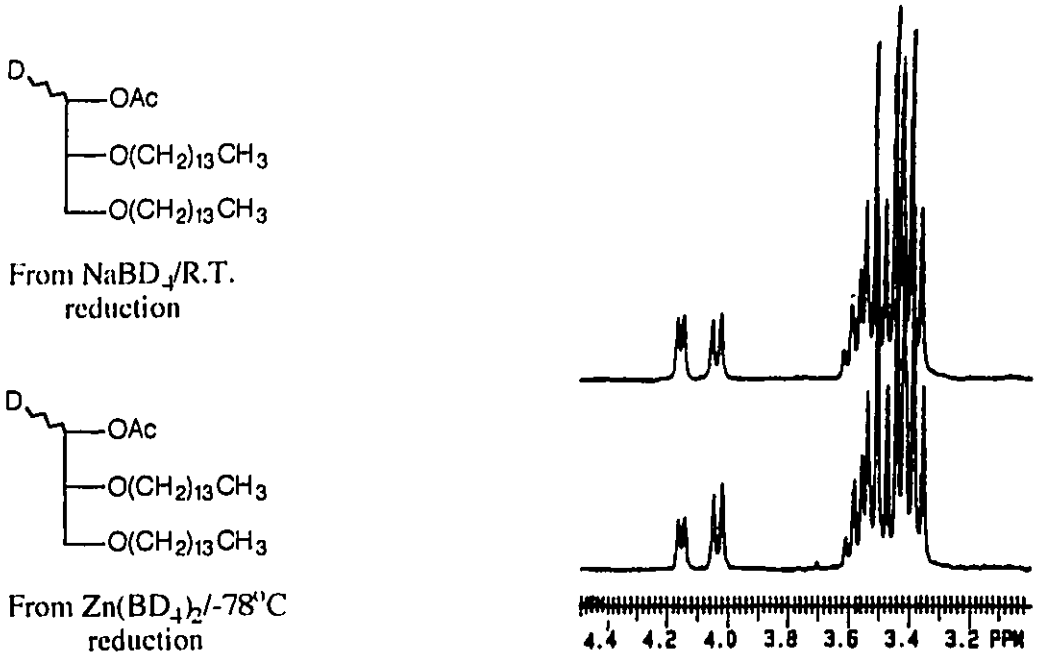


Figure VII. Comparison of the ^1H NMR spectra of the two Deuteriated Acetates obtained from the Room Temperature Sodium Borodeuteride and the -78°C Zinc Borodeuteride Reductions.

To verify that the stereoselectivity of the deuteriation reaction performed at -78°C in the presence of zinc borodeuteride was not only a factor of the reduced temperature and was indeed, at least in part, caused by the addition of the chelating agent, the reduction with zinc borodeuteride was repeated at room temperature. The reaction was completed within a period of one hour. Following the reduction, the diastereomers obtained were acetylated. As expected, the reduction was also observed to be stereoselective. The ^1H NMR spectrum of the diastereomeric mixture of acetates displayed the H-3 protons at $\delta = 4.15$ ppm (dd, 0.4 H, $J_{2,3} = 3.9$ Hz) and 4.04 ppm (dd, 0.6 H, $J_{2,3} = 5.6$ Hz) and their integration confirmed that the extent of the deuteriation was slightly lower than that obtained for the reaction performed at -78°C. The stereoselectivity of the reduction was found to be 60% in favour of the pro-R deuteron and the level of induction for the pro-S deuteron was of 40%.

1.2.5.2 Tentative Assignment of the Stereochemistry of the Deuteriated Glycerolipid Obtained in Reductions Performed in the Presence of Chelating Agents

For both reactions performed in the presence of chelating agents some diastereofacial stereoselectivity was observed. In all cases, the deuteriation favored the same *re*-face attack providing the pro-R diastereomer preferentially. The tentative assignment of the stereochemistry was made using Newman projections of the chelate and by comparison to a publication by Uzawa et al.[46] wherein the synthesis and characterization of chirally deuteriated glycerols was made.

- **Newman Projections used in the Assignment of the Stereochemistry of the Deuteriated Glycerolipid**

The prediction of the chirality induced during the reduction reaction required comparison of the level of hindrance of the two faces of the carbonyl group of the aldehyde starting material. No stereoselectivity was observed when the deuteriation was performed in the absence of chelating agent. Therefore, it was reasonable to assume that the stereoselectivity observed during the reduction performed in the presence of a chelating agent was caused by the addition of the agent. The chelation was believed to take place between the carbonyl oxygen and the oxygen of the tetradecyloxy group located at C-2 carbon relative to aldehyde to form a transient organometallic complex (Figure VIII).

46 H. Uzawa, Y. Nishida, S. Hanada, H. Ohrui and H.Meguro, J. Chem. Soc., Chem. Commun., (1989) 862.

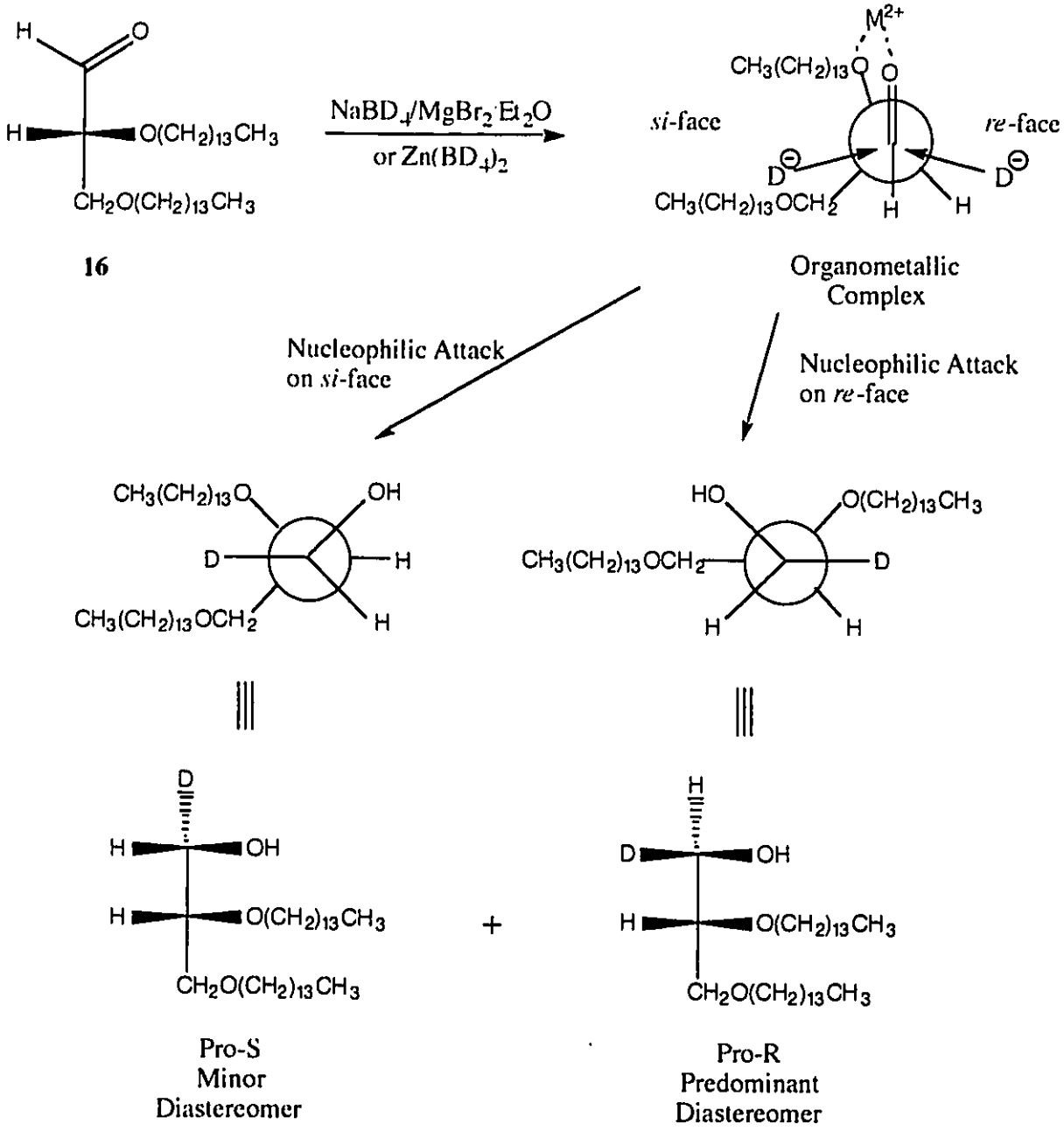


Figure VIII. Prediction of the Chirality of the Diastereomers using Newman Projections.

As it can be seen on Figure VIII, the addition of the metal cation to aldehyde **16** may form a 5-membered ring with the carbonyl oxygen and the oxygen of the tetradecyl group located on C-2 of the glyceraldehyde moiety. Because of the formation of the chelate, the rotation about the C1-C2 bond of the aldehyde which could occur freely before chelation was reduced. The spacial

orientation of the three groups substituting the 2-position of the glyceraldehyde was therefore fixed. The two faces of the carbonyl group were thereby sterically different since the groups encumbering the faces, a tetradecyloxymethyl group and a hydrogen atom, were of considerably different size. Because of the size differences, it was expected that the reduction would proceed preferentially on the least hindered face of the carbonyl group. It was therefore possible to conclude that the deuteriation occurred preferentially from the *re*-face of the carbonyl group and that the deuteron would be located at the pro-R position of the C-3 carbon. From the ^{1}H NMR spectrum of **17a**, it was possible to observe that the signal appearing at δ = 4.17 ppm was indicative of the favored position of deuteriation. Thus, it was possible to assign the signals displayed at δ = 4.17 ppm and at 4.05 ppm respectively to the pro-R and pro-S positions of the glycerol moiety.

- **Assignment of Stereochemistry of the Deuteriated Glycerolipid by Comparison to Published Characterization of Chirally Deuteriated Glycerols[4-6].**

The publication by Uzawa et al.[46] described a synthesis of the chirally deuteriated *sn*-glycerols (1-pro-S)-deutero-*sn*-glycerol and (1-pro-R)-deutero-*sn*-glycerol using as key intermediate (6S)-deuteriated 1,6-anhydro-β-D-galactopyranose.

The ^{1}H NMR spectra of the deuteriated-*sn*-glycerol derivatives allowed, by comparison with the spectrum of *sn*-glycerol, the unequivocal assignment of the four prochiral protons of *sn*-glycerol (H-1-pro-R, H-1-pro-S, H-3-pro-R, H-3-pro-S). The ^{1}H NMR spectrum of *sn*-glycerol displayed a multiplet at δ = 3.78 ppm (confirming the presence of the H-2 proton), a doublet of doublets at δ = 3.65 ppm and another doublet of doublets at δ = 3.56 ppm. The comparison of the ^{1}H NMR spectra revealed that the proton signals for the equivalent H-1-pro-S and H-3-pro-R protons were at lower field than the equivalent H-1-pro-R and H-3-pro-S protons. This conclusion was made because the intensity of the signal found at δ = 3.65 ppm diminished for the (1-pro-S)-deuteriated *sn*-glycerol derivative and similarly, the intensity of the signal found at δ = 3.56 ppm diminished for the (1-pro-R)-deuteriated *sn*-glycerol derivative.

The conclusion of the above study can easily be applied to the interpretation of the ^{1}H NMR spectrum of **17a** and to the determination of the prochirality of the C-3 deuterium labels placed on the *sn*-glycerol moiety of the deuteriated glycerolipids. The conclusion of the study was that the H-3-pro-R protons were found at a lower field than the H-3-pro-S protons in glycerol. But first, the assumption that the presence of the acetyl group which was placed on the C-3 hydroxy group of the free glycerolipid to shift the H-3 signals downfield to simplify its ^{1}H NMR

spectrum, influenced the chemical shifts of the two H-3 protons in a similar manner. Therefore, the downfield shift of the H-3 protons of the acetylated glycerol derivative owed to the presence of the acetyl group should not change the position of the two H-3 glycerol proton signals relative to each another. The signals displayed at δ = 4.17 ppm and 4.05 ppm can therefore respectively be assigned to the pro-R and pro-S positions of the glycerol moiety of **17** and **17a**. Experimentally it was observed that the ^{1}H NMR signal found at δ = 4.17 ppm indicated the most favored position of deuteriation. It was therefore possible to conclude that aldehyde **16** favored preferential deuteriation at the C-3 pro-R position of the glycerolipid.

The two methods used to perform the tentative assignment of the stereochemistry of the deuteriated glycerolipid gave the same final result. The stereochemistry of the favored position of deuteriation was found to be the pro-R position of the C-3 carbon atom of the glycerol moiety of the labeled glycerolipid.

1.3 Conclusion

The synthesis of the glycerolipid 1,2-di-O-tetradecyl-*sn*-glycerol was successfully accomplished. The procedure developed overcame the numerous inconveniences of the previous synthetic route. The novel synthesis required less synthetic steps (6 compared to 7) and the glycerolipid was obtained in a slightly improved overall yield.

Furthermore the synthetic route had the required flexibility to allow for the introduction of a deuterium label in the last step of the procedure to prepare the 3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol. The previous synthetic route did not have such flexibility since, to obtain the labeled glycerolipid in addition to the plain glycerolipid, the time consuming and costly repetition of the four last steps of the synthesis would have to be performed. It was demonstrated that the introduction of the deuteron proceeded without any particular stereoselectivity but it was found that some chiral induction could be obtained by performing the deuteriation in the presence of various chelating agents.

The reaction intermediates and reaction conditions were safe against decomposition, polymerization, oxidation and racemization. The advantage of using stable reaction intermediates was confirmed by the optical activity measurement of the final glycerolipid. The glycerolipid obtained possessed a higher optical rotation than the glycerolipids prepared by the previous procedure ($[\alpha]_D$ -9.3° (c1.0, chloroform) compared to -7.79° (c0.95, chloroform)[44]). Since the

retention of configuration at the C-2 stereocenter of the glycerol moiety was critical for the synthesis of glycerolipids that mimic naturally occurring lipids, the development of a synthetic route which utilized stable intermediates and safe reaction conditions was a substantial advance in the synthesis of such lipids. The versatility of the novel procedure could easily be adapted to the synthesis of other glycerolipids bearing various lipophilic groups.

1.4 Experimental

1.4.0 General Methods

Melting points were determined using a Gallenkamp melting point apparatus and were uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter and were run at 23-25°C for 0.9-1.0% solutions in chloroform unless otherwise stated. Elemental analysis were performed by Guelph Chemical Laboratories Ltd. (Ontario) or M-H-W Laboratories (Phoenix, AZ). Mass spectra, obtained by the E.I. or C.I. mode, or by negative or positive FAB, were recorded on a VG 7070-E spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-300 and on a Varian Gemini 200 at 300 and 200 MHz respectively for protons and at 75.4 and 50.3 MHz respectively for carbons. The proton chemical shifts (δ) are given relative to internal deuterochloroform at 7.24 ppm for deuterochloroform solutions, to internal methanol (3.34 ppm) for methanol-d_4 solutions and to internal acetone (2.216 ppm) for deuterium oxide solutions. The carbon chemical shifts are given relative to the central line of deuterochloroform (77.0 ppm) or of methanol at (49.0 ppm) and to internal acetone at 31.1 ppm for D_2O solutions. Assignments of ^{13}C peaks were aided by ADEPT experiments. The analysis were done as a first order approximation. Thin Layer Chromatography (TLC) was performed by using silica gel coated plates (Kieselgel 60 F-254). The developed plates were visualized either by U.V. irradiation and/or by dipping in a solution of ceric sulfate (1%) ammonium molybdate (2.5%) in a 10% aqueous sulfuric acid and heated at ≈150°C. Purifications were performed by gravity chromatography on silica gel columns, by chromatography on a Harrison Research Chromatotron model 7924 using silica gel 60-F254 rotors (1, 2 or 4 mm thickness) by preparative TLC plates (0.25 or 1 mm thickness) or by flash chromatography on silica gel 60 (230-400 mesh, E. Merck No. 9385). All solvents and reagents used were reagent grade and, where required, further purifications were accomplished following published procedures[47].

47 D.D. Perrin, W.L. Armarego, D.R. Perrin, "Purification of Laboratory Compounds", 2nd Edition, Pergamon Press, London (1980).

1.4.1 Synthesis of 1,2-Di-O-tetradecyl-*sn*-glycerol 4

1,2:3,4:5,6-Tri-O-isopropylidene-D-mannitol 12 - A solution of concentrated sulfuric acid (60 ml) and glacial acetic acid (30 ml) in dry acetone (1200 ml) was prepared at 0°C. The solution was warmed to room temperature and anhydrous D-mannitol **5** (0.500 g, 2.74 mmol) and 4Å molecular sieves (50 mol) were added. The reaction mixture was stirred at room temperature for 20 hours. The mixture was filtered and poured into a vigorously stirred cooled suspension of sodium carbonate (500 g) in water (700 ml). The slurry was stirred until neutralization, the precipitated salts were filtered off and washed thoroughly with acetone. The filtrate and washings were combined and evaporated to give a semi-solid residue. Ether (500 ml) was added to the solids and the phases were separated. The etherial layer was washed with water (2 x 500 ml) and brine (1 x 500 ml), dried over anhydrous sodium sulfate and evaporated to dryness. The crude oil was recrystallized from 95% ethanol to yield the pure tri-O-isopropylidene **12** in a 67% yield (0.556 g); m.p. 70.3-72.0°C; $[\alpha]_D$ +14.2° (c1.0, chloroform), +13.4° (c5.0, 95% ethanol); lit.[40] yield 65%; m.p. 69-70°C; $[\alpha]_D$ +11.5° (c5.0, 95% ethanol); lit.[41] yield 66.7%; m.p. 70-71°C; $[\alpha]_D$ +13.8° (c1.0, chloroform); ^{1}H NMR ($CDCl_3$): δ (ppm) 3.89-4.20 (m, 8H, CH_2, CH), 1.39, 1.35, 1.31 (3s, 18H, CH_3); lit.[40] ^{1}H NMR ($CDCl_3$): δ (ppm) 3.85-4.45 (m, 8H, CH_2, CH), 1.43, 1.40, 1.37 (3s, 18H, CH_3); ^{13}C NMR ($CDCl_3$): δ (ppm) 110.1, 109.5 (3C, $\underline{C}(CH_3)_2$), 79.3, 76.2 (4C, CH), 66.1 (2C, CH_2), 27.2, 26.3, 25.1 (6C, CH_3); M.S. (C.I. ether) m/z: 303 (100%, $[M + H]^+$), 287 (38%, $[M - Me]^+$), 245 (70%, $[M - acetone]^+$); Anal. calcd for $C_{15}H_{26}O_6$: C, 59.58; H, 8.67; Found: C, 59.46; H, 8.80.

3,4-O-Isopropylidene-D-mannitol 13 - A solution of tri-O-isopropylidene **12** (10.3 g, 34.0 mmol) in 70% acetic acid (200 ml) was stirred at 40°C. After 1.75 hours, the clear solution was evaporated in vacuo and coevaporated several times with toluene to remove residual acetic acid. Dry acetone (70 ml) was added and the mixture was thoroughly stirred with anhydrous potassium carbonate (10 g) and filtered. The insoluble materials were washed with acetone and the combined filtrate and washings were evaporated in vacuo. The oil obtained was dissolved in a small volume of acetone and benzene (70 ml) was added. The clear solution was warmed to drive off the acetone and allowed to crystallize, at room temperature. White crystals of tetrol **13** were obtained in 86% yield (6.50 g); m.p. 85.2-86.6°C; $[\alpha]_D$ +30.2° (c1.0, water); lit.[40] yield 70-80%; m.p. 83°C; $[\alpha]_D$ +30° (c1.0, water); ^{1}H NMR (D_2O): δ (ppm) 4.11 (m, 2H, H-3, H-4 (tentative)), 3.82 (m, 2H, H-2, H-5 (tentative)), 3.79 (q, 2H, $J_{1a, 2} = J_{5, 6a} = 2.9$ Hz, $J_{1a, 1b} = J_{6a, 6b} = 12.4$ Hz, H-1a, H-6a), 3.62 (q, 2H, $J_{1b, 2} = J_{5, 6b} = 7.2$ Hz, H-1b, H-6b), 1.42 (s, 6H, CH_3); ^{13}C NMR (D_2O): δ (ppm) 113.5 (1C, $\underline{C}(CH_3)_2$), 81.6, 75.2 (4C, CH) 65.4 (2C, CH_2), 29.0 (2C, CH_3); M.S. (Positive FAB) m/z : 223 (100%, $[M + H]^+$), 207 (22%, $[M - Me]^+$), 165

(100%, [M+H-acetone]$^+$); Anal. calcd for $C_9H_{18}O_6$: C, 48.64; H, 8.16; Found: C, 48.54; H, 8.36.

3,4-O-Isopropylidene-1,2,5,6-tetrakis-O-tetradecyl-D-mannitol 14

Method A - Alkylation Method - A solution of tetrol **13** (5.56 g, 25 mmol) in dry DMF (50 ml) was added dropwise to a stirred suspension of NaH (50% dispersion in oil, 8 eq, 9.60 g) in dry DMF (100 ml) over a period of 30 minutes. The addition of a solution of 1-bromotetradecane (4.4 eq, 32.7 ml, 30.5 g, 110 mmol) in dry DMF (100 ml) followed. The reaction mixture was further diluted with DMF (200 ml) to allow for more efficient stirring. The reaction mixture was stirred at room temperature over a period of 2 days. The reaction was quenched with water (200 ml) and ether (500 ml) was poured into the mixture. The phases were separated and the organic layer was washed with water (4 x 500 ml), dried over anhydrous sodium sulfate and evaporated. The oil obtained, still containing DMF, was dissolved in hexanes (250 ml) and extracted with water (3 x 250 ml). The organic phase was concentrated and chromatographed on silica gel. The column was first eluted with hexanes in order to remove the excess of 1-bromotetradecane and then with a 1:9 ethyl acetate:hexanes solution which was used to recuperate tetraalkylated product **14**. The solvent was evaporated and the tetraalkylated compound **14** was obtained in 76% yield as a colorless oil (19.1 g). It was used directly in the next step, without any further purifications.

Method B - Phase Transfer Catalysis Method - In a 25 ml round bottom flask were placed powdered $NaOH/K_2CO_3$ (1/4 w/w, 0.96 g), benzene (8 ml), amyl alcohol (0.2 ml), tetrabutylammonium hydrogen sulfate (0.2 mmol, 0.068 g), bromotetradecane (1.2 eq per OH group, 2.85 ml, 2.67 g, 9.6 mmol) and a solution of tetrol **13** (0.45 g, 2.0 mmol) in DMSO (1.6 ml) and the mixture was vigorously stirred at room temperature. The reaction was monitored by TLC and thereby the reaction appeared to be very slow. After 7 days, some product was detected by TLC and the reaction was stopped. The reaction mixture was diluted with hexanes (25 ml) and water (25 ml) and the phases were separated. The organic layer was washed with water (3 x 10 ml) and brine (1 x 10 ml), dried over anhydrous sodium sulfate and concentrated in vacuo. The colorless oil obtained was chromatographed on silica gel by gravity chromatography using hexanes as eluent, until removal of the excess bromotetradecane. Elution with a 10% ethyl acetate in hexanes solution followed. The tetraalkylated compound **14** was obtained as a colorless oil, yield 33% (0.67 g): $[\alpha]_D$ +8.0° (c1.0, chloroform); ^1H NMR ($CDCl_3$): δ (ppm) 3.67-3.36 (m, 16H, $OC\underline{H}_2(CH_2)_{12}CH_3$, H-1a, 1b, 2, 3, 4, 5, 6a, 6b), 1.53 (m, 8H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.35 (s, 6H, $C(CH_3)_2$), 1.22 (bs, 88H, $OCH_2CH_2(C\underline{H}_2)_{11}CH_3$), 0.85 (t, 12H, $J_{13',14'}$ = 6.5 Hz,

$O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 109.5 (1C, $\underline{C}(CH_3)_2$), 79.8, 78.4 (4C, CH), 71.5, 71.1, 70.9 (6C, C-1, C-6, $O\underline{C}H_2(CH_2)_{12}CH_3$) 31.7, 29.9, 29.5, 29.4, 29.3, 29.2, 26.0, 22.5 (48C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 26.9 (2C, $C(\underline{C}H_3)_2$), 13.9 (4C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Positive FAB) m/z: 949 (12%, $[M - acetone]^+$), 197 (100%, $[CH_3(CH_2)_{13}]^+$); Anal. calcd for $C_{65}H_{130}O_6$: C, 77.47; H, 13.00; Found: C, 77.60; H, 12.89.

1,2,5,6-Tetrakis-O-tetradecyl-D-mannitol 15 - Tetraalkylated compound **14** (2.25 g, 2.24 mmol) was dissolved in chloroform (50 ml) and a 1M solution of HCl (20 ml) was added. Methanol was then incorporated into the stirred two phase system until homogeneity of the mixture was obtained. The reaction mixture was refluxed for 4 days, until completion of the reaction. The volatile solvents were evaporated and ethyl acetate (50 ml) and water (30 ml) were added to the mixture. The phases were separated and the organic layer was washed with water (50 ml), saturated sodium bicarbonate (50 ml) and brine (50 ml), dried over anhydrous sodium sulfate and evaporated in vacuo. Diol **15** was obtained as a clear, colorless oil in 91% yield (1.97 g). The oil was crystallized from ethanol to afford pure white crystals of diol **15**; m.p. 41.3-42.2°C; $[\alpha]_D$ -8.1° (c1.0, chloroform); ^1H NMR ($CDCl_3$): δ (ppm) 3.83-3.38 (m, 16H, $OC\underline{H}_2(CH_2)_{12}CH_3$, H-1a, 1b, 2, 3, 4, 5, 6a, 6b), 3.28 (d, 2H, J = 5.5 Hz, OH), 1.53 (m, 8H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.22 (bs, 88H, $OCH_2CH_2(C\underline{H}_2)_{11}CH_3$), 0.85 (t, 12H, $J_{13',\ 14'}$ = 6.4 Hz, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 79.6 (2C, $\underline{C}HO(CH_2)_{13}CH_3$), 71.7, 71.2, 70.6 (6C, C-1, C-6, $O\underline{C}H_2(CH_2)_{12}CH_3$), 70.0 (2C, CHOH), 31.7, 29.9, 29.5, 29.5, 29.3, 29.2, 25.9, 22.5 (48C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 13.9 (4C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 967 (33%, $[M]^-$); Anal. calcd for $C_{62}H_{126}O_6$: C, 76.96; H, 13.12; Found: C, 76.63; H, 13.40.

2,3-Di-O-tetradecyl-D-glyceraldehyde 16 - Diol **15** (6.19 g, 6.4 mmol) and periodic acid (1.1 eq, 1.60 g, 7.04 mmol) were stirred in dry ether (120 ml) at room temperature for 20 hours. Water (100 ml) was added to the reaction mixture and the phases were separated. The organic layer was washed with saturated sodium bicarbonate (3 x 200 ml) and brine (200 ml), dried over anhydrous sodium sulfate and evaporated to dryness. The oil obtained solidified spontaneously to give aldehyde **16** as a white solid, 88% yield (5.44 g); m.p. 28.0-29.6°C; $[\alpha]_D$ +9.4° (c1.0, chloroform); ^1H NMR ($CDCl_3$): δ (ppm) 9.69 (d, 1 H, $J_{1,\ 2}$ = 1.43 Hz, CHO), 3.81-3.36 (m, 7 H, H-2, 3a, 3b, $OC\underline{H}_2(CH_2)_{12}CH_3$), 1.53 (m, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.22 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.85 (t, 6H, $J_{13',\ 14'}$ = 6.4 Hz, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 203.4 (1C, C=O), 83.8 (1C, C-2), 71.9, 71.2, 69.7 (3C, C-3), 31.7, 29.5, 29.2, 29.2, 25.9, 25.8, 22.4 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 13.9 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S.

(C.I. ether) m/z: 483 (78%, $[M + H]^+$), 197 (100%, $[CH_3(CH_2)_{13}]^+$); Anal. calcd for $C_{31}H_{62}O_3$: C, 77.12; H, 12.94; Found: C, 76.90; H, 13.21.

1,2-Di-O-tetradecyl-*sn*-glycerol 4 - Aldehyde **16** (4.36 g, 9.0 mmol) was dissolved in methanol (100 ml) and sodium borohydride (2 eq, 0.68 g, 18.0 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 6 hours. The reaction was quenched by the addition of 1M HCl until the pH of the reaction mixture became slightly acidic (pH < 6). The solution was evaporated in vacuo and the solids obtained were dissolved in ether (150 ml) and water (150 ml). The phases were separated and the organic layer was washed with 1M HCl (150 ml), saturated sodium bicarbonate (2 x 150 ml) and brine (1 x 150 ml). The etherial solution was dried over anhydrous sodium sulfate and evaporated to dryness. The solids obtained were recrystallized from methanol/ether to yield the pure alcohol **4** in a 93% yield (4.06 g); m.p. 42.0-42.6°C; $[\alpha]_D$ -9.3° (c1.0, chloroform); lit.[44] m.p. 42-43°C; $[\alpha]_D$ -7.79° (c 0.95, chloroform); ^{1}H NMR ($CDCl_3$): δ (ppm) 3.66 (t, 1H, J = 11.4 Hz, OH), 3.64-3.37 (m, 9H, H-1a, 1b, 2, 3a, 3b, $OC\underline{H}_2(CH_2)_{12}CH_3$), 1.52 (m, 4 H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.22 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.84 (t, 6H, $J_{13',\ 14'}$ = 6.3 Hz, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 78.1 (1C, C-2), 71.7, 70.8, 70.3 (3C, C-1, $O\underline{C}H_2(CH_2)_{12}CH_3$), 62.9 (1C, C-3), 31.7, 29.9, 29.5, 29.4, 29.3, 29.2, 25.8, 22.5 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 13.9 (2C, $O(CH_2)_{13}C\underline{H}_3$); lit.[44] ^{13}C NMR (DMSO-d_6) 79.0 (1C, C-2), 70.6, 70.6 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 69.0 (1C, C-1), 61.1 (1C, C-3); M.S. (C.I. ether) m/z: 485 (62%, $[M + H]^+$); Anal. calcd for $C_{31}H_{64}O_3$: C, 76.80; H, 13.31; Found: C, 76.59; H, 13.22.

1.4.2 Synthesis of 3-Deutero-1,2-di-O-tetradecyl-*sn*-glycerol 4a

3-Deutero-1,2-di-O-tetradecyl-*sn*-glycerol 4a - Aldehyde **16** (0.20 g, 0.42 mmol) was dissolved in methanol (20 ml) and sodium borodeuteride (2 eq, 32 mg, 0.84 mmol) was added. The reaction mixture was stirred at room temperature for 6 hours. The reaction was quenched by the addition of 1 M HCl until the pH of the reaction mixture became slightly acidic (pH <6) and evaporated in vacuo. The solids obtained were dissolved in ether (25 ml) and water was added (25 ml). The phases were separated and the organic layer was washed with 1M HCl (2 x 25 ml), saturated sodium bicarbonate (2 x 25 ml) and brine (25 ml), dried over anhydrous sodium sulfate and evaporated. The oil obtained was crystallized from ether/methanol and alcohol **4a** was obtained as white crystals in 91% yield (0.184 g); m.p. 42.4-43.0°C; $[\alpha]_D$ -9.0° (c1.0, chloroform); ^{1}H NMR ($CDCl_3$): δ (ppm) 3.66 (d, 1H, J = 8.7 Hz, OH), 3.61-3.37 (m, 8H, H-1a, 1b, 2, 3, $OC\underline{H}_2(CH_2)_{12}CH_3$), 1.54 (m, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.22 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.84 (t, 6H, $J_{13',\ 14'}$ = 6.4 Hz, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ

(ppm) 78.1 (1C, C-2), 71.7, 70.8, 70.3 (3C, C-1, $O\underline{C}H_2(CH_2)_{12}CH_3$), 31.7, 29.9, 29.5, 29.4, 29.3, 29.2, 25.9, 22.5 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 13.9 (1C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (C.I. ether) m/z: 486 (69%, $[M + H]^+$); Anal. calcd for $C_{31}H_{63}DO_3$: C, 76.64; H, D, 13.48; Found: C, 76.72; H, D, 13.38.

1.4.3 Methods to Assign the H-3 Protons of Alcohols 4 and 4a

Method A - Acetylation Method - 3-O-Acetyl-1,2-di-O-tetradecyl-*sn*-glycerol 17 and 3-O-Acetyl-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol 17a - Alcohol **4** or **4a** (50 mg, 0.1 mmol) was dissolved in dry pyridine (5 ml) and acetic anhydride (2 ml) was added to the cooled solution. The reaction mixture was stirred for 1 hour at room temperature and the reaction was quenched with methanol (10 ml). The reaction mixture was evaporated and co-evaporated several times with toluene. The oil obtained was dissolved in ether (20 ml) and the organic solution was washed with 0.1M HCl (2 x 20 ml), saturated sodium bicarbonate (2 x 20 ml) and brine (20 ml) and dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo and acetate **17** or **17a** was obtained as a colorless oil, yield 99% (52 mg).

3-O-Acetyl-1,2-di-O-tetradecyl-*sn*-glycerol 17 - $[\alpha]_D$ +0.4° (c1.0, chloroform); ^1H NMR ($CDCl_3$): δ (ppm) 4.18 (dd, 1H, $J_{2,\,3a}$ = 4.1 Hz, $J_{3a,\,3b}$ = 11.6 Hz, H-3a), 4.04 (dd, 1H, $J_{2,\,3b}$ = 5.6 Hz, H-3b), 3.55 (m, 1H, H-2), 3.50 (t, 2H, $J_{1,\,2}$ = 6.2 Hz, H-1a, H-1b), 3.44 (t, 2H, $J_{1',\,2'}$ = 6.6 Hz, $OC\underline{H}_2(CH_2)_{12}CH_3$), 3.38 (t, 2H, $J_{1',\,2'}$ = 6.6 Hz, $OC\underline{H}_2(CH_2)_{12}(CH_3)$, 2.02 (s, 3H, $CH_3C(O)$), 1.50 (m, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.20 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.83 (t, 12H, $J_{13',\,14'}$ = 6.4 Hz, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 171.1 (1C, C=O), 76.3 (1C, C-2), 71.7, 70.5, 70.1 (3C, C-1, $O\underline{C}H_2(CH_2)_{12}CH_3$), 64.0 (1C, C-3), 31.7, 29.8, 29.5, 29.4, 29.3, 29.1, 25.9, 25.8, 22.5, 20.7 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 13.9 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (C.I. ether) m/z: 527 (100%, $[M + H]^+$), 313 (87%, $[M - O(CH_2)_{13}CH_3]^+$); Anal. calcd for $C_{33}H_{66}O_4$: C, 75.23; H, 12.63; Found: C, 75.38; H, 12.69.

3-O-Acetyl-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol 17a - $[\alpha]_D$ +0.5°, (c1.0, chloroform), ^1H NMR ($CDCl_3$): δ (ppm) 4.17 (d, 0.5H, $J_{2,\,3a}$ = 4.1 Hz, H-3a), 4.05 (d, 0.5H, $J_{2,\,3b}$ = 5.7 Hz, H-3b), 3.56 (m, 1H, H-2), 3.52 (t, 2H, $J_{1,\,2}$ = 6.2 Hz, H-1a, H-1b), 3.46 (t, 2H, $J_{1',\,2'}$ = 6.6 Hz, $OCH_2(C\underline{H}_2)_{12}CH_3$), 3.40 (t, 2H, $J_{1',\,2'}$ = 6.6 Hz, $OC\underline{H}_2(CH_2)_{12}CH_3$), 2.04 (s, 3H, $C(O)CH_3$), 1.52 (m, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.22 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.85 (t, 6H, $J_{13',\,14'}$ = 6.4 Hz, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ

(ppm) 171.1 (1C, C=O), 76.3 (1C, C-2), 71.7, 70.5, 70.1 (3C, C-1, $O\underline{C}H_2(CH_2)_{12}CH_3$), 31.7, 29.8, 29.5, 29.3, 29.2, 25.9, 25.8, 22.5, 20.7 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 13.9 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (C.I. ether) m/z: 528 (100%, $[M + H]^+$), 314 (78%, $[M - O(CH_2)_{13}CH_3]^+$); Anal. calcd for $C_{33}H_{65}DO_4$: C, 75.08; H,D, 12.79; Found: C, 74.92; H,D, 12.68.

Stereoselectivity of Deuteriation:

Integration ratio: Integration of ^1H signal at δ = 4.17 ppm: Integration of ^1H signal at δ = 4.05 ppm = 41 : 39 = 1 : 1

Deuterium stereoselectivity: 50% Pro-R: 50% Pro-S.

Method B - Mesylation Method - 3-O-Methylsulfonyl-1,2-di-O-tetradecyl-*sn*-glycerol 18 - Alcohol **4** (0.50 g, 1.0 mmol) was dissolved in dry pyridine (5 ml) and methanesulfonyl chloride (1.2 eq, 0.12 g, 80 µl, 1.2 mmol) was added to the solution. The reaction mixture was stirred at room temperature for a period of 2 hours, after which the reaction was quenched with water (2 ml). The mixture was evaporated in vacuo and coevaporated several times with toluene. The solids obtained were dissolved in ether (50 ml) and the etherial solution was washed with 0.1M HCl solution (2 x 50 ml), saturated sodium bicarbonate solution (2 x 50 ml) and brine (50 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo. The solids obtained were recrystallized from ether/methanol to afford white crystals of the methylsulfonate **18** in an 85% yield (0.48 g); m.p. 141.1°C (sint'd 106.3-107.7°C); $[\alpha]_D$ -0.9° (c1.0, chloroform); ^1H NMR ($CDCl_3$): δ (ppm) 4.36 (dd, 1H, $J_{2,\,3a}$ = 3.6 Hz, $J_{3a,\,3b}$ = 10.9 Hz, H-3a), 4.22 (dd, 1H, $J_{2,\,3b}$ = 5.6 Hz, H-3b), 3.64 (m, 1H, H-2), 3.53 (t, 2H, $J_{1',\,2'}$ = 6.6 Hz, $OC\underline{H}_2(CH_2)_{12}CH_3$), 3.47 (m, 2H, H-1a, H-1b), 3.40 (t, 2H, $J_{1',\,2'}$ = 6.6 Hz, $OC\underline{H}_2(CH_2)_{12}CH_3$), 3.01 (s, 3H, $S(O)_2CH_3$), 1.54 (bs, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.22 (bs, 44 H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.85 (t, $J_{13',\,14'}$ = 6.4 Hz, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 76.3 (1C, C-2), 71.8, 70.7, 69.6, 68.9 (4C, C-1, C-3, $O\underline{C}H_2(CH_2)_{12}CH_3$), 37.2 (1C, $S(O)_2CH_3$), 31.7, 29.7, 29.5, 29.3, 29.2, 25.9, 25.8, 22.48 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 13.9 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (C.I. ether) m/z: 563 (65%, $[M + H]^+$), 467 (16%, $[M - MeSO_3]^+$); Anal. calcd. for $C_{32}H_{66}O_5S$: C, 68.28; H, 11.82; S, 5.70; Found: C, 68.15; H, 11.80; S, 5.63.

1.4.4 Study of Stereoselectivity of Borodeuteride Reduction

1.4.4.1 Procedure for Low Temperature Reduction using Sodium Borodeuteride

A solution of aldehyde **16** (0.097 g, 0.20 mmol) in methanol was cooled either at 0°C using an ice bath or at -78°C using a dry ice/acetone bath prepared in a Dewar flask (low form). At these temperatures, the solubility of the aldehyde was very poor; it was crystallizing out of the solution. In spite of the solubility problem, sodium borodeuteride (1.2 eq) was added to the suspension but also appeared to be insoluble. The reaction mixture was stirred for 2 days. After that time period no product was detected by TLC and the reaction was quenched with 1M HCl (the pH of the reaction mixture was adjusted to 6). The mixture was evaporated in vacuo, ether (10 ml) and water (10 ml) were added to the semi-solid mixture obtained and the phases were separated. The organic layer was washed with 1M HCl (2 x 10 ml), saturated sodium bicarbonate (2 x 10 ml) and brine (10 ml), dried over anhydrous sodium sulfate and evaporated to dryness. A clear oil was obtained and it appeared by ^{1}H NMR that only the starting aldehyde was present; it was recuperated in 90-95% yield (0.095-0.10 g). The mixture obtained was further acetylated according to the above described acetylation method in order to render any trace of deuteriated product **17a** detectable by ^{1}H NMR as the acetate contrary to the alcohol possesses protons which are not overlapped by those of aldehyde 16. ^{1}H NMR following the acetylation revealed no trace of acetate.

1.4.4.2 Reduction using Magnesium Bromide Etherate as Chelating Agent

To a solution of aldehyde **16** (0.049 g, 0.10 mmol) in dry tetrahydrofuran (10 ml) was added solid sodium hydrogen carbonate (3 eq) and powdered 4Å molecular sieves. The suspension was cooled to -78°C under nitrogen and magnesium dibromide etherate (1.5 eq) was added with stirring. After 15 minutes, sodium borodeuteride was added as a solid. The mixture was stirred at -78°C for 10 hours, until completion of the reaction. The mixture was allowed to warm to room temperature, water (2 ml) was added and the mixture was evaporated to dryness. The solids obtained were partitioned between methylene chloride (10 ml) and water (10 ml). The phases were separated and the organic layer was washed with 0.1M HCl (2 x 10 ml), saturated sodium bicarbonate (2 x 10 ml) and brine (10 ml), dried (anhydrous sodium sulfate) and evaporated. The semi-solid mixture was acetylated according to the above-described acetylation method. Acetate **17a** was obtained as a clear oil, yield 90% (0.048 g).

Stereoselectivity of Deuteriation:

^{1}H NMR ($CDCl_3$): δ (ppm) 4.17 (d, 0.4H, $J_{2,3}$ = 4.1Hz, H-3 Pro R), 4.05 (d, 0.6H, $J_{2,3}$ = 5.7 Hz, H-3 Pro S).

Integration ratio: Integration of ^{1}H signal at δ = 4.17 ppm: Integration of ^{1}H signal at δ = 4.05 ppm = 38: 62 = 2: 3

Deuterium Stereoselectivity: 60% Pro R, 40% Pro S

1.4.4.3 Reduction using Zinc Borodeuteride as Reducing and Chelating Agent.

Preparation of Zinc Borodeuteride - Dried zinc chloride (1.00 g, 7.3 mmol) was refluxed in ether (20 ml) until most of the solid had dissolved. The mixture was allowed to stand and the supernatant zinc chloride solution was added dropwise to a solution of sodium borodeuteride (0.41 g, 9.8 mmol) in ether (5 ml). The mixture was stirred at room temperature for 10 hours and the precipitate obtained was left to settle. The etherial solution containing the zinc borodeuteride was used as such.

Borodeuteriation Reaction using Zinc Borohydride as Chelating and Reducing Agent - General Method - The aldehyde (0.20 g, 0.42 mmol) was dissolved in THF or ether (5 ml). The solution was kept at room temperature or cooled at -78°C. The zinc borodeuteride solution (20 ml) was added dropwise and the solution was stirred 1 hour for the room temperature reaction and 72 hours for -78°C reaction. After completion of the reaction, water (2 ml) was added to the mixture to quench the reaction. The reaction mixture was evaporated and the solids were partitioned between ether (25 ml) and water (25 ml). The phases were separated and the organic layer was washed with 1M HCl (2 x 25 ml), saturated sodium bicarbonate (2 x 25 ml) and brine (25 ml), dried over anhydrous sodium sulfate and evaporated. The resulting mixture was acetylated according to the above-described acetylation method. The acetate was obtained as a clear oil, (room temperature, 0.20 g, yield 90%, -78°C, 0.15g, yield 70%).

<u>Stereoselectivity of Deuteriation:</u>

- Room Temperature Reaction:

^{1}H NMR ($CDCl_3$): δ (ppm) 4.15 (d, 0.4H, $J_{2,3}$ = 3.9 Hz, H-3 pro R), 4.04 (d, 0.6H, $J_{2,3}$ = 5.6 Hz, H-3 pro S).

Integration ratio: Integration of ^1H signal at δ = 4.15 ppm: Integration of ^1H signal at δ = 4.04 ppm = 37: 63 = 2: 3

Deuterium Stereoselectivity: 63% Pro R: 37% Pro S

- -78°C Reaction :

^1H NMR ($CDCl_3$): δ (ppm) 4.15 (d, 0.33H, $J_{2,3}$ = 4.0 Hz, H-3 Pro R), 4.04 (d, 0.67 H, $J_{2,3}$ = 5.6 Hz, H-3 Pro S)

Integration ratio: Integration of ^1H signal at δ = 4.15 ppm: Integration of ^1H signal at δ = 4.04 ppm = 17: 34 = 1: 2

Deuterium Stereoselectivity: 67% Pro R: 33% Pro S

CHAPTER 2 Synthesis of Labeled and Unlabeled Sialic Acid Precursors

2.1 Introduction

The solution structure of sialic acids have been extensively studied[18]. However, to fully appreciate the biological function of these molecules as they exist in nature, their conformational properties must be determined. Deuterium NMR has been of tremendous value in the study of conformational properties of molecules at membrane surfaces[48]. Applications of deuterium NMR include the use of simple deuterium-labeled carbohydrates and glycolipids in media that mimic a biological membrane environment[10,13]. Because of the low natural abundance of deuterium, enrichment is a prerequisite for application of these methods.

Several procedures for the deuteriation of N-acetylneuraminic acid have been reported. A procedure by Thomson et al.[49] provides a method to introduce a deuterium label at several sites within the sialic acid molecule in one step. The method involves the use of Raney nickel as catalyst in deuterium oxide coupled with sonication resulting in the substitution of deuterium for hydrogen directly bonded to hydroxylated carbon atoms. The procedure yields N-acetylneuraminic acid deuteriated at the 8 and 9 positions, the rate of Raney nickel-catalyzed hydrogen-deuterium exchange at the 4 and 7 positions being very slow.

Julina et al.[50] described a 13 step synthesis of N-acetylneuraminic acid designed to permit modifications, particularly at C-6 to C-9 of sialic acid. It is based upon an extension of N-acetyl-1-deoxy-1-nitro-D-glucosamine at C-1 by a C_3 unit corresponding to C-7 to C-9 of NeuAc. The C-6 deutero N-acetylneuraminic acid was synthesized.

48 R.E. Jacobs and E. Oldfield, Progr. NMR Spectrosc., **14** (1981) 113.
49 D.S. Thomson and J.H. Prestegard, Carbohydr. Res., **196** (1990) 206.
50 R. Julina, I. Muller, A. Vasella and R. Wyler, Carbohydr. Res., **164** (1987) 415.

Friebolin[51,52] et al. have reported the deuteriation of sialic acid at C-3 of N-acetyl-α-neuraminic acid. In alkaline deuterium oxide solution, both H-3 protons of NeuAc are exchanged by deuterium whereby the exchange rate was found to be faster for the axial proton than for the H-3 equatorial proton. The exchange reaction was found to be highly specific and the incorporated label was retained under neutral and acidic conditions.

Since the ultimate goal of this deuterium NMR study is the determination of conformational properties of sialyl-containing glycolipids at membrane surfaces, specifically labeled model sialylglycerolipids are necessary. To prepare these glycolipids, labeled sialosyl donors which will be used as starting materials for the glycosylation of the glycerolipid moieties, have to be synthesized. The synthesis of sialosyl donors from labeled N-acetylneuraminic acid derivatives will be discussed in the following pages.

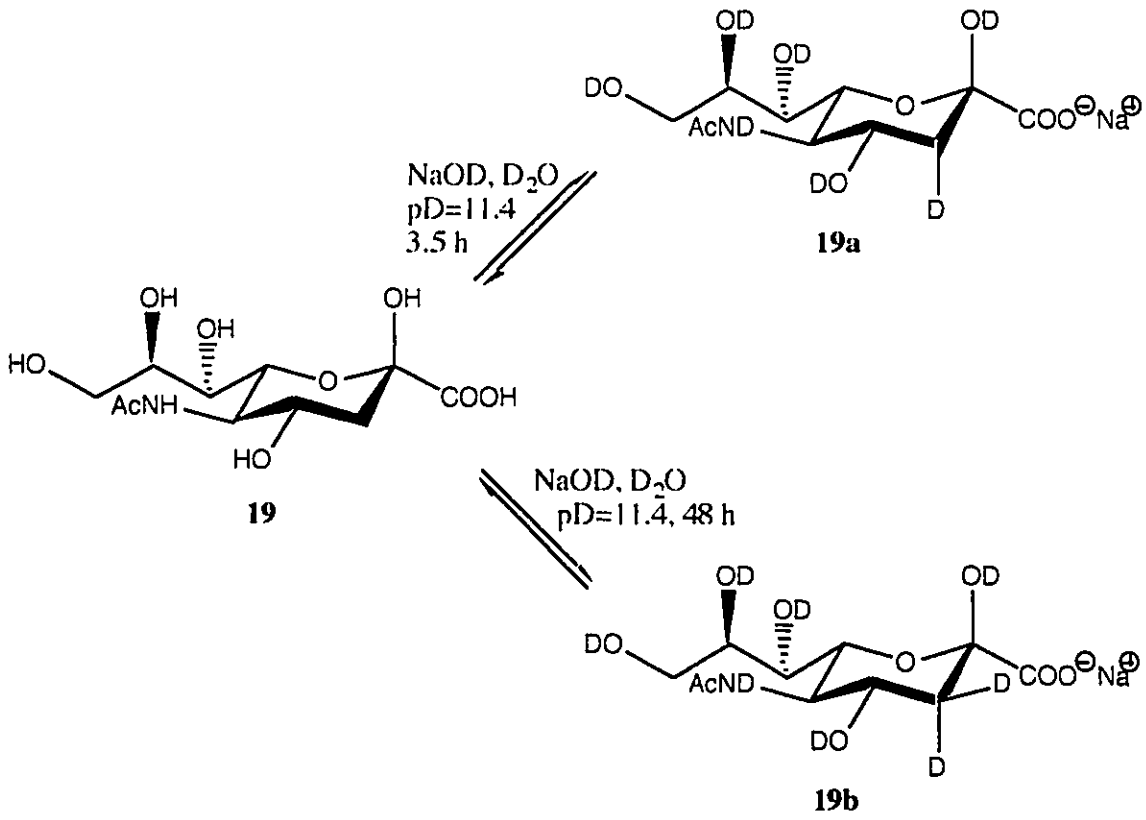
2.2 Discussion

2.2.1 Synthesis of Deuterium Labeled N-Acetylneuraminic Acid

- Synthesis of Mono- and Dilabeled N-Acetylneuraminic Acids

Simplicity, efficiency and specificity are desired for the deuteriation of the N-acetyl neuraminic acid starting material. These attributes were found in the method described by Friebolin et al.[51,52]. In contrast with the deuteriation procedures of Thomson[49] and Julina[50], this method has the advantage of accomplishing a selective and site-specific deuteriation on the cyclic moiety of the sugar residue in a single step. The deuteriation was therefore accomplished following a slight modification of the procedure by Friebolin et al. and is depicted in Scheme 11.

51 H. Friebolin, H. Schmidt and M. Supp, Tetrahedron Lett., **2 2** (1981) 5171.
52 H. Schmidt and H. Friebolin, J. Carbohydr. Chem., **2** (1983) 405.



Scheme 11. Site Specific Deuteriation of Sialic Acid **19**

The pD of the solution of N-acetylneuraminic acid **19** in D_2O was adjusted to 11.4 using a 2M solution of NaOD. Then, in accordance with the method of Friebolin et al., the clear solution obtained was stirred at room temperature, under nitrogen, for periods of 3.5 and 48 hours for the monodeuteriation and the dideuteriation respectively. Unlike for the published procedure, the neutralization of the reaction mixtures was advantageously performed with acidic resin thus avoiding the risk of using too much acid or incomplete neutralization. Labeled N-acetylneuraminic acids **19a** and **19b** were obtained after lyophilization of the filtered solutions in yields of 90% and 92% respectively. The melting points and optical rotation values obtained for the labeled derivatives **19a** and **19b** (**19a**: m.p. 186.0-187.0°C, $[\alpha]_D$ -33.6° (c1.0, H_2O); **19b**: m.p. 186.2-187.1°C, $[\alpha]_D$ -33.5° (c1.0, H_2O)) are comparable to those found in the literature[53,54] for unlabeled sialic acid.

53 M.N. Sharma and R. Eby, Carbohydr. Res., **127** (1984) 201.
54 H. Ogura and K. Furuhata, Carbohydr. Res., **158** (1986) 37.

The best results in the exchange reaction were obtained when all the starting materials and reagents used were previously treated with deuterium oxide to remove any trace of exchangeable hydrogen atoms. Therefore the sialic acid, the NaOH used to adjust the basicity of the NeuAc solution and the resin used to neutralize the reaction mixture after completion of the reaction were exchanged before use. The sialic acid and the sodium hydroxide were exchanged through three cycles of dissolution in deuterium oxide-freezing-lyophilizing and the resin was simply exchanged by contact with deuterium oxide before use.

- ^{1}H NMR Spectroscopy of Labeled Sialic Acids

Evidence of the regiospecificity of the deuteriation reaction and of the extent of deuteriation of sialic acid **19** is readily available from the comparison of the ^{1}H NMR spectra of N-acetylneuraminic acid **19** and of labeled derivatives **19a** and **19b.** ^{1}H NMR spectra (200 MHz, D_2O) of sialic acid **19** and of the labeled derivatives **19a** and **19b** consist generally of a complex multiplet located at δ = 4.06-3.54 ppm, of a singlet found at about δ = 2.07 ppm and of signals found at δ = 2.24 ppm and 1.84 ppm respectively for the H-3eq and H-3ax protons if present. The ^{1}H NMR spectra of sialic acid **19** and of the mono- and dilabeled derivatives **19a** and **19b** are compared in Figure IX.

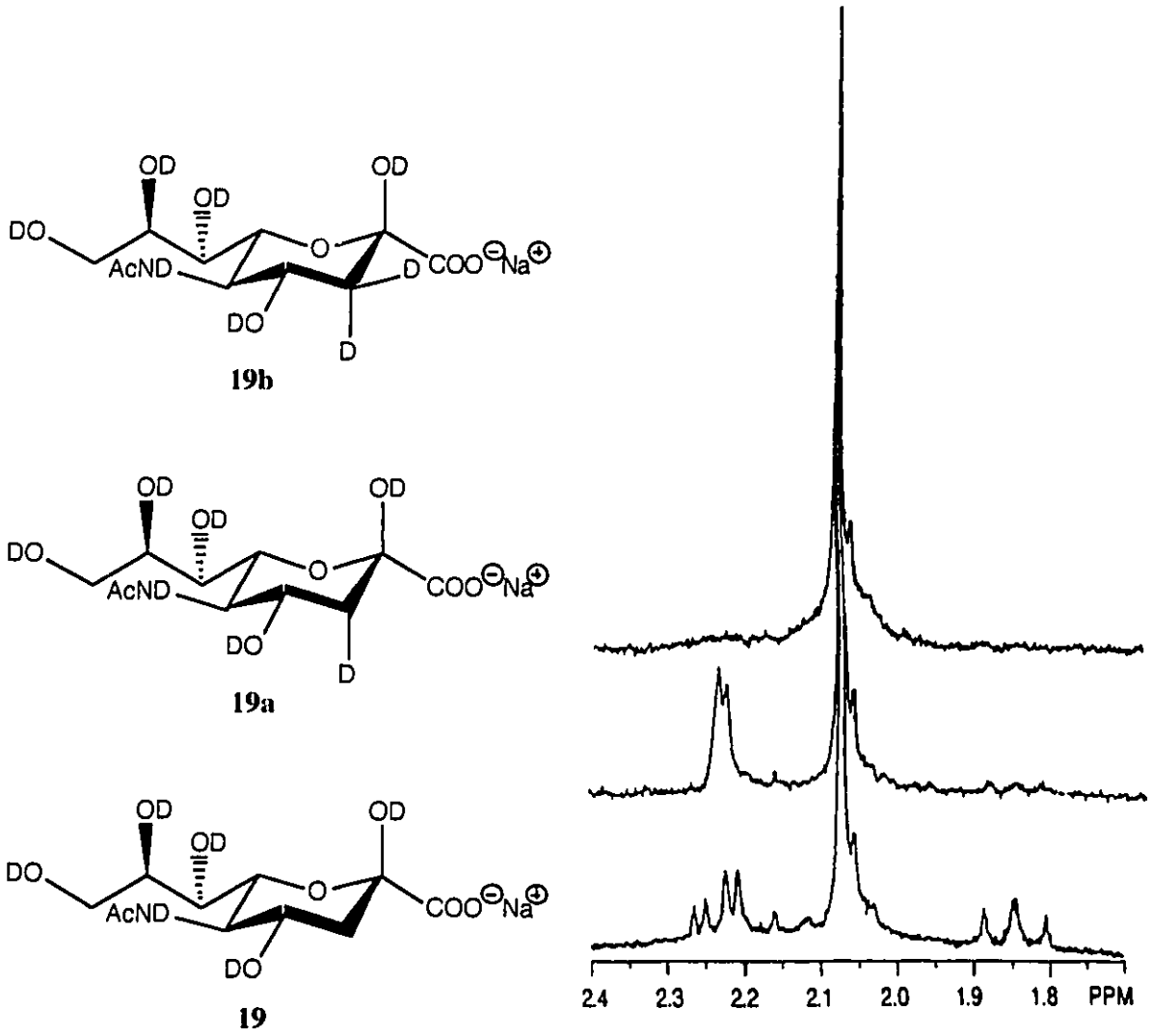


Figure IX. ^{1}H NMR Spectra of Unlabeled Sialic Acid, Mono- and Dilabeled **19**, **19a**, **19b** Derivatives Showing the Sialyl H-3ax, H-3eq Region.

In Figure IX, the spectrum of unlabeled sialic acid **19** displays the two H-3 proton signals as doublets of doublets at δ = 2.24 ppm ($J_{3ax,3eq}$ = 12.6 Hz, $J_{3eq,4}$ = 4.6 Hz) and at 1.84 ppm ($J_{3ax,4}$ = 11.4 Hz) for the H-3eq and H-3ax protons respectively. The two peaks appearing at δ = 2.24 ppm and 1.84 ppm can be respectively assigned to the H-3eq and H-3ax protons because of the difference in the magnitude of the $J_{3,4}$ coupling constants. The largest of the two $J_{3,4}$ coupling constant, 11.4 Hz, is typical of the dihedral angle found between two vicinal protons in a trans relationship to one another, such as that found between the H-3ax and H-4 protons.

In the spectrum of the monolabeled N-acetylneuraminic acid **19a**, the disappearance of the H-3eq proton signal is the first indication of the introduction of the deuteron in the sialic acid molecule. The H-3ax proton which appeared as a doublet of doublets in the ^{1}H NMR spectrum of the unlabeled molecule **19** converged to a doublet appearing at δ = 2.24 ppm ($J_{3eq,4}$ = 4.8 Hz). The disappearance of the geminal coupling constant $J_{3ax,3eq}$ is more evidence of the monodeuteriation. With the integration of the spectrum it was possible to determine that the extent of the deuteriation was above 92%.

The ^{1}H NMR spectrum of dilabeled N-acetylneuraminic acid **19b** does not display any peaks for the H-3 protons indicating that the exchange reaction of the two H-3 protons was complete.

The ^{1}H NMR spectra of mono- and dilabeled N-acetylneuraminic acid **19a** and **19b** confirm the regio- and stereoselective introduction of deuterium labels in the sugar molecules.

- **Mechanism of Deuteriation**

The regio- and stereoselectivity of the deuteriation reaction, which favors the exchange of the axial H-3ax proton rather than the equatorial proton H-3eq, can be explained by the mechanism of the reaction (Figure X).

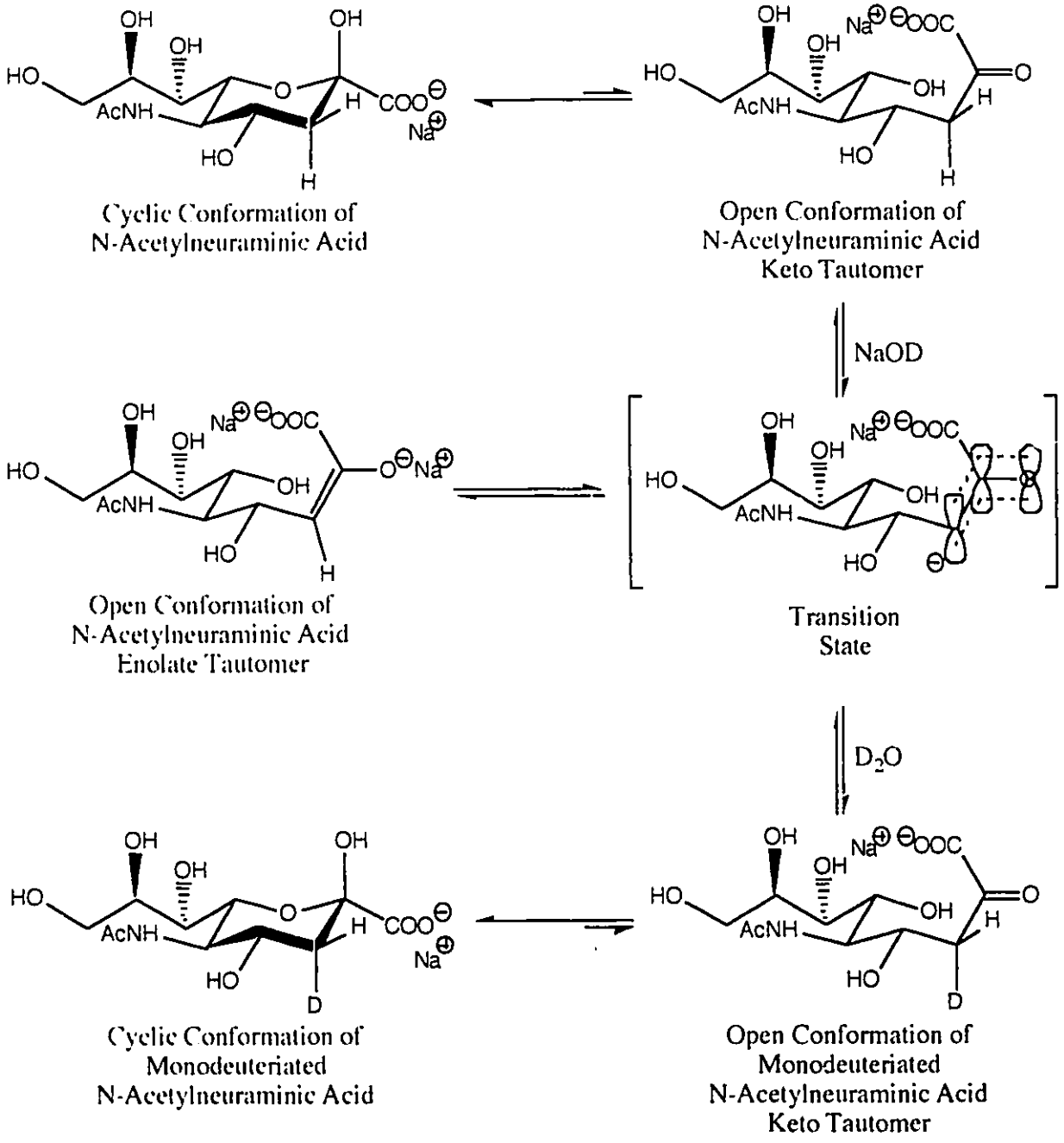


Figure X. Mechanism of Deuteriation Reaction.

The exchange reaction proceeds through a base catalyzed enolization of N-acetylneuraminic acid in its open-chain form. The formation of an enolate transition state wherein overlapping of the p-orbitals of the C-3 carbon and π-orbitals of the carbonyl group occurs is believed to favor the removal of the axial proton over the equatorial proton. The introduction of the deuteron occurs in the enolate.

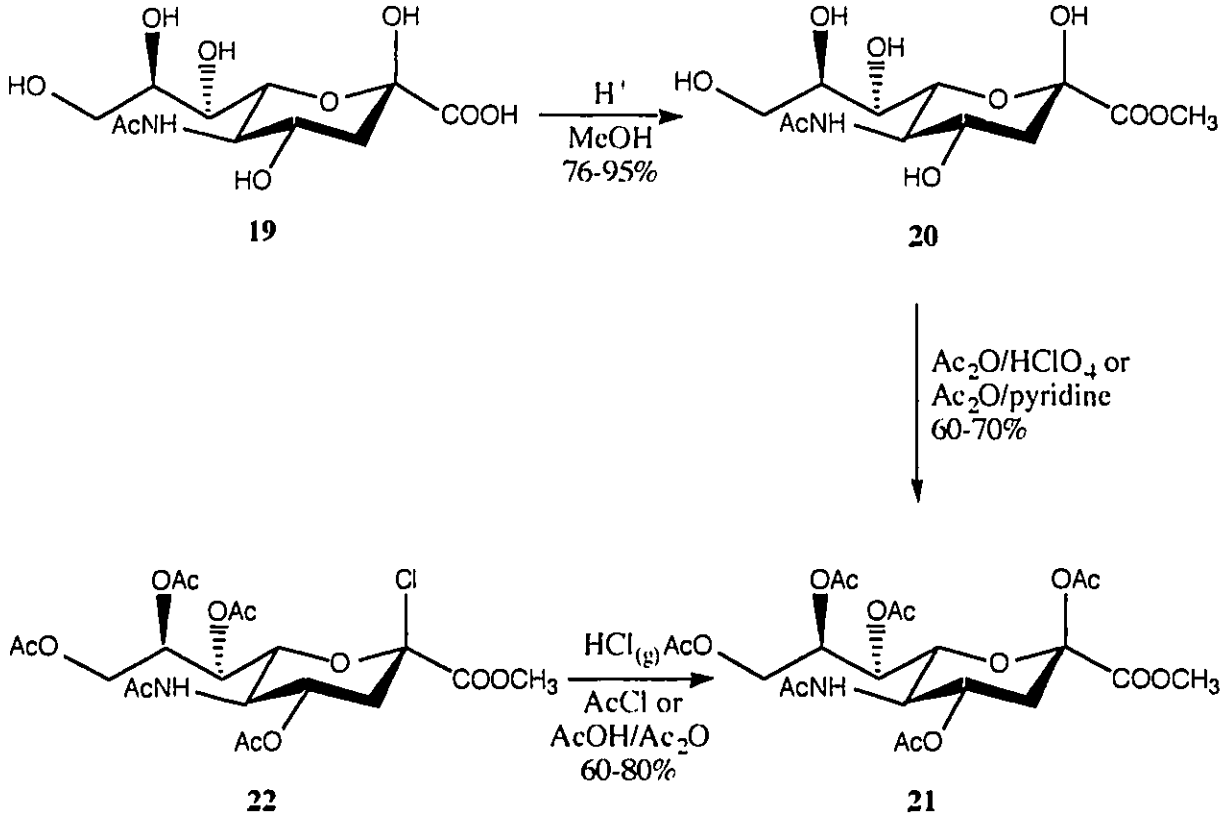
The reaction rate of the deuteriation at the equatorial position was thought to be slower than that of the axial proton because, in order to permit the formation of a transition state, a rotation about the C-2-C-3 bond has to happen. The exchange of the proton at the equatorial position is therefore less favored resulting in slower reaction kinetics.

2.2.2 Synthesis of Unlabeled and Labeled Sialosyl Donors

Glycosidation of N-acetylneuraminic acid has been widely investigated by many groups[55]. Many syntheses of N-acetylneuraminic acid glycosides have been performed using classical Koenigs-Knorr methodology. Although the anomeric selectivity and the yields of these glycosidations are rather unpredictable and vary greatly depending on reaction conditions, these reactions are considered to be useful with primary alcohols, phenols and sugar alcohols[55]. Since the glycosyl acceptor used in the synthesis of the labeled sialylglycerolipids is a primary alcohol, the Koenigs-Knorr methodology appeared to be appropriate to accomplish the synthesis. The glycosyl donors used in Koenigs-Knorr reaction conditions are activated glycosyl halides therefore the synthesis of unlabeled and labeled acetochloroneuraminic acid was required.

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate is the most commonly used sialosyl donors in Koenigs-Knorr reaction conditions[55]. It was first synthesized by Kuhn et al.[56] from N-acetylneuraminic acid in a three step procedure, modifications of which are still currently used today. The procedure involved the protection of the carboxylic acid and of the hydroxy groups of N-acetylneuraminic acid followed by the activation of the protected NeuAc derivative with HCl gas. It is depicted in Scheme 12.

55 K. Okamoto and T. Goto, Tetrahedron, **46** (1990) 5835.
56 R. Kuhn, P. Luts and D. L. MacDonald, **99** (1966) 611.

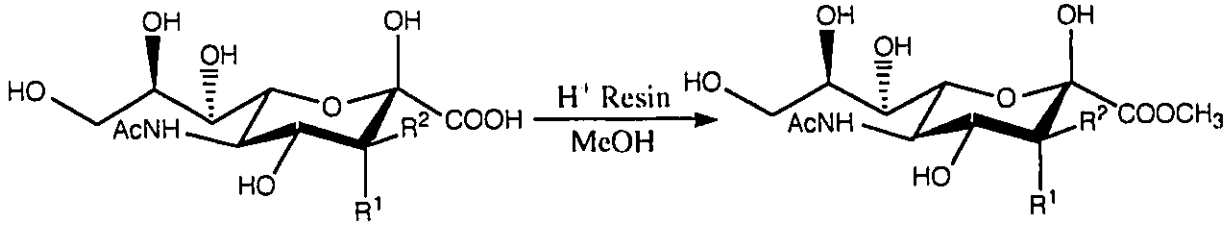


Scheme 12. Classical Synthesis of Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate **22**

This procedure is hindered by the relatively low overall yields of final compound which vary from 20-53% and the cumbersome use of HCl gas. In order to improve this procedure and to synthesize the necessary unlabeled and labeled sialosyl donors, a simple two-step synthetic route was suggested.

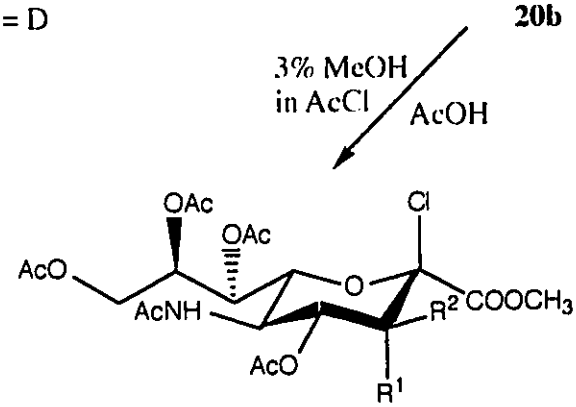
- **Synthesis of Unlabeled and Labeled Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate 22, 22a and 22b**

After consideration of the various inconveniences of the previous procedure, a two-step synthetic route was developed. Similarly to the previous procedure, the first step of the synthetic route involves the protection of the carboxylic acid of N-acetylneuraminic acid. The next and final step was a simultaneous acetylation/activation reaction which permits a one-pot synthesis of the acetochloroneuraminic acid from the protected NeuAc ester. The procedure for the synthesis of the unlabeled, mono- and dilabeled sialosyl donors is shown in Scheme 13.



19 $R^1 = R^2 = H$
19a $R^1 = D, R^2 = H$
19b $R^1 = R^2 = D$

20 $R^1 = R^2 = H$
20a $R^1 = D, R^2 = H$
20b $R^1 = R^2 = D$

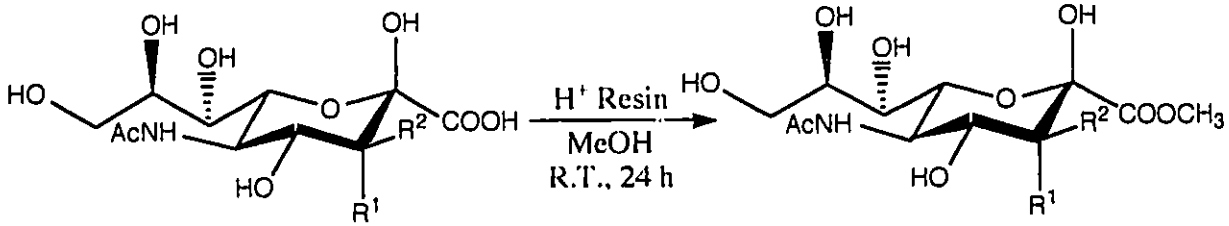


22 $R^1 = R^2 = H$
22a $R^1 = D, R^2 = H$
22b $R^1 = R^2 = D$

Scheme 13. Synthesis of the Unlabeled, Mono- and Dilabeled Sialosyl Donors **22**, **22a** and **22b**.

- **Synthesis of Unlabeled, Mono- and Dilabeled Methyl Esters of N-Acetylneuraminic Acid 20, 20a and 20b**

The esterification of unlabeled, mono- and dilabeled N-acetylneuraminic acid **19**, **19a** and **19b** was performed according to a slight modification of the method by Kuhn et al.[56] and is depicted in Scheme 14.



19 $R^1 = R^2 = H$
19a $R^1 = D, R^2 = H$
19b $R^1 = R^2 = D$

20 $R^1 = R^2 = H$
20a $R^1 = D, R^2 = H$
20b $R^1 = R^2 = D$

Scheme 14. Esterification of Unlabeled, Mono- and Dilabeled N-Acetylneuraminic Acid **19**, **19a** and **19b**.

The esterification was performed in a methanolic solution of the unlabeled, mono- or dilabeled N-acetylneuraminic acid **19**, **19a** or **19b** at room temperature in the presence of H^+ resin (Amberlite IR-120) as catalyst. Thin layer chromatography indicated that after a period of 24 hours the reaction was complete therefore the resin was then removed from the reaction mixture by filtration. The solid obtained from evaporation of the methanolic solution was recrystallized from methanol/ether and yielded the unlabeled mono- and dilabeled methyl esters **20**, **20a** and **20b** in yields ranging from 85 to 95%. The melting points and optical rotation values measured for the products were equivalent to those found in the literature for the unlabeled methyl ester of N-acetylneuraminic acid.

Generally, the ^1H NMR spectra (200 MHz, D_2O) of methyl esters **20**, **20a** and **20b** displayed a complex multiplet located at δ = 4.09 to 3.52 ppm indicating the presence of the H-4,5,6,7,8,9a,9b sialic acid protons, a singlet found at about 3.56 ppm confirming the esterification of N-acetylneuraminic acid, a second singlet at 2.07 ppm corresponding to the acetamido methyl protons and the H-3 protons, if present, appeared at δ = 2.35 (H-3eq) and at 1.95 ppm (H-3ax). The ^1H NMR spectroscopic data for the methyl esters **20**, **20a** and **20b** are summarized in Table 3.

Table 3. ^{1}H NMR Spectroscopic Data for the Methyl Esters **20**, **20a** and **20b**.

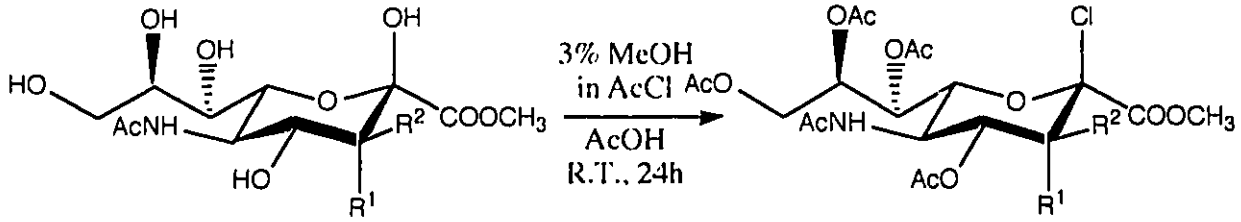
Compound	**20**	**20a**	**20b**	**lit.**[18]
Protons	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	1.95 (13.0, 11.5)	-	-	1.91 (13.0, 11.5)
H-3eq ($J_{3eq,4}$)	2.35 (4.7)	2.34 (4.7)	-	2.32 (5.0)
H-4 to H-9	4.09-3.54	4.07-3.55	4.08-3.52	4.07-3.55
$OC\underline{H}_3$	3.87	3.85	3.84	3.84
$C\underline{H}_3C(O)$	2.08	2.06	2.04	2.05

The integration of the H-3 proton of monolabeled methyl ester **20a**, and the absence of the H-3 proton of dilabeled methyl ester **20b** allowed the verification that full retention of the deuterium labels was achieved during the esterification reaction.

The ^{13}C NMR spectra (50.3 MHz, D_2O) of the methyl esters also confirmed the esterification of the unlabeled and labeled N-acetylneuraminic acid as a signal characteristic of the methyl carbon of the ester moiety was found in the region of δ = 53.4 ppm.

- **Synthesis of Fully Protected Unlabeled Mono- and Dilabeled Sialyl Donors**

  - **Acetylation/Activation Procedure**



20 $R^1 = R^2 = H$
20a $R^1 = D, R^2 = H$
20b $R^1 = R^2 = D$

22 $R^1 = R^2 = H$
22a $R^1 = D, R^2 = H$
22b $R^1 = R^2 = D$

Scheme 15. Acetylation/Activation of Methyl Esters **20**, **20a** and **20b**

The acetylation/activation reaction first involved the preparation of a 3% methanol solution in acetyl chloride. This solution, prepared at 0°C, was stirred overnight in a sealed reaction vessel. The purpose of this preparation is the generation of HCl in situ according to the following reaction.

$$\mathbf{CH_3OH + CH_3C(O)Cl \rightarrow CH_3OC(O)CH_3 + HCl}$$

The cooled HCl saturated acetyl chloride solution was then added to a suspension of the unlabeled, mono- or dilabeled methyl ester **20**, **20a** or **20b** in acetic acid and the mixture was stirred at room temperature in a sealed reaction vessel. The reaction was monitored by TLC and was deemed complete after a 24 hours period. The chlorides **22**, **22a** and **22b** were isolated from the reaction mixture by evaporation of the volatile solvents followed by several co-evaporations of the residual acetic acid with toluene. Chlorides **22**, **22a** and **22b** were obtained as clear oils which could be recrystallized from methylene chloride/diethyl ether/petroleum ether with difficulty to yield white crystals. The yields of the crude chlorides were almost quantitative and the purity was estimated at 90-98% by ^{1}H NMR spectroscopy. The impurity exhibited faint fluorescence on thin layer chromatographic plates and ^{1}H NMR spectroscopy revealed that the main impurity contained a double bond. These clues lead to the conclusion that the impurity was a 2,3-dehydro derivative of N-acetylneuraminic acid resulting from dehydrochlorination. Because of the high purity of the crude oil, the difficulty of crystallization and the short shelf-life of the product, the crude chlorides **22**, **22a** and **22b** were freshly prepared and used as such, without any further purifications, in any further reactions.

The ^{1}H NMR spectra (200 MHz, $CDCl_3$) of chlorides **22**, **22a** and **22b** confirmed the presence of the four acetyl groups which appeared as singlets between δ = 2.12 to 1.88 ppm.

Compared to the ^{1}H NMR spectra of the corresponding methyl esters, an upfield shift of the H-3eq proton was observed in the spectra of unlabeled and monolabeled chlorides **22** and **22a**, indicating the proximity of the electronegative chloride atom on the anomeric carbon. The ^{1}H NMR spectroscopic data of chlorides **22**, **22a** and **22b** is summarized in Table 4.

Table 4. ^{1}H NMR Spectroscopic Data for the Chlorides **22**, **22a** and **22b**.

Compound	**22**	**22a**	**22b**	**lit.**[53]
Protons	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	2.25 (13.9, 11.2)	- (- , -)	- (- , -)	2.28 (13.8, 10.6)
H-3eq ($J_{3eq,4}$)	2.76 (4.8)	2.77 (4.9)	- (-)	2.80 (4.9)
H-4 ($J_{4,5}$)	5.35 (9.7)	5.36 (9.7)	5.35 (9.6)	5.40 (10.7)
H-5 ($J_{5,6}$, $J_{NH,5}$)	4.20 (10.2, 9.6)	4.21 (10.2, 9.5)	4.20 (10.1,9.6)	4.20 (10.4, -)
H-6 ($J_{6,7}$)	4.32 (2.2)	4.32 (2.1)	4.31 (2.1)	4.30 (2.1)
H-7 ($J_{7,8}$)	5.44 (7.1)	5.46 (7.1)	5.44 (7.0)	5.47 (6.8)
H-8 ($J_{8,9a}$, $J_{8,9b}$)	5.15 (2.7, 5.7)	5.15 (2.8, 5.7)	5.15 (2.8, 5.7)	5.18 (2.5, 5.9)
H-9a ($J_{9a,9b}$)	4.39 (13.9)	4.40 (13.9)	4.39 (13.8)	4.40 (12.5)
H-9b	4.03	4.03	4.02	4.08
N<u>H</u>	5.52	5.50	5.50	5.60
OC<u>H</u>$_3$	3.85	3.80	3.84	3.85
C<u>H</u>$_3$C(O)NH C<u>H</u>$_3$C(O)O	2.09 2.05 2.03 2.02 1.88	2.11 2.06 2.04 2.02 1.89	2.12 2.07 2.03 2.01 1.88	

The ^{13}C NMR spectra (50.3 MHz, $CDCl_3$) of chlorides **22**, **22a** and **22b** also contained convincing evidence of the full acetylation of methyl esters **20**, **20a** and **20b**. Compared to the ^{13}C NMR spectra of the methyl esters, the spectra of the chlorides included four additional carbonyl signals (from δ = 171.2 to 170.1 ppm) as well as four more methyl signals (between δ = 23.1 to 20.8 ppm) characteristic of the presence of acetylated hydroxyl groups.

The mass spectra (C.I., ether) of chlorides **22**, **22a** and **22b** included the most convincing evidence of the chloride substitution of the N-acetylneuraminic acid esters **20**, **20a** and **20b**. The mass spectra display two molecular ion peaks, one for each of the two isotopes of chlorine. Furthermore, the spectra also confirmed the presence of the deuterium labels on mono- and dilabeled chlorides **22a** and **22b**. Accordingly, the mass spectra of chlorides **22**, **22a** and **22b** displayed respectively peaks at 510 and 512, 511 and 513 as well as at 512 and 514.

The sialosyl donors **22**, **22a** and **22b** were successfully synthesized in a two step procedure from sialic acids **19**, **19a** and **19b**. This method was quite advantageous by comparison to the classical procedure since the product was obtained in significantly higher yields (76-93%) than when the previous procedure was used (20-44%). Although the above method was very efficient and is noteworthy for its simplicity, it was the result of a time-consuming and careful optimization of reaction conditions. The optimization was performed by the qualitative examination of the reaction mechanism and the identification of the various reaction intermediates and side-products of the reaction.

- **Study of Mechanism**

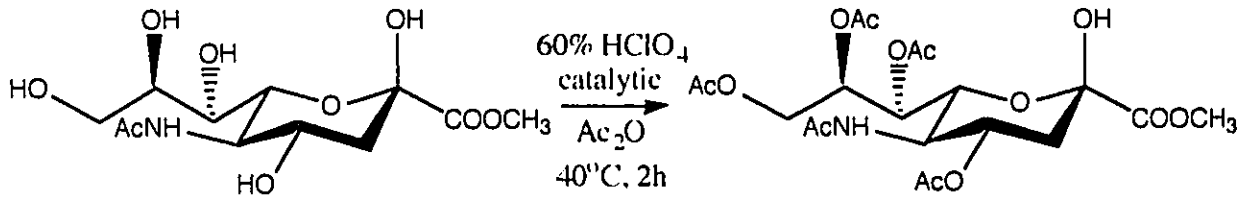
The study of the mechanism of the acetylation/activation reaction began with a hypothesis concerning the identity of the various reaction intermediates. The acetylation of the methyl ester of N-acetylneuraminic acid was assumed to proceed relatively stepwise, the primary hydroxy group being acetylated first, followed by the secondary hydroxy groups and finally the anomeric hydroxy group. Thin layer chromatography (2 x EtOAc) supported this assumption since early TLC's showed several polar spots which over time evolved into five more non-polar and distinct spots at rf's of 0.24, 0.32, 0.38, 0.41 and 0.45. The three lower spots, further converged to the spot found at 0.41, representing the chloride. Tentatively, the spots appearing at 0.24, 0.32, 0.38 and 0.45 were assigned to the tetra-O-acetyl methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (0.24), the α and β penta-O-acetates methyl 5-acetamido-2,4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*- α or β -D-*galacto*-2-

nonulopyranosonate (0.45 and 0.32 respectively) and to the 2,3-dehydro derivative of N-acetylneuraminic acid methyl ester, methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (0.38). The latter assignment was made in view of the faint fluorescence exhibited by the spot when visualized by U.V. light. In order to confirm the hypotheses, the different acetylated derivatives of the methyl ester of N-acetylneuraminic acid were synthesized, and various synthetic procedures were evaluated.

- Synthesis of Reaction Intermediates

- a - Synthesis of β-Tetra-O-Acetate 23

The synthesis of the tetra-O-acetate, methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate **23**, was accomplished using a procedure pioneered by Kuhn et al.[56]. This procedure, then believed to produce the β-penta-O-acetate, has been shown to yield the tetra-O-acetate by Baggett and Marsden[57]. The confusion concerning the procedure was clarified by Marra et al.[58] who determined that the process did indeed yield the tetra-O-acetate (Scheme 16).



20 **23**

Scheme 16. Synthesis of Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate **23**

For the purpose of comparison, the synthesis of the tetra-O-acetyl **23** was performed under the same reaction conditions to that pioneered by Kuhn et al.[56]. A catalytic amount of perchloric acid in acetic anhydride was used to acetylate methyl ester **20**. The reaction was performed at 40°C over a period of 2 hours. In our hands, the reaction yielded two products, the tetra-O-acetate **23** and another side-product believed to be penta-O-acetate **21** in a 2:1 ratio. Tetra-O-acetate **23**

57 N. Baggett and B.J. Marsden, Carbohydr. Res., **110** (1982) 11.
58 A. Marra and P. Sinaÿ, Carbohydr. Res., **190** (1989) 317.

was recrystallized from ethyl acetate/hexanes to give white crystals (yield 58%, m.p. 147.0-148.3°C, $[\alpha]_D$ -2.3° (c1.0, chloroform), lit.[58] m.p. 147-148°C, $[\alpha]_D$ -2.1° (c1, chloroform)). The spectroscopic characterization data were consistent with the one published by Marra et al.[58] and are summarized in Table 5 (page 65).

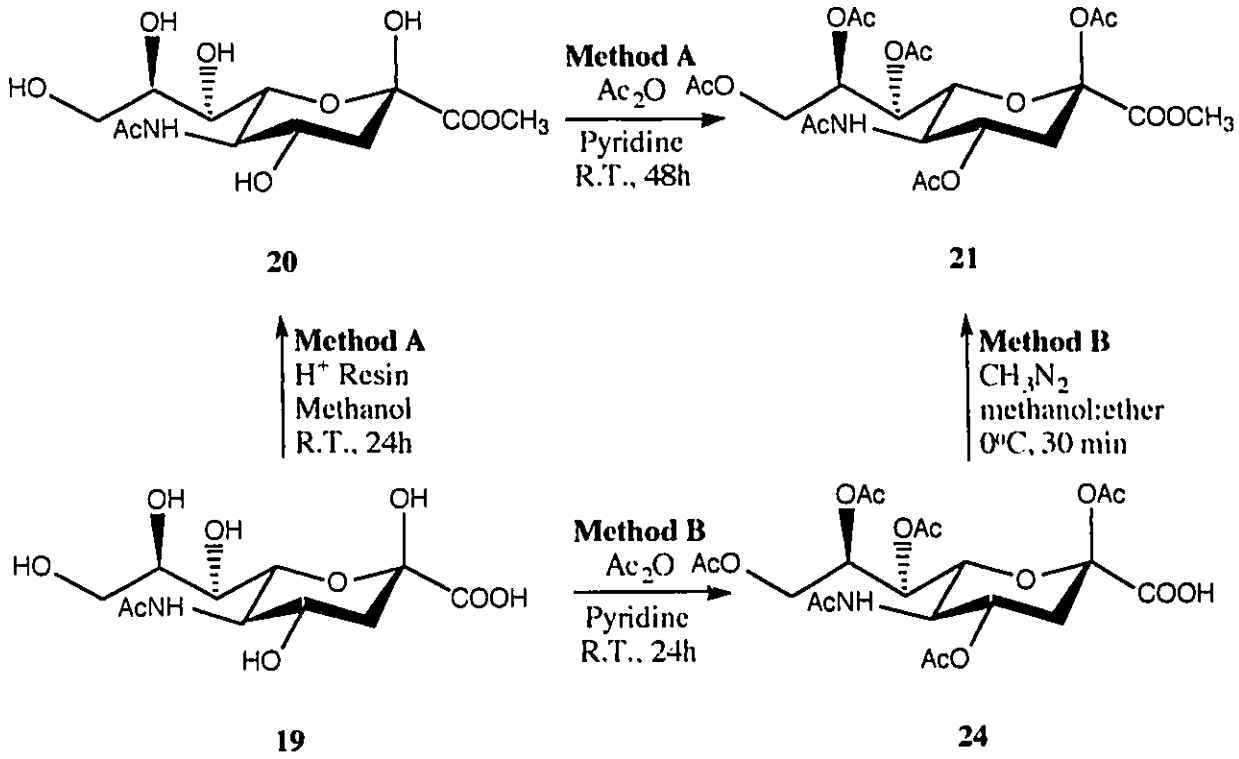
The formation of two products rather than only tetra-O-acetate **23** can be explained by the apparent high sensitivity of the acetylation to reaction conditions such as concentration of the catalyst, temperature and reaction time. As the concentration of catalyst to be used was not clearly described in the publications by Kuhn[56] and Marra[58], it was found that the use of different concentrations of catalyst influenced greatly the yields of both the tetra- and the penta-O-acetates **23** and **21**. As expected, a five times greater concentration of catalyst yielded, over the same period of time, a 1:1 molar ratio of the tetra- and penta-O-acetates. When the reaction was performed at room temperature, rather than at 40°C, after a period of two hours, in addition to tetra-O-acetate **23** only a trace of the penta-O-acetate was observed but the acetylation of the starting material was not complete. Leaving the reaction at room temperature for over two days was sufficient to complete the synthesis of tetra-O-acetate **23** but, by that time, penta-O-acetate **21** was predominant in the reaction mixture. In order to attempt producing exclusively penta-O-acetate **21**, the reaction was performed at 40°C, left to react overnight and yielded the penta-O-acetate in a 70% yield. Traces of tetra-O-acetate **23** could still be detected by TLC. In light of the above experiments it appeared evident that the tetra-O-acetate was an intermediate in the acetylation reaction and that its isolation was a delicate balance of reaction conditions. The reaction conditions preferred were those proposed by Kuhn et al.[56] although carefully monitoring the reaction by TLC to determine the optimum time to stop the reaction was recommended.

Thin layer chromatography of the tetra-O-acetate **23** displayed a spot at a rf of 0.24 confirming the initial assumption as to the identity of the compound indicated by that spot in the TLC of the acetylation/activation reaction.

- b - Synthesis of β-Penta-O-acetate 21

Two different methods are currently used to synthesize β-penta-O-acetate, methyl 5-acetamido-2,4,7,8,9-penta-O-acetyl-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate **21**. Kuhn et al.[56] developed a method involving the acetylation of the methyl ester of N-acetylneuraminic acid **20** using acetic anhydride in pyridine. A two step procedure, reported by

Sugiyama et al.[59] requires the acetylation of N-acetylneuraminic acid **19** followed by esterification with diazomethane. In order to synthesize β-penta-O-acetate **21** and to compare the usefulness of both methods, both procedures were used. The procedures are depicted in Scheme 17.



Scheme 17. Synthesis of Methyl 5-Acetamido-2,4,7,8,9-penta-O-acetyl-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate **21**

In the literature, there are conflicting reports concerning the results of acetylation Method A. Kuhn et al[56] who pioneered the method suggested that acetylation of the methyl ester of N-acetylneuraminic acid **20** gave an anomeric mixture of the penta-O-acetates but the mixture was not analyzed. Using these conditions, Baggett and Marsden[57] reported the formation of the crystalline tetra-O-acetate **23**, Sharma and Eby[53] reported that β-penta-O-acetate **21** was formed, but the physical properties reported appear to be those of tetra-O-acetate **23** (lit.[53] m.p. 157-158°C, $[\alpha]_D$ -3.4° (c1.0, chloroform)) and Warner et al.[60] also reported the formation of amorphous β-penta-O-acetate **21**. When we applied Method A, that is treating methyl ester **20**, previously obtained from

59 N. Sugiyama, K. Sugai, N. Yamada, M. Goto, C. Ban, K. Furuhata, H. Takayanagi and H. Ogura, Chem. Pharm. Bull., **36** (1988) 1147.

60 T.G. Warner and L.A. Lee, Carbohydr. Res., **176** (1988) 211.

the esterification of N-acetylneuraminic acid, with acetic anhydride/pyridine for 48 hours at room temperature, TLC of the reaction mixture indicated that two products were present. A minor product was found at a rf of 0.36 and the major product was found at 0.32. In addition, no trace of tetra-O-acetate **23** was detected on TLC. Chromatography of the crude products (60:1 chloroform:methanol) yielded β-penta-O-acetate **21** as a clear oil (yield 66%, $[\alpha]_D$ -31.1° (c1.0, chloroform), lit.[58] $[\alpha]_D$ -32° (c1, chloroform)). The ^1H NMR, ^{13}C NMR and mass spectra confirmed the identity of the major product of the reaction and are consistent with published spectroscopic data[58]. ^1H NMR data is summarized in Table 5 (page 65). Since TLC of the reaction indicated no trace of tetra-O-acetate **23** and the spot found at 0.36 exhibited no faint fluorescence, the spot was tentatively assigned to the α-penta-O-acetate (yield, 25%). Verification of this tentative assignment will be done in the following section.

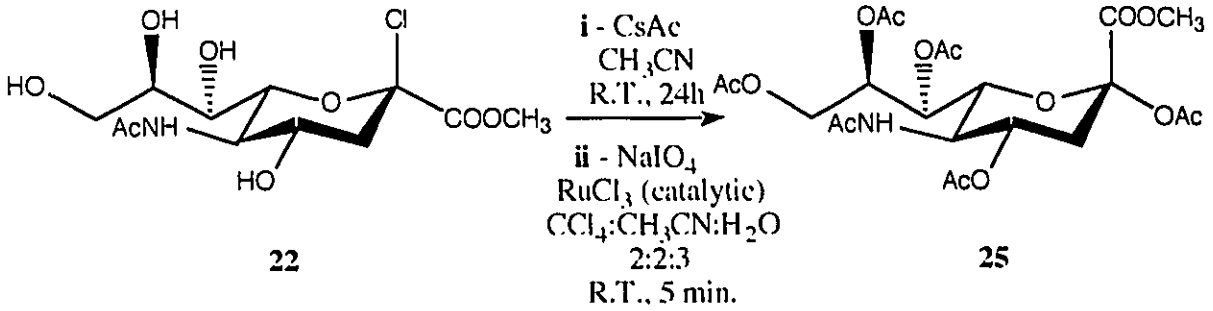
Acetylation by Method B reversed the order of the steps of Method A. The acetylation of N-acetylneuraminic acid **20** was performed first and was followed by the esterification of the intermediate with diazomethane. The acetylation was performed at room temperature in a mixture of acetic anhydride/pyridine and yielded, after 24 hours, penta-O-acetylated intermediate **24**. After evaporation and several toluene coevaporations, a methanolic solution of crude intermediate **24** was treated with etherial diazomethane at 0°C. The reaction was deemed complete after the evolution of nitrogen gas stopped and starting material could no longer be detected by TLC. Thin layer chromatography indicated the presence of β-penta-O-acetate **21** at rf = 0.32 and a trace of a secondary product was seen at rf = 0.36. Only β-penta-O-acetate **21** was isolated from the mixture of compounds (yield 90%).

To fairly compare the two acetylation Methods A and B the yields of β-penta-O-acetate have to be based on the same starting material. Therefore, the yields of β-penta-O-acetate by Methods A and B were respectively of 56-63% (when considering that the yields of esterification of N-acetylneuraminic acid vary from 85-95%) and 90%. This comparison clearly showed the superiority of the Method B.

The initial hypothesis concerning the identity of the various compounds displayed on TLC of the acetylation/activation of methyl ester **20** was confirmed for the β-penta-O-acetate intermediate; the spot found at 0.32 does correspond to β-penta-O-acetate **21**.

- c - Synthesis of α-Penta-O-acetate 25

The synthesis of methyl 5-acetamido-2,4,7,8,9-penta-O-acetyl-2,3,5-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate **25** was accomplished according to a procedure by Marra et al.[58] (Scheme 18).



Scheme 18 Synthesis of Methyl 5-Acetamido-2,4,7,8,9-penta-O-acetyl-2,3,5-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate **25**

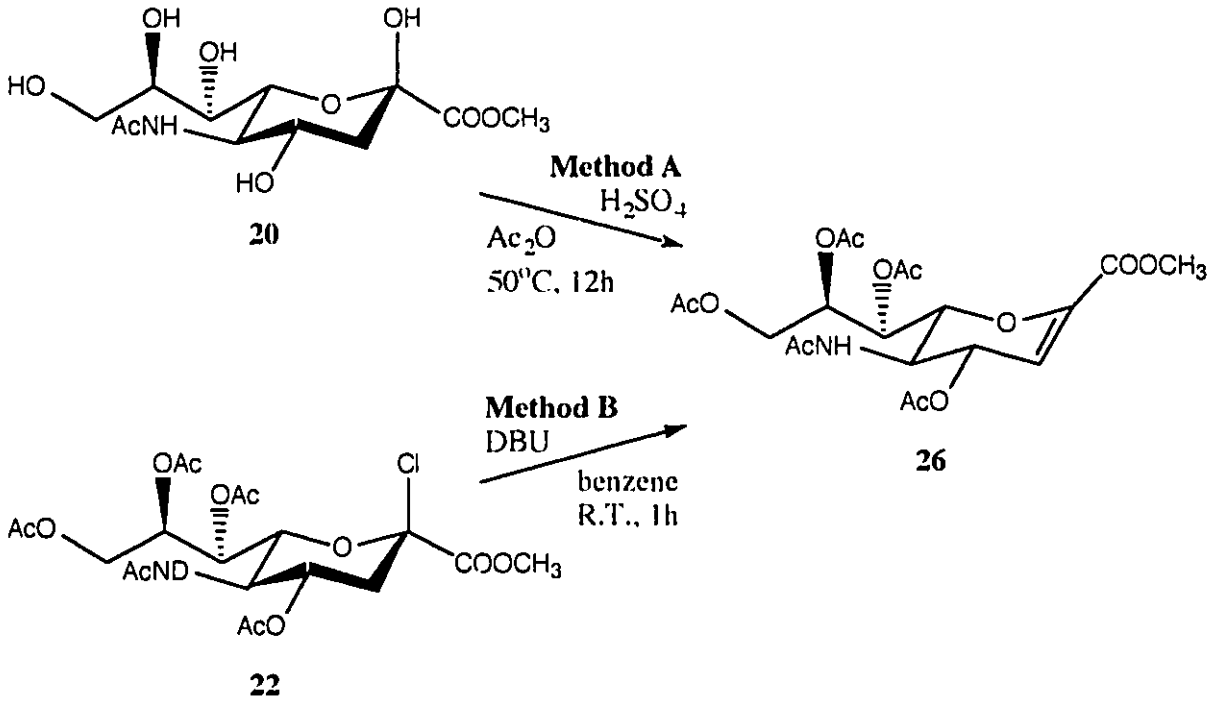
An acetonitrile solution of freshly prepared chloride **22** was stirred at room temperature in the presence of cesium acetate. After 24 hours, TLC of the reaction mixture revealed two spots; the major product, presumably the desired α-penta-O-acetate **25** appeared at a rf of 0.36 and the second spot found at a rf of 0.38 was assigned to the 2,3-dehydro derivative. Because of the proximity of the two spots and the probable difficulty of chromatographic separation of the mixture, the 2,3-dehydro derivative was destroyed in situ using an oxidizing agent. Thus the mixture obtained from the acetylation was dissolved in a 2:2:3 carbon tetrachloride: acetonitrile: water mixture, sodium metaperiodate and a catalytic amount of ruthenium trichloride were added. The biphasic mixture was vigorously stirred at room temperature for 5 minutes. After separation of the phases, several washes with water and chromatography on silica gel (ethyl acetate), α-penta-O-acetate **25** was obtained as a clear oil in 60% yield ($[\alpha]_D$ +13.2° (c1.0, chloroform), lit.[58] $[\alpha]_D$ +13° (c1, chloroform)). The spectroscopic data of the α-penta-O-acetate **25** are consistent with published results[58]. The ^1H NMR data is summarized in Table 5 (page 65).

The rf of the α-penta-O-acetate **25** was calculated to be 0.36. The initial hypothesis which tentatively attributed the formation of the spot at rf = 0.45 to α-penta-O-acetate **25** was incorrect. Therefore, there was no evidence regarding the presence of α-penta-O-acetate **25** as an

intermediate in the synthesis of chloride **22**. Furthermore, the identity of the compound found at a rf of 0.45 on TLC plates of the acetylation/activation of methyl ester **20** was not known.

- d - Synthesis of 2,3-Dehydro Derivative 26

Procedures for the synthesis of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate **26** have been developed by Kumar[61] (Method A) and Okamoto[62] (Method B) (Scheme 19).



Scheme 19. Synthesis of Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate **26**

The procedure by Kumar et al.[61] (Method A) involves a one-pot acetylation/elimination of N-acetylneuraminic acid methyl ester **20**. Treatment of N-acetylneuraminic acid methyl ester **20** with acetic anhydride in the presence of a catalytic amount of concentrated sulfuric acid at 50°C

61 V. Kumar, J. Kessler, M.E. Scott, B.H. Patwardhan, S.W. Tanenbaum and M. Flashner, Carbohydr. Res., **94** (1981) 123.
62 K. Okamoto, T. Kondo and T. Goto, Bull. Chem. Soc. Jpn, **60** (1987) 631.

yielded, as demonstrated by TLC, one single product, at a rf of 0.38. Although TLC appeared to indicate the formation of only one product, the complexity of the ^{1}H NMR spectrum disproved that assumption. The presence of two olefinic doublets in the spectrum were indicative of the possibility of an acid catalyzed epimerization of 2,3-dehydro derivative **26**. This mixture could not be separated.

The base catalyzed elimination procedure of Okamoto et al.[62], Method B, was much more successful than Method A. The 2,3-dehydro derivative **26** was easily synthesized from freshly prepared sialyl chloride **22**. The reaction took place in the presence of a base (DBU), in benzene and was complete within one hour. As determined by TLC, and confirmed by ^{1}H NMR spectroscopy, the 2,3-dehydro derivative **26** was the only product of the reaction. The spot, as previously observed, exhibited faint fluorescence, and was found at a rf of 0.38. The product was isolated as a syrup which was recrystallized from benzene/acetone to give the pure 2,3-dehydro derivative **26**, as white needles (yield 76%). The spectroscopic data for the 2,3-dehydro derivative **26** were consistent with published results[62] (^{1}H NMR spectroscopic data included in Table 5, page 65).

Of the two methods compared, only the elimination methodology gave satisfactory results. The procedure was also successfully used with triethylamine but the yield was slightly lower (61%).

The hypothesis regarding the identity of the compound forming the spot at a rf of 0.38 on TLC of the acetylation/activation of N-acetylneuraminic acid methyl ester was confirmed. The spot correspond to the 2,3-dehydro derivative **26**.

Table 5. ^{1}H NMR Spectroscopic Data of Acetylated Derivatives of Methyl Ester **20**

Compound	**21**	**23**	**24**	**26**
Protons	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	2.07 (13.6, 11.5)	2.23 (12.6, 11.4)	2.06 (13.2, 12.0)	-
H-3eq ($J_{3eq,4}$)	2.52 (5.0)	2.18 (4.9)	2.54 (4.7)	-
H-3 ($J_{3,4}$)	-	-	5.59 (3.2)	5.59 (3.2)
H-4 ($J_{4,5}$)	5.23 (6.4)	5.19 (-)	4.99 (10.2)	5.47 (7.5)
H-5 ($J_{5,6}$, $J_{NH,5}$)	4.13-4.06 (- , 9.6)	4.16-4.11 (- , -)	4.13 (10.7, 10.4)	4.35 (- , -)
H-6 ($J_{6,7}$)	4.13-4.06 (2.0)	4.16-4.11 (1.6)	4.67 (2.4)	4.37 (3.4)
H-7 ($J_{7,8}$)	5.35 (5.2)	5.31 (6.0)	5.35 (6.9)	5.49 (4.7)
H-8 ($J_{8,9a}$, $J_{8,9b}$)	5.05 (2.6, 6.7)	5.22 (2.4, 7.4)	5.15 (2.4, 5.8)	5.34 (3.3, 6.9)
H-9a ($J_{9a,9b}$)	4.47 (12.4)	4.44 (12.4)	4.33 (12.4)	4.57 (12.3)
H-9b	4.10	4.00	4.02	4.17
O<u>H</u>	4.47 (0.8)	4.47 (0.8)	-	-
N<u>H</u>	5.30	5.53	5.36	5.52
OC<u>H</u>$_3$	3.77	3.84	3.73	3.79
C<u>H</u>$_3$C(O)NH C<u>H</u>$_3$C(O)O	2.13 2.12 2.04 2.03 2.02 1.87	2.12 2.08 2.01 2.00 1.88	2.11 2.07 2.07 2.03 2.02 1.88	2.11 2.06 2.05 2.03 1.92

- **Mechanism**

TLC plates taken during the acetylation/activation of N-acetylneuraminic acid methyl ester **20** revealed, in addition to the presence of chloride **22**, four other products. These products were positively identified as being the tetra-O-acetate **23** (rf = 0.24), the β-penta-O-acetate **21** (rf = 0.32) and the 2,3-dehydro derivative **26** (rf = 0.38). A spot, at a rf of 0.45 remained unidentified.

Tetra-O-acetate **23**, β-penta-O-acetate **21** and the 2,3-dehydro derivative **26** were confirmed as intermediates or side products of the acetylation/activation reaction. Furthermore, TLC of the reaction did not show the presence of the α-penta-O-acetate **25**. Although rigorous determination of reaction mechanisms require extensive kinetic studies, the knowledge acquired during the qualitative study of the acetylation/activation reaction was used to establish the following tentative reaction mechanism (Figure XI).

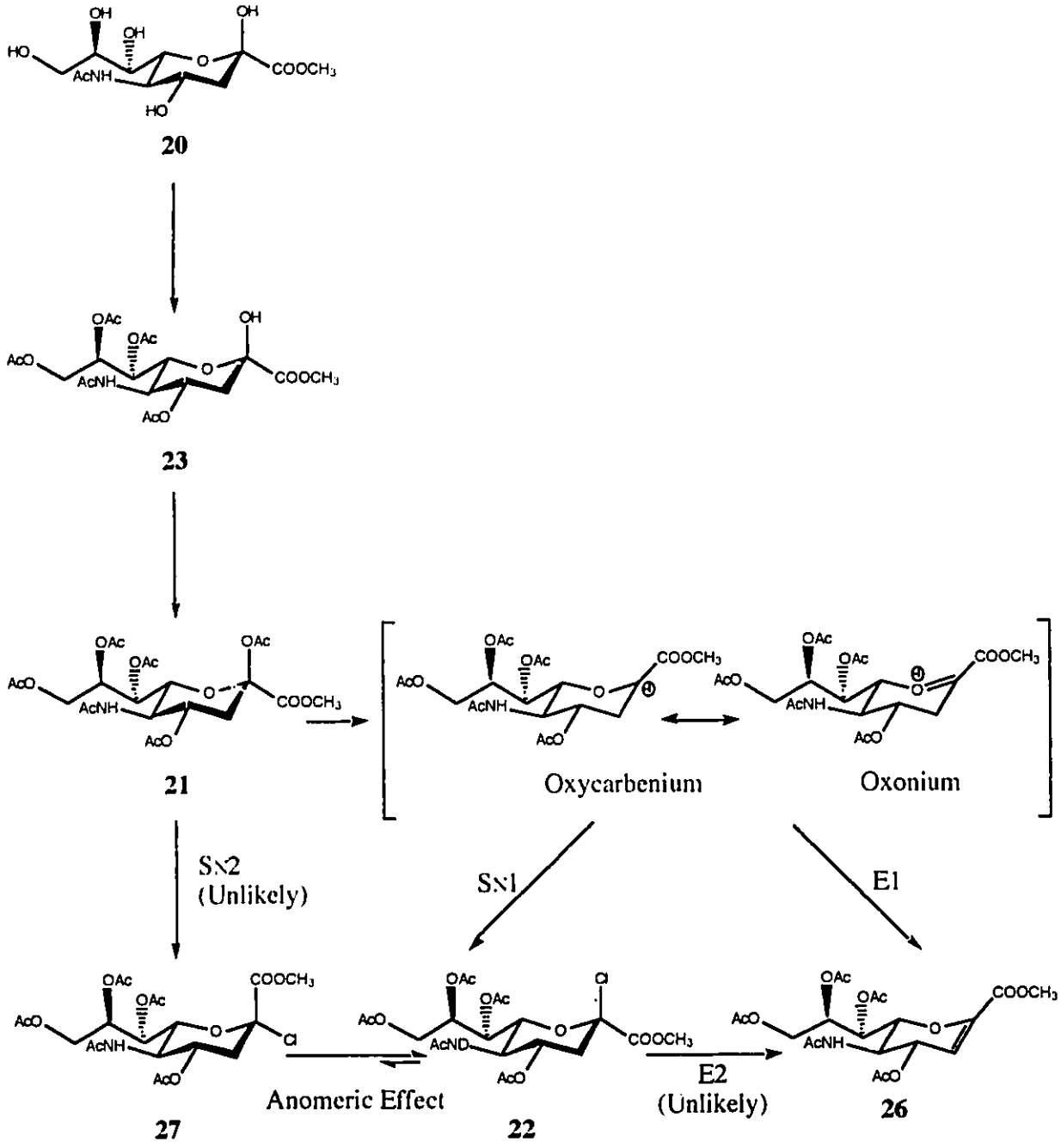


Figure XI. Mechanism of Acetylation/activation of N-Acetylneuraminic Acid Methyl Ester of **20**

The acetylation of the primary hydroxyl group of N-acetylneuraminic acid methyl ester **20** was believed to occur first, followed by the acetylation of the secondary hydroxyl groups, to form tetra-O-acetate **23**. The tertiary hydroxyl group, located on the anomeric carbon was then acetylated, yielding β-penta-O-acetate **21**.

Two possible mechanisms may thereafter participate to the chloride substitution of β-penta-O-acetate **21**. A bimolecular nucleophilic substitution (S_N2) at the anomeric carbon produced the α-anomer of the chloride **27** with full inversion of configuration. Although possible, the contribution of this substitution mechanism to the formation of the chloride, was believed to be unlikely because the steric hindrance of the anomeric carbon disfavored the formation of the required transition state. The preferred substitution mechanism was through an unimolecular nucleophilic substitution mechanism (S_N1). The formation of the carbocation intermediate, produced by the acid catalyzed loss of acetic acid, was resonance stabilized (oxycarbenium ion ↔ oxonium anion) and therefore was highly favored. The nucleophilic attack of the chloride anion could proceed on either side of the pyranose ring to give the α and β chlorides **27** and **22** respectively. Then, because of the anomeric effect, that is the conformational preference for the axial position of anomeric substituents, the formation of β chloride **22** was favored.

The 2,3-dehydro derivative **26** was believed to be formed by an E1 mechanism, which competes with the substitution mechanisms. The loss of the axial H-3 proton after the formation of the carbocation resulted in the formation of side-product **26**. Although the axial H-3 proton and the chloride atom of β-chloride **22** are in a trans relationships to one another, conformation usually favorable to elimination mechanisms (E2), the formation of 2,3-dehydro derivative **26** was not believed to occur from β-chloride **22** since, as observed in the synthesis derivative **26**, a base is required to remove the H-3ax axial proton. Therefore, the only mechanism explaining adequately the formation of 2,3-dehydro derivative **26** was a E1 mechanism involving the formation of a carbocation intermediate.

The proposed mechanism provided some information about the identity of the unidentified product detected on TLC of the acetylation/activation reaction. It was concluded that the product was the α anomer of chloride **27**. This conclusion was confirmed by visualization of a TLC plate of the acetylation/activation reaction mixture in a silver nitrate solution. Charring revealed the presence of two spots, at rf = 0.45 and 0.41, representing respectively the α and β-anomers of chloride **27** and **22**.

- **Improvement of Acetylation/Activation Reaction**

Guided by the mechanism of the reaction, improvements to the acetylation/activation reaction were made in the following manner.

Initially, the goal was to provide a procedure that would allow the full acetylation of methyl ester of N-acetylneuraminic acid **20** followed by the activation of acetate **21** produced in one pot. Elimination of the use of external HCl was also desired.

Acetyl chloride was selected as acetylating agent in the hope that the HCl produced from acetylation (5 eq per molecule) would be sufficient to activate the acetylated intermediate. In such conditions, it was difficult to reach completion of the reaction. Since, as observed by TLC, the reaction appeared to loiter at the formation of tetra-O-acetate **23** additional acetylating agents were introduced into the reaction mixture. The addition of acetic anhydride or the combination of acetic anhydride/acetic acid had no effect on the reaction rate.

Since addition of further acetylating agents did not appear to give any positive result, a different approach was taken to drive the reaction to completion. In situ generation of HCl was considered. The generation of HCl in the reaction system would facilitate the formation of chloride **22**, thereby consuming the β-penta-O-acetate. In turn, the equilibrium formed between tetra-O-acetate **23** and β-penta-O-acetate **21** would have to shift towards the formation the penta-O-acetate. In order to generate the HCl in situ, a proton donor, which would react with the acetyl chloride, was needed. Water and methanol were tested but the violence and rapidity of the reaction of water with acetyl chloride, quickly eliminated that option. Various concentration of methanol in acetyl chloride were tested (1%, 3%, 10%). The solutions were prepared at 0°C and left to react overnight. The 3% methanol solution was found to be the most practical. It contained a sufficient amount of HCl, to drive the acetylation/activation reaction to completion. More concentrated solutions of methanol in acetyl chloride were difficult to contain in closed reaction systems and were hazardous to handle.

Thus, the procedure developed for the one pot acetylation/activation of methyl 5-acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate **20** involved the preparation of a 3% solution of methanol in acetyl chloride which was left to react overnight in a closed reaction vessel to allow for the formation of HCl, followed by the addition of that solution to a suspension of methyl ester **20** in acetic acid. After a 24 h period, chloride **22** was obtained in nearly quantitative yields and the purity was estimated at 90-98%.

2.3 Conclusion

The synthesis of unlabeled, mono and dilabeled sialosyl donors (**22**, **22a** and **22b**) was successfully accomplished in a two step procedure from unlabeled and labeled sialic acids **19**, **19a** and **19b**. This method is quite advantageous by comparison to the classical procedure since the product is obtained in significantly higher yields (76-93%) than when the previous procedure is used (20 -44%). A careful optimization of the acetylation/activation reaction was performed by the examination of the mechanism of the reaction. Synthesis of the various reaction intermediates provided sufficient information to determine the mechanism of the reaction which, when clearly established, revealed the best approach to optimize the reaction. Furthermore, the synthesis of the various intermediates allowed the comparison of existing procedures for their preparation, allowed clarification of the outcome of several of the procedures and will be practical standards to evaluate the identity of side-products produced in the forthcoming glycosidation of the sialosyl donors.

2.4 Experimental

2.4.0 General Methods

See Section **1.4.0** in Chapter 1.

2.4.1 Synthesis of Deuteriated N-Acetylneuraminic Acid Derivatives.

5-Acetamido-3ax-deutero-3,5-dideoxy-β-*glycero*-D-*galacto*-2-nonulosonic acid 19a - The synthesis was performed following a slight modification of previously described procedures[51,52] and consisted generally of adjusting the pD of a solution of N-acetylneuraminic acid **19** (0.150 g, 0.5 mmol, previously lyophilized in deuterium oxide) in D_2O (20 ml) to 11.4 with a 2M solution of NaOD in deuterium oxide. The reaction vessel was flushed with nitrogen gas and sealed, the reaction mixture was stirred at room temperature for 3.5 hours and was subsequently quenched using D^+ resin (Rexyn 101, previously washed in D_2O). The solution was filtered and lyophilized to yield the monolabeled acid **19a** as a white powder (0.135 g, yield 90%). Extent of deuteriation as determined by ^1H NMR: 92%; m.p. 186.0-187.0°C; $[\alpha]_D$ -33.6° (c1.0, H_2O); lit.[54] m.p. 186-187°C dec.; $[\alpha]_D$ -33.8° (c1.0, H_2O); lit.[53] m.p. 188-189°C; ^1H NMR (200 MHz, D_2O) δ (ppm) 4.06-3.56 (m, 7H, H-4, 5, 6, 7, 8, 9a, 9b), 2.24 (d, 1H, $J_{3eq,4}$ = 4.8 Hz, H-3eq), 2.07 (s, 3H, $CH_3C(O)ND$); Trace of α anomer 8%; lit.[18] ^1H NMR of N-Acetylneuraminic acid (500 MHz, D_2O, pH = 7.0) δ (ppm) 4.024 (ddd, 1H, $J_{3ax,4}$ = 11.4 Hz, $J_{3eq,4}$ = 4.6 Hz, $J_{4,5}$ = 10.2 Hz, H-4), 3.984 (dd, 1H, $J_{5,6}$ = 10.2 Hz, $J_{6,7}$ = 1.0 Hz, H-6), 3.899 (dd, 1H, H-5), 3.835 (dd, 1H, $J_{8,9a}$ = 2.8 Hz, $J_{9a,9b}$ = 11.4 Hz, H-9a), 3.753 (ddd, 1H, $J_{7,8}$ = 9.0 Hz, $J_{8,9b}$ = 6.2 Hz, H-8), 3.608 (dd, 1H, H-9b), 3.514 (dd, 1H, H-7), 2.208 (dd, 1H, $J_{3ax,3eq}$ = 12.6 Hz, H-3eq), 2.050 (s, 3H, $CH_3C(O)ND$), 1.827 (dd, 1H, H-3ax); ^{13}C NMR (50.3 MHz, D_2O) δ (ppm) 176.1 (1C, C-1), 174.6 (1C, $CH_3\underline{C}(O)$), 96.5 (1C, C-2), 71.6 (1C, C-8), 71.4 (1C, C-6), 69.5 (1C, C-7), 67.9 (1C, C-4), 64.4 (1C, C-9), 53.3 (1C, C-5), 23.4 (1C, $\underline{C}H_3C(O)$); lit.[18] ^{13}C NMR of N-Acetylneuraminic acid (500 MHz, D_2O, pH = 7.0) δ (ppm) 177.87 (1C, C-1), 175.98 (1C, $CH_3\underline{C}(O)$), 97.61 (1C, C-2), 71.59 (1C, C-8), 71.45 (1C, C-6), 69.82 (1C, C-7), 68.51 (1C, C-4), 64.55 (1C, C-9), 53.50 (1C, C-5), 40.63 (1C, C-3), 23.34 (1C, $\underline{C}H_3C(O)$); M.S. (Negative FAB) of **19a** exchanged in H_2O, m/z : 309 (100%, $[M - H]^-$).

5-Acetamido-3,5-dideoxy-3ax,3eq-dideutero-β-*glycero*-D-*galacto*-2-nonulosonic acid 19b - The synthesis was performed following a slight modification of previously described procedures[51,52] and consisted generally of adjusting the pD of a solution of N-acetylneuraminic acid **19** (0.150 g, 0.5 mmol, previously lyophilized in deuterium oxide) in 20 ml of deuterium oxide to 11.4 with a 2M solution of NaOD in D_2O. The reaction vessel was flushed with nitrogen gas and, sealed. The reaction mixture was stirred at room temperature for 48 hours. The reaction was quenched using H^+ resin previously washed in deuterium oxide, and the mixture was filtered and lyophilized. The dilabeled acid **19b** was obtained as a white powder (0.138 g, yield 92%). Extent of deuteriation, as determined by ^1H NMR ≈ 99%; m.p. 186.2-187.1°C; $[\alpha]_D$ -33.5° (c1.0, H_2O); lit.[54] m.p. 186-187°; $[\alpha]_D$ -33.8° (c1.0, H_2O); lit.[53] m.p. 188-189°C; ^1H NMR (200 MHz, D_2O): δ (ppm) 4.04-3.54 (m, 7H, H-4, 5, 6, 7, 8, 9a, 9b), 2.07 (s, 3H, $CH_3C(O)ND$); ^{13}C NMR (50.3 MHz, D_2O): δ (ppm) 176.1 (1C, C-1), 174.6 (1C, $CH_3\underline{C}(O)$), 96.6 (1C, C-2), 71.7 (1C, C-8), 71.4 (1C, C-6), 69.5 (1C, C-7), 68.0 (1C, C-4), 64.4 (1C, C-9), 53.4 (1C, C-5), 23.4 (1C, $\underline{C}H_3C(O)$); M.S. (Negative FAB) of **19b** exchanged in H_2O, m/z: 310 (100%, $[M - H]^-$).

2.4.2 General Method for the Synthesis of Methyl 5-Acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate, (unlabeled, mono- and dilabeled derivatives 20, 20a or 20b).

A solution of the acid **19**, **19a** or **19b** (3.23 g, 10.0 mmol) in methanol (50 ml) containing Amberlite IR-120 (H^+) resin was stirred at room temperature for 24 hours, until completion of the reaction (TLC 3:7 H_2O:isopropyl alcohol). The clear solution was filtered on a filter paper and evaporated to dryness. The solids obtained were recrystallized from methanol/ether and the ester **20**, **20a** or **20b** respectively was obtained as white crystals in nearly quantitative yields (2.75 g-3.07 g, ≈ 85-95% yields).

Methyl 5-Acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate 20 - 3.07 g, Yield 95%; m.p. 181.0-182.3°C; $[\alpha]_D$ -28.0° (c1.0, water); lit.[54] m.p. 180-182°C; $[\alpha]_D$ -28.0° (c1.0, water); lit.[56] m.p. 179-180°C; $[\alpha]_D$ -28° (c1.0, water); ^1H NMR (200 MHz, D_2O): δ (ppm) 4.09-3.54 (m, 7H, H-4, 5, 6, 7, 8, 9a, 9b), 3.87 (s, 3H, OMe), 2.35 (dd, 1H, $J_{3ax,3eq}$ = 13.0 Hz, $J_{3eq,4}$ = 4.7 Hz, H-3eq), 2.08 (s, 3H, $CH_3C(O)$), 1.95 (dd, 1H, $J_{3ax,4}$ = 11.5 Hz, H-3ax); lit.[18] ^1H NMR (500 MHz, D_2O): δ (ppm) 4.067 (dd, 1H, $J_{3ax,4}$ = 11.5 Hz, $J_{3eq,4}$ = 5.0 Hz, H-4), 4.067 (dd, 1H, H-6), 3.916 (dd, 1H, H-5), 3.838 (s, 3H, OMe), 3.834 (dd, 1H, H-9a), 3.731 (ddd, 1H, H-8), 3.619 (dd, 1H, H-9b), 3.552 (dd, 1H, H-

7), 2.315 (dd, 1H, $J_{3ax,3eq}$ = 13.0 Hz, H-3eq), 2.051 (s, 3H, $CH_3C(O))ND$), 1.913 (dd, 1H, H-3ax); ^{13}C NMR (50.3 MHz, D_2O): δ (ppm) 176.1 (1C, $CH_3\underline{C}(O)ND$), 172.0 (1C, C-1), 98.1 (1C, C-2), 71.7 (1C, C-8), 71.3 (1C, C-6), 69.4 (1C, C-7), 67.8 (1C, C-4), 64.6 (1C, C-9), 53.4 (1C, OMe), 53.3 (1C, C-5), 40.3 (1C, C-3), 23.3 (1C, $\underline{C}H_3C(O)$); lit.[18] ^{13}C NMR (500 MHz, $CDCl_3$): δ (ppm) 176.1 (1C, $CH_3\underline{C}(O)ND$), 174.1 (1C, C-1), 100.6 (1C, C-2), 71.8 (1C, C-8), 71.2 (1C, C-6), 69.4 (1C, C-7), 67.8 (1C, C-4), 64.7 (1C, C-9), 53.1 (1C, C-5), 52.1 (1C, OMe), 40.6 (1C, C-3), 23.3 (1C, $\underline{C}H_3C(O)$); M.S. (C.I., ether) m/z: 324 (5%, $[M + H]^+$), 308 (10%, $[M - Me]^+$), 292 (25%, $[M-OMe]^+$); Anal. calcd for $C_{12}H_{21}NO_9 \cdot H_2O$: C, 42.23; H, 6.79; N, 4.10; Found: C, 42.18; H, 6.85; N, 4.06.

Methyl 5-Acetamido-3,5-dideoxy-3ax-deutero-β–D-*glycero*-D-*galacto*-2-nonulopyranosonate 20a - 444 mg, Yield 85%; m.p. 179.0-181.2°C: $[\alpha]_D$ -28.2° (c1.0, water); ^1H NMR (200 MHz, D_2O): δ (ppm) 4.07-3.55 (m, 7H, H-4, 5, 6, 7, 8, 9a, 9b), 3.85 (s, 3H, OMe), 2.34 (d, 1H, $J_{3eq,4}$ = 4.7 Hz, H-3eq), 2.06 (s, 3H, $CH_3C(O)$); ^{13}C NMR (50.3 MHz, D_2O): δ (ppm) 176.1 (1C, $CH_3\underline{C}(O)ND$), 172.1 (1C, C-1), 98.1 (1C, C-2), 71.7 (1C, C-8), 71.3 (1C, C-6), 69.4 (1C, C-7), 67.9 (1C, C-4), 64.7 (1C, C-9), 53.4 (1C, OMe), 53.3 (1C, C-5), 23.3 (1C, $\underline{C}H_3C(O)$); M.S. (C.I., ether) m/z: 325 (5%, $[M + H]^+$); Anal. calcd for $C_{12}H_{20}DNO_9 \cdot H_2O$: C, 42.10; H, D, 7.07; N, 4.09; Found: C, 42.20; H, D, 7.00; N, 4.00.

Methyl 5-Acetamido-3,5-dideoxy-3ax,3eq-dideutero-β–D-*glycero*-D-*galacto*-2-nonulopyranosonate 20b - 226 mg,Yield 86%; m.p. 178.5-180.0°C; $[\alpha]_D$ -28.1° (c1.0, water); ^1H NMR (200 MHz, D_2O): δ (ppm) 4.08-3.52 (m, 7H, H-4, 5, 6, 7, 8, 9a, 9b), 3.84 (s, 3H, OMe), 2.04 (s, 3H, $CH_3C(O)$); ^{13}C NMR (50.3 MHz, D_2O): δ (ppm) 176.1 (1C, $CH_3\underline{C}(O)ND$), 172.1 (1C, C-1), 98.2 (1C, C-2), 71.8 (1C, C-8), 71.3 (1C, C-6), 69.4 (1C, C-7), 67.9 (1C, C-4), 64.7 (1C, C-9), 53.5 (1C, OMe), 53.4 (1C, C-5), 23.3 (1C, $\underline{C}H_3C(O)$); M.S. (C.I. ether) m/z: 326 (4%, $[M + H]^+$); Anal. calcd for $C_{12}H_{19}D_2NO_9 \cdot H_2O$: C, 41.98; H, D, 7.33; N; 4.08; Found: C, 42.09; H, D, 7.39; N; 3.99.

2.4.3. General Method for the Synthesis of Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate, (unlabeled, mono- or dilabeled derivatives 22, 22a and 22b).

A 3% solution of methanol in acetyl chloride (5 ml) was prepared at 0°C in a small round bottom flask. The flask was sealed, the solution was warmed to room temperature and stirred

overnight. The methyl ester **20**, **20a** or **20b** (100 mg, 0.31 mmol) was suspended in glacial acetic acid (1.0 ml) and the cooled 3% methanol in acetyl chloride solution was added to the suspension. The flask was sealed and upon stirring the solution rapidly became clear. The reaction was monitored by TLC (eluent 2 x 100% ethyl acetate) and after 24 hours, the reaction was deemed complete. The clear solution was evaporated and after several coevaporations with dry toluene, the chloride was obtained as a colorless foam. The yields (≈ 150 mg) of crude chlorides **22**, **22a** or **22b** are usually quasi-quantitative and by ^{1}H NMR, the purity is estimated to range from 90 to 98%. The main impurity consists mostly of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate as determined by TLC and confirmed by ^{1}H NMR. The crude chloride is usually used without any further purification in the subsequent reactions but it can also be purified by crystallization from methylene chloride/diethyl ether/petroleum ether whereby white crystals are obtained.

Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate 22 - m.p. 107.1-108.5°C (sint'd 94.0°C); $[\alpha]_D$ -68.0° (c1.0, chloroform); lit.[53] m.p. 84-86°C (sint'd), 105°C (dec.); $[\alpha]_D$ -68.3° (c1.0, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 5.52 (d, 1H, $J_{NH,\,5}$ = 9.8 Hz, NH), 5.44 (dd, 1H, $J_{6,\,7}$ = 2.2 Hz, $J_{7,\,8}$ = 7.1 Hz, H-7), 5.35 (ddd, 1H, $J_{3ax,\,4}$ = 11.2 Hz, $J_{3eq,\,4}$ = 4.8 Hz, $J_{4,\,5}$ = 9.7 Hz, H-4), 5.15 (ddd, 1H, $J_{8,\,9a}$ = 2.7 Hz, $J_{8,\,9b}$ = 5.7 Hz, H-8), 4.39 (dd, 1H, $J_{9a,\,9b}$ = 13.9 Hz, H-9a), 4.32 (dd, 1H, $J_{5,\,6}$ = 10.2 Hz, H-6), 4.20 (m, 1H, H-5), 4.03 (dd, 1H, H-9b), 3.85 (s, 3H, OMe), 2.76 (dd, 1H, $J_{3ax,\,3eq}$ = 13.9 Hz, H-3eq), 2.25 (dd, 1H, H-3ax), 2.09, 2.05, 2.03, 2.02, 1.88 (s, 15H, $CH_3C(O)$); lit.[53] ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm) 5.60 (d, 1H, NH), 5.47 (dd, 1H, $J_{6,\,7}$ = 2.1 Hz, $J_{7,\,8}$ = 6.8 Hz, H-7), 5.40 (ddd, 1H, $J_{3ax,\,4}$ = 10.6 Hz, $J_{3eq,\,4}$ = 4.9 Hz, $J_{4,\,5}$ = 10.7 Hz, H-4), 5.18 (ddd, 1H, $J_{8,\,9a}$ = 2.5 Hz, $J_{8,\,9b}$ = 5.9 Hz, H-8), 4.40 (dd, 1H, $J_{9a,\,9b}$ = 12.5 Hz, H-9a), 4.30 (dd, 1H, $J_{5,\,6}$ = 10.4 Hz, H-6), 4.20 (m, 1H, H-5), 4.08 (dd, 1H, H-9b), 3.85 (s, 3H, OMe), 2.80 (dd, 1H, $J_{3ax,\,3eq}$ = 13.8 Hz, H-3eq), 2.28 (dd, 1H, H-3ax); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.2, 171.1, 170.5, 170.1, 170.0, (5C, $CH_3\underline{C}(O)$), 165.9 (1C, C-1), 96.9 (1C, C-2), 74.1 (1C, C-6), 70.3 (1C, C-8), 69.0 (1C, C-4), 67.3 (1C, C-7), 62.3 (1C, C-9), 54.0 (1C, OMe), 49.0 (1C, C-5), 40.8 (1C, C-3), 23.1, 21.1, 20.9, 20.8, 20.8 (5C, $\underline{C}H_3C(O)$); lit.[53] ^{13}C NMR (90 MHz, $CDCl_3$): δ (ppm) 171.1-170.0 (5C, $CH_3\underline{C}(O)$), 165.9 (1C, C-1), 96.9 (1C, C-2), 74.1 (1C, C-6), 70.3 (1C, C-8), 69.0 (1C, C-4), 67.2 (1C, C-7), 62.3 (1C, C-9), 53.8 (1C, OMe), 48.8 (1C, C-5), 40.8 (1C, C-3), 23.0, 20.7, 20.7, 20.7, 20.7 (5C, $\underline{C}H_3C(O)$); M.S. (C.I. ether) m/z: 512 (33%, $[M(^{37}Cl) + H]^+$), 510 (100%, $[M(^{35}Cl) + H]^+$), 474 (19%, $[M - Cl]^+$), 473 (61%, $[M - HCl]^+$).

Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-3ax-deutero-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate 22a - $[\alpha]_D$ -68.0° (c1.0, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 5.50 (d, 1H, $J_{NH,\,5}$ = 9.5 Hz, NH), 5.46 (dd, 1H, $J_{6,\,7}$ = 2.1 Hz, $J_{7,\,8}$ = 7.1 Hz, H-7), 5.36 (dd, 1H, $J_{3eq,\,4}$ = 4.9 Hz, $J_{4,\,5}$ = 9.7 Hz, H-4), 5.15 (ddd, 1H, $J_{8,\,9a}$ = 2.8 Hz, $J_{8,\,9b}$ = 5.7 Hz, H-8), 4.40 (dd, 1H, $J_{9a,\,9b}$ = 13.9 Hz, H-9a), 4.32 (dd, 1H, $J_{5,\,6}$ = 10.2 Hz, H-6), 4.21 (m, 1H, H-5), 4.03 (dd, 1H, H-9b), 3.80 (s, 3H, OMe), 2.77 (d, 1H, H-3eq), 2.11, 2.06, 2.04, 2.02, 1.89 (s, 15H, $CH_3C(O)$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.2, 171.2, 170.6, 170.1, 170.1 (5C, $CH_3\underline{C}(O)$), 166.0 (1C, C-1), 96.9 (1C, C-2), 74.1 (1C, C-6), 70.4 (1C, C-8), 69.0 (1C, C-4), 67.3 (1C, C-7), 62.4 (1C, C-9), 54.0 (1C, OMe), 49.0 (1C, C-5), 23.1, 21.1, 20.9, 20.9, 20.8 (5C, $\underline{C}H_3(CO)$); M.S. (C.I., ether) m/z: 513 (20%, $|M(^{37}Cl) + H|^+$), 511 (62%, $|M(^{35}Cl) + H|^+$), 475 (17%, $|M - Cl|^+$), 474 (51%, $|M - HCl|^+$).

Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-3ax,3eq-dideutero-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate 22b - $[\alpha]_D$ -67.9° (c1.0, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$) : δ (ppm) 5.50 (d, 1H, $J_{NH,\,5}$ = 9.6 Hz, NH), 5.44 (dd, 1H, $J_{6,\,7}$ = 2.1 Hz, $J_{7,\,8}$ = 7.0 Hz, H-7), 5.35 (d, 1H, $J_{4,\,5}$ = 9.6 Hz, H-4), 5.15 (ddd, 1H, $J_{8,\,9a}$ = 2.8 Hz, $J_{8,\,9b}$ = 5.7 Hz, H-8), 4.39 (dd, 1H, $J_{9a,\,9b}$ = 13.8 Hz, H-9a), 4.31 (dd, 1H, $J_{5,\,6}$ = 10.1 Hz, H-6), 4.20 (m, 1H, H-5), 4.02 (dd, 1H, H-9b), 3.84 (s, 3H, OMe), 2.12, 2.07, 2.03, 2.01, 1.88 (s, 15H, $CH_3C(O)$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.2, 171.1, 170.5, 170.1, 170.0 (5C, $CH_3\underline{C}(O)$), 165.9 (1C, C-1), 96.9 (1C, C-2), 74.1 (1C, C-6), 70.3 (1C, C-8), 69.0 (1C, C-4), 67.3 (1C, C-7), 62.4 (1C, C-9), 54.1 (1C, OMe), 49.0 (1C, C-5), 23.1, 21.2, 20.9, 20.8, 20.8 (5C, $\underline{C}H_3C(O)$); M.S. (C.I., ether) m/z: 514 (29%, $|M(^{37}Cl) + H|^+$), 512 (90%, $|M(^{35}Cl) + H|^+$), 476 (23%, $|M - Cl|^+$), 475 (70%, $|M - HCl|^+$).

2.4.4. Synthesis of Acetylated Derivatives of Methyl 5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate 20

Synthesis of Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate 23 - The procedure used was a procedure designed by Kuhn et al.[56] and repeated by Marra and Sinay[58] and consisted generally of adding to a stirred, warmed (40°C) solution of acetic anhydride (0.5 ml) and aqueous perchloric acid (60% solution, 2.5 µl), methyl ester **20** (101 mg, 0.32 mmol) in small portions over a period of 30 min. After 2 hours of stirring at 40°C, all the starting material seemed to have reacted and 2 spots (rf = 0.24 and 0.32) were observed on TLC (eluent 2 x 100% ethyl acetate). The reaction mixture was

cooled to room temperature, diluted with water (5 ml), saturated with ammonium chloride and extracted with methylene chloride (3 x 20 ml). The organic extracts were combined, washed with saturated sodium bicarbonate solution (2 x 20 ml), water (20 ml), brine (20 ml), dried (anh. sodium sulfate) and concentrated in vacuo. The oil obtained was chromatographed on silica gel using 60:1 chloroform: methanol as eluent. The first product eluted from the column was identified as the penta-O-acetate **21** in 26% yield (for full characterization, see next synthesis) and the second product obtained was the tetra-O-acetate **23**. It was obtained as a clear oil after evaporation of the combined fractions and was recrystallized from ethyl acetate/hexanes to give white needles (91 mg, yield 58%): m.p. 147.0-148.3°C; $[\alpha]_D$ -2.3° (c1.0, chloroform); lit.[58] m.p. 147-148°C; $[\alpha]_D$ -2.1° (c1, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.53 (d, 1H, NH), 5.31 (dd, 1H, $J_{6,7}$ = 1.6 Hz, $J_{7,8}$ = 6.0 Hz, H-7), 5.22 (ddd, 1H, $J_{8,9a}$ = 2.4 Hz, $J_{8,9b}$ = 7.4 Hz, H-8), 5.19 (m, 1H, $J_{3ax,4}$ = 11.4 Hz, $J_{3eq,4}$ = 4.9 Hz, H-4), 4.44 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.41 (d, 1H, $J_{OH,3ax}$ = 0.9 Hz, OH), 4.16-4.11 (m, 2H, H-4, 5), 4.00 (dd, 1H, H-9b), 3.84 (s, 3H, OMe), 2.23 (dd, 1H, $J_{3ax,3eq}$ = 12.6 Hz, H-3ax), 2.18 (dd, 1H, H-3eq), 2.12, 2.08, 2.01, 2.00, 1.88 (s, 15H, $CH_3C(O)$); lit.[3] ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.71 (m, 1H, NH), 5.36 (dd, 1H, $J_{6,7}$ = 1.5 Hz, $J_{7,8}$ = 5.6 Hz, H-7), 5.25 (ddd, 1H, $J_{8,9a}$ = 2.4 Hz, $J_{8,9b}$ = 7.5 Hz, H-8), 5.22 (ddd, 1H, $J_{3ax,4}$ =11.4 Hz, $J_{3eq,4}$ = 5.4 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 4.51 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.47 (d, 1H, $J_{OH,3ax}$ ≈ 0.8 Hz, OH), 4.21-4.13 (m, 2H, H-5, 6), 4.03 (dd, 1H, H-9b), 3.86 (s, 3H, OMe), 2.26 (ddd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, H-3ax), 2.19 (dd, 1H, H-3eq), 2.15, 2.11, 2.03, 2.02, 1.91 (5s, 15H, 5Ac); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ (ppm) 171.0, 170.9, 170.8, 170.2, 170.0 (5C, $CH_3\underline{C}(O)$), 168.9 (1C, C-1), 94.8 (1C, C-2), 71.6 (1C, C-6), 71.2 (1C, C-8), 69.2 (1C, C-4), 68.2 (1C, C-7), 62.5 (1C, C-9), 53.4 (1C, OMe), 49.4 (1C, C-5), 36.2 (1C, C-3), 23.2 21.1, 21.0, 20.8, 20.8 (5C, $\underline{C}H_3C(O)$); lit.[58] ^{13}C NMR (100.6 MHz, $CDCl_3$): δ (ppm) 171.43, 171.04, 170.72, 170.30, 170.12 and 168.93 (6C, C=O), 94.84 (1C, C-2), 72.07, 71.32, 69.12, 68.30 (4C, C-4, 6, 7, 8), 62.50 (1C, C-9), 53.18 (1C, OMe), 49.04 (1C, C-5), 36.11 (1C, C-3), 22.93, 20.93, 20.75, 20.65 (5C, $\underline{C}H_3C(O)$); M.S. (C.I. ether) m/z : 492 (94%, $[M + H]^+$), 474 (25%, $[M - OH]^+$), 432 (67%, $[M - OAc]^+$).

Synthesis of Methyl 5-Acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate 21

Method A : Acetylation Procedure using Acetic Anhydride/Pyridine[56]

Acetic anhydride (4.5 ml) was added to a stirred cooled solution of methyl ester **20** (100 mg, 0.31 mmol) in pyridine (4.0 ml). The mixture was further stirred for 48 hours at room temperature. The reaction mixture was diluted with methanol, evaporated in vacuo and

coevaporated several times with toluene. The resulting oil was chromatographed on silica gel (eluent = 60:1, chloroform: methanol). The first product eluted was identified as the α-penta-O acetate **25** (rf = 0.36) by ^{1}H NMR (full characterization, see next synthesis) and was obtained in a 25% yield as a clear colorless oil. The second product obtained, also as a clear oil, was the β penta-O-acetate **21** (109 mg, yield 66%).

Method B : Two-Steps in One-pot Acetylation/Esterification Procedure[58]

To a stirred ice-cooled suspension of the acid **19** (101 mg, 0.32 mmol) in pyridine (1.5 ml) was added acetic anhydride (1.5 ml). The mixture was stirred for 24 hours at room temperature then concentrated and co-evaporated several times with toluene to leave crude 5-acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyra-nosonic acid **24**, a solution of which in 2:1 methanol:ether (3 ml) was treated with etherial diazomethane at 0°C. After the evolution of nitrogen stopped and the starting material had completely disappeared (by TLC 2 x 100% ethyl acetate, 2 spots; rf = 0.32 and rf = 0.36 (trace)) the solution was concentrated in vacuo and the oily residue obtained was eluted from a column of silica gel with 60:1 chloroform: methanol to give a 90% yield (153 mg) of β-penta-O-acetyl **21**;

Methyl 5-Acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate 21 - $[\alpha]_D$ -31.1° (c1.0, chloroform); lit.[58] $[\alpha]_D$ -32° (c1, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.35 (dd, 1H, $J_{6,7}$ = 2.0 Hz, $J_{7,8}$ = 5.2 Hz, H-7), 5.30 (d, 1H, NH), 5.23 (ddd, 1H, $J_{3ax,4}$ = 11.5 Hz, $J_{3eq,4}$ = 5.0 Hz, $J_{4,5}$ = 6.4 Hz, H-4), 5.05 (ddd, 1H, $J_{8,9a}$ = 2.6 Hz, $J_{8,9b}$ = 6.7 Hz, H-8), 4.47 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.13-4.06 (m, 2H, H-5, 6), 4.10 (dd, 1H, H-9b), 3.77 (s, 3H, OMe), 2.52 (dd, 1H, $J_{3ax,3eq}$ = 13.6 Hz, H-3ax), 2.07 (dd, 1H, H-3eq), 2.13, 2.12, 2.04, 2.03, 2.02, 1.87 (s, 18H, $CH_3C(O)$); lit.[58] ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.38 (dd, 1H, $J_{6,7}$ = 1.5 Hz, $J_{7,8}$ = 5.3 Hz, H-7), 5.33 (d, 1H, NH) 5.26 (m, 1H, $J_{3ax,4}$ = 11.5 Hz, $J_{3eq,4}$ = 5.0 Hz, H-4), 5.08 (ddd, 1H, $J_{8,9a}$ = 2.6 Hz, $J_{8,9b}$ = 6.6 Hz, H-8), 4.50 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, H-9a), 4.17-4.08 (m, 2H, H-5, 6), 4.12 (dd, 1H, H-9b), 3.80 (s, 3H, OMe), 2.55 (dd, 1H, $J_{3ax,3eq}$ = 13.5 Hz, H-3eq), 2.10 (dd, 1H, H-3ax), 2.15, 2.14, 2.07, 2.04, 2.03, 1.90 (s, 18H, $CH_3C(O)$); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ (ppm) 170.8, 170.4, 170.1, 170.1, 170.1, 170.1 (6C, $CH_3\underline{C}(O)$), 170.0 (1C, C-1), 97.4 (1C, C-2), 72.8 (1C, C-6), 71.3 (1C, C-8), 68.3 (1C, C-4), 67.8 (1C, C-7), 62.1 (1C, C-9), 53.2 (1C, OMe), 49.4 (1C, C-5), 37.0 (1C, C-3), 23.2, 21.0, 20.9, 20.9, 20.9, 20.9 (6C, $\underline{C}H_3C(O)$); lit.[58] ^{13}C NMR (100.6 MHz, $CDCl_3$): δ (ppm) 170.80, 170.46, 170.20, 170.19, 170.13, 168.13, 167.24 (7C, $CH_3\underline{C}(O)$, C-1), 97.39 (1C, C-2), 72.72, 71.37, 68.26, 67.71 (4C, C-4, 6, 7, 8), 62.04 (1C, C-9), 53.06 (1C, OCH_3), 49.05 (1C, C-5), 35.82 (1C, C-

3), 22.97, 20.75, 20.70, 20.64, 20.62, 20.57 (6C, $\underline{C}H_3C(O)$); M.S. (C.I. ether) m/z: 534 (2%, $[M + H]^+$), 474 (59%, $[M - OAc]^+$).

Synthesis of Methyl 5-Acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosonate 25 - The procedure followed is as described in Marra and Sinay[58] and consisted generally of adding anhydrous cesium acetate (92 mg, 0.48 mmol, 1.5 eq) to a solution of chloride **22** (101 mg, 0.32 mmol) in anhydrous acetonitrile (5 ml) and stirring the solution at room temperature for 24 hours. The mixture was concentrated, diluted with methylene chloride (50 ml), washed with water (10 ml) and concentrated to yield an oily residue. A catalytic amount of ruthenium trichloride hydrate (≈ 2 mg) was added to a vigorously stirred biphasic solution of the residue and sodium metaperiodate (68 mg, 0.32 mmol, 1 eq) in carbon tetrachloride (2 ml), acetonitrile (2 ml), and water (3 ml). After 5 minutes at room temperature, the yellow mixture was diluted with methylene chloride (50 ml), and the phases were separated. The organic layer was washed with water (1 x 20 ml) and brine (20 ml), dried over anhydrous sodium sulfate and evaporated. The residue obtained was eluted from a column of silica gel (eluent 100% ethyl acetate) to give the pure α-penta-O-acetyl **25** as a clear oil in 60% yield (102 mg); $[\alpha]_D$ +13.2° (c1.0, chloroform); lit.[58] $[\alpha]_D$ +13° (c1.0, chloroform); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.36 (d, 1H, $J_{5,NH}$ = 10.4 Hz, NH), 5.35 (dd, 1H, $J_{6,7}$ = 2.4 Hz, $J_{7,8}$ = 6.9 Hz, H-7), 5.15 (ddd, 1H, $J_{8,9a}$ = 2.4 Hz, $J_{8,9b}$ = 5.8 Hz, H-8), 4.99 (ddd, 1H, $J_{3ax,4}$ = 12.0 Hz, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 10.2 Hz, H-4), 4.67 (dd, 1H, $J_{5,6}$ = 10.7 Hz, H-6), 4.33 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.13 (ddd, 1H, H-5), 4.02 (dd, 1H, H-9b), 3.73 (s, 3H, OMe), 2.54 (dd, 1H, $J_{3ax,3eq}$ = 13.2 Hz, H-3eq), 2.06 (dd, 1H, H-3ax), 2.11, 2.07, 2.07, 2.03, 2.02, 1.88 (s, 18H, $CH_3C(O)$); lit.[58] ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.39 (d, 1H, $J_{5,NH}$ = 10.5 Hz, NH), 5.38 (dd, 1H, $J_{6,7}$ = 2.5 Hz, $J_{7,8}$ = 7.0 Hz, H-7), 5.20 (ddd, 1H, $J_{8,9a}$ = 2.5 Hz, $J_{8,9b}$ = 5.7 Hz, H-8), 5.02 (ddd, 1H, $J_{3ax,4}$ = 12.0 Hz, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 10.3 Hz, H-4), 4.70 (dd, 1H, $J_{5,6}$ = 10.7 Hz, H-6), 4.36 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, H-9a), 4.16 (ddd, 1H, H-5), 4.06 (dd, 1H, H-9b), 3.76 (s, 3H, OMe), 2.57 (dd, 1H, $J_{3ax,3eq}$ = 13.2 Hz, H-3eq), 2.08 (dd, 1H, H-3ax), 2.14, 2.10, 2.10, 2.05, 2.04, 1.90 (s, 18H, $CH_3C(O)$); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ (ppm) 170.8, 170.6, 170.3, 179.9, 179.9, 168.5 (6C, $CH_3\underline{C}(O)$), 168.2 (1C, C-1), 95.4 (1C, C-2), 73.8 (1C, C-6), 70.1 (1C, C-8), 68.4 (1C, C-4), 67.4 (1C, C-7), 62.1 (1C, C-9), 52.8 (1C, OMe), 48.8 (1C, C-5), 23.0 (1C, C-3), 23.0 20.7, 20.6, 20.6, 20.6, 20.6 (6C, $\underline{C}H_3C(O)$); lit.[58] ^{13}C NMR (100.6 MHz, $CDCl_3$): δ (ppm) 170.81, 170.68, 170.30, 170.00, 170.00, 168.49 (6C, $CH_3\underline{C}(O)$), 168.22 (1C, C-1), 95.41 (1C, C-2), 73.84 (1C, C-6), 70.14 (1C, C-8), 68.45 (1C, C-4), 67.43 (1C, C-7), 62.17 (1C, C-9), 52.81 (1C, OCH_3), 48.88 (1C, C-5), 23.06 (1C, C-3), 23.05 20.78, 20.71, 20.71, 20.66, 20.66 (6C, $\underline{C}H_3C(O)$); M.S. (C.I. ether) m/z: 534 (5%, $[M + H]^+$), 474 (61%, $[M - OAc]^+$).

Synthesis of Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate 26

Method A: Concentrated Sulfuric Acid/Acetic Anhydride Procedure[61].

A 2% (w/w) solution of sulfuric acid in acetic anhydride (0.5 ml) was slowly added to a solution of methyl ester **20** (100 mg, 0.31 mmol) in acetic anhydride (1.5 ml). After being stirred for 12 hours at room temperature, the reaction mixture was poured into ice-water (5 ml). The mixture was stirred, saturated with ammonium chloride and extracted with chloroform (3 x 10 ml). The organic extracts were combined, washed with cold saturated sodium bicarbonate (2 x 20 ml) dried over anhydrous sodium sulfate and evaporated in vacuo. The oily mixture was chromatographed on silica gel (eluent 60:1 chloroform: methanol) and the first compounds eluted contained a mixture of products (epimeric mixture of double bond containing compounds as detected by ^{1}H NMR: δ = 5.99 ppm and 5.95 ppm) which could not be separated by chromatography. Yield of 1:1 mixture of epimers (rf = 0.38): 80% (117 mg).

Method B: Elimination Procedure[62]

A solution of DBU (1.2 eq, 55 µl, 56 mg, 0.37 mmol) in benzene (5 ml) was added to a solution of chloride **22** (159 mg, 0.31 mmol) in benzene (5 ml) and the mixture was stirred for 1 hour at room temperature under a nitrogen atmosphere. The reaction mixture was then washed with water (10 ml) and brine (10 ml), dried over anhydrous sodium sulfate and evaporated in vacuo to give a syrup which was crystallized from benzene/acetone to give compound **26** as needles (111 mg, yield 76%).

Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate 26 - m.p. 125.5-126.9°C; $[\alpha]_D$ +78.0° (c1.0, chloroform); lit.[62] m.p. 126-127°C; $[\alpha]_D$ +79.9° (c1.3, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.99 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-3), 5.52 (d, 1H, NH), 5.49 (dd, 1H, $J_{6,7}$ = 3.4 Hz, $J_{7,8}$ = 4.7 Hz, H-7), 5.47 (dd, 1H, $J_{4,5}$ = 7.5 Hz, H-4), 5.34 (ddd, 1H, $J_{8,9a}$ = 3.3 Hz, $J_{8,9b}$ – 6.9 Hz, H-8), 4.37 (dd, 1H, $J_{9a,9b}$ = 12.3 Hz, H-9a), 4.35 (dd, 1H, H-6), 4.35 (m, 1H, H-5), 4.17 (dd, 1H, H-9b), 3.79 (s, 3H, OCH_3), 2.11, 2.06, 2.05, 2.03, 1.92 (s, 15H, $CH_3C(O)$); lit.[62] ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.00 (d, 1H, $J_{3,4}$ = 3.4 Hz, H-3), 5.55 (d, 1H, NH), 5.55 (dd, 1H, $J_{4,5}$ = 7.0 Hz, H-4), 5.51 (dd, 1H, $J_{6,7}$ = 3.5 Hz, $J_{7,8}$ = 4.3 Hz, H-7), 5.37 (ddd, 1H, $J_{8,9a}$ = 3.2 Hz, $J_{8,9b}$ = 7.0 Hz, H-8), 4.60 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, H-9a), 4.41 (ddd,

1H, $J_{5,6}$ = 8.9 Hz, H-6), 4.38 (m, 1H, H-5), 4.20 (dd, 1H, H-9b), 3.81 (s, 3H, OMe), 2.13, 2.08, 2.07, 2.06, 1.94 (5s, 15H, $CH_3C(O)$); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ (ppm) 170.6, 170.6, 170.4, 170.0, 170.0 (5C, $CH_3\underline{C}(O)$), 161.4 (1C, C-1), 144.9 (1C, C-2), 107.9 (1C, C-3), 76.6 (1C, C-6), 70.8 (1C, C-8), 67.9 (1C, C-4), 67.6 (1C, C-7), 61.9 (1C, C-9), 52.6 (1C, OCH_3), 46.6 (1C, C-5), 23.2, 20.9, 20.9, 20.8, 20.8 (5C, $\underline{C}H_3C(O)$); M.S. (C.I., ether) m/z: 474 (7%, $[M + H]^+$), 414 (87%, $[M - OAc]^+$).

CHAPTER 3 Synthesis of Regio- and Stereo-selectively Deuteriated Sialyl Glycerolipids

3.1 Introduction

Recent developments in carbohydrate synthetic methodology has opened the possibility for practical synthesis of sialic acid containing glycoconjugates. Most of the research involved in this pursuit has centered around the formation the critical glycosidic bond found in all sialyl glycoconjugates. Although major advances have been registered in the synthesis of simple disaccharides[63-70], the synthesis of N-acetylneuraminic acid glycosides has proven to be much more complex, complexity which can be attributed to three factors inherent in the molecule (Figure XII, next page).

First, the carboxylic acid function at C2 electronically disfavors oxonium ion formation, an intermediate necessary for almost all known glycosidation reactions. Secondly, the carboxy group sterically restricts glycoside formation. Thirdly, the lack of a substituent at C3 precludes the possible assisting and/or directing effect of an adjacent functional group. These combined factors reduce the reactivity of sialyl donors in glycosidation, disfavor glycoside formation and promote an elimination pathway to produce a 2,3-dehydro derivative. Despite these drawbacks, major advances in sialosides syntheses have been achieved in recent years[55,71].

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70 P. Sinaÿ, Pure & Appl. Chem., **6 3** (1991) 519.
71 M.P. De Nunno, Synthesis, (1991) 583.

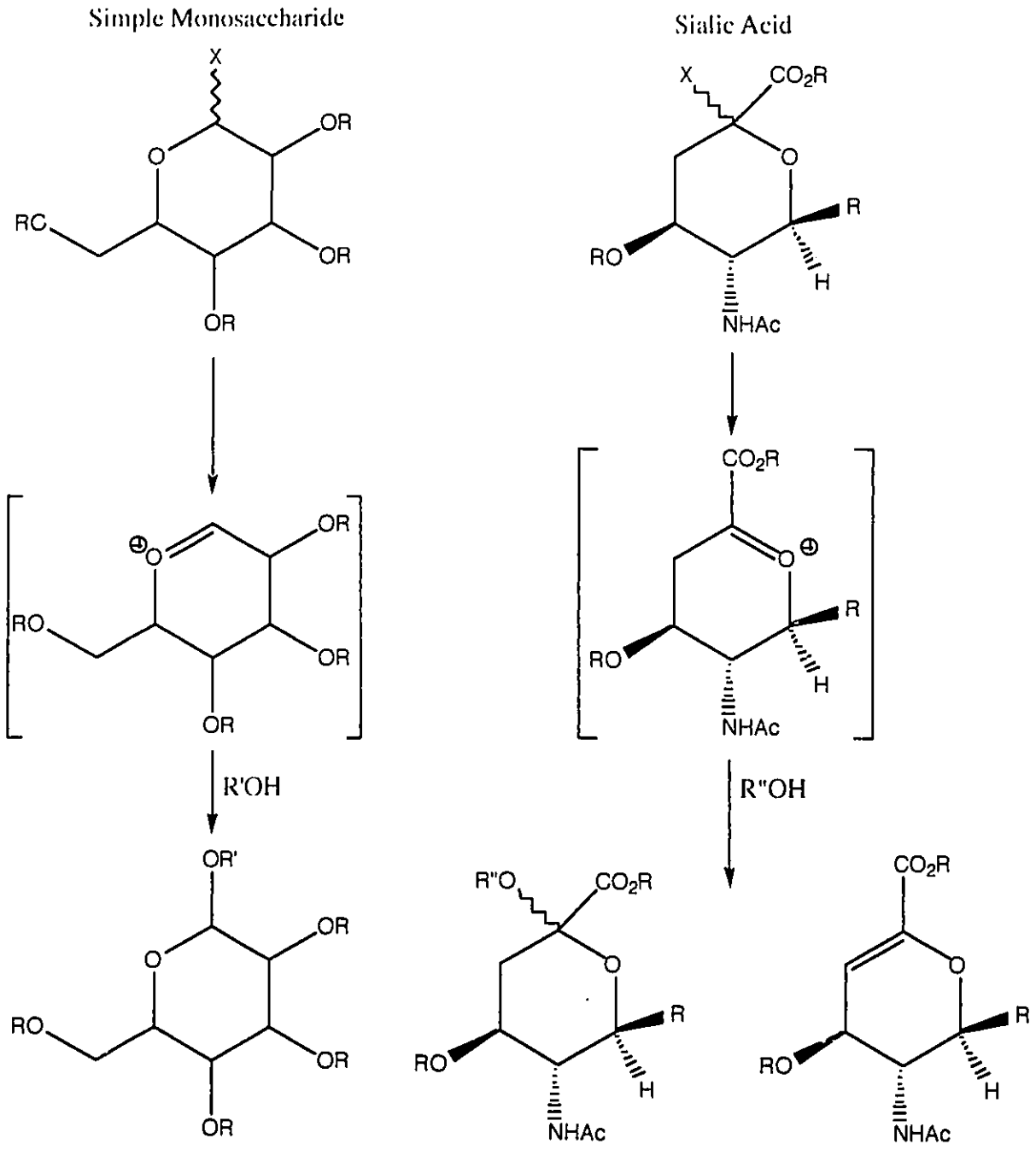

Figure XII. Comparison Between the Glycosidation of Monosaccharides vs Sialic Acid.

Investigations in the area of sialoside synthesis began in 1965 when Meindel et al.[72] who used 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-β-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid as donor succeeded in the synthesis (30-50%) of various sialosides using Koenigs-Knorr reaction conditions. Subsequently, the free acid was found to create problems so the corresponding methyl ester, first prepared by Kuhn et al.[56], has found wide use in glycosidation of N-acetylneuraminic acid[55]. Early work in the synthesis of sialosides focused on the use of simple primary alcohols (non-carbohydrate) as glycosyl acceptors. Both α and β anomers could be prepared selectively in good yields. Thus the β-methyl glycoside of N-acetyl neuraminic acid was prepared by the acid-catalyzed procedure of Fischer[73] and the corresponding α anomer was prepared under classical Koenigs-Knorr conditions[56]. The latter reaction is of some significance since N-acetylneuraminic acid is α-glycosidically linked in all isolated sialosides containing biomolecules.

Work in the area of sialyl conjugates began in 1971 when Khorlin et al.[74] reported the silver carbonate catalyzed condensation of acetochloroneuraminic acid **22** with several carbohydrate acceptors. The yields of the disaccharides ranged from 8% to 18%. It took over a decade for further interest in this area to develop. The emergence was no doubt spurred by the increasing biological significance attributed to sialic acids as well as the challenge of the goal. Early research was directed to the improvement of the yields of the glycosidation of N-acetylneuraminic acid with primary sugar alcohols. By employing modified Koenigs-Knorr conditions, improved yields of the α linked disaccharides could be obtained. Unfortunately, in the case of less reactive acceptors, such as secondary or hindered hydroxyl groups of sugars, the main product was the 2,3-dehydro derivative of N-acetylneuraminic acid. The discovery of the important role of promoters in the glycosidation of N-acetylneuraminic acids coupled with the difficulty to glycosylate hindered acceptors instigated much interest in the development of new promoters[75,76]. In addition, the acceptors have been activated[77] and the protecting groups of N-acetylneuraminic acid were changed[78,79], all in order to improve the glycosidation of N-acetylneuraminic acid.

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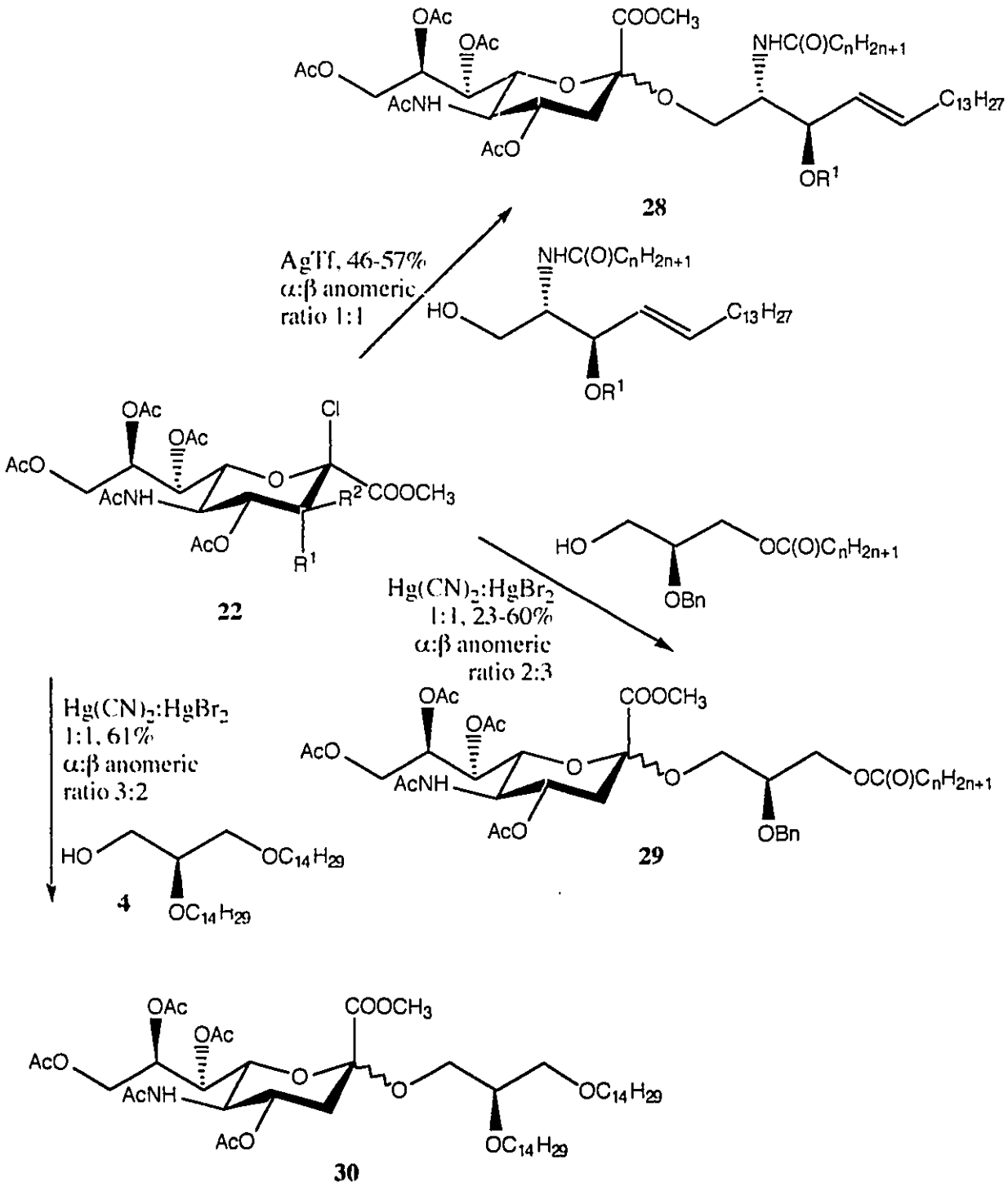
78 H. Kunz and H. Waldman, J. Chem. Soc., Chem Comm.,(1985) 638.
79 H. Kunz, H. Waldman and U. Klenkhammer, Helv. Chim. Acta, **71** (1988) 1868.

Among many sialosides obtained by the Koenigs-Knorr methods, several sialyl lipids have been synthesized (Scheme 20).



Scheme 20. Previous Synthesis of Sialyl Lipids

Kiso et al.[80] reported a synthesis of 1-O-(N-acetyl-α and β-O-neuraminyl)-ceramides **28**. The silver triflate promoted glycosylation of 3-O-protected ceramides gave approximately 1:1 mixtures of the α and β glycosides in 46 to 57% yields.

The glycerolipid of N-acetyl neuraminic acid **29** has been synthesized by Shimizu et al [81]. The 1-O-acyl-2-O-benzyl-*sn*-glycerols were glycosylated in the presence of a 1:1 mixture of mercuric bromide and cyanide and produced anomeric mixtures of the desired glycerolipids in 23 60% yields.

Ogawa et al.[82] also synthesized a glycerolipid of N-acetylneuraminic acid. The glycosylation of di-O-tetradecyl-*sn*-glycerol **4** with the glycosyl donor methyl 5-acetamido 4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate **22** was performed in the presence of $Hg(CN)_2$:$HgBr_2$ as promoter and gave a 3:2 mixture of the α and β anomers in 61% yield.

The procedure developed by Ogawa et al.[82] was an attractive possibility for the synthesis of the regio- and stereo-selectively deuteriated sialyl glycerolipids **1a**, **1b** and **1c**. It will be included in a study of the glycosylation of 1,2-di-O-tetradecyl-*sn*-glycerol **4** generally with the methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto* 2 nonulopyranosonate **22** under various reaction conditions. The necessity of such a study became apparent because of the known great sensitivity of the glycosidation of N-acetylneuraminic acid to reaction conditions. The results of the study were applied to the synthesis of the deuteriated methyl esters of acetylated sialyl glycerolipid which, after deprotection, yielded the desired regio- and stereo-selectively deuteriated sialyl glycerolipids **1a**, **1b** and **1c**.

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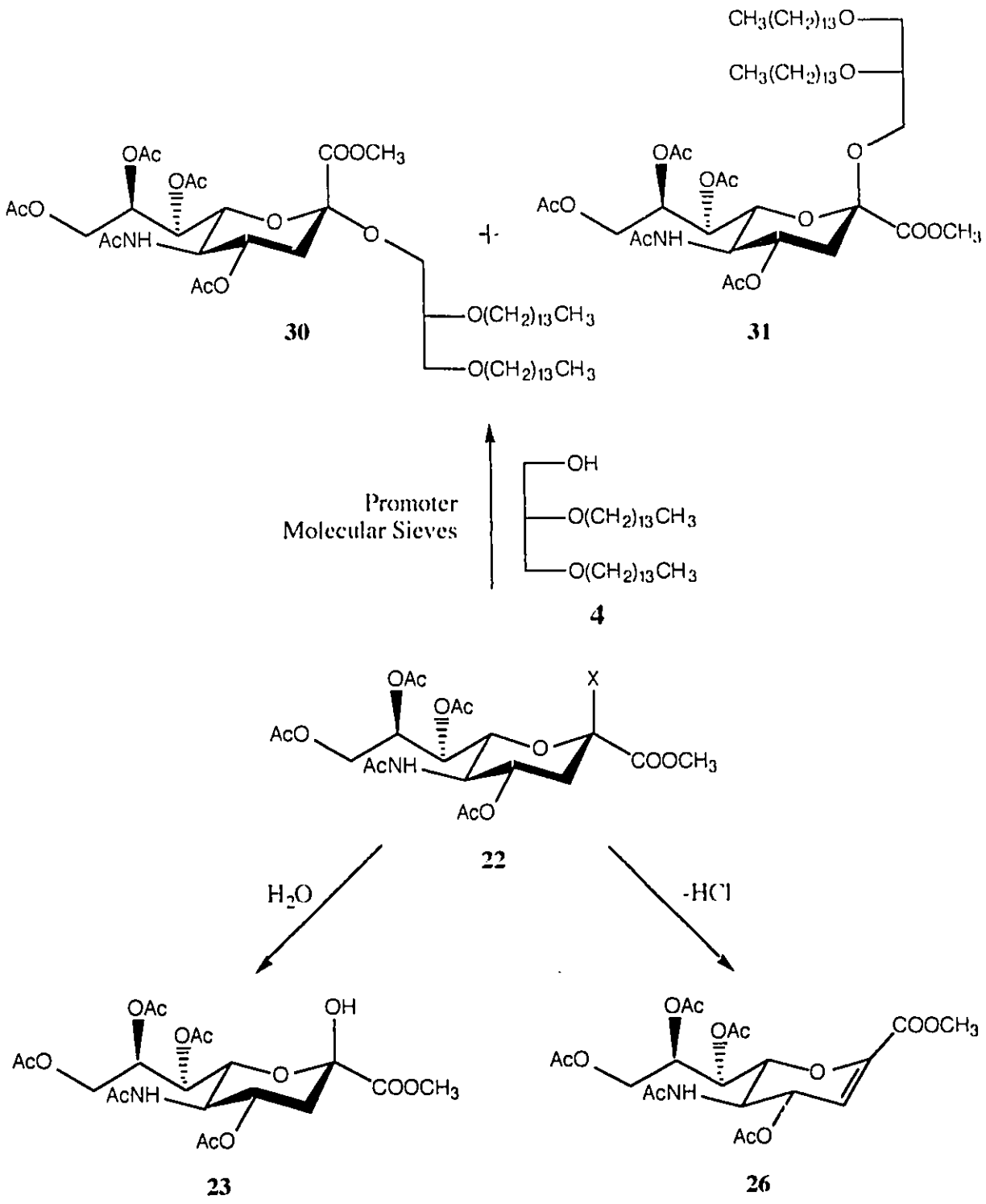
3.2 Discussion

3.2.1 Study of the Glycosidation of Sialosyl Donors.

Since most naturally occurring sialyl containing glycoconjugates include α glycosidically linked sialic acid molecules, the aim of the glycosidation study of sialic acid donors was to determine the best promoter and reaction conditions to favor the formation of the α anomer of the sialyl glycoside. The outcome of glycosidations of sialyl donors is known to be sensitive to the nature of the promoter selected to catalyze the reaction. therefore, various promoters and reaction conditions were considered.

Prior to the study of the glycosidation of N-acetylneuraminic acid donors, it was necessary to anticipate the various side-reactions that could hinder the synthesis of the desired glycosides (Scheme 21, next page).

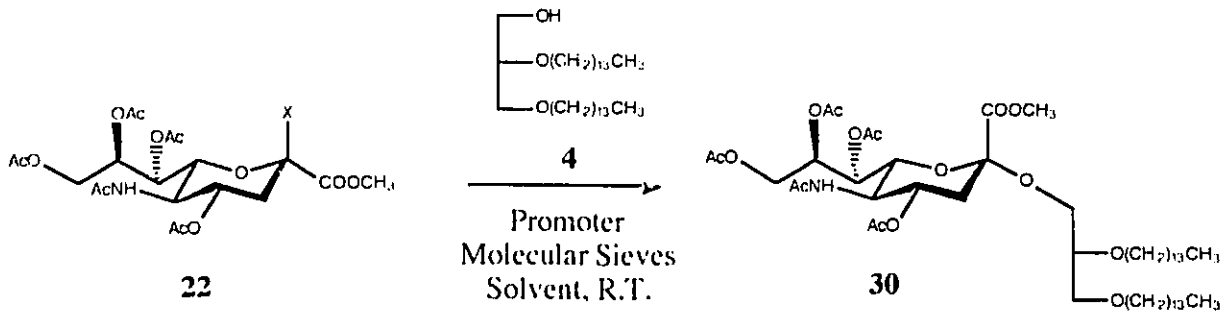
The known lack of stereoselectivity of the glycosidation of N-acetylneuraminyl donors was evidence that simultaneous production of the α and β anomers **30** and **31** of the desired glycoside was to be expected. Furthermore the production of 2,3-dehydro derivative **26** was an eventuality and was known to occur in various conditions. Finally any presence of water in the reaction mixture could lead to hydrolysis product **23** rather than to the desired glycoside. Therefore, all reactions were conducted under strictly anhydrous conditions and all reagents used needed to be thoroughly dried before use. The reactions were all performed in the presence of molecular sieves and when chloride **22** was required as starting material, it was freshly prepared.



Scheme 21. Expected Side-Reactions in the Glycosidation of N-Acetylneuraminic Acid Donors

The various attempts to glycosylate glycerolipid **4** with a glycosyl donor are summarized in Table 6.

Table 6. Glycosylation of Glycerolipid **4**



+ β-Anomer **31**

Donor X	Promoter	Solvent	Reaction Time	Yield of Glycoside 30 and 31	Anomeric Ratio α:β	Side-Product (Yield)
Cl	$HgBr_2$:$Hg(CN)_2$ 1:1	CH_2Cl_2	48	62%	3:2	**26** (15%)
Cl	$HgBr_2$:$Hg(CN)_2$ 1:1	$C_2H_4Cl_2$	48	62%	3:2	**26** (15%)
Cl	Ag(salicylate)	CH_2Cl_2	24	<15%	4:1	**34** (68%)
Cl	Ag(salicylate)	toluene	24	<10%	9:2	**34** (70%)
Cl	Ag(triflate) collidine	CH_2Cl_2	8	55%	1:3	**26** (30%)
Cl	Ag(triflate) collidine	THF	24	<10%	11:9	**26** (55%)
OAc	TMSiOTf	CH_2Cl_2	8	55%	4:7	**26** (15%)

- **Mercuric Cyanide/Mercuric Bromide as Promoter.**

The glycosylation of glycerolipid **4** with chloride **22**, in the presence of mercuric cyanide/mercuric bromide promoter was performed according to Ogawa et al[82]. Thus a solution of freshly prepared chloride **22** was added to a suspension of glycerolipid **4**, $HgBr_2$:$Hg(CN)_2$ and molecular sieves in the same solvent. The reaction was performed at room temperature, under nitrogen and, by Thin Layer Chromatography, was deemed complete within 48 hours. The mixture of anomers obtained from the reaction was separated by flash chromatography on silica gel using a 2% solution of ethanol in chloroform This solvent system found after an extensive search for an adequate eluent to perform the chromatography of the anomeric mixture represented a significant improvement over the solvent system used by Ogawa et al (1:1 toluene: EtOAc). The use of the 2% solution of ethanol in methylene chloride in place of the published 1:1 toluene:EtOAc reduced the number of fractions collected from 300 to 40 with absolutely no mixed fractions. The α and β anomers were obtained in yields of 37 and 25% respectively based on methyl ester **20**. The α and β.anomers were obtained as clear oils which could not be recrystallized (**30** α anomer: $[\alpha]_D$ -8.6° (c1.0, chloroform), lit.[82] $[\alpha]_D$ -9.3° (c1.04, chloroform); **31** β anomer: $[\alpha]_D$ -15.2° (c1.0, chloroform), lit.[82] $[\alpha]_D$ -13.0° (c1.0, chloroform)). The α and β anomers of the sialoside **30** and **31** were fully characterized by ^{1}H NMR, mass spectrometry and chemical analysis.

The ^{1}H NMR spectrum (200 MHz, $CDCl_3$) of sialosides **30** and **31** confirmed the successful glycosylation of glycerolipid **4**. The spectra of the glycerolipids generally displayed multiple signals from 5.34 to 3.78 ppm, a sharp singlet at about 3.76 ppm, a multiplet found between 3.62 to 3.28 ppm, 5 sharp singlets around 2 ppm and broad peaks at 1.53, 1.23 and 0.86 ppm. The multiple signals found between 5.34 and 3.78 ppm, the sharp singlet found at 3.76 ppm and the 5 sharp singlets appearing at about 2 ppm represent respectively the H-4 to H-9, the methyl ester and the acetyl groups protons of the sialyl moiety. The multiplet found between 3.62 to 3.28 ppm and broad signals at 1.53, 1.23 and 0.86 ppm are characteristic of the H-1 to H-3 glycerol and tetradecyl protons of the glycerolipid moiety. Furthermore, the ^{1}H NMR spectrum of α anomer showed characteristic peaks at δ =2.55 for H-3ax and at δ = 1.95 ppm for H-3eq. The H-3ax and H-3eq protons signals the β-anomer appeared at δ = 2.41 and 1.84 ppm.

The detailed ^{1}H NMR data for the α and β sialosides **30** and **31** is summarized in Table 7.

Table 7. ^{1}H NMR Data for the α and β Sialyl Glycosides **30** and **31**.

Compound	**30**	**30** lit.[82]	**31**	**31** lit.[82]
Protons	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	1.95 (12.7,10.1)	1.97 (13.1, 12.8)	1.84 (12.9,11.8)	1.90 (12.5, 12.2)
H-3eq ($J_{3eq,4}$)	2.58 (4.7)	2.60 (4.8)	2.41 (4.8)	2.45 (4.8)
H-4 ($J_{4,5}$)	4.83 (9.9)	4.85 (m)	5.19	5.23 (m)
H-5 ($J_{5,6}$, $J_{NH,5}$)	4.05 (10.5,10.0)		4.04-412 (- ,9.8)	
H-6 ($J_{6,7}$)	3.78 (1.6)		4.04-4.12	
H-7 ($J_{7,8}$)	5.30 (8.6)		5.34 (6.1)	
H-8 ($J_{8,9a}$, $J_{8,9b}$)	5.33 (2.4,5.0)		5.21 (2.2,5.0)	
H-9a ($J_{9a,9b}$)	4.27 (12.4)		4.68 (12.3)	
H-9b	4.08		4.11	
NH	5.10		5.12	
OCH_3	3.77		3.75	
$CH_3C(O)NH$ $CH_3C(O)O$	2.12 2.11 2.02 2.00 1.86		2.10 2.02 1.98 1.97 1.84	
H-1', H-2', H-3'	3.62-3.28		3.61-3.31	
$CH_2(CH_2)_{12}CH_3$ $CH_2CH_2(CH_2)_{11}$ CH_3	1.53 (6.5)		1.51 (6.0)	
$CH_2CH_2(CH_2)_{11}$ CH_3	1.23		1.21	
$(CH_2)_{13}CH_3$	0.86 (6.7)		0.83 (6.2)	

The anomeric configurations of the α and β anomers **30** and **31** have been assigned according to well established empirical rules. These rules and the result of their application to the determination of the anomeric configuration of the glycoside are summarized in Table 8.

Table 8. Determination of Anomeric Configuration of Glycosides **30** and **31**

Rule #	α anomer **30**		β anomer **31**
1[83]	H-3eq	>	H-3eq
	2.58 ppm	>	2.41 ppm
2[76,84]	H-4	<	H-4
	4.83 ppm	<	5.19 ppm
3[85-91]	$J_{7,8}$	>	$J_{7,8}$
	8.6 Hz	>	6.1 Hz
4[85-91]	|H-9a - H-9b|	<	|H-9a - H-9b|
	(4.27-4.08) ppm		(4.69-4.11) ppm
	0.19ppm	<	0.58ppm

Evidence of the successful glycosidation of sialyl donor **22** was also available from the ^{13}C NMR spectra (50.3 MHz, $CDCl_3$) of the α and β anomers of the obtained glycerolipids. The ^{13}C spectra of the α and β anomers confirm the presence of the four O-acetyl and acetamido group (5 carbonyl signals at δ = 171.2-170.0 ppm), the methyl ester (52.4 ppm (α anomer **30**) and 52.5 ppm (β anomers **31**)) and of the glycerolipid (strong alkylene signal at about 23.0-20.6 ppm).

Mass spectra (Negative FAB) of the α and β glycosides **30** and **31** displayed weak molecular ion peaks at 958 (relative abundance: 4% for α-glycoside and 9% for β-glycoside).

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84 H. Paulsen and H. Tietz, Carbohydr. Rev., **125** (1984) 47.
85 K. Okamoto, T. Kondo and T. Goto, Chem. Lett (1986) 1449.
86 K. Okamoto, T. Kondo and T. Goto, Tetrahedron Lett., **27** (1986) 5229.
87 K. Okamoto, T. Kondo and T. Goto, Tetrahedron Lett., **27** (1986) 5233.
88 K. Okamoto, T. Kondo and T. Goto, Tetrahedron, **43** (1987) 5909.
89 K. Okamoto, T. Kondo and T. Goto, Bull. Chem. Soc. Jpn, **60** (1987) 637.
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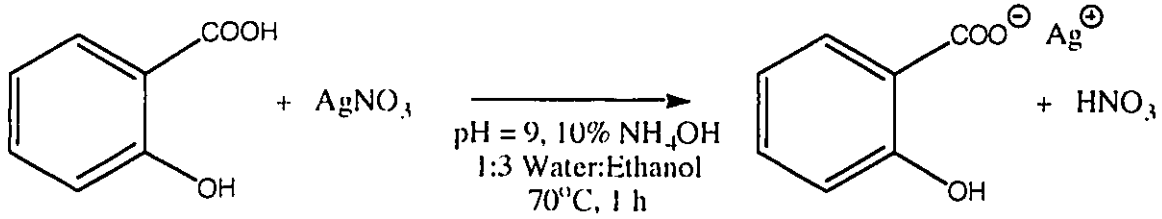
91 K. Okamoto, T. Kondo and T. Goto, Tetrahedron, **44** (1988) 1291.

- **Silver Salicylate as Promoter**

The promoter, silver salicylate, was prepared according to the method published by Wulff et al.[92] depicted in reaction Scheme 22.

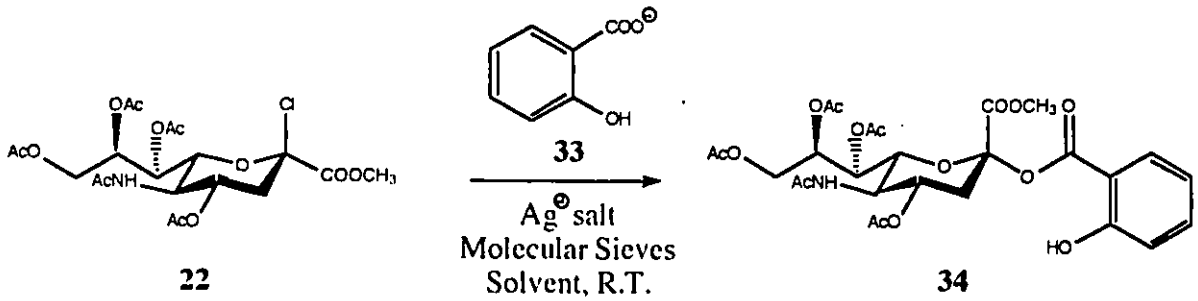


32 **33**

Scheme 22. Synthesis of Silver Salicylate **33**

It involved the addition of a solution of silver nitrate in aqueous ethanol to a basic solution of salicylic acid **32**. Silver salicylate **33** precipitated out of the solution and was recovered as a pinkish powder which was diligently dried.

The use of silver salicylate as promoter was very promising since various authors[93,94,95] had observed its remarkable selectivity towards the α anomers of sialyl glycosides. Glycosidation reactions were performed in anhydrous conditions in the presence of molecular sieves, under nitrogen and in the absence of light. Freshly prepared chloride **22** was used as glycosyl donor. Unfortunately the reaction produced only a very small yield of the desired glycosides (<10%), the major product being the 2-O-salicylyl derivative **34** (Scheme 23).



Scheme 23. Side-Reaction Observed with the Use of Silver Salicylate as Promoter.

92 G. Wulff, W. Kruger and G. Rohle, Chem. Ber., **104** (1971) 1387.

93 R. Roy, C. A. Laferrière, A. Gamian and H.J. Jennings, J. Carbohydr. Chem., **6** (1987)161.

94 D.J.M. Van der Vleugel, F.R. Wassenburg, J.W. Zwikker and J.F.G. Vliegenthart, Carbohydr. Res., **104** (1982) 221.

95 V. Pozsgay, H.J. Jennings and D.L. Kasper, J. Carbohydr. Chem., **6** (1987) 41.

Apparently, the salicylate anion of the promoter acted as a glycosyl acceptor and competed with glycerolipid **4** in the reaction to produce good yields of the 2-O-salicylyl derivative (68-70 %). The identity of that side-product, first suspected because of its intense fluorescence on TLC plates was confirmed by ^{1}H NMR and mass spectrometry. The ^{1}H NMR of the salicylyl derivative displayed signals at δ = 7.84, 7.45, 6.96 and 6.89 ppm characteristic of aromatic protons. The ortho substitution of the aryl group was also determined by the pattern of the signals and the coupling constants calculated therefrom. The mass spectrum (C.I., ether) of salicylyl derivative displayed a molecular ion peak at 612.

The most unfortunate and irritating result of this glycosidation reaction was that, as predicted, the procedure has a marked selectivity for the desired α anomer **30**. As confirmed by ^{1}H NMR, the anomeric ratio of the α vs the β glycosides was 4:1 in favor of the α anomer. Therefore several attempts to improve the yields of the reaction were made. Because the problem appeared to be due to the competition between glycerolipid **4** and the salicylate anion, modifications to the molar ratio of glycerolipid relative to the salicylate ions were made. An increase of the ratio from 2 to 10 only increased the yield from $\approx$ 10% to about 15%. Modifications of the number of equivalents of promoter relative to that of the donor were also made but the use of less than 1.1 equivalent of promoter resulted in unreacted glycosyl donor. The reaction was also conducted in various solvents such as methylene chloride or toluene but no significant improvements in the yields of glycosidation were obtained. All efforts to improve the yield of the reaction failed.

- **Silver Triflate as Promoter.**

When silver triflate was used as promoter in the glycosylation of glycerolipid **4**, with freshly prepared chloride **22**, the reaction was performed in methylene chloride at room temperature, in the dark and in the presence of a base, collidine or tetramethylurea (TMU). The reaction was monitored by TLC and quenched after the disappearance of the chloride. TLC of the reaction mixture showed the presence of the α and β glycosides but also indicated the presence of the elimination product **26**. Isolation of the reaction products revealed a 55% yield of the α and β anomeric mixture of glycosides **30** and **31** and a 30% yield of derivative **26**. The anomeric ratio was 1:3 in favor of the β-glycoside as determined by ^{1}H NMR. On the other hand, when the reaction was performed in THF, only traces of the glycosides were found but a 55% yield of the elimination product **26** was obtained.

To verify whether the presence of the base was one of the causes for the increased production of derivative **26**, the reaction was also attempted in methylene chloride without any base. Unfortunately the results of this reaction were inconclusive since much decomposition occurred. It was therefore not possible to determine whether the base increased the possibility of formation of the derivative **26** but it definitely played an important role as proton scavenger. Since derivative **26** can be synthesized by a base-catalyzed elimination reaction using the chloride as starting material, it was likely that its presence had an influence on the outcome of the reaction.

- **Trimethylsilyl Triflate as Promoter.**

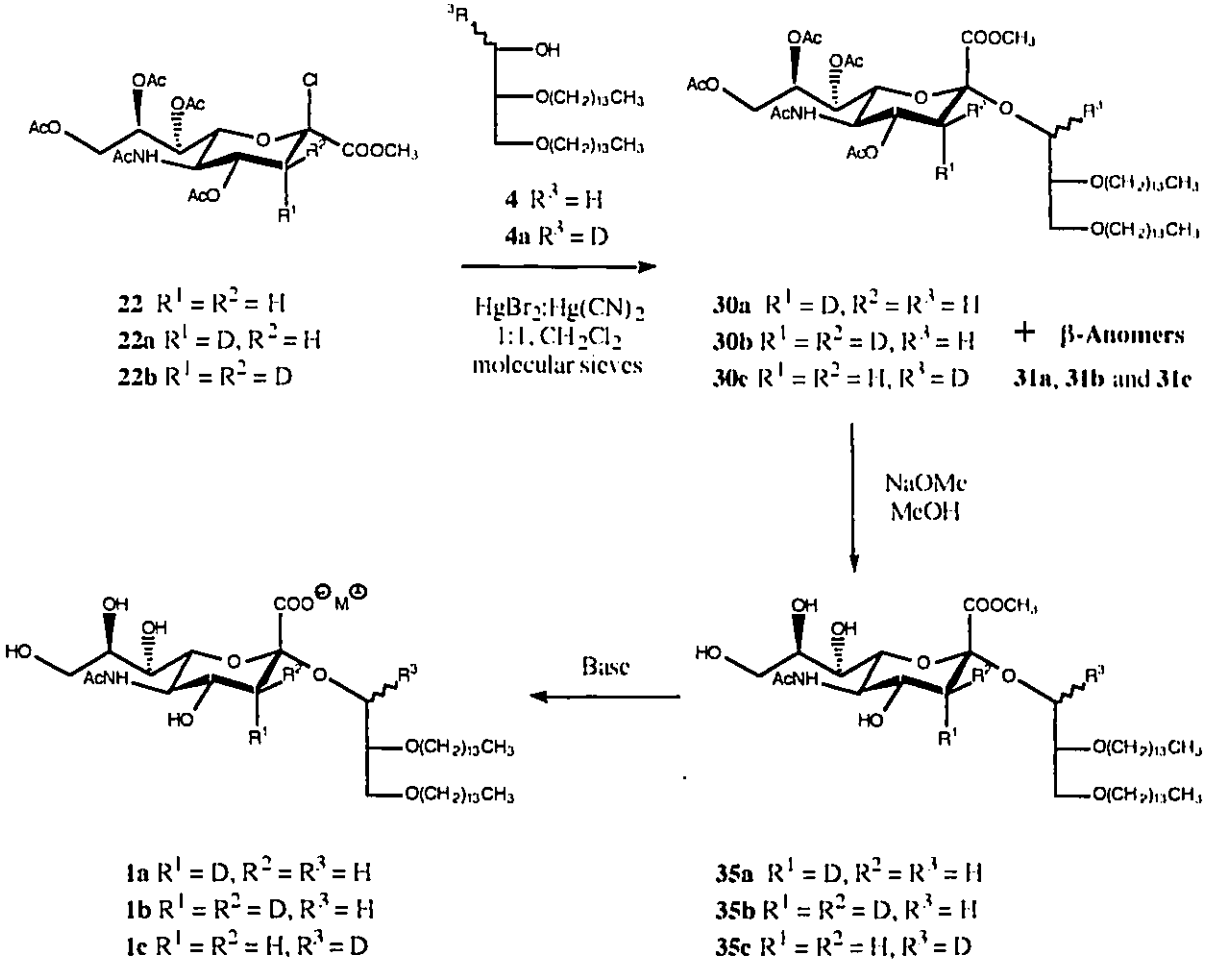
Trimethylsilyl triflate (TMSiOTf) is a Lewis acid used as promoter in Fisher glycosidation reactions. Contrary to the previous methods proposed for the synthesis of the glycosides this method uses a Lewis acid as promoter rather than a heavy metal salt. It proceeds under Fisher rather than Koenigs-Knorr reaction conditions. The starting material used was penta-O acetate **21**. A solution of TMSiOTf in methylene chloride was added to a suspension of glycerolipid **4**, penta-O-acetate **21** and molecular sieves. The reaction was monitored by TLC and, upon disappearance of the penta-O-acetate starting material, the products were extracted from the reaction mixture. The α and β glycosides were obtained in respective yields of 10% and 30% but the major product of the reaction was the 2,3-dehydro derivative **26**. Although the reaction produced a relatively reasonable yield of the glycoside, it unfortunately did not favor the desired α-anomer **30**.

Through the study of various promoters, the numerous difficulties of the glycosidation of N-acetylneuraminic acid have become evident. The difficulty of controlling the selectivity of the glycosidation was apparent in the unpredictable anomeric ratios obtained. The necessity of having good nucleophiles to perform the reaction on the sterically hindered sialosyl donor was demonstrated when the salicylate anion successfully competed with glycerolipid **4** to yield an unwelcomed side-product. The difficulty to avoid competitive elimination was apparent as the 2,3-dehydro derivative **26** was a product in many of the attempted glycosidations.

After all the various attempts to find a very efficient method to synthesize the desired α-glycosides, the procedure published by Ogawa et al.[82] remained superior and was selected to perform the synthesis of the labeled glycoglycerolipids **1a**, **1b**, and **1c**.

3.2.2 Synthesis of the Regio- and Stereo-selectively Deuteriated Sialyl Glycerolipids 1a, 1b and 1c.

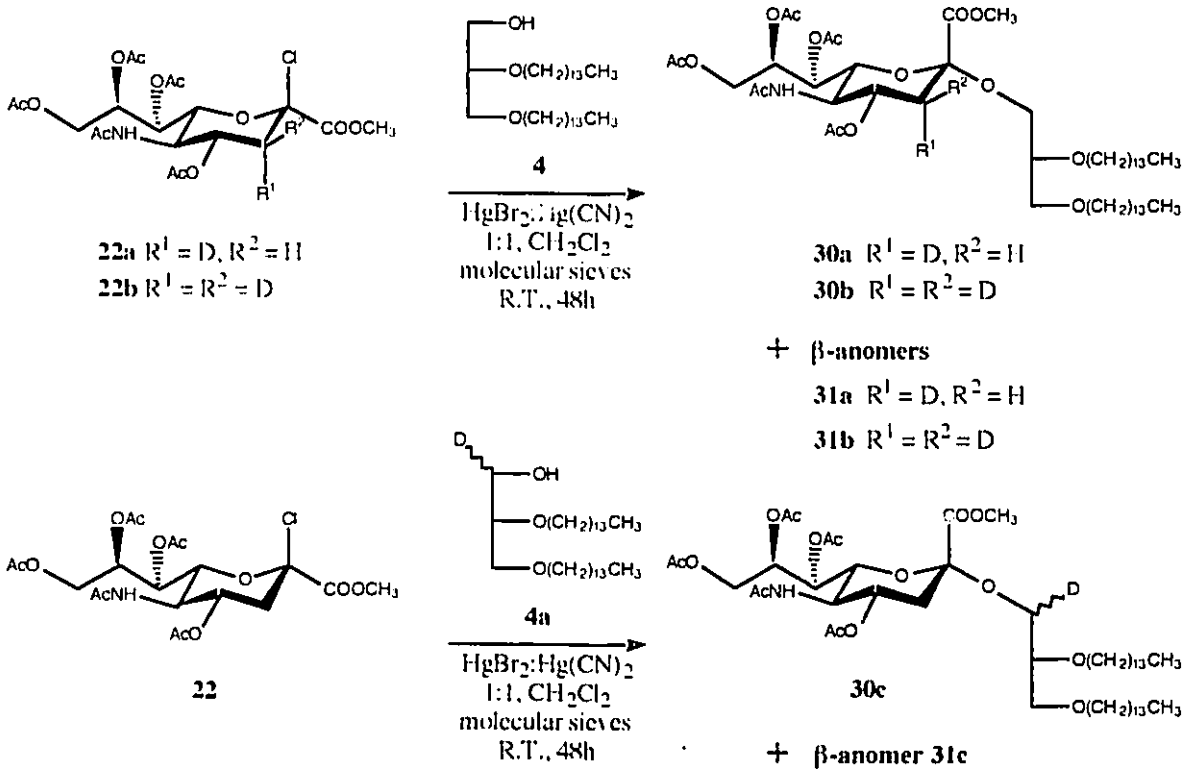
The regio- and stereo-selectively deuteriated sialyl glycerolipids **1a**, **1b** and **1c** were synthesized using a three step synthetic route involving the Koenigs-Knorr glycosylation of glycerolipid **4** and **4a** with the appropriate glycosyl donor **22**, **22a** or **22b** followed by Zemplen deacetylation and saponification. The synthetic route is illustrated in Scheme 24.



Scheme 24. Synthesis of Regio- and Stereo-selectively Deuteriated Sialyl Glycerolipids **1a**, **1b** and **1c**

- **Glycosylation of Glycerolipids 4 and 4a.**

The method selected to perform the glycosylation of glycerolipids **4** and **4a** was the Helferich modification of the Koenigs-Knorr method developed by Ogawa et al.[82]. It involves the use of $Hg(Br)_2$:$Hg(CN)_2$ as promoter. The procedure is depicted in Scheme 25.



Scheme 25. Glycosylation of Glycerolipids **4** and **4a** with Sialyl Chlorides **22**, **22a** and **22b**

Glycosidations were performed as described by Ogawa et al.[82] with the exception that the reactions were conducted in methylene chloride rather than in dichloroethane. The difference of solvent did not influence the outcome of the reaction as the yields and anomeric ratios were consistent with those obtained by Ogawa et al[82]. The α and β.anomers were obtained as clear oils which could not be recrystallized (α-anomers; **30a:** yield 31%; **30b:** yield 37%; **30c:** yield 40%, $[\alpha]_D$ -8.6° (c1.0, chloroform); β-anomers; **31a:** yield 20%; **31b:** yield 25%; **31c:** yield 27%.

The ^{1}H NMR spectra (200 MHz, $CDCl_3$) of the α and β anomers confirmed the presence of the labels on the sugar moiety of the anomers. The spectra of the monodeuteriated α and β derivative **30a** and **31b** did not display a H-3ax signal and the dideuteriated α and β derivative **30b** and **31b** did not exhibit any H-3 signals. The presence of the deuteron on the glycerolipid moiety was verified by the integration of the multiplets found at δ = 3.55–3.30 ppm and at 3.61-3.32 ppm in the ^{1}H NMR spectra of the α and β anomers **30c** and **31c** respectively. The ^{1}H NMR spectroscopic data for the α anomers **30a**, **30b** and **30c** and the β anomers **31a**, **31b** and **31c** are summarized in Table 9.

Table 9. ^{1}H NMR Spectroscopic Data for the α Anomers **30a**, **30b** and **30c** and the β Anomers **31a**, **31b** and **31c**

Compound	**30a**	**30b**	**30c**	**30 lit.**[82]
Protons	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	- (- , -)	- (- , -)	1.96 (12.7,10.1)	1.97 (13.1, 12.8)
H-3eq ($J_{3eq,4}$)	2.55 (4.7)	- (-)	2.58 (4.7)	2.60 (4.8)
H-4 ($J_{4,5}$)	4.82 (10.0)	4.80 (10.1)	4.81 (10.1)	4.85 (m)
H-5 ($J_{5,6}$, $J_{NH,5}$)	4.05 (10.5, 9.9)	4.04 (10.6,9.9)	4.04 (10.6,9.9)	
H-6 ($J_{6,7}$)	3.79 (1.0)	3.78 (1.8)	3.78 (1.6)	
H-7 ($J_{7,8}$)	5.29 (9.1)	5.29 (8.6)	5.29 (8.8)	
H-8 ($J_{8,9a}$, $J_{8,9b}$)	5.15 (2.2,4.5)	5.34 (2.0,4.7)	5.34 (2.1,4.6)	
H-9a ($J_{9a,9b}$)	4.26 (12.4)	4.29 (12.4)	4.28 (12.4	
H-9b	4.07	4.07	4.07	
NH	5.09	5.17	5.12	
OCH_3	3.76	3.75	3.76	
$CH_3C(O)NH$	2.10	2.10	2.11	
$CH_3C(O)O$	2.10	2.09	2.10	
	2.01	2.00	2.01	
	1.99	1.99	1.99	
	1.85	1.84	1.85	
H-1', H-2', H-3'	3.56-3.28	3.54-3.30	3.55-3.30	
$CH_2(CH_2)_{12}CH_3$				
$CH_2CH_2(CH_2)_{11}$ CH_3	1.52 (6.5)	1.52 (6.5)	1.52 (6.5)	
$CH_2CH_2(CH_2)_{11}$ CH_3	1.22	1.21	1.22	
$(CH_2)_{13}CH_3$	0.84 (6.4)	0.84 (6.5)	0.85 (6.5)	

Table 9. (Cont'd)

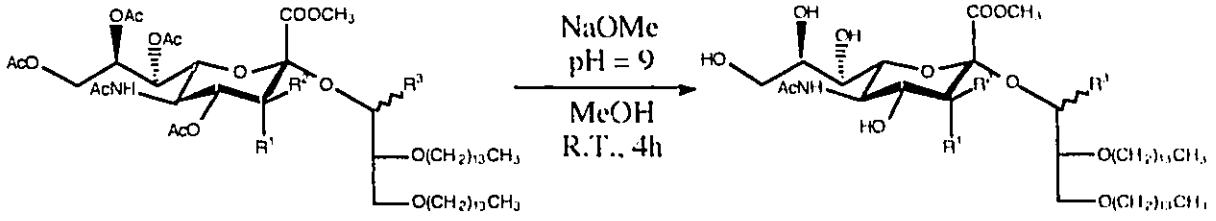
Compound	31a	31b	31c	31 lit.[82]
Protons	δ (ppm) J (Hz)	δ (ppm) J (Hz)	δ (ppm) J (Hz)	δ (ppm) J (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	- (- , -)	- (- , -)	1.86 (12.8,11.9)	1.90 (12.5, 12.2)
H-3eq ($J_{3eq,4}$)	2.38 (4.8)	- (-)	2.39 (4.8)	2.45 (4.8)
H-4 ($J_{4,5}$)	5.19	5.19	5.19	5.23 (m)
H-5 ($J_{5,6}$, $J_{NH,5}$)	4.03-4.10 (- , 9.8)	4.03-4.11 (- ,9.8)	4.04-411 (- ,9.7)	
H-6 ($J_{6,7}$)	4.03-4.10	4.03-4.11	4.04-4.11	
H-7 ($J_{7,8}$)	5.33 (6.2)	5.33 (6.0)	5.34 (6.1)	
H-8 ($J_{8,9a}$, $J_{8,9b}$)	5.23 (2.3,4.5)	5.22 (2.24.6)	5.21 (2.2,4.7)	
H-9a ($J_{9a,9b}$)	4.67 (12.3)	4.67 (12.3)	4.67 (12.3)	
H-9b	4.10	4.10	4.10	
N<u>H</u>	5.09	5.17	5.11	
O<u>CH</u>$_3$	3.74	3.74	3.75	
<u>CH</u>$_3$C(O)NH <u>CH</u>$_3$C(O)O	2.08 2.01 1.97 1.96 1.83	2.09 2.01 1.97 1.96 1.83	2.10 2.01 1.98 1.97 1.84	
H-1', H-2', H-3'	3.60-3.33	3.60-3.33	3.61-3.32	
<u>CH</u>$_2$(CH$_2$)$_{12}$CH$_3$ CH$_2$<u>CH</u>$_2$(CH$_2$)$_{11}$ CH$_3$	1.49 (6.0)	1.50 (5.9)	1.51 (6.1)	
CH$_2$CH$_2$(<u>CH</u>$_2$)$_{11}$ CH$_3$	1.20	1.21	1.21	
(CH$_2$)$_{13}$<u>CH</u>$_3$	0.82 (6.4)	0.82 (6.3)	0.83 (6.4)	

The ^{13}C NMR spectrum (50.3 MHz, $CDCl_3$) included further evidence of the presence of the labels in the α and β anomers. Signals of carbon atoms bearing one or two labels should respectively be triplets or quintets . Therefore, such signals were expected to be lost in the baseline noise. In the spectra of the unlabeled α and β anomers **30** and **31**, the C-3 signal of the sialyl moiety and the C-3 signal of the glycerolipid moiety appeared respectively, for the α anomer, at 37.7 and 64.1 ppm and at 37.2 and 64.1 ppm for the β anomers. These signals should not be detectable in the ^{13}C NMR spectra of the corresponding deuteriated anomers since they are expected to be lost in baseline noise. Indeed, in the spectra of the mono- and dilabeled α and β anomers **30a**, **30b**, **31a** and **31b** no signals were detectable for C-3 of the sialyl moiety. Furthermore in the spectra of the deuteriated α and β anomers **30c** and **31c** no signals were observed for the C-3 of glycerol.

Mass spectra (negative FAB) of the α and β anomers showed very weak molecular ion signals but peaks occurring respectively at 475, 476 and 474 in the spectra of the mono- (**30a**, **31a**) di- (**30b**, **31b**) deuteriated and glycerolipid deuteriated (**30c**, **31c**) α and β anomers, were very strong. The fragments resulted from the loss of the aglycon.

All the evidence obtained from the full characterization of the α and β anomers of the mono-(**30a** and **31a**), di-(**30b** and **31b**) and the glycerolipid labeled (**30c** and **31c**) sialyl glycerolipids clearly demonstrated the success of the glycosidation.

- **Zemplen Deacetylation of α-Anomers 30a, 30b and 30c**



30a $R^1 = D, R^2 = R^3 = H$
30b $R^1 = R^2 = D, R^3 = H$
30c $R^1 = R^2 = H, R^3 = D$

35a $R^1 = D, R^2 = R^3 = H$
35b $R^1 = R^2 = D, R^3 = H$
35c $R^1 = R^2 = H, R^3 = D$

Scheme 26. Zemplen Deacetylation of α-Anomers **30a**, **30b** and **30c**

The Zemplen deacetylation (Scheme 26) of the α-anomers **30a, 30b** and **30c** proceeded at room temperature and was complete within 4 hours. Neutralization of the reaction mixture with H^+ resin followed by filtration yielded methyl esters **35a, 35b** and **35c** as clear oils which were recrystallized from methanol/ether to give the pure α anomers of sialylglycerolipid methyl esters **35a, 35b** and **35c** as white crystals (**35a**: yield 95%; **35b**: yield 91%; **35c** yield 96%).

^{1}H NMR spectra (200 MHz, CD_3OD) of sialyl glycerolipids **35a, 35b** and **35c** consisted generally of a singlet found at δ = 3.84 ppm, a complex multiplet found between 3.82 and 3.41 ppm, a second singlet appearing at 2.00 ppm and broad signals at 1.55, 1.29 and 0.90 ppm. The singlets displayed at 3.84 and at 2.00 ppm indicated respectively the presence of the methyl esters and acetamido protons. The complex multiplet integrated for 16 (**35a** and **35b**) or 15 protons (**35c**) and represented the H-4 to H-9 sialyl moiety protons, the H-1 to H-3 glycerol protons and the $OC\underline{H_2}(CH_2)_{12}CH_3$ protons of the two tetradecyl chains. The broad signals at 1.55, 1.22 and 0.90 ppm were evidence of the alkyl chain protons $OCH_2C\underline{H_2}(CH_2)_{11}CH_3$, $O(CH_2)_2(C\underline{H_2})_{11}CH_3$ and $O(CH_2)_{13}C\underline{H_3}$. Furthermore, the spectra of the monolabeled sialyl glycerolipid ester **35a** and of the sialyl glycerolipid **35c** also respectively displayed a doublet and a doublet of doublet at δ = 2.67 ppm, indicating the presence of H-3eq protons. The spectra of sialyl glycerolipid **35c** also contained a doublet of doublets at δ =1.74 ppm, representing the H-3ax proton. The 1.5-3.0 ppm portions of the ^{1}H NMR spectra of the α sialyl glycerolipids methyl esters **35a, 35b** and **35c** are illustrated in Figure XIII.

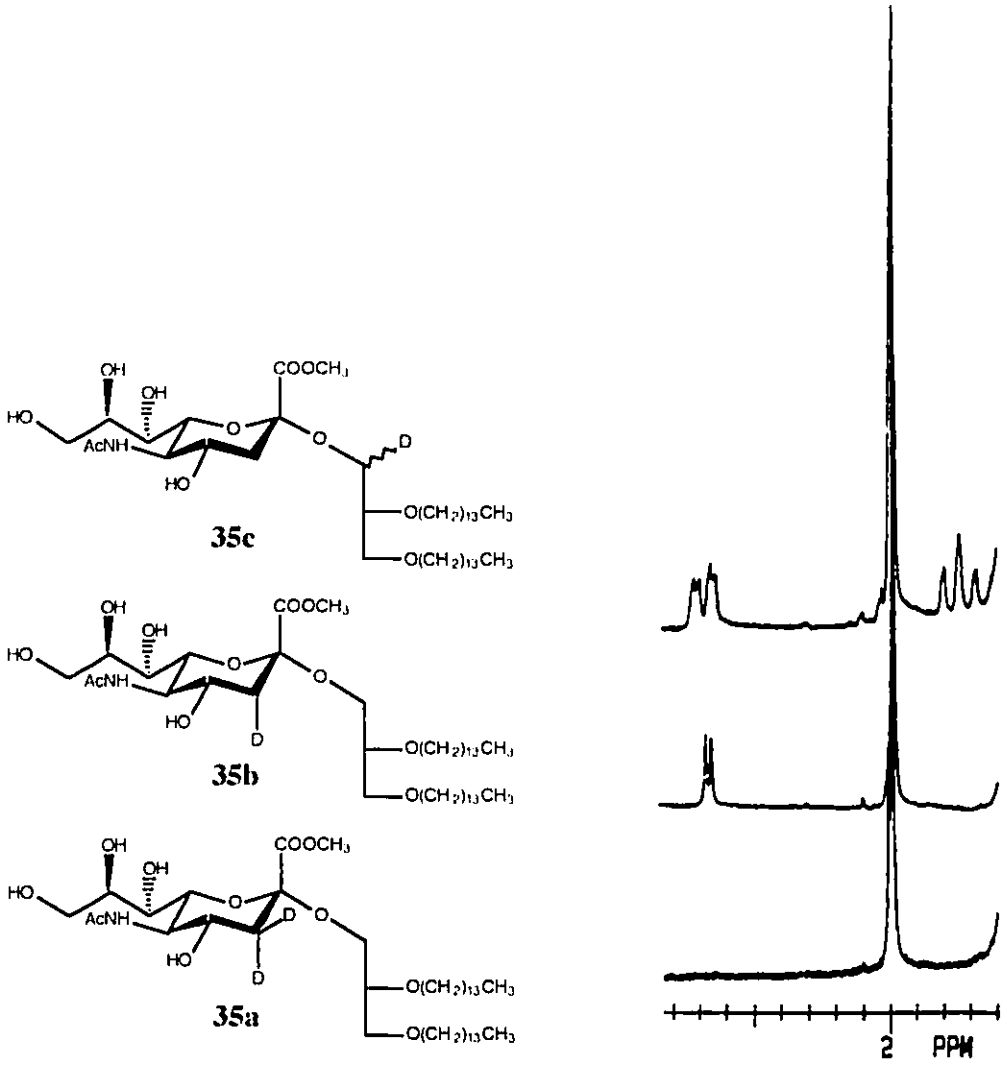
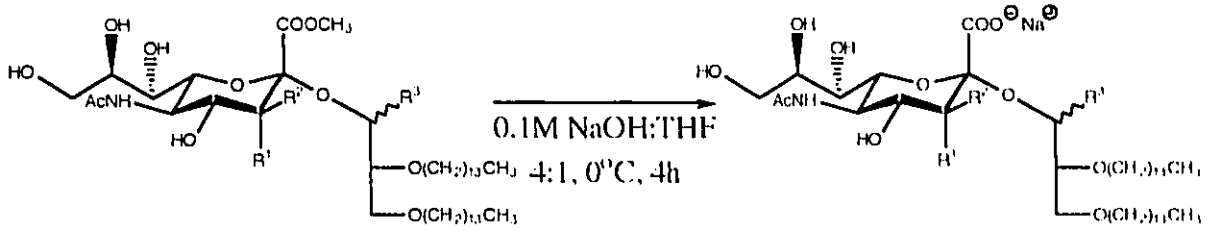


Figure XIII. Partial ^{1}H NMR Spectra of the α Sialyl Glycerolipid Methyl Esters **35a**, **35b** and **35c** showing the Sialyl H-3ax, H-3eq Region. The Singlet belongs to the N-Acetyl Residue.

Mass spectra (negative FAB) of the α sialyl glycerolipids methyl esters **35a**, **35b** and **35c** did not exhibit strong molecular ion peaks, but the fragment formed by the loss of the glycerolipid moiety were the base peaks in the spectra of all the α sialyl derivatives (**35a**; m/z 307, **35b**; m/z, 308, **35c**; m/z, 306, relative intensity, 100%).

- **Saponification of α Sialyl Glycerolipid Methyl Esters 35a, 35b and 35c.**

The base catalyzed saponification of the α sialyl glycerolipid methyl esters **35a**, **35b** and **35c** was performed according to Scheme 27



35a $R^1 = D, R^2 = R^3 = H$
35b $R^1 = R^2 = D, R^3 = H$
35c $R^1 = R^2 = H, R^3 = D$

1a $R^1 = D, R^2 = R^3 = H$
1b $R^1 = R^2 = D, R^3 = H$
1c $R^1 = R^2 = H, R^3 = D$

Scheme 27. Saponification of α Sialyl Glycerolipid Methyl Esters **35a**, **35b** and **35c**

The saponification of the α sialyl glycerolipid methyl esters **35a**, **35b** and **35c** was conducted in a mixture of THF:0.1M NaOH (1:4) to avoid the formation of emulsions. The reaction mixture was stirred at 0°C for a period of 4 hours. Neutralization of the base with H^+ resin, filtration and adjustment of the pH of the solution to 7 (1M NaOH or 1M NH_4OH) yielded the desired mono-, di- or aglycon labeled sialyl glycerolipids **1a**, **1b** and **1c** (**1a** (Na^+ salt): yield 90 %; **1b** (Na^+ salt): yield 95%; **1c** (Na^+ salt): yield 85%.

The regio- and stereo-selectively deuteriated α-sialyl glycerolipids **1a**, **1b** and **1c** were fully characterized by ^1H NMR, ^{13}C NMR and M.S. (Negative FAB).

^1H NMR (200 MHz, CD_3OD) spectroscopic data are summarized in Table 10

Table 10. ^{1}H NMR Spectroscopic Data for the α-Sialyl Glycerolipids **1a**, **1b** and **1c**

Compound	**1a**	**1b**	**1c**
Protons	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	- (- , -)	- (- , -)	1.63 (12.2,11.5)
H-3eq ($J_{3eq,4}$)	2.77 (3.7)	- (-)	2.78 (3.6)
H-4,5,6,7,8,9a,9b H-1', H-2', H-3' $CH_2(CH_2)_{12}CH_3$	3.88-3.42	3.85-3.41	3.85-3.41
$CH_3C(O)NH$	2.01	2.00	1.99
$CH_2CH_2(CH_2)_{11}CH_3$	1.54	1.55	1.55
$CH_2CH_2(CH_2)_{11}CH_3$	1.29	1.29	1.29
$(CH_2)_{13}CH_3$	0.90	0.89	0.88

The ^{1}H NMR (200 MHz, CD_3OD) spectroscopic data confirmed the presence of the labels in the mono- and di-labeled sialyl glycerolipids **1a** and **1b**. The absence of H-3ax and H-3eq signals on the spectrum of the dilabeled sialyl glycerolipid **1b** was evidence for the replacement of the H-3 protons by deuterons. The ^{1}H NMR spectrum of monolabeled sialyl glycerolipid **1a** displayed a doublet representing the H-3 equatorial proton. The absence of the H-3 axial proton signal coupled with the loss of the geminal $J_{3ax,3eq}$ coupling constant were proofs of the presence of the label on the C-3 carbon. For the sialyl glycerolipid **1c** the only evidence of the presence of the label on the glycerolipid was the integration of the multiplet found at 3.85-2.41 ppm. The multiplet integrated for 15 protons: 7 protons from the sialyl moiety, 4 glycerol protons and 4 alkyl chain protons. The ^{1}H NMR spectra of **1a**, **1b** and **1c** also confirmed the completion of the saponification since the ester signals found in the ^{1}H NMR spectra of the corresponding sialyl glycerolipid methyl esters **35a**, **35b** and **35c** were not present in the spectra of the acids.

This last deprotection step concluded the synthesis of the regio and stereo-selectively deuteriated sialyl glycerolipid **1a**, **1b** and **1c**.

3.3 Conclusion

The synthesis of the regio- and stereo-selectively deuteriated sialyl glycerolipids **1a**, **1b** and **1c** was successfully accomplished using a modified Koenigs-Knorr glycosidation procedure followed by the deprotection of the α-glycosides to yield the desired sialosides[96]. These deuteriated sialyl glycerolipids have been used by Fenske et al.[97] in ^2H NMR study of sialyl glycerolipids in model membranes.

The study of the glycosidation of sialyl donors under various reaction conditions confirmed the challenge represented by the synthesis of sialosides. The difficulty of controlling the selectivity of the glycosidation was apparent in the unpredictable anomeric ratios of glycosides obtained. The necessity of having good nucleophiles to glycosidate the sterically hindered sialyl donors was demonstrated when the salicylate ion of a promoter successfully competed with the glycosyl acceptor. The difficulty to avoid competitive elimination was also observed. The 2,3-dehydro derivative of N-acetylneuraminic acid was frequently a side-product of the attempted glycosidation.

The above problems with Koenigs-Knorr glycosidations prompted a search for more efficient glycosidation methods. The recent popularity of thioglycosides as glycosyl donors and the various mild methods available for their activation, was the motivation for the following investigation in the synthesis of thioglycosides of N-acetylneuraminic acid and their usefulness as glycoside donors.

96 R.Roy, M.Letellier, D. Fenske and H.C.Jarrell, J. Chem. Soc., Chem. Comm., (1990) 378

97 D.Fenske, M.Letellier, R.Roy, D.C.P. Smith, H.C.Jarrell, Biochem. **30** (1991) 10542

3.4 Experimental

3.4.0 General Methods

See Section **1.4.0** in Chapter 1.

3.4.1 Synthesis of O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol (labeled and unlabeled derivatives 30, 30a, 30b, and 30c).

Method A - Mercuric Cyanide/Mercuric Bromide as Promoters

Glycerolipid **4** or **4a** (179 mg, 0.37 mmol, 1.2 eq), the mercuric cyanide (93 mg, 0.37 mmol, 1.2 eq), the mercuric bromide (133 mg, 0.37 mmol, 1.2 eq) and 4Å powdered molecular sieves were suspended in dry methylene chloride or dichloroethane (5 ml) and the mixture was stirred for 1 hour at room temperature under nitrogen. A solution of freshly prepared chloride **22**, **22a** or **22b** (158 mg, 0.31 mmol) in methylene chloride or dichloroethane (5 ml) was added and the mixture was stirred at room temperature for 48 hours, until complete disappearance of the chloride as detected by TLC. The α and β acetylated glycoglycerolipid esters appeared at rf's of 0.63 and 0.69 respectively using 2 x 100% ethyl acetate as eluent. The reaction mixture was diluted to 20 ml with methylene chloride, filtered through Celite and evaporated in vacuo. The oil obtained was chromatographed on silica gel (eluent: 2% ethanol in methylene chloride) and the α and β anomers were obtained as clear oils in yields ranging from 31-40% (92-120 mg) and 20-37% (59-110 mg) respectively.

Method B - Silver Salicylate as Promoter

Synthesis of Silver Salicylate 33

The pH of a solution of salicylic acid **32** (6.84 g, 50.0 mmol) in 95% ethanol (25 ml) was adjusted to 9 with a 10% solution of NH_4OH. A solution of silver nitrate (8.16 g, 48.0 mmol) in 50% ethanol was added dropwise to the basic solution at 70°C and in the absence of light. The reaction mixture was stirred at 70°C for 1 more hour, in the dark, and was subsequently cooled.

The reaction mixture was filtered and the pinkish powder obtained was dried in vacuo in a dessicator (P_2O_5). Silver Salicylate **33** was obtained in 94% yield (11.0 g), as a slightly pink powder.

Glycosidation Method using Silver Salicylate.

Glycerolipid **4** (378 mg (0.77 mmol, 2.5 eq.), 756 mg (1.55 mmol, 5 eq.) or 1.5 g (3.1 mmol, 10 eq.)), silver salicylate **33** (91 mg, 0.37 mmol, 1.2 eq) and 4Å powdered molecular sieves were suspended in dry methylene chloride, dichloroethane or toluene (5 ml). The mixture was stirred at room temperature for 1 hour, under nitrogen, and a solution of chloride **22** (158 mg, 0.31 mmol) in methylene chloride or dichloroethane (5 ml) was added to the mixture. Thin Layer Chromatography (TLC, eluent 2 x 100% ethyl acetate) showed complete disappearance of the chloride after 24 hours of stirring at room temperature and the presence of a strongly fluorescent compound at rf = 0.45 and traces of α and β sialyl glycerolipid **30** and **31**. The reaction mixture was diluted to 20 ml with the appropriate reaction solvent and the mixture was filtered through Celite. The clear solution was washed with water (1 x 20 ml), saturated sodium bicarbonate (2 x 20 ml) and brine (20 ml), dried (anhydrous sodium sulfate) and evaporated in vacuo. ^{1}H NMR of the crude oil obtained revealed that traces of α and β anomers were present, but they were not the major product. The α:β anomeric ratios of the traces of sialyl glycerolipids **30** and **31** produced by the reaction performed in methylene chloride dichloroethane and toluene were 4:1, 3:1 and 9:2 respectively. The crude oil was chromatographed by flash chromatography on silica gel (eluent: 2% ethanol in methylene chloride). A glassy compound was recuperated in 68% yield (129 mg, CH_2Cl_2 reaction), 58% yield (110 mg, $C_2H_4Cl_2$ reaction) or 70% yield (132 mg, toluene reaction) and was identified as methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-O-salicylyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosonate **34**; $[\alpha]_D$ +21° (c1.0, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.84 (dd, 1H, $J_{4',6'}$ = 1.5 Hz, $J_{5',6'}$ = 8.5 Hz, H-6'), 7.48 (ddd, 1H, $J_{3',4'}$ = 8.5 Hz, $J_{4',5'}$ = 7.3 Hz, H-4'), 6.96 (d, 1H, H-3'), 6.89 (dd, 1H, H-5'), 5.36 (dd, 1H, $J_{6,7}$ = 1.3 Hz, $J_{7,8}$ = 8.0 Hz, H-7), 5.28 (d, 1H, $J_{NH,5}$ = 10.0 Hz, NH), 5.21 (ddd, 1H, $J_{8,9a}$ = 2.4 Hz, $J_{8,9b}$ = 5.2 Hz, H-8), 5.06 (ddd, 1H, $J_{3ax,4}$ = 11.5 Hz, $J_{3eq,4}$ = 4.5 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 4.75 (dd, 1H, $J_{5,6}$ = 10.6 Hz, H-6), 4.35 (dd, 1H, $J_{9a,9b}$ = 12.8 Hz, H-9a), 4.06 (ddd, 1H, H-5), 4.04 (dd, 1H, H-9b), 3.77 (s, 3H, OMe), 2.69 (dd, 1H, $J_{3ax,3eq}$ = 13.0 Hz, H-3eq), 2.29 (dd, 1H, H-3ax), 2.10, 2.04, 2.02, 2.00, 1.90 (s, 15H, $CH_3C(O)$); M.S. (C.I., ether) m/z: 612 (1.5%, $[M + H]^+$), 474 (20%, $[M - OC(O)PhOH]^+$).

Method C - Silver Triflate as Promoter

Glycerolipid **4** (56 mg, 0.115 mmol, 1.5 eq), silver triflate (23 mg, 0.092 mmol, 1.2 eq), collidine (12 µl, 11 mg, 0.092 mmol, 1.2 eq) or TMU (11 µl, 11 mg, 0.092 mmol, 1.2 eq) and 4Å molecular sieves were stirred in methylene chloride or tetrahydrofuran (5 ml) at room temperature for 1 hour under a nitrogen atmosphere. A solution of chloride **22** (40 mg, 0.077 mmol) in the appropriate solvent (5 ml) was added to the reaction mixture. After stirring for 8 and 24 hours for the reaction in methylene chloride and in THF respectively, TLC showed complete disappearance of the chloride **22** starting material. The mixture was filtered through Celite and the solvent was evaporated. The oily residue obtained was dissolved in ethyl acetate (10 ml) and washed with a 2 M sodium thiosulfate solution (15 ml), saturated sodium bicarbonate (15 ml) and brine (15 ml), dried (anhydrous sodium sulfate) and evaporated. The crude oil obtained was chromatographed on preparative TLC plates using 100% ethyl acetate as eluent. For the reaction performed in methylene chloride the yield of sialylglycerolipids **30** and **31** was 55% (41 mg) and α:β anomer ratio was 1:3 (determined by ^{1}H NMR). For the glycosylation conducted in tetrahydrofuran, only a trace of sialyl glycerolipids **30** and **31** was detected by TLC (<10%, α:β anomer ratio obtained form ^{1}H NMR of crude mixture: 11:9 but a 55% yield (20 mg) of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-2-enopyranosonate **26** was obtained.

Method D - Trimethylsilyl Triflate as Promoter

Methyl 5-acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate **21** (41 mg, 0.077 mmol), glycerolipid **4** (45 mg, 0.092 mmol, 1.2 eq) and 4Å powdered molecular sieves were stirred in methylene chloride (8 ml) at room temperature for 1 hour. A solution of TMSiOTf (17 µl, 20 mg, 0.092 mmol, 1.2 eq) in methylene chloride (2 ml) was added dropwise to the reaction mixture and it was stirred at room temperature until TLC indicated complete disappearance of penta-O-acetate **21**. Triethylamine was added to the reaction mixture and it was filtered through Celite. The solution was washed with 0.1M HCl (1 x 20 ml), saturated sodium bicarbonate solution (2 x 20 ml) and brine (20 ml), dried (anhydrous sodium sulfate) and evaporated in vacuo. The crude oil obtained was chromatographed by preparative TLC using a 100% ethyl acetate as eluent. The acetylated sialylglycerolipid esters (α and β) were obtained in 55% yield (40 mg), in an α:β ratio of 4:7 (determined by ^{1}H NMR). A 15% yield (6 mg) of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-2-enopyrano-sonate **26** was also obtained.

O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 30- Yield 37%; $[\alpha]_D$ -8.6° (c1.0, chloroform); $[\alpha]_D$ -9.3° (c1.04 chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.33 (ddd, 1H, $J_{7,8}$ = 8.6 Hz, $J_{8,9a}$ = 2.4 Hz, $J_{8,9b}$ = 5.0 Hz, H-8), 5.30 (dd, 1H, $J_{6,7}$ = 1.6 Hz, H-7), 5.10 (d, 1H, $J_{NH,5}$ = 10.0 Hz, NH), 4.83 (ddd, 1H, $J_{3ax,4}$ = 10.1 Hz, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 4.27 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.08 (dd, 1H, H-9b), 4.05 (ddd, 1H, $J_{5,6}$ = 10.5 Hz, H-5), 3.78 (dd, 1H, H-6), 3.77 (s, 3H, OMe), 3.62-3.28 (m, 9H, $OC\underline{H}_2(CH_2)_{12}CH_3$, 5H of glycerol), 2.58 (dd, 1H, $J_{3ax,3eq}$ = 12.7 Hz, H-3eq), 2.12, 2.11, 2.02, 2.00, 1.86 (s, 15H, $CH_3C(O)$), 1.95 (dd, 1H, H-3ax), 1.53 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.23 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.86 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); lit.[82] ^{1}H NMR ($CDCl_3$): δ (ppm) 4.85 (m, 1H, $J_{3ax,4}$ = 12.8 Hz, $J_{3eq,4}$ = 4.8 Hz, H-4), 2.60 (dd, 1H, $J_{3ax,3eq}$ = 13.1 Hz, H-3eq), 1.97 (dd, 1H, H-3ax); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.0, 170.6, 170.3, 170.1, 170.0 (5C, $CH_3\underline{C}(O)$), 168.3 (1C, C-1), 98.8 (1C, C-2), 77.4 (1C, C-2'), 72.4 (1C, C-6), 71.5 (1C, C-1'), 70.6, 70.5 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 69.1 (1C, C-8), 68.7 (1C, C-4), 67.3 (1C, C-7), 64.8 (1C, C-3'), 62.2 (1C, C-9), 52.4 (1C, OMe), 49.2 (1C, C-5), 37.7 (1C, C-3), 31.7, 29.8, 29.4, 29.3, 29.1, 25.9, 22.4 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 22.9, 20.8, 20.5, 20.4, 20.4 (5C, $\underline{C}H_3C(O)$), 13.8 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 958 (4%, $[M]^-$), 483 (9%, $[aglycon]^-$), 474 (100%, $[M - aglycon]^-$); Anal. calcd for $C_{51}H_{91}NO_{15}$: C, 63.92; H, 9.57; N, 1.46; Found: C, 62.85; H, 9.46; N, 1.71.

O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 31 - Yield 25%; $[\alpha]_D$ -15.2° (c1.0, chloroform); $[\alpha]_D$ -13.0° (c1.0, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 5.34 (dd, 1H, $J_{7,8}$ = 6.1 Hz, H-7), 5.21 (ddd, 1H, $J_{8,9a}$ = 2.2 Hz, $J_{8,9b}$ = 5.0 Hz, H-8), 5.19 (ddd, 1H, $J_{3ax,4}$ = 11.8 Hz, $J_{3eq,4}$ = 4.8 Hz, H-4), 5.12 (d, 1H, $J_{NH,5}$ = 9.8 Hz, NH), 4.68 (dd, 1H, $J_{9a,9b}$ = 12.3 Hz, H-9a), 4.11 (dd, 1H, H-9b), 4.12-4.04 (m, 2H, H-5, 6), 3.75 (s, 3H, OMe), 3.61-3.31 (m, 9H, $OC\underline{H}_2(CH_2)_{12}CH_3$, 5H of glycerol), 2.41 (dd, 1H, $J_{3ax,3eq}$ = 12.9 Hz, H-3eq), 2.10, 2.02, 1.98, 1.97, 1.84 (s, 15H, $CH_3C(O)$), 1.84 (dd, 1H, H-3ax), 1.51 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.21 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.83 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); lit.[82] ^{1}H NMR ($CDCl_3$): δ (ppm) 5.23 (m, 1H, $J_{3ax,4}$ = 12.2 Hz, $J_{3eq,4}$ = 4.8 Hz, H-4), 2.45 (dd, 1H, $J_{3ax,3eq}$ = 12.5 Hz, H-3eq), 1.90 (dd, 1H, H-3ax); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.2, 170.7, 170.7, 170.3, 170.3 (5C, $CH_3\underline{C}(O)$), 167.4 (1C, C-1), 98.1 (1C, C-2), 77.3 (1C, C-2'), 71.6 (1C, C-1'), 71.4 (1C, C-6'), 71.1 (1C, C-8), 70.9, 70.0 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 68.8 (1C, C-4), 68.1 (1C, C-7), 64.1 (1C, C-3'), 62.3 (1C, C-9), 52.5 (1C, OMe), 49.2 (1C, C-5), 37.2 (1C, C-3), 31.7, 30.1, 30.0, 29.5, 29.4, 29.3, 29.2,

26.0, 25.9, 22.5 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 23.0, 20.9, 20.7, 20.6, 20.6 (5C, $\underline{C}H_3C(O)$), 13.9 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 958 (9%, $[M]^-$), 483 (15%, $[aglycon]^-$), 474 (100%, $[M\text{-}aglycon]^-$).

O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3ax-deutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 30a- Yield 31%; $[\alpha]_D$ -8.5° (c1.0, chloroform); ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 5.33 (ddd, 1H, $J_{7,8}$ = 9.1 Hz, $J_{8,9a}$ = 2.2 Hz, $J_{8,9b}$ = 4.5 Hz, H-8), 5.29 (dd, 1H, $J_{6,7}$ = 1.0 Hz, H-7), 5.09 (d, 1H, $J_{NH,5}$ = 9.9 Hz, NH), 4.82 (dd, 1H, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 10.0 Hz, H-4), 4.26 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.07 (dd, 1H, H-9b), 4.05 (ddd, 1H, $J_{5,6}$ = 10.5 Hz, H-5), 3.79 (dd, 1H, H-6), 3.76 (s, 3H, OMe), 3.56-3.28 (m, 9H, $OC\underline{H}_2(CH_2)_{12}CH_3$, 5H of glycerol), 2.55 (d, 1H, H-3eq), 2.10, 2.10, 2.01, 1.99, 1.85 (s, 15H, $CH_3C(O)$), 1.52 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.22 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.84 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.2, 170.7, 170.3, 170.2, 170.0 (5C, $CH_3\underline{C}(O)$), 168.2 (1C, C-1), 98.7 (1C, C-2), 77.4 (1C, C-2'), 72.3 (1C, C-6), 71.6 (1C, C-1'), 70.6 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 69.0 (1C, C-8), 68.4 (1C, C-4), 67.1 (1C, C-7), 64.8 (1C, C-3'), 62.1 (1C, C-9), 52.6 (1C, OMe), 49.2 (1C, C-5), 31.7, 29.8, 29.5, 29.4, 29.2, 29.1, 25.8, 22.5 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 23.0, 20.9, 20.9, 20.5, 20.5 (5C, $\underline{C}H_3C(O)$), 13.9 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 959 (4%, $[M]^-$), 483 (9%, $[aglycon]^-$), 475 (94%, $[M\text{-}aglycon]^-$); Anal. calcd for $C_{51}H_{90}DNO_{15}$: C, 63.86; H, D, 9.67; N, 1.46; Found: C, 62.76; H, D, 10.11; N, 1.81.

O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3ax-deutero-β-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 31a - Yield 20%; $[\alpha]_D$ -15.0° (c1.0, chloroform); ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 5.33 (dd, 1H, $J_{7,8}$ = 6.2 Hz, H-7), 5.23 (ddd, 1H, $J_{8,9a}$ = 2.3 Hz, $J_{8,9b}$ = 4.5 Hz, H-8), 5.19 (dd, 1H, $J_{3eq,4}$ = 4.8 Hz, H-4), 5.09 (d, 1H, $J_{NH,5}$ = 9.8 Hz, NH), 4.67 (dd, 1H, $J_{9a,9b}$ = 12.3 Hz, H-9a), 4.10 (dd, 1H, H-9b), 4.10-4.03 (m, 2H, H-5, 6), 3.74 (s, 3H, OMe), 3.60-3.33 (m, 9H, $OC\underline{H}_2(CH_2)_{12}CH_3$, 5H of glycerol), 2.38 (d, 1H, H-3eq), 2.08, 2.01, 1.97, 1.96, 1.83 (s, 15H, $CH_3C(O)$), 1.49 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.20 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.82 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.1, 170.6, 170.6, 170.3, 170.3 (5C, $CH_3\underline{C}(O)$), 167.4 (1C, C-1), 98.0 (1C, C-2), 77.3 (1C, C-2'), 71.6 (1C, C-1'), 71.3 (1C, C-6), 71.1 (1C, C-8), 70.9, 70.0 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 68.8 (1C, C-4), 68.0 (1C, C-7), 64.0 (1C, C-3'), 62.3 (1C, C-9), 52.5 (1C, OMe), 49.2 (1C, C-5), 31.7, 30.1, 29.5, 29.3, 29.1, 25.9, 25.8, 22.4 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 22.9, 20.9, 20.6,

20.6, 20.6 (5C, $\underline{C}H_3C(O)$), 13.9 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 959 (9%, $[M]^-$), 483 (5%, $[aglycon]^-$), 475 (89%, $[M - aglycon]^-$).

O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3ax,3eq-dideutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 30b - Yield 37%; $[\alpha]_D$ -8.6° (c1.0, chloroform); ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 5.34 (ddd, 1H, $J_{7,8}$ = 8.6 Hz, $J_{8,9a}$ = 2.0 Hz, $J_{8,9b}$ = 4.7 Hz, H-8), 5.29 (dd, 1H, $J_{6,7}$ = 1.8 Hz, H-7), 5.17 (d, 1H, $J_{NH,5}$ = 9.9 Hz, NH), 4.80 (d, 1H, $J_{4,5}$ = 10.1 Hz, H-4), 4.29 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.07 (dd, 1H, H-9b), 4.04 (ddd, 1H, $J_{5,6}$ = 10.6 Hz, H-5), 3.78 (dd, 1H, H-6), 3.75 (s, 3H, OMe), 3.54-3.30 (m, 9H, $OC\underline{H}_2(CH_2)_{12}CH_3$, 5H of glycerol), 2.10, 2.09, 2.00, 1.99, 1.84 (s, 15H, $CH_3C(O)$), 1.52 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.21 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.84 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.1, 170.6, 170.3, 170.2, 170.0 (5C, $CH_3\underline{C}(O)$), 168.3 (1C, C-1), 98.7 (1C, C-2), 77.4 (1C, C-2'), 72.4 (1C, C-6), 71.6 (1C, C-1'), 70.6 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 69.0 (1C, C-8), 68.6 (1C, C-4), 67.2 (1C, C-7), 64.8 (1C, C-3'), 62.1 (1C, C-9), 52.5 (1C, OMe), 49.2 (1C, C-5), 31.7, 29.8, 29.5, 29.3, 29.1, 25.9, 25.8, 22.4 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 23.0, 20.8, 20.6, 20.6, 20.5 (5C, $\underline{C}H_3C(O)$), 13.8 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 960 (10%, $[M]^-$), 483 (11%, $[aglycon]^-$), 476 (92%, $[M - aglycon]^-$); Anal. calcd for $C_{51}H_{89}D_2NO_{15}$: C, 63.79; H, D, 9.76; N, 1.46; Found : C, 62.51; H, D, 10.22; N, 1.73.

O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3ax,3eq-dideutero-β-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 31b- Yield 25%; $[\alpha]_D$ -14.8° (c1.0, chloroform); ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 5.33 (dd, 1H, $J_{7,8}$ = 6.0 Hz, H-7), 5.22 (ddd, 1H, $J_{8,9a}$ = 2.2 Hz, $J_{8,9b}$ = 4.6 Hz, H-8), 5.19 (d, 1H, H-4), 5.17 (d, 1H, $J_{NH,5}$ = 9.8 Hz, NH), 4.67 (dd, 1H, $J_{9a,9b}$ = 12.3 Hz, H-9a), 4.10 (dd, 1H, H-9b), 4.11-4.03 (m, 2H, H-5, 6), 3.74 (s, 3H, OMe), 3.60 3.33 (m, 9H, $OC\underline{H}_2(CH_2)_{12}CH_3$, 5H of glycerol), 2.09, 2.01, 1.97, 1.96, 1.83 (s, 15H, $CH_3C(O)$), 1.50 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.21 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.82 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.1, 170.6, 170.6, 170.3, 170.2 (5C, $CH_3\underline{C}(O)$), 167.4 (1C, C-1), 98.0 (1C, C-2), 77.2 (1C, C-2'), 71.6 (1C, C-1'), 71.4 (1C, C-6), 71.1 (1C, C-8), 70.8, 69.9 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 68.7 (1C, C-4), 68.0 (1C, C-7), 64.0 (1C, C-3'), 62.3 (1C, C-9), 52.4 (1C, OMe), 49.1 (1C, C-5), 31.7, 30.0, 29.4, 29.3, 29.1, 25.9, 25.8, 22.4 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 22.9, 20.8, 20.6, 20.5, 20.5 (5C, $\underline{C}H_3C(O)$), 13.8 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 960 (9%, $[M]^-$), 483 (15%, $[aglycon]^-$), 476 (83%, $[M - aglycon]^-$).

O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol 30c - Yield 40%; $[\alpha]_D$ -8.6° (c1.0, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 5.34 (ddd, 1H, $J_{7,8}$ = 8.8 Hz, $J_{8,9a}$ = 2.1 Hz, $J_{8,9b}$ = 4.6 Hz, H-8), 5.29 (dd, 1H, $J_{6,7}$ = 1.6 Hz, H-7), 5.12 (d, 1H, $J_{NH,5}$ = 9.9 Hz, NH), 4.81 (ddd, 1H, $J_{3ax,4}$ = 10.1 Hz, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 10.1 Hz, H-4), 4.28 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.07 (dd, 1H, H-9b), 4.04 (ddd, 1H, H-5), 3.78 (dd, 1H, H-6), 3.76 (s, 3H, OMe), 3.55-3.30 (m, 8H, OC$\underline{H}_2$(CH_2)$_{12}CH_3$, 4H of glycerol), 2.58 (dd, 1H, $J_{3ax,3eq}$ = 12.7 Hz, H-3eq), 2.11, 2.10, 2.01, 1.99, 1.85 (s, 15H, CH_3C(O)), 1.96 (dd, 1H, H-3ax), 1.52 (t, 4H, OCH_2C$\underline{H}_2$(CH_2)$_{11}CH_3$), 1.22 (bs, 44H, O(CH_2)$_2$(C$\underline{H}_2$)$_{11}CH_3$), 0.85 (t, 6H, O(CH_2)$_{13}$C$\underline{H}_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.0, 170.6, 170.2, 170.1, 170.0 (5C, $CH_3\underline{C}$(O)), 168.3 (1C, C-1), 98.7 (1C, C-2), 77.4 (1C, C-2'), 72.3 (1C, C-6), 71.5 (1C, C-1'), 70.6, 70.5 (2C, O$\underline{C}H_2$(CH_2)$_{12}CH_3$), 69.0 (1C, C-8), 68.7 (1C, C-4), 67.2 (1C, C-7), 62.1 (1C, C-9), 52.4 (1C, OMe), 49.2 (1C, C-5), 37.6 (1C, C-3), 31.6, 29.8, 29.4, 29.3, 29.1, 25.9, 22.4 (24C, OCH_2(CH_2)$_{12}CH_3$), 22.8, 20.8, 20.5, 20.4, 20.4 (5C, $\underline{C}H_3$C(O)), 13.8 (2C, O(CH_2)$_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 959 (2%, $[M]^-$), 484 (13%, $[aglycon]^-$), 474 (97%, $[M - aglycon]^-$); Anal. calcd for $C_{51}H_{90}DNO_{15}$: C, 63.86; H, D, 9.67; N, 1.46; Found : C, 63.03; H, D, 10.1; N, 1.53.

O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol 31c- Yield 27%; $[\alpha]_D$ -15.2° (c1.0, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 5.34 (dd, 1H, $J_{7,8}$ = 6.1 Hz, H-7), 5.21 (ddd, 1H, $J_{8,9a}$ = 2.2 Hz, $J_{8,9b}$ = 4.7 Hz, H-8), 5.19 (ddd, 1H, $J_{3ax,4}$ = 11.9 Hz, $J_{3eq,4}$ = 4.8 Hz, H-4), 5.11 (d, 1H, $J_{NH,5}$ = 9.7 Hz, NH), 4.67 (dd, 1H, $J_{9a,9b}$ = 12.3 Hz, H-9a), 4.10 (dd, 1H, H-9b), 4.11-4.04 (m, 2H, H-5, 6), 3.75 (s, 3H, OMe), 3.61-3.32 (m, 8H, OC$\underline{H}_2$(CH_2)$_{12}CH_3$, 4H of glycerol), 2.39 (dd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, H-3eq), 2.10, 2.01, 1.98, 1.97, 1.84 (s, 15H, CH_3C(O)), 1.86 (dd, 1H, H-3ax), 1.51 (t, 4H, OCH_2C$\underline{H}_2$(CH_2)$_{11}CH_3$), 1.21 (bs, 44H, O(CH_2)$_2$(C$\underline{H}_2$)$_{11}CH_3$), 0.83 (t, 6H, O(CH_2)$_{13}$C$\underline{H}_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.2, 170.7, 170.7, 170.3, 170.3 (5C, $CH_3\underline{C}$(O)), 167.4 (1C, C-1), 98.1 (1C, C-2), 77.3 (1C, C-2'), 71.6 (1C, C-1'), 71.4 (1C, C-6), 71.1 (1C, C-8), 70.9, 70.0 (2C, O$\underline{C}H_2$(CH_2)$_{12}CH_3$), 68.8 (1C, C-4), 68.0 (1C, C-7), 62.3 (1C, C-9), 52.5 (1C, OMe), 49.2 (1C, C-5), 37.2 (1C, C-3), 31.7, 30.1, 30.0, 29.5, 29.3, 29.2, 29.0, 28.9, 26.0, 25.9, 22.5 (24C, OCH_2(CH_2)$_{12}CH_3$), 23.0, 20.9, 20.7, 20.6, 20.6 (5C, $\underline{C}H_3$C(O)), 13.9 (2C, O(CH_2)$_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 959 (2%, $[M]^-$), 484 (11%, $[aglycon]^-$), 474 (87%, $[M - aglycon]^-$).

3.4.2 O-[Methyl-(5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol (labeled and unlabeled derivatives 35a, 35b and 35c)

General Zemplen Deacetylation Procedure.

The pH of a methanolic solution (10 ml) of acetylated glycoglycerolipid ester **30a**, **30b** or **30c** (134 mg, 0.14 mmol) was adjusted to 9 with a 1M solution of sodium methoxide in methanol. After stirring for 4 hours at room temperature, the reaction was quenched with H+ resin. The mixture was filtered and evaporated to dryness in vacuo. The oil obtained was crystallized from methanol/ether to give the pure α anomer of sialylglycerolipid esters **35a**, **35b** or **35c** as white crystals. The yields varied from 91 to 96% (101-106 mg).

O-[Methyl-(5-acetamido-3,5-dideoxy-3ax-deutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 35a - Yield 95%; m.p. 106.3°C (sint'd), 135.4-136.4°C (melted); $[\alpha]_D$ -5.0° (c1.0, chloroform); lit.[82] **35** $[\alpha]_D$ -5.30° (c1.0, chloroform); ^{1}H NMR (200 MHz, CD_3OD): δ (ppm) 3.84 (s, 3H, OCH_3), 3.80-3.41 (m, 16H, H-4, 5, 6, 7, 8, 9a, 9b, 1', 2', 3', $OC\underline{H}_2(CH_2)_{12}CH_3$), 2.67 (d, 1H, $J_{3eq,4}$ = 4.6 Hz, H-3eq), 2.00 (s, 3H, $NDC(O)CH_3$), 1.55 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.29 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.90 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR (50.3 MHz, CD_3OD): δ (ppm) 175.6 (1C, $ND\underline{C}(O)CH_3$), 171.2 (1C, C-1), 100.3 (1C, C-2), 79.2 (1C, C-2'), 75.0 (1C, C-6), 72.7 (1C, C-1'), 72.5 (1C, C-8), 71.9, 71.8 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 70.2 (1C, C-4), 68.6 (1C, C-7), 65.4 (1C, C-3'), 64.7 (1C, C-9), 53.8 (1C, OMe), 53.5 (1C, C-5), 33.1, 31.1, 30.9, 30.7, 30.6, 27.3, 27.2, 23.8 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 22.7 (1C, $NDC(O)\underline{C}H_3$), 14.5 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 790 (1%, $[M + H]^-$), 731 (21%, $[M - COOMe]^-$), 307 (100%, $[M - Oglycerolipid]^-$); Anal. calcd for $C_{43}H_{82}DNO_{11}$: C, 65.28; H, D, 10.70; N, 1.77; Found : C, 65.71; H, D, 10.71; N, 1.70.

O-[Methyl-(5-acetamido-3,5-dideoxy-3ax,3eq-dideutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 35b - Yield 91%; m.p. 106.0°C (sint'd), 139.0-140.8°C (melted); $[\alpha]_D$ -4.9° (c1.0, chloroform); ^{1}H NMR (200 MHz, CD_3OD): δ (ppm) 3.83 (s, 3H, OCH_3), 3.82-3.41 (m, 16H, H-4, 5, 6, 7, 8, 9a, 9b, 1', 2', 3', $OC\underline{H}_2(CH_2)_{12}CH_3$), 1.99 (s, 3H, $NDC(O)CH_3$), 1.55 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.29 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.90 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR (50.3 MHz, CD_3OD): δ (ppm) 175.5 (1C, $ND\underline{C}(O)CH_3$), 171.2 (1C, C-1), 100.4

(1C, C-2), 79.2 (1C, C-2'), 75.1 (1C, C-6), 72.7 (1C, C-1'), 72.5 (1C, C-8), 71.9, 71.7 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 70.3 (1C, C-4), 68.5 (1C, C-7), 65.4 (1C, C-3'), 64.8 (1C, C-9), 53.8 (1C, OCH_3), 53.4 (1C, C-5), 33.1, 31.1, 31.0, 30.8, 30.6, 30.5, 27.3, 27.2, 23.7 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 22.7 (1C, $NDC(O)\underline{C}H_3$), 14.4 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Positive FAB) m/z: 792 (6%, $|M + H|^+$), 732 (4%, $|M - COOMe|^+$), 308 (100%, |M - Oglycerolipid|$^+$); Anal. calcd for $C_{43}H_{81}D_2NO_{11}$: C, 65.20; H, D, 10.81; N, 1.77; Found: C, 65.79; H, D, 10.51; N, 1.62.

O-[Methyl-(5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol 35c - Yield 96%; m.p. 106.1°C (sint'd), 138.5-139.9°C (melted); $|\alpha|_D$ -5.2° (c1.0, chloroform); ^1H NMR (200 MHz, CD_3OD): δ (ppm) 3.83 (s, 3H, OCH_3), 3.81-3.41 (m, 15H, H-4, 5, 6, 7, 8, 9a, 9b, 1', 2', 3' (1H), $OC\underline{H}_2(CH_2)_{12}CH_3$), 2.69 (dd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, $J_{3eq,4}$ = 4.3 Hz, H-3eq), 2.00 (s, 3H, $NDC(O)CH_3$), 1.74 (dd, 1H, $J_{3ax,4}$ = 11.7 Hz, H-3ax), 1.54 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.29 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.90 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR (50.3 MHz, CD_3OD): δ (ppm) 175.5 (1C, $ND\underline{C}(O)CH_3$), 171.2 (1C, C-1), 100.3 (1C, C-2), 79.2 (1C, C-2'), 75.1 (1C, C-6), 72.7 (1C, C-1'), 72.5 (1C, C-8), 71.9, 71.8 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 70.2 (1C, C-4), 68.6 (1C, C-7), 64.8 (1C, C-9), 53.9 (1C, OMe), 33.1, 31.1, 30.9, 30.6, 30.6, 27.3, 27.2, 23.7, (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 22.6 (1C, $NDC(O)\underline{C}H_3$), 14.4 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (C.I., ether) m/z: 791 (4%, $|M + H|^+$), 731 (7%, $|M - COOMe|^+$), 306 (100%, |M - HOglycerolipid|$^+$); Anal. calcd for $C_{43}H_{82}DNO_{11}$: C, 65.28; H, D, 10.70; N, 1.77; Found: C, 64.78 ; H, D, 10.54 ; N, 1.53.

3.4.3 Ammonium or Sodium O-[5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol - (labeled and unlabeled derivatives 1a, 1b and 1c).

General Saponification Procedure.

A sodium hydroxide solution (0.1M, 8 ml) was added to a stirred solution of the methyl ester **35a**, **35b** or **35c** (100 mg, 0.13 mmol) in tetrahydrofuran (2 ml). The cloudy mixture slowly became clear and the solution was stirred at room temperature for 4 hours until completion of the reaction. Acidic resin (Amberlite IR-120 (H^+)) was used to quench the reaction and the mixture was filtered and evaporated in order to remove the THF. The pH of the clear solution was

adjusted to 7 with an ammonium hydroxide solution (0.1M) and was lyophilized to yield the ammonium salt (**1a**, **1b** or **1c**) as a white fluffy powder (85-95 mg, 85 to 95% yields). If the sodium salt was desired, the ammonium salt could be converted to the acid using H^+ resin and was subsequently neutralized with a dilute sodium hydroxide solution.

Ammonium O-[(5-acetamido-3,5-dideoxy-3ax-deutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 1a - Yield 90%; m.p. 137.0-138.7°C ; $[\alpha]_D$ +10.7° (c1.0, methanol); ^{1}H NMR (200 MHz, CD_3OD): δ (ppm) 3.88-3.42 (m, 16H, H-4, 5, 6, 7, 8, 9a, 9b, 1', 2', 3', $OC\underline{H}_2(CH_2)_{12}CH_3$), 2.77 (d, 1H, $J_{3eq,4}$ = 3.7 Hz, H-3eq), 2.01 (s, 3H, $NDC(O)CH_3$), 1.54 (t, 2H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.29 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.90 (t, 6H, $O(CH_2)_{13}C\underline{H}_3$); ^{13}C NMR (50.3 MHz, CD_3OD): δ (ppm) 175.9 (1C, $ND\underline{C}(O)CH_3$), 169.9 (1C, C-1), 110.7 (1C, C-2), 79.4 (1C, C-2'), 74.6 (1C, C-6), 73.0 (1C, C-8), 72.7, 72.6 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 71.6 (1C, C-1'), 70.3 (1C, C-4), 69.4 (1C, C-7), 65.0 (1C, C-3'), 64.6 (1C, C-9), 54.1 (1C, C-5), 33.1, 31.1, 30.9, 30.7, 30.6, 27.3, 27.2, 23.8 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 22.6 (1C, $NDC(O)\underline{C}H_3$), 14.5 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 775 (100%, $[M - NH_4^+]^-$).

Ammonium O-[(5-acetamido-3,5-dideoxy-3ax,3eq-dideutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol 1b - Yield 95%; m.p. 137.0-139.0°C ; $[\alpha]_D$ +10.5° (c1.0, methanol); ^{1}H NMR (200 MHz, CD_3OD): δ (ppm) 3.85-3.41 (m, 16H, H-4, 5, 6, 7, 8, 9a, 9b, 1', 2', 3', $OC\underline{H}_2(CH_2)_{12}CH_3$), 2.00 (s, 3H, $NDC(O)CH_3$), 1.55 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.29 (bs, 44H, $O(CH_2)_2(C\underline{H}_2)_{11}CH_3$), 0.89 (t, 6H, $O(CH_2)_{13}CH_3$); ^{13}C NMR (50.3 MHz, CD_3OD): δ (ppm) 175.9 (1C, $ND\underline{C}(O)CH_3$), 171.0 (1C, C-1), 110.6 (1C, C-2), 79.4 (1C, C-2'), 74.7 (1C, C-6), 73.0 (1C, C-8), 72.7, 72.6 (2C, $O\underline{C}H_2(CH_2)_{12}CH_3$), 71.6 (1C, C-1'), 70.3 (1C, C-4), 69.2 (1C, C-7), 65.0 (1C, C-3'), 64.6 (1C, C-9), 54.0 (1C, C-5), 33.1, 31.1, 30.8, 30.6, 30.5, 27.3, 27.2, 23.7 (24C, $OCH_2(\underline{C}H_2)_{12}CH_3$), 22.6 (1C, $NDC(O)\underline{C}H_3$), 14.4 (2C, $O(CH_2)_{13}\underline{C}H_3$); M.S. (Negative FAB) m/z: 776 (100%, $[M - NH_4^+]^-$).

Ammonium O-[(5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol 1c - Yield 85%; m.p. 137.8-139.2°C ; $[\alpha]_D$ +10.2° (c1.0, methanol); ^{1}H NMR (200 MHz, CD_3OD): δ (ppm) 3.85-2.41 (m, 16H, H-4, 5, 6, 7, 8, 9a, 9b, 1', 2', 3', $OC\underline{H}_2(CH_2)_{12}CH_3$), 2.78 (dd, 1H, $J_{3ax,3eq}$ = 12.2 Hz, $J_{3eq,4}$ = 3.6 Hz, H-3eq), 1.99 (s, 3H, $NDC(O)CH_3$), 1.63 (dd, 1H, $J_{3ax,4}$ = 11.5 Hz, H-3ax), 1.55 (t, 4H, $OCH_2C\underline{H}_2(CH_2)_{11}CH_3$), 1.29 (bs, 44H,

O(CH$_2$)$_2$(C$\underline{H}_2$)$_{11}$CH$_3$), 0.88 (t, 6H, O(CH$_2$)$_{13}$C$\underline{H}_3$); ^{13}C NMR (50.3 MHz, CD$_3$OD): δ (ppm) 175.8 (1C, NDC(O)CH$_3$), 169.9 (1C, C-1), 110.7 (1C, C-2), 79.4 (1C, C-2'), 74.7 (1C, C-6), 73.0 (1C, C-8), 72.7, 72.6 (2C, O$\underline{C}$H$_2$(CH$_2$)$_{12}$CH$_3$), 71.6 (1C, C-1'), 70.3 (1C, C-4), 69.3 (1C, C-7), 64.6 (1C, C-9), 54.1 (1C, C-5), 42.1 (1C, C-3), 33.1, 31.1, 30.9, 30.7, 30.5, 27.4, 27.2, 23.8 (24C, OCH$_2$(C$\underline{H}_2$)$_{12}$CH$_3$), 22.6 (1C, NDC(O)C$\underline{H}_3$), 14.4 (2C, O(CH$_2$)$_{13}$C$\underline{H}_3$); M.S. (Negative FAB) m/z: 775 (100%, [M - NH_4^+]$^-$).

CHAPTER 4 Stereoselective α-Thiosialoside Synthesis under PTC Conditions

4.1 Introduction

Thioglycosides constitute an important family of carbohydrate derivatives. They have recently gained enormous synthetic and biochemical interest due to their intrinsic versatility. They have been recognized as enzyme inhibitors and have thus been used as ligands for affinity chromatography[98]. They are presently used as glycosyl donors in block-oligosaccharide synthesis[99,100]. Their recent popularity is no doubt due to the variety of mild methods available for their activation[64,101].

Recently, various types of important biological functions of sialic acid containing glycoconjugates have been reported by many groups[102-104]. In view of these facts, the synthesis of a variety of gangliosides and their various types of analogs is of critical importance to investigate the structure-function relationship of gangliosides. Furthermore the involvement of sialic acid in a wide number of biological phenomena and their receptor binding properties to human influenza virus hemagglutinin (HA), in particular, have been well documented[24,105-108]. Recent interest for

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106 J.C. Paulson, "The Receptors", Ed. M. Conn, Academic Press, New York, (1985)

107 T.J. Pritchett, R. Brossmer, U. Rose, J.C. Paulson, Virology, **160** (1987) 502

108 N.K. Sauter, M.D. Bednarski, B.A. Wurzburg, J.E. Hanson, G.M. Whitesides, J.J. Skehel, D.C. Wiley, Biochemistry **28** (1989) 8388

the synthesis of multivalent influenza virus HA inhibitors[109-115] together with rational design of suitable α-stereoselective N-acetylneuraminic acid glycosyl donors in blockwise oligosaccharide synthesis have stimulated widespread research activities towards sialic acid containing carbohydrate derivatives[55,71].

The use of thioglycosides as sialyl donors methodology has not escaped the attention of those seeking alternate methods for sialyl glycoside synthesis[116-120]. Several groups have investigated the synthesis of thiosialosides (Scheme 28).

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119 A. Marra and P. Sinaÿ, Carbohydr. Res., **195** (1990) 303.
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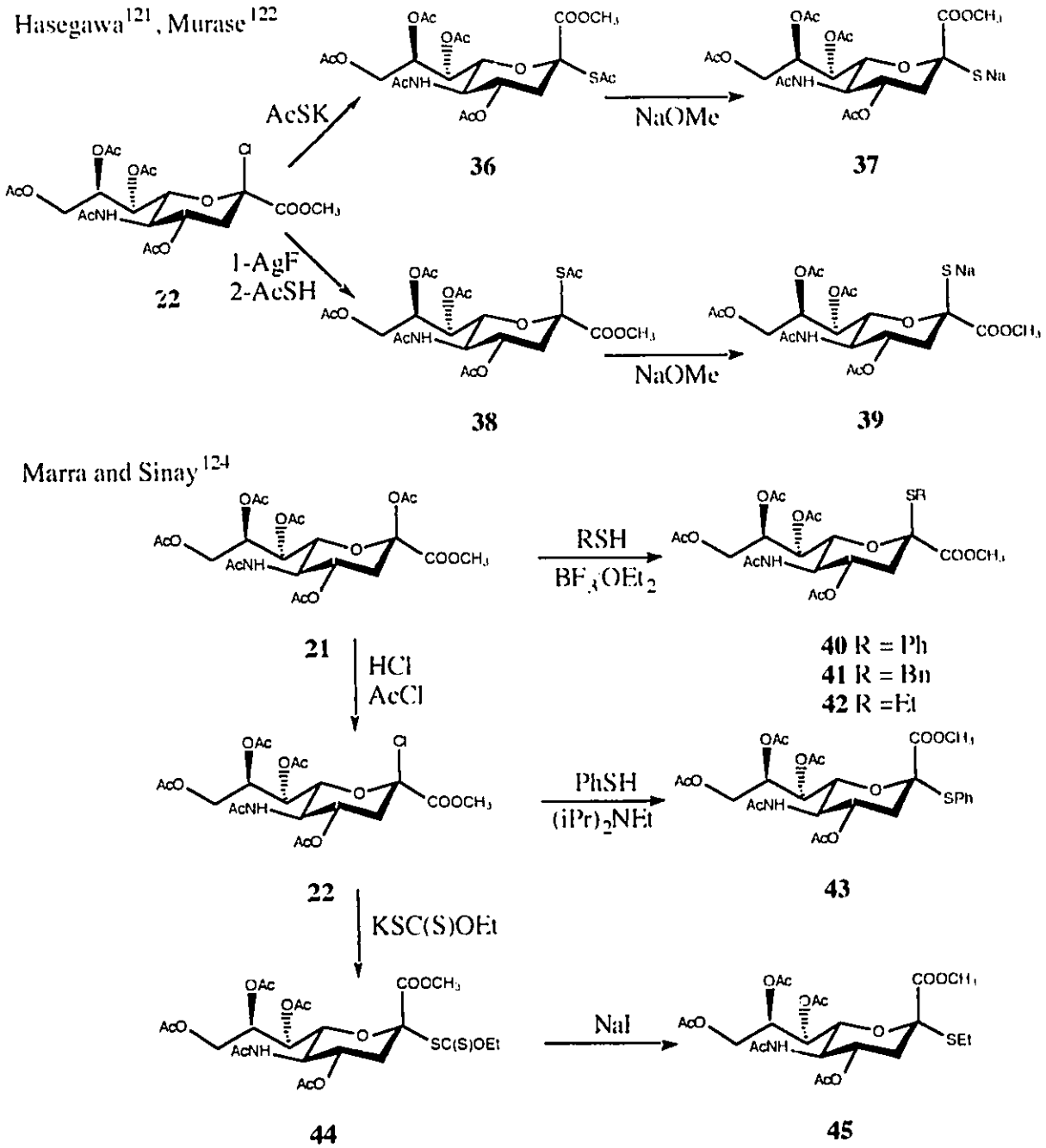

Scheme 28. Previous Syntheses of Thiosialosides

In order to synthesize stereoselectively thioglycosides of N-acetylneuraminic acid, Hasegawa et al.[121] developed a new method which involves the use of the sodium salts of 2α- and 2β-thio-N-acetylneuraminic acids **37** and **39**. Treatment of chloride **22** with potassium thioacetate followed by selective S-deacetylation yielded the sodium salt of 2α-thio-N-acetylneuraminic acid **37**. Subsequent alkylation of that sodium salt furnished α-alkyl glycosides of 2-thio-N-acetylneuraminic acid[121,122]. The 2β-thio-N-acetylneuraminic acid **39** was obtained in an analogous matter with the exception that chloride **22** was treated with silver fluoride prior to thioacetoxylation with thioacetic acid. β-Thiosialosides have also been obtained after alkylation of sodium salt 2β-thiosialoside **39**[123].

Marra and Sinay[124] have also investigated the stereoselective synthesis 2-thio-α and β glycosides of N-acetylneuraminic acid. Treatment of penta-O-acetate **21** with various thiols in the presence of boron trifluoride etherate gave β-thiosialosides **40**, **41** and **42** in 72-84% yields. Phenyl α-thiosialoside **43** was obtained by the treatment of chloride **22** with thiophenol in the presence of a base (65% yield). Under these conditions, ethanediol was unreactive towards chloride **22**, therefore, in order to obtain ethyl α-thiosialoside **45**, chloride **22** was transformed into the α-xanthate **44** which on heating in acetone in the presence of sodium iodide yielded the ethyl α-thiosialoside **45** in nearly quantitative yield.

A facile synthesis of an anomeric mixture of methyl 2-thioglycosides **46** and **47** was developed by Hasegawa et al[125]. The method involved the replacement of the anomeric acetoxy group of **21** with methylthio group by heating with (methylthio)trimethylsilane in the presence of trimethylsilyl trifluoromethane sulfonate. The methyl thioglycosides **46** and **47** were obtained in 96% yield as a 1:1 anomeric mixture.

In order to evaluate the possible extension of thioglycoside methodology and in view of the recent interest in the synthesis of sialic acid-containing oligosaccharides and multivalent influenza virus HA inhibitors and of our efforts in the synthesis of sialyl glycerolipids, the stereoselective synthesis of a series of 2-thiosialosides of N-acetylneuraminic acid has been investigated.

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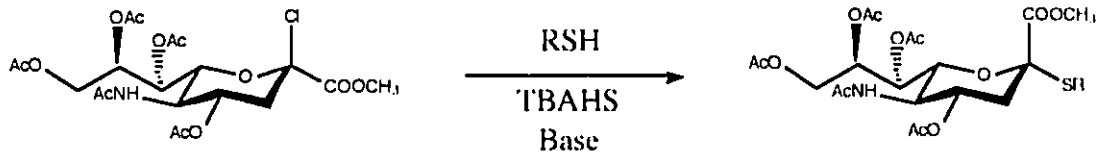
125 A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida and M. Kiso, Carbohydr. Res., **212** (1991) 277.

The recent popularity of the application of phase transfer catalysis to carbohydrate chemistry[126-130] and particularly to the synthesis of thioglycosides[131-135] prompted us to investigate its utility in the synthesis of thiosialosides.

4.2 Discussion

4.2.1 Synthesis of α-Thiosialosides

Phase transfer catalysis was used to synthesize a family of thiosialosides (Scheme 29).



22

43 R = Ph
45 R = Et
46 R = Me
48 R = MePh
49 R = MeOPh
50 R = NO_2Ph
51 R = 2-Py
52 R = 2-(1-MeIm)

Scheme 29. Synthesis of a Series of α-Thiosialosides

Treatment of freshly prepared chloride **22** at room temperature with tetrabutylammonium hydrogen sulfate (1.2 eq) as catalyst in methylene chloride or benzene with thiols (1.2 to 4.5 eq) and a 1M solution of NaOH afforded α-thiosialosides in 63 to 81% yields (crystalline) for the aryl thioglycosides and 23 to 47% yields (crystalline) for the alkyl thioglycosides after purification by silica gel chromatography (Table 11).

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131 J. Bogusiak and W. Szeja, Pol. J. Chem., **59** (1985) 293.
132 F. Chrétien, P. DiCesare and B. Gross, J. Chem. Soc. Perkin Trans. 1, (1988) 3297.
133 R. Roy and F.D. Tropper, Can. J. Chem., **69** (1991) 817.
134 F.D. Tropper, F.O. Andersson, C. Grand-Maître and R. Roy, Synthesis, (1991) 734.
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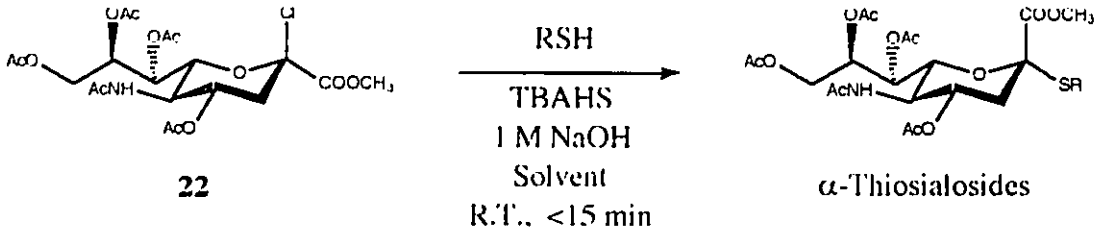
Table 11. Synthesis of α-Thiosialosides from Chloride **22** under PTC Conditions.



22 α-Thiosialosides

Compound #	R	Yields % (a)	Solvent	m.p. °C	$[\alpha]_D$ (c1.0, $CHCl_3$)
43	Ph	74	CH_2Cl_2	143.7-144.5	+23° (lit.[124] +21°)
43	Ph	81	benzene	-	-
45	Et	47	CH_2Cl_2	80.1 (sint'd 65.0-66.2)	+21° (lit.[124] +21°)
46	Me	23	CH_2Cl_2	80.1-81.3 (lit.[121] 80-82)	+51° (lit.[121] +17.8°, c0.5)
48	MePh	70	CH_2Cl_2	114.1-114.7	+24°
49	MeOPh	63	CH_2Cl_2	132.0-133.1	+17°
49	MeOPh	70	benzene	-	-
50	NO_2Ph	68	CH_2Cl_2	173.1-173.3	+35°
51	Py	69	CH_2Cl_2	150.5-152.5	+29°
52	MeIm	68	CH_2Cl_2	140.5-142.5	+28°

(a) Isolated crystalline material.

All of the above reactions were complete within 15 minutes. TLC and ^1H NMR spectroscopy of the crude reaction mixture revealed the absolute stereospecificity of the reaction in favor of α-thiosialosides. The reactions occurred with complete inversion of configuration at the anomeric carbon, indicative of an S_N2 type reaction mechanism.

The only side-product in the synthesis of aryl thiosialosides was the 2,3-dehydro derivative **26** produced by the base catalyzed dehydrochlorination of chloride **22** (5-10% yields). The situation was quite different for the synthesis of alkyl thiosialosides **45** and **46**. In addition to the formation of 2,3-dehydro derivative **26**, hydrolyzed products were detected and deacetylation products were recovered from the aqueous phase. These side-reactions were reflected in lower yields of the alkyl thiosialosides reactions. Furthermore, the synthesis of methyl thiosialoside **46** was complicated by the fact that the thiol necessary to accomplish the reaction, methanethiol, is a gas. Thus to accomplish the reaction, the sodium thiomethoxide salt was used instead of the thiol. The inevitable loss of thiomethoxide as methanethiol gas was compensated by the incremental addition of sodium thiomethoxide during the reaction. In spite of all the difficulties hindering the synthesis of methyl thioglycoside **46**, a yield of 23% was still obtained.

Generally, the organic solvent used as lipophilic phase was methylene chloride but in control experiments, products resulting from displacement of one or two of the chloride anions from methylene chloride were detected. The use of solvents more stable to reaction conditions was recommended to avoid consumption of the nucleophiles. Indeed, the use of benzene as lipophilic phase resulted in a slight improvement of the yields of the reactions (7%). Ethyl acetate has also been advantageously used[136].

The base generally used in the phase transfer catalyzed reaction was 1M NaOH but 1M sodium bicarbonate or saturated sodium hydrogen carbonate were found to be as efficient.

The α-thiosialosides **43**, **45** and **46** are known compounds[122,124] and for these, the physical data were in good agreement with reported values (Table 11). The family of α thiosialosides synthesized was fully characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry and chemical analysis. The ^{1}H NMR spectroscopic data for the series of α-thiosialosides is summarized in Table 12.

136 F.D. Tropper, F.O. Andersson, S. Cao and R. Roy, J. Carbohydr. Chem., 11 (1992) 741.

Table 12. ^{1}H NMR Spectroscopic Data for the Series of α-Thiosialosides

Compound	**45**	**46**	**46** lit.[122]
Protons	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	1.96 (12.8, 11.9)	1.97 (12.7, 11.9)	- (12.8, -)
H-3eq ($J_{3eq,4}$)	2.70 (4.7)	2.71 (4.8)	2.73 (4.8)
H-4 ($J_{4,5}$)	4.84 (10.4)	4.86 (10.4)	4.88 (10.2,)
H-5 ($J_{5,6}$, $J_{NH,5}$)	4.03 (10.7, 10.3)	4.04 (10.7, 10.3)	4.08 (10.2, 10.2)
H-6 ($J_{6,7}$)	3.81 (2.1)	3.80 (2.2)	3.84 (1.8)
H-7 ($J_{7,8}$)	5.30 (8.3)	5.31 (8.5)	5.31 (8.4)
H-8 ($J_{8,9a}$, $J_{8,9b}$)	5.36 (2.6, 4.9)	5.37 (2.7, 5.0)	5.38 (2.4, 5.1)
H-9a ($J_{9a,9b}$)	4.29 (12.4)	4.29 (12.4)	4.33 (11.7)
H-9b	4.09	4.08	4.11
N<u>H</u>	5.13	5.13	5.18
OC<u>H</u>$_3$	3.78	3.79	3.81
C<u>H</u>$_3$C(O)NH C<u>H</u>$_3$C(O)O	2.15, 2.12 2.02, 2.01 1.86	2.15, 2.12 2.02, 2.01 1.86	2.17, 2.14 2.04, 2.03 1.88
C<u>H</u>$_2$CH$_3$ ($J_{1',2'}$)	2.76 (7.5)		
C<u>H</u>$_3$	1.17	2.09	2.11

Table 12. (Cont'd)

Compound	43	48	49	50
Protons	δ (ppm) J (Hz)	δ (ppm) J (Hz)	δ (ppm) J (Hz)	δ (ppm) J (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	2.00 (12.8, 11.7)	2.02 (12.9, 11.8)	2.01 (12.8, 11.7)	2.08 (12.8, 11.9)
H-3eq ($J_{3eq,4}$)	2.80 (4.7)	2.76 (4.6)	2.76 (4.6)	2.85 (4.7)
H-4 ($J_{4,5}$)	4.82 (10.1)	4.81 (10.0)	4.81 (10.2)	4.86 (9.9)
H-5 ($J_{5,6}$, $J_{NH,5}$)	3.97 (10.7, 9.8)	3.95 (10.7, 9.3)	3.95 (10.9, 9.8)	3.99 (10.3, 9.5)
H-6 ($J_{6,7}$)	387 (1.6)	3.85 (1.6)	3.83 (1.8)	4.10 (2.2)
H-7 ($J_{7,8}$)	5.28 (7.3)	5.27 (8.1)	5.27 (6.8)	5.30 -
H-8 ($J_{8,9a}$, $J_{8,9b}$)	5.25 (2.2, 5.1)	5.25 (2.4, 5.1)	5.22 (2.6, 5.5)	5.27 (1.2, 2.1)
H-9a ($J_{9a,9b}$)	4.37 (12.5)	4.39 (12.4)	4.37 (12.3)	4.30 (12.5)
H-9b	4.18	4.19	4.17	4.06
N<u>H</u>	5.10	5.08	5.15	5.14
O<u>CH</u>$_3$	3.55	3.58	3.59	3.59
<u>CH</u>$_3$C(O)NH <u>CH</u>$_3$C(O)O	2.12, 2.04 2.02, 2.00 1.84	2.12, 2.04 2.04, 2.00 1.84	2.12, 2.04 2.02, 2.00 1.84	2.14, 2.04 2.04, 2.02 1.87
H-2' ($J_{2',3'}$)	7.49 (6.7)	7.12 (7.9)	6.84 (8.8)	7.63 (9.0)
H-3' ($J_{3',4'}$)	7.32 (7.2)	7.37	7.40	8.17
H-4'	7.36			
<u>CH</u>$_3$		2.34	3.81	

Table 12. (Cont'd)

Compound	51	52
Protons	δ (ppm) **J** (Hz)	δ (ppm) **J** (Hz)
H-3ax ($J_{3ax,3eq}$, $J_{3ax,4}$)	2.18 (12.8, 11.8)	2.17 (13.0, 11.9)
H-3eq ($J_{3eq,4}$)	2.86 (4.7)	2.85 (4.7)
H-4 ($J_{4,5}$)	4.88 (9.9)	4.82 (9.9)
H-5 ($J_{5,6}$, $J_{NH,5}$)	4.03 (10.7, 9.6)	3.87 (9.7, 9.8)
H-6 ($J_{6,7}$)	4.10 (1.8)	3.82 (1.5)
H-7 ($J_{7,8}$)	5.29 (7.9)	5.19 (9.9)
H-8 ($J_{8,9a}$, $J_{8,9b}$)	5.21 (2.8, 5.2)	5.16 (2.3, 5.8)
H-9a ($J_{9a,9b}$)	4.27 (12.5)	4.23 (12.3)
H-9b	4.09	3.99
N<u>H</u>	5.15	5.12
OC'<u>H</u>$_3$	3.65	3.75
C'<u>H</u>$_3$C(O)NH C'<u>H</u>$_3$C(O)O	2.11, 2.05 2.02, 2.01 1.86	2.09, 2.04 2.01, 2.01 1.85
H-3' ($J_{3',4'}$, $J_{3',5'}$, $J_{3',6'}$)	7.54 (7.4, 1.1, 0.8)	
H-4' ($J_{4',5'}$, $J_{4',6'}$)	7.66 (7.9, 1.9)	7.12 (1.2)
H-5' ($J_{5',6'}$)	7.18 (4.8)	7.10
H-6'	8.46	
C'<u>H</u>$_3$		3.82

The determination of the configuration of the sialosides was accomplished according to well established empirical rules. The assignment of anomeric configurations of sialosides is usually performed by a comparison of selected features in the ^{1}H NMR spectra of the α- and β-sialosides. Because of the absolute stereoselectivity of the PTC reaction, no β-anomers were available to perform this comparison. Thus for the purpose of comparison the phenyl β-thio-sialoside **40** was synthesized according to Marra et al.[124] in 71% yield. The physical data for the β-thiosialoside **40** was in good agreement with reported values[124]. The result of the application of the empirical rules to the determination of the anomeric configuration of phenyl α-thioglycoside **43** is summarized in Table 13.

Table 13. Determination of Anomeric Configuration of Phenyl Thioglycosides **30** and **31.**

Rule #	α anomer **43**		β anomer **40**
1[83]	H-3eq	>	H-3eq
	2.96 ppm	>	2.83 ppm
2[76,84]	H-4	<	H-4
	4.87 ppm	<	5.35 ppm
3[85-91]	$J_{7,8}$	>	$J_{7,8}$
	6.7 Hz	>	2.0 Hz
4[85-91]	|H-9a - H-9b|	<	|H-9a - H-9b|
	(4.73-4.44) ppm		(5.03-4.38) ppm
	0.29 ppm	<	0.64 ppm

The configuration of the other members of the family of thiosialosides synthesized was inferred to be α by comparison with the assignment of the α and β phenyl thiosialosides.

Phase transfer catalysis has proved to be a highly stereoselective and practical method for the synthesis of thiosialosides. It has the advantages of being a completely stereoselective one-step entry to useful α-thiosialosides. In addition to being potential glycosyl donors, these compounds are of biological interest, either as competitive inhibitors of neuraminidase[126] or as affinity probes of sialidases and sialic acid binding proteins[123], presumably because of the resistance of the thio-α-glycosidic linkage to enzyme degradation.

The success of the phase transfer catalysis methodology for the synthesis of α-thiosialosides prompted the investigation of the applicability of the method to the synthesis of aryl, heteroaryl and phosphotriester sialosides.

4.2.2 Applications of Phase Transfer Catalysis

The versatility of the PTC methodology, originally developed for the synthesis of thiosialosides, was demonstrated by its application to the synthesis of neoglycoprotein precursors, fluorogenic substrates and sialyl phosphotriester.

4.2.2.1 Synthesis of p-Formylphenyl Sialoside, Neoglycoprotein Precursor.

Artificial carbohydrate protein conjugates, so-called neoglycoproteins, continue to attract the attention of biochemists and immunochemists because of the large number of events in which they are implicated. Among these, fundamental aspect of binding, such as the evaluation of threshold sugar densities on glycoprotein for the expression of antigenicities are of special interest. In order to evaluate the implication of the carbohydrate content on the antigenicity of model neoglycoprotein, simple glycoconjugates can be used in serological studies.

The preparation of neoglycoproteins has been previously reviewed[137,138]. Among the various methodologies, p-aminophenyl glycosides[139] and alditol derivatives[140,141] find widespread applications in spite of evident drawbacks. For instance, the diazotization reaction required noxious reagents and is not chemoselective, while the isothiocyanate method requires labile thiophosgene reagent. Recently, reductive amination[142] with both reducing sugars[143] and aglycons containing terminal aldehyde functionalities[109,144] has been shown to be highly efficient. Therefore it was realized that the synthesis of p-formylphenyl sialosides would provide an important precursor for the expedient synthesis of sialylated neoglycoproteins.

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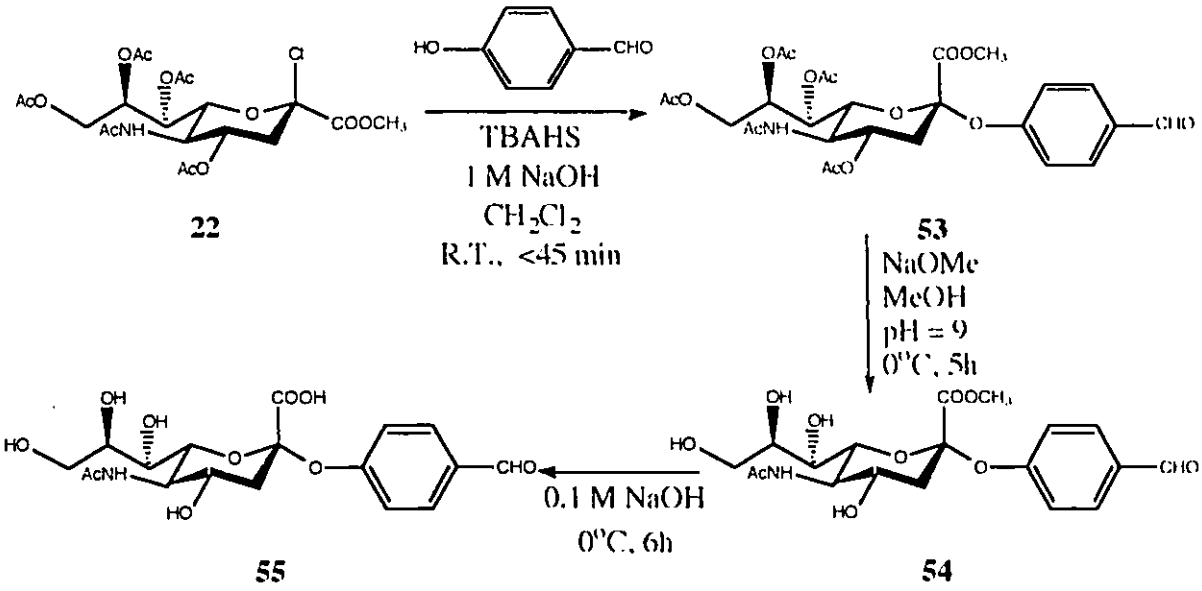
The p-formylphenyl sialoside was synthesized stereospecifically under phase transfer catalysis followed by two deprotection steps according to Scheme 30.



Scheme 30. Synthesis of p-Formyl Sialoside **55**

Thus, treatment of chloride **22** with p-hydroxybenzaldehyde and tetrabutylammonium hydrogen sulfate (TBAHS) as phase transfer catalyst at room temperature yielded the aldehyde **53** in 65% yield after silica gel chromatography. ^{1}H NMR of the crude reaction product revealed the stereoselectivity of the reaction since it was determined that the α-ketoside was the only glycosidic product obtained. The only by-product of the reaction was the 2,3-dehydro derivative **26** which was obtained in 10-15% yield. Aldehyde **53** was obtained as an oil which was recrystallized from benzene/hexanes to give white crystals (m.p. 90.4-91.6°C, $[\alpha]_D$ + 32.4 ° (c1.0, chloroform)).

Aldehyde **53** was characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry. The ^{1}H NMR (300 MHz, CDCl3) spectrum of aldehyde **53** displayed a characteristic aldehyde proton signal at δ = 9.90 ppm, and aromatic proton signals at δ = 7.81 and 7.15 ppm, confirming the synthesis of the p-formylphenyl α-sialoside **53**.

Zemplén de-O-acetylation of aldehyde **53** furnished the deacetylated aldehyde **54** (m.p. 109.2-110.1°C, $[\alpha]_D$ + 56.3° (c1.0, water)) which, after saponification afforded the free aldehyde **55** (m.p. 180-184°C (dec.), $[\alpha]_D$ + 72.4° (c1.0, water)) in 84% yield.

Direct reductive animation of the p-formyl-phenyl sialoside **55** onto bovine serum albumin (BSA) in various molar ratios was accomplished by Roy et al.[145] and gave a series of sialylated neoglycoproteins having a wide distribution of sugar contents with different levels of antigenic expression.

In conclusion, phase transfer catalysis represents an expeditious route towards the synthesis of sialosides having reactive aldehyde functionalities which can be directly coupled to proteins by reductive animation.

4.2.2.2 Synthesis of the Fluorogenic Substrate 2'-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid.

Sialidosis is a hereditary liposomal storage disease transmitted as an autosomal recessive trait resulting from a profound deficiency of N-acetylneuraminidase[146-151]. Near absence of neuraminidase activity accounts for storage in tissues and urinary excretion of massive amounts of sialyl oligosaccharides[146,147,149,152-154].

In addition to its involvement in sialidosis, human neuraminidases are of great interest because of their potential involvement in ganglioside metabolism[155,156], mammalian reproduction, blood cloth formation, hormone-membrane interactions and neurotransmission[157,158], their elevated levels in viral transformed cells[159] and the presence of naturally occurring antibodies against desialated human lymphocytes[160]. Detection and assays of human neuraminidase activity

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have been difficult due to the low levels of enzymatic activity and the lack of a suitable chromophoric substrate whose cleavage product can be sensitively detected with low background values.

Most assays currently employed are based on the determination of liberated sialic acids or of radiolabeled N-acetylneuraminic acids from oligosaccharides or glycoprotein substrates or on the determination of liberated aglycons and require long incubation times or are extremely involved and complex[161,162]. Assays based on the liberation of sialic acids include for example the colorimetric methods of Warren[169] and Aminoff[170] which do not always give reliable results with crude enzyme preparations wherein the free sialic acids must be separated from interfering substances by time consuming chromatographic methods prior to colorimetric determination[171] Neuraminidase assay methods based on the determination of radiolabeled N-acetylneuraminic acid were developed by Tallman et al.[172] Bhavanandan et al.[173] and Schraven et al.[174] who used radiolabels in the N-acetylneuraminic acid moiety of gangliosides, α-D-N-acetylneuraminosyl (2→3)lactit(^{3}H)ol and ^{3}H-gangliosides G_{D1a} respectively. Although sensitive, these methods involved chromatographic separations of the labeled products from unhydrolyzed substrate. Simpler assay procedures based on the determination of a liberated aglycon used phenyl-[72], 3 methoxyphenyl-[175] and p-nitrophenyl-[176] ketosides of N-acetylneuraminic acid as substrates. The liberated phenol or 3-methoxyphenol were determined colorimetrically with the Folin-Ciocalteu reagent and p-nitrophenol by alkanization of the incubation medium. For these reasons, the diagnosis of sialidosis and the detection of carriers of the trait have been difficult. Hence, a more rapid and quantitative measure of N-acetylneuraminidase activity was warranted.

Thomas et al[177] recognizing the higher sensitivity of fluorometric over colorimetric substrates, designed and synthesized such a substitute. The fluorogenic substrate designed was 2'-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid (Figure XIV) because the product of neuraminidase hydrolysis is the highly fluorescent 4-methyl-umbelliferone (7-hydroxy 4-methyl coumarin).

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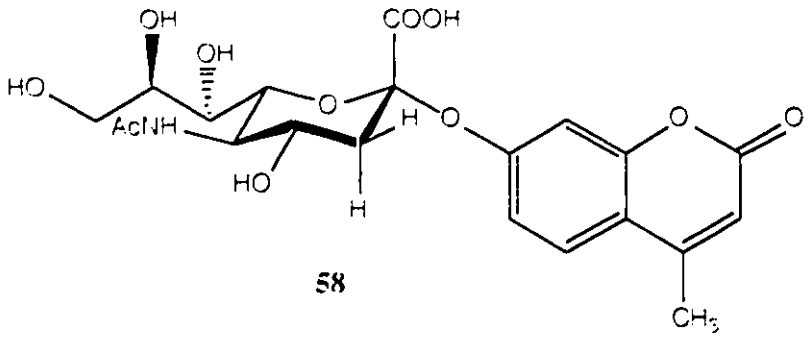

58

Figure XIV. Fluorogenic Substrate 2'-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid **58**

The synthetic procedure of Thomas[177] et al. involved the reaction of sialyl chloride **22** with 7-hydroxy-4-methylcoumarin in the presence of cadmium carbonate as the key coupling-step. Further investigations of this method revealed that yields were very dependent on batch variations in the catalyst[178]. Thus, in order to improve the yield of the reaction, the promoter was changed to silver carbonate[179], the 4-methyl-umbelliferone was transformed into its sodium[180] or tetrabutyl ammonium[57] salt or DMF was used as reaction solvent[181]. In spite of the various procedures developed for the synthesis of fluorogenic substrate **58**, the overall yields were very low (37-54%) and the lack of stereoselectivity of the key coupling step gave rise to difficult anomeric mixture separations.

In view of the importance of the fluorogenic substrate **58** and the low yields obtained in the available literature procedures, it became apparent that a more efficient and reliable synthetic procedure was required. This necessity provided us with the opportunity to demonstrate the versatility of phase transfer catalysis originally developed for the synthesis of α-thiosialosides.

Thus, phase transfer catalysis was used to perform the key coupling-step in the synthesis of the 4-umbelliferyl α-ketoside **58**. The formation of the α-sialoside was followed by Zemplén deacetylation and saponification (Scheme 31).

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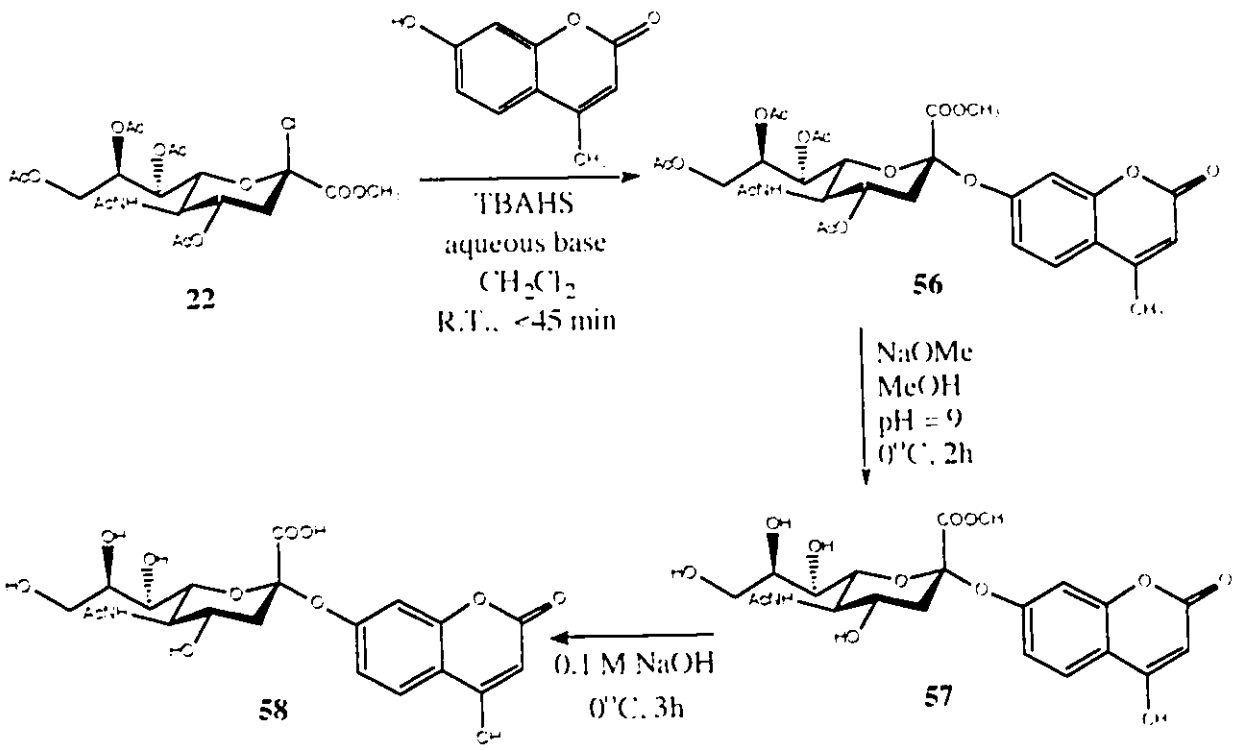

Scheme 31. Synthesis of Fluorogenic Substrate 2'-(4-Methylumbelliferyl)-α-D N-acetylneuraminic Acid **58**.

Treatment of freshly prepared chloride **22** with 7-hydroxy-4-methylcoumarin under catalytic two phase system using methylene chloride and either 1M NaOH or saturated sodium bicarbonate and the tetrabutylammonium hydrogen sulfate afforded the 4-methylcoumarin-7-yl α sialoside **56** in 78% (NaOH) or 85% ($NaHCO_3$) yields. The reaction, performed at room temperature, occurred with complete stereocontrol by nucleophilic displacement with inversion of configuration. No β-sialoside could be detected from the crude reaction mixtures by TLC or ^1H NMR spectroscopy. Again the only side-product obtained was a trace of the 2,3 dehydro-derivative **26** (yield 5%). The 4-methylcoumarin-7-yl α-sialoside **56** was obtained as a clear oil after silica gel chromatography which was recrystallized from benzene to yield white crystals (m.p. 108.6-110.8°C (100°C sint'd), $[\alpha]_D$ +51° (c1.0, chloroform, lit.[57] 98-100°C, $[\alpha]_D$ +52° (c1.0, chloroform)). The physical and spectroscopic data for the known 4-methyl-coumarin-7-yl α-sialoside **56** are in full agreement with reported values[57].

The PTC reaction used to perform the key coupling step in the synthesis of the fluorogenic substrate **58** is noteworthy for its stereoselectivity and its high yield. It is much more efficient than

the previous procedure which involved the use of expensive Koenigs-Knorr catalysts, gave difficult to separate anomeric mixtures and low yields.

The synthesis of fluorogenic substrate **58** was completed by two deprotection steps. Thus, Zemplén deacetylation of the 4-methyl-coumarin-7-yl α-sialoside **56** performed et 0°C yielded the deacetylated methyl ester **57** in 95% yield. The product was obtained as a solid which was recrystallized from methanol/ether to give white crystals of methyl ester **57**.

The saponification of methyl ester **57** proceeded in 0.1M NaOH at 0°C. The fluorogenic substrate **58** was obtained in 95% yield as an amorphous powder. The physical and spectroscopic data for the fluorogenic substrate **58** are in good agreement with reported values[57].

Phase transfer catalysis was found to be very efficient for the synthesis of the fluorogenic substrate 2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid **58**. The key coupling reaction occurred with complete stereoselectivity to give high yields of the α-sialoside. The overall yield for the procedure was 71%, a noteworthy improvement over previous results (37-54%). Furthermore, the stereoselectivity of the PTC reaction rendered the purification of the α-sialoside very easy since difficult anomeric mixture separations were avoided.

4.2.2.3 Synthesis of Sialyl Phosphotriester

Carbohydrate phosphates are important intermediates in a wide variety of biosynthetic processes[182]. They constitute integral components of the core and lipid A region of lipopolysaccharides, sugar nucleotides, teichoic acids, bacterial polysaccharides, glycophospholipids and phosphoinositides.

Previous methods for the synthesis of glycosyl 1-phosphates are essentially based on two fundamental concepts. In the first one, phosphoric acids, their salts or dialkyl phosphates were used as nucleophiles on glycose peracetates[183,184], 1,2-orthoesters[185,186], halogenoses[187,188,189],

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[190], 1,2-oxazolines[191], Brigl anhydrides[192] or on trichloroacetimidates[193,194]. In the second approach, the anomeric hydroxyl groups were activated as lithium[195] or thallium[196] salts and reacted with electrophiles dialkyl phosphochloridates. More recently, the phosphoramidite and H-phosphonate methods have also attracted interest[197]. These methods and the occurrences of carbohydrate phosphates have been reviewed[198].

The development of the phase transfer catalysis for the synthesis of α-thiosialosides, p-formylphenyl- and 4-methylumbelliferyl-α-sialosides stimulated us to attempt the application of this methodology to the synthesis of anomeric α-sialoside phosphate. It was anticipated that dibenzylphosphate would be a potent nucleophile under a catalytic two-phase system. This procedure would give access to a sialic acid phosphotriester which in turn would be a practical precursor of complex polysialyl phospholipids such as the α-(2→9)-linked polysialyl phospholipid surface antigen of the group C meningococcal polysaccharide[199] (Figure XV).

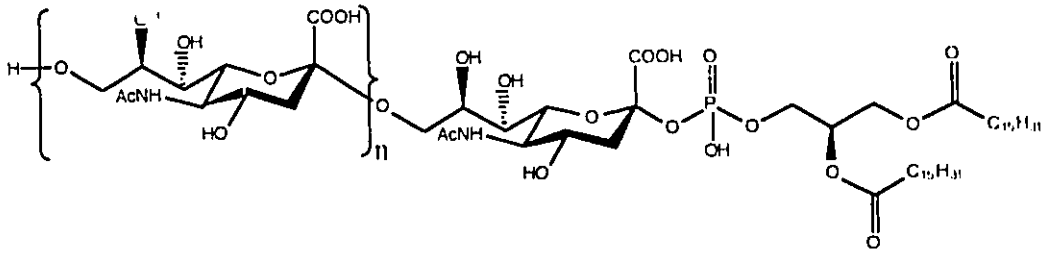

Figure XV. Polysialyl Phospholipid Surface Antigen of the Group C Meningococcal Polysaccharide

Chloride 22 was subjected to phase transfer catalysis reaction conditions according to Scheme 32.

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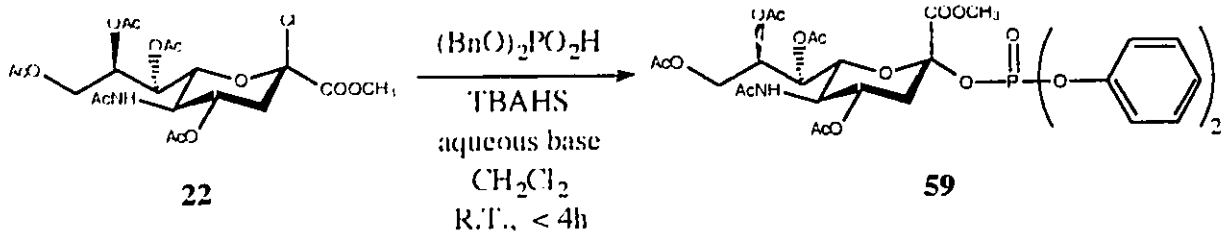

22 **59**

Scheme 32. Synthesis of Sialyl Phosphotriester **59**

Treatment of β-chloride **22** in methylene chloride and either 1M NaOH or saturated sodium hydrogen carbonate with dibenzyl phosphate (2eq) and tetrabutylammonium hydrogen sulfate (1eq) at room temperature afforded crude α-sialyl phosphotriester **59**. By TLC and ^{1}H NMR, spectroscopy (200 MHz, $CDCl_3$), no β-phosphotriester was detected. The 2,3-dehydro derivative **26** was the only side-product of the reaction. From the ^{1}H NMR spectrum of the crude reaction mixture, it was possible to determine that the ratio of phosphotriester to 2,3-dehydro derivative **26** was 3:1. Unfortunately, purification of the crude product was not possible. Various attempts at the chromatographic separation of the reaction mixture yielded either tetra-O-acetate **23** when ethyl acetate was used as eluent or the 2,3-dehydro-derivative **26** when a dilute solution of triethylamine in ethyl acetate was used as eluent. Purification by crystallization was also attempted but was equally unsuccessful. Although the procedure did yield the α-sialyl phosphotriester **59**, the apparent good leaving group ability of the phosphate aglycon prevented an adequate purification of the desired product.

Despite the lack of purity of the α-sialyl phosphotriester, the ^{1}H NMR spectroscopic data for that product was obtained from the spectrum of the crude reaction mixture. The ^{1}H NMR spectra displayed characteristic signals for the aromatic benzyl protons at 7.27 ppm and the methylene protons of the benzyl groups appeared as doublets at 5.03 and 4.97 ppm. Characteristic signals for the H-3eq and H-3ax protons were found respectively at δ = 2.54 ppm ($J_{3ax,3eq}$ = 12.9 Hz, $J_{3eq,4}$ = 4.7 Hz) and 2.13 ppm ($J_{3ax,4}$ = 11.9 Hz).

In spite of the difficulty of purification, it was possible to determine that the PTC procedure was successfully used to produce the α-sialyl phosphotriester **59**. Isolation of the pure phosphotriester was prevented by purification problems needing further investigation.

4.3 Conclusion

In conclusion, a mild, highly stereoselective and practical entry into useful α-thiosialosides has been performed under phase transfer catalysis. In addition to being potential sialyl donors useful in blockwise oligosaccharide synthesis, the α-thiosialosides are of biological interest, either as competitive inhibitors of neuraminidase[126] or as affinity probes of sialidase and sialic acid binding proteins[123]. Thus the α-thiosialosides have a dual role as they could be used as glycosylating reagents and they could act as sialidase-stable human influenza virus HA inhibitors[200].

The versatility of phase transfer catalysis has been convincingly demonstrated through the synthesis of important aryl and heteroaryl sialosides and of a sialyl phosphotriester derivative. p-Formylphenyl sialoside, a precursor to sialylated neoglycoproteins was efficiently synthesized. The synthesis of the fluorogenic substrate 2'-(4-methylumbelliferyl)-α-D-N-acetylneuramine acid was successfully accomplished using the phase transfer methodology and constitutes a significant improvement over the previously used synthetic methods. The phase transfer catalysis was also applied to the synthesis of a sialyl phosphotriester which can be a useful precursor for the synthesis of complex polysialyl phospholipids.

The evaluation of the α-thiosialosides as thioglycosyl donors will be presented in Chapter 5.

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4.4 Experimental

4.4.0 General Methods

See Section **1.4.0** in Chapter 1.

4.4.1 Synthesis of Methyl (Alkyl or Aryl 5-Acetamido-4,7,5,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate Derivatives.

General Phase Transfer Catalysis Method

To a stirred solution of a thiol (# of equivalent used, see specific compound) and tetrabutylammonium hydrogen sulfate (126 mg, 0.37 mmol, 1.2 eq) in an aqueous base (1M NaOH, 1M sodium carbonate or saturated sodium bicarbonate, 5 ml) was added a freshly prepared solution of chloride **22** (158 mg, 0.31 mol) in dry methylene chloride or benzene (5 ml). The reaction mixture was stirred at room temperature, until complete transformation of the starting material was observed by TLC (eluent : 2 x 100 % ethyl acetate), was subsequently diluted with ethyl acetate (10 ml) and saturated sodium bicarbonate (10 ml) and the phases were separated. The organic layer was washed with saturated hydrogen carbonate (2 x 10 ml) and brine (10 ml), dried over anh. sodium sulfate and evaporated in vacuo to dryness. The oil obtained was purified by radial chromatography on a Chromatotron (1 mm silica gel plate) using pure ethyl acetate as eluent.

Methyl (Phenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 43 - The reaction was performed using 1.3 eq of the thiol (41µl, 44 mg, 0.40 mmol). The 2-thioglycoside obtained was crystallized from ether/benzene. Yield 74% (133 mg, NaOH, CH_2Cl_2) or yield 81% (147 mg, NaOH, benzene); m.p. 143.7-144.5°C; $[\alpha]_D$ + 23° (c1.0, chloroform); lit.[124] m.p. 139-140°C; $[\alpha]_D$ + 21° (c1.0, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.49 (d, 2H, $J_{2',3'}$ = 6.7 Hz, H-meta), 7.36 (t, 1H, $J_{3',4'}$ = 7.2 Hz, H-para), 7.32 (dd, 2H, H-ortho), 5.28 (dd, 1H, $J_{6,7}$ = 1.6 Hz, $J_{7,8}$ = 7.3 Hz, H-7), 5.25 (ddd, 1H, $J_{8,9a}$ = 2.2 Hz, $J_{8,9b}$ = 5.1 Hz, H-8), 5.10 (d, 1H, $J_{NH,5}$ = 9.8 Hz, NH), 4.82 (ddd, 1H, $J_{3ax,4}$ = 11.7 Hz, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 10.1 Hz, H-4), 4.37 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, H-9), 4.18 (dd, 1H, H-9b), 3.97 (ddd, 1H, $J_{5,6}$ = 10.7 Hz, H-5), 3.87 (dd, 1H, H-6), 3.55 (s, 3H, OMe), 2.80 (dd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, H-3ax), 2.00 (dd, 1H, H-3ax), 2.12, 2.04, 2.02, 2.00, 1.84 (s, 15H, $C(O)CH_3$); ^{1}H NMR (200 MHz, C_6D_6); δ (ppm) 7.61 (dd, 2H, $J_{2',3'}$ = 7.6 Hz, $J_{2',4'}$ = 1.5 Hz, H-2'), 7.11 (dd, 1H, $J_{3',4'}$ = 6.7 Hz, H-4'), 7.02

(dd, 2H, H-3'), 5.61 (ddd, 1H, $J_{7,8}$ = 6.7 Hz, $J_{8,9a}$ = 2.6 Hz, $J_{8,9b}$ = 5.8 Hz, H-8), 5.53 (dd, 1H, $J_{6,7}$ = 2.0 Hz, H-7), 5.08 (d, 1H, $J_{NH,5}$ = 10.4 Hz, NH), 4.87 (ddd, 1H, $J_{3ax,4}$ = 11.4 Hz, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 10.5 Hz, H-4), 4.73 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.44 (dd, 1H, H-9b), 4.35 (ddd, 1H, $J_{5,6}$ = 10.7 Hz, H-5), 4.01 (dd, 1H, H-6), 3.25 (s, 3H, OMe), 2.96 (dd, 1H, $J_{3ax,3eq}$ = 12.7 Hz, H-3eq), 2.03 (dd, 1H, H-3ax), 1.97, 1.95, 1.77, 1.67, 1.62 (s, 15H, $CH_3C(O)$); lit.[124] ^1H NMR (400 MHz, C_6D_6): δ (ppm) 7.64, 7.16, 7.07 (3m, 5H, Ph), 5.67 (ddd, 1H, $J_{7,8}$ = 7.0 Hz, $J_{8,9a}$ = 2.7 Hz, $J_{8,9b}$ = 5.8 Hz, H-8), 5.51 (dd, 1H, $J_{6,7}$ = 2.2 Hz, H-7), 4.81 (ddd, 1H, $J_{3ax,4}$ = 12.0 Hz, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 10.5 Hz, H-4), 4.77 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, H-9a), 4.46 (dd, 1H, H-9b), 4.35 (ddd, 1H, $J_{5,6}$ = 10.7 Hz, $J_{5,NH}$ = 10.5 Hz, H-5), 4.10 (d, 1H, NH), 3.94 (dd, 1H, H-6), 3.21 (s, 3H, OMe), 2.98 (dd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, H-3eq), 2.03 (dd, 1H, H-3ax), 1.96, 1.94, 1.79, 1.57, 1.56 (s, 15H, 5 $CH_3C(O)$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.1, 170.8, 170.3, 170.2, 170.2 (5C, $CH_3\underline{C}(O)$), 168.0 (1C, C-1), 136.5 (2C, C-meta), 129.9 (2C, C-ortho), 128.9 (1C, C ipso), 128.3 (1C, C-para), 87.4 (1C, C-2), 74.6 (1C, C-6), 69.8 (1C, C-8), 69.6 (1C, C-4), 67.6 (1C, C-7), 61.9 (1C, C-9), 52.6 (1C, CH_3O), 49.1 (1C, C-5), 38.0 (1C, C-3), 23.0, 20.8, 20.6, 20.6, 20.6 (5C, $\underline{C}H_3C(O)$); M.S. (C.I., ether) m/z: 584 (39%, $[M + H]^+$), 524 (26%, $[M - OAc]^+$), 474 (14%, $[M - SPh]^+$); Anal. calcd for $C_{26}H_{33}NO_{12}S$: C, 53.51; H, 5.70; N, 2.44; S, 5.49; Found: C, 53.74; H, 5.83; N, 2.40; S, 5.66.

Methyl (Ethyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 45 - The reaction was performed using 1.3 eq of the thiol (30µl, 24 mg, 0.40 mmol). The 2-thioglycoside obtained was crystallized from benzene/hexanes. Yield 47% (78 mg, NaOH, CH_2Cl_2); m.p. 80.1°C (sint'd 65.0-66.2°C) ; $[\alpha]_D$ +21° (c1.0, chloroform); lit.[124] m.p. 80° (softening at 65°C); $[\alpha]_D$ +21° (c1.0, chloroform); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.36 (ddd, 1H, $J_{7,8}$ = 8.3 Hz, $J_{8,9a}$ = 2.6 Hz, $J_{8,9b}$ = 4.9 Hz, H-8), 5.30 (dd, 1H, $J_{6,7}$ = 2.1 Hz, H-7), 5.13 (d, 1H, $J_{NH,5}$ = 10.3 Hz, NH), 4.84 (ddd, 1H, $J_{3ax,4}$ = 11.9 Hz, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 10.4 Hz, H-4), 4.29 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.09 (dd, 1H, H-9b), 4.03 (ddd, 1H, $J_{5,6}$ = 10.7 Hz, H-5), 3.81 (dd, 1H, H-6), 3.78 (s, 3H, OCH_3), 2.76 (q, 2H, J = 7.5 Hz, $SC\underline{H}_2CH_3$), 2.70 (dd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, H-3eq), 2.15, 2.12, 2.02, 2.01, 1.86 (s, 15H, $CH_3C(O)$), 1.96 (dd, 1H, H-3ax), 1.17 (t, 2H, $SCH_2C\underline{H}_3$); lit.[124] (400 MHz, C_6D_6): δ (ppm) 5.81 (ddd, 1H, $J_{7,8}$ = 8.2 Hz, $J_{8,9a}$ = 2.7 Hz, $J_{8,9b}$ = 5.7 Hz, H-8), 5.48 (dd, 1H, $J_{6,7}$ = 2.4 Hz, H-7), 4.81 (ddd, 1H, $J_{3ax,4}$ = 11.6 Hz, $J_{3eq,4}$ = 4.6 Hz, $J_{4,5}$ = 10.7 Hz, H-4), 4.68 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, H-9a), 4.39 (ddd, 1H, $J_{5,6}$ = 10.8 Hz, $J_{NH,5}$ = 10.4 Hz, H-5), 4.32 (dd, 1H, H-9b), 4.00 (d, 1H, NH), 3.83 (dd, 1H, H-6), 3.37 (s, 3H, OMe), 2.99, 2.70, (2m, 2H, $SC\underline{H}_2CH_3$), 2.83 (dd, 1H, $J_{3ax,3eq}$ = 12.6 Hz, H-3eq), 2.01 (dd, 1H, H-3ax), 2.16, 1.90, 1.77, 1.59, 1.58 (5s, 15H, $5CH_3C(O)$), 1.12 (t, 3H, J

= 7.5 Hz, $SCH_2\underline{C}H_3$); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 171.1, 170.8, 170.3, 170.3, 170.3 (5C, $CH_3C(O)$), 168.6 (1C, C-1), 83.2 (1C, C-2), 74.0 (1C, C-6), 69.6 (1C, C-8), 68.6 (1C, C-4), 67.2 (1C, C-7), 62.0 (1C, C-9), 52.8 (1C, OMe), 49.2 (1C, $S\underline{C}H_2CH_3$), 49.2 (1C, C-5), 37.8 (1C, C-3), 23.0, 21.0, 20.6, 20.6 (5C, $\underline{C}H_3C(O)$), 13.8 (1C, $S\underline{C}H_2CH_3$); M.S. (C.I., ether) m/z: 536 (96%, $|M + H|^+$), 476 (47%, $|M - COOMe|^+$), 474 (18%, $|M - SEt|^+$); Anal. calcd for $C_{22}H_{33}NO_{12}S$: C, 49.34; H, 6.21; N, 2.62; S, 5.98; Found: C, 49.56; H, 6.31; N, 2.69; S, 5.80.

Methyl (Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 46 - The reaction was performed using 3 x 1.5 eq of sodium thiomethoxide (220 mg, 1.4 mmol,). The 2-thioglycoside solidified after evaporation with ethyl ether. Yield 23% (37 mg, NaOH, CH_2Cl_2): m.p. 80.1-81.3°C: $|\alpha|_D$ +27° (c1.0, chloroform); lit.[121] m.p. 80-82°; $|\alpha|_D$ +26° (c1.0, chloroform); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.37 (ddd, 1H, $J_{7,8}$ = 8.5 Hz, $J_{8,9a}$ = 2.7 Hz, $J_{8,9b}$ = 5.0 Hz, H-8), 5.31 (dd, $J_{6,7}$ = 2.2 Hz, H-7), 5.13 (d, 1H, $J_{NH,5}$ = 10.3 Hz, NH), 4.86 (ddd, 1H, $J_{3ax,4}$ = 11.9 Hz, $J_{3eq,4}$ = 4.8 Hz, $J_{4,5}$ = 10.4 Hz, H-4), 4.29 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.08 (dd, 1H, H-9b), 4.04 (ddd, 1H, $J_{5,6}$ = 10.7 Hz, H-5), 3.80 (dd, 1H, H-6), 3.79 (s, 3H, OCH_3), 2.71 (dd, 1H, $J_{3ax,3eq}$ = 12.7 Hz, H-3eq), 1.97 (dd, 1H, H-3ax), 2.15, 2.12, 2.02, 2.01, 1.86 (s, 15H, $CH_3C(O)$), 2.11 (s, 3H, SCH_3); lit.[122] ^1H NMR (270 MHz, $CDCl_3$): δ (ppm) 5.38 (ddd, 1H, $J_{7,8}$ = 8.4 Hz, $J_{8,9a}$ = 2.4 Hz, $J_{8,9b}$ = 5.1 Hz, H-8), 5.31 (dd, 1H, $J_{6,7}$ = 1.8 Hz, H-7), 5.18 (d, 1H, $J_{NH,5}$ = 10.2 Hz, NH), 4.88 (ddd, 1H, $J_{3eq,4}$ = 4.8 Hz, $J_{4,5}$ = 10.2 Hz, H-4), 4.33 (dd, 1H, $J_{9a,9b}$ = 11.7 Hz, H-9a), 4.11 (dd, 1H, H-9b), 4.08 (ddd, 1H, $J_{5,6}$ = 10.2 Hz, H-5), 3.84 (dd, 1H, H-6), 3.81 (s, 3H, OCH_3), 2.73 (dd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, H-3ax), 2.17, 2.14, 2.04, 2.03, 1.86 (s, 15H, $CH_3C(O)$), 2.11 (s, 3H, SCH_3); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.1, 170.8, 170.3, 170.2, 170.2 (5C, $CH_3\underline{C}(O)$), 168.2 (1C, C-1), 82.7 (1C, C-2), 74.0 (1C, C-6), 69.7 (1C, C-8), 68.7 (1C, C-4), 67.3 (1C, C-7), 62.2 (1C, C-9), 52.6 (1C, $\underline{O}Me$), 49.2 (1C, C-5), 37.7 (1C, C-3), 23.1, 21.1, 20.7, 20.6, 20.6 (5C, $\underline{C}H_3C(O)$), 11.9 (1C, SMe); M.S. (C.I., ether) m/z: 522 (96%, $|M + H|^+$), 462 (31%, $|M - COOMe|^+$), 474 (13%, $|M - SMe|^+$).

Methyl (4-Methylphenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 48 - The reaction was performed using 1.3 eq of the thiol (50 mg, 0.40 mmol). The 2-thioglycoside obtained was crystallized from ether/benzene. Yield 70% (130 mg, NaOH, CH_2Cl_2): m.p. 114.1-114.7°C; $|\alpha|_D$ + 24° (c1.0, chloroform); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.37 (d, 2H, $J_{m,o}$ = 7.9 Hz, H-ortho), 7.12 (d, 2H, H-meta), 5.27 (dd, 1H, $J_{6,7}$ = 1.6 Hz, $J_{7,8}$ = 8.1 Hz, H-7), 5.25 (ddd, 1H, $J_{8,9a}$ = 2.4

Hz, $J_{8,9b}$ = 5.1 Hz, H-8), 5.08 (d, 1H, $J_{NH,5}$ = 9.3 Hz, NH), 4.81 (ddd, 1H, $J_{3ax,4}$ = 11.8 Hz, $J_{3eq,4}$ = 4.6 Hz, $J_{4,5}$ = 10.0 Hz, H-4), 4.39 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.19 (dd, 1H, H-9b), 3.95 (ddd, 1H, $J_{5,6}$ = 10.7 Hz, H-5), 3.85 (dd, 1H, H-6), 3.58 (s, 3H, OCH_3), 2.76 (dd, 1H, $J_{3ax,3eq}$ = 12.9 Hz, H-3eq), 2.34 (s, 3H, CH_3), 2.12, 2.04, 2.04, 2.00, 1.84 (s, 15H, $C(O)CH_3$), 2.02 (dd, 1H, H-3ax); ^{13}C NMR (50.3 MHz, $CDCl_3$) : δ (ppm) 171.1, 170.8, 170.3, 170.3, 170.1 (5C, $CH_3\underline{C}(O)$), 168.2 (1C, C-1), 140.3 (1C, C-ipso), 136.6 (2C, C-meta), 129.7 (1C, C-ortho), 125.0 (2C, C-para), 87.4 (1C, C-2), 74.7 (1C, C-6), 70.1 (1C, C-8), 69.6 (1C, C-4), 67.6 (1C, C-7), 61.9 (1C, C-9), 52.6 (1C, OMe), 49.1 (1C, C-5), 37.8 (1C, C-3), 22.9, 20.8, 20.6, 20.6, 20.5 (5C, $\underline{C}H_3C(O)$), 21.1 (1C, $Ph\underline{C}H_3$); M.S. (C.I., ether) m/z: 598 (29%, $[M + H]^+$), 538 (26%, $[M - COOMe]^+$), 474 (16%, $[M - SPhMe]^+$); Anal. calcd for $C_{27}H_{35}NO_{12}S$: C, 54.26; H, 5.90; N, 2.34; S, 5.36; Found: C, 54.43; H, 6.02; N, 2.40; S, 5.14.

Methyl (4-Methoxyphenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 49 - The reaction was performed using 1.3 eq of the thiol (56 mg, 0.40 mmol). The 2-thioglycoside obtained was crystallized from ether/benzene. Yield 63% (118 mg, NaOH, CH_2Cl_2), yield 70% (132 mg, NaOH, CH_2Cl_2); m.p. 132.0-133.1°C; $[\alpha]_D$ +17° (c1.0, chloroform); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.40 (d, 2H, $J_{o,m}$ = 8.8 Hz, H-meta), 6.84 (d, 2H, H-ortho), 5.27 (dd, 1H, $J_{6,7}$ = 1.8 Hz, $J_{7,8}$ = 6.8 Hz, H-7), 5.22 (ddd, 1H, $J_{8,9a}$ = 2.6 Hz, $J_{8,9b}$ = 5.5 Hz, H-8), 5.15 (d, 1H, $J_{NH,5}$ = 9.8 Hz, NH), 4.81 (ddd, 1H, $J_{3ax,4}$ = 11.7 Hz, $J_{3eq,4}$ = 4.6 Hz, $J_{4,5}$ = 10.2 Hz, H-4), 4.37 (dd, 1H, $J_{9a,9b}$ = 12.3 Hz, H-9a), 4.17 (dd, 1H, H-9b), 3.95 (ddd, 1H, $J_{5,6}$ = 10.9 Hz, H-5), 3.83 (dd, 1H, H-6), 3.81 (s, 3H, $PhOC\underline{H}_3$), 3.59 (s, 3H, OCH_3), 2.76 (dd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, H-3eq), 2.12, 2.04, 2.02, 2.00, 1.84 (s, 15H, $CH_3C(O)$), 2.01 (dd, 1H, H-3ax); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.1, 170.8, 170.3, 170.3, 170.2 (5C, $CH_3\underline{C}(O)$), 168.2 (1C, C-1), 161.3 (1C, C-ipso), 138.4 (2C, C-meta), 119.1 (1C, C-para), 114.4 (2C, C-ortho), 87.4 (1C, C-2), 74.7 (1C, C-6), 70.0 (1C, C-8), 69.6 (1C, C-4), 67.6 (1C, C-7), 61.9 (1C, C-9), 55.2 (1C, $PhO\underline{C}H_3$), 52.6 (1C, OCH_3), 49.1 (1C, C-5), 37.7 (1C, C-3), 23.0, 20.8, 20.6, 20.6, 20.6 (5C, $\underline{C}H_3C(O)$); M.S. (C.I. ether) m/z: 614 (26%, $[M + H]^+$), 554 (32%, $[M - COOMe]^+$), 474 (21%, $[M - SPhOCH_3]^+$); Anal. calcd for $C_{27}H_{35}NO_{13}S$: C, 52.85; H, 5.75; N, 2.28; S, 5.22; Found: C, 53.06; H, 5.76; N, 2.35; S, 5.14.

Methyl (4-Nitrophenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 50 - The reaction was performed using 1.3 eq of the thiol (62 mg, 0.40 mmol). The 2-thioglycoside obtained was crystallized from ether. Yield 68% (133 mg, NaOH, CH_2Cl_2); m.p. 173.1-173.3°C; $[\alpha]_D$ +35° (c1.0, chloroform);

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.17 (d, 2H, $J_{o,\ m}$ = 9.0 Hz, H-meta), 7.63 (d, 2H, H-ortho), 5.30 (dd, 1H, $J_{6,\ 7}$ = 2.2 Hz, H-7), 5.27 (ddd, 1H, $J_{8,\ 9a}$ = 1.2 Hz, $J_{8,\ 9b}$ = 2.1 Hz, H-8), 5.14 (d, 1H, $J_{NH,\ 5}$ = 9.5 Hz, NH), 4.86 (ddd, 1H $J_{3ax,\ 4}$ = 11.9 Hz, $J_{3eq,\ 4}$ = 4.7 Hz, $J_{4,\ 5}$ = 9.9 Hz, H-4), 4.30 (dd, 1H, $J_{9a,\ 9b}$ = 12.5 Hz, H-9a), 4.10 (dd, 1H, $J_{5,\ 6}$ = 11.3 Hz, H-6), 4.06 (dd, 1H, H-9b), 3.99 (ddd, 1H, H-5), 3.59 (s, 3H, OCH_3), 2.85 (dd, 1H, $J_{3ax,\ 3eq}$ = 12.8 Hz, H-3eq), 2.14, 2.04, 2.04, 2.02, 1.87 (s, 15H, $C\underline{H}_3C(O)$), 2.08 (dd, 1H, H-3ax); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.0, 170.9, 170.3, 170.2, 169.8 (5C, $CH_3\underline{C}(O)$), 167.5 (1C, C-1), 148.3 (1C, C-ipso), 137.8 (1C, C-para), 135.1 (2C, C-meta), 123.9 (2C, C-ortho), 86.9 (1C, C-2), 74.4 (1C, C-6), 69.1 (1C, C-8), 68.6 (1C, C-4), 67.2 (1C, C-7), 62.0 (1C, C-9), 52.9 (1C, OCH_3), 49.1 (1C, C-5), 38.2 (1C, C-3), 22.9, 20.7, 20.6, 20.6, 20.6 (5C, $\underline{C}H_3C(O)$).M.S. (C.I., ether) m/z: 629 (39%, $[M + H]^+$), 568 (31%, $[M - COOMe]^+$), 474 (11%, $[M - SPhNO_2]^+$); Anal. calcd for $C_{26}H_{32}N_2O_{14}S$: C, 49.68; H, 5.13; N, 4.46; S:, 5.10; Found: C, 49.87; H, 5.38; N, 4.28; S:, 4.96.

Methyl (Pyridin-2-yl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 51 - The reaction was performed using 1.3 eq of the thiol (45 mg, 0.40 mmol). The 2-thioglycoside obtained was crystallized from ether/benzene. Yield 69% (127 mg, NaOH, CH_2Cl_2); m.p. 150.5-152.5°C; $[\alpha]_D$ +29° (c1.0, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.46 (ddd, 1H, $J_{3',\ 6'}$ = 0.8 Hz, $J_{4',\ 6'}$ = 1.9 Hz, $J_{5',\ 6'}$ = 4.8 Hz, H-6'), 7.66 (ddd, 1H, $J_{3',\ 4'}$ = 7.4 Hz, $J_{4',\ 5'}$ = 7.9 Hz, H-4'), 7.54 (ddd, 1H, $J_{3',\ 5'}$ = 1.1 Hz, H-3'), 7.18 (ddd, 1H, H-5'), 5.29 (dd, 1H, $J_{6,\ 7}$ = 1.8 Hz, $J_{7,\ 8}$ = 7.9 Hz, H-7), 5.21 (ddd, 1H, $J_{8,\ 9a}$ = 2.8 Hz , $J_{8,\ 9b}$ = 5.2 Hz, H-8), 5.15 (d, 1H, $J_{NH,\ 5}$ = 9.6 Hz, NH), 4.88 (ddd, 1H, $J_{3ax,\ 4}$ = 11.8 Hz, $J_{3eq,\ 4}$ = 4.7 Hz, $J_{4,\ 5}$ = 9.9 Hz, H-5), 4.27 (dd, 1H, $J_{9a,\ 9b}$ = 12.5 Hz, H-9a), 4.10 (dd, 1H, $J_{5,\ 6}$ = 10.7 Hz, H-6), 4.09 (dd, 1H, H-9b), 4.03 (ddd, 1H, H-5), 3.65 (s, 3H, OCH_3), 2.86 (dd, 1H, $J_{3ax,\ 3eq}$ = 12.8 Hz, H-3eq), 2.18 (dd, 1H, H-3ax), 2.11, 2.05, 2.02, 2.01, 1.86 (s, 15H, $CH_3C(O)$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.0, 170.7, 170.3, 170.2, 170.1 (5C, $CH_3\underline{C}(O)$), 168.3 (1C, C-1), 153.0 (1C, C-ipso), 149.9 (1C, -$\underline{C}$=N-), 137.3 (1C, C-meta), 129.2 (1C, C-para), 122.8 (1C, C-ortho), 86.1 (1C, C-2), 74.5 (1C, C-6), 69.3 (1C, C-8), 69.3 (1C, C-4), 67.3 (1C, C-7), 61.8 (1C, C-9), 52.9 (1C, OMe), 49.1 (1C, C-5), 38.2 (1C, C-3), 23.0, 20.8, 20.6, 20.6, 20.6 (5C, $CH_3C(O)$); M.S. (C.I., ether) m/z: 585 (100%, $[M + H]^+$), 525 (1.5%, $[M - COOMe]^+$), 474 (8%, $[M - SPy]^+$); Anal. calcd for $C_{25}H_{32}N_2O_{12}S$: C, 51.36; H, 5.52; N, 4.79; S, 5.48; Found: C, 51.55; H, 5.69; N, 4.59; S, 5.29.

Methyl (1-Methylimidazol-2-yl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 52 - The reaction

was performed using 1.3 eq of the thiol (46 mg, 0.40 mmol). The 2-thioglycoside obtained was crystallized from ether/benzene. Yield 68% (124 mg, NaOH, CH_2Cl_2): m.p. 140.5-142.5°C; $[\alpha]_D$ +28° (c1.0, chloroform); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.12 (d, 1H, $J_{4',5'}$ = 1.2 Hz, H-4'), 7.10 (d, 1H, H-5'), 5.19 (dd, 1H, $J_{6,7}$ = 1.5 Hz, $J_{7,8}$ = 9.9 Hz, H-7), 5.16 (ddd, 1H, $J_{8,9a}$ = 2.3 Hz, $J_{8,9b}$ = 5.8 Hz, H-8), 5.12 (d, 1H, $J_{NH,5}$ = 9.8 Hz, NH), 4.82 (ddd, 1H, $J_{3ax,4}$ = 11.9 Hz, $J_{3eq,4}$ = 4.7 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 4.23 (dd, 1H, $J_{9a,9b}$ = 12.3 Hz, H-9a), 3.99 (dd, 1H, H-9b), 3.87 (ddd, 1H, $J_{5,6}$ = 9.7 Hz, H-5), 3.82 (dd, 1H, H-6), 3.82 (s, 3H, 1 CH_3-imidazolyl), 3.75 (s, 3H, OCH_3), 2.85 (dd, 1H, $J_{3ax,3eq}$ = 13.0 Hz, H-3eq), 2.17 (dd, 1H, H-3ax), 2.09, 2.04, 2.01, 2.01, 1.85 (s, 15H, $CH_3C(O)$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.1, 170.9, 170.3, 170.0, 169.9 (5C, $CH_3\underline{C}(O)$), 167.7 (1C, C-1), 134.1 (1C, C ipso), 130.7 (1C, =N$\underline{C}$H=), 125.2 (1C, =CHN(CH_3)), 88.0 (1C, C-2), 74.4 (1C, C-6), 69.3 (1C, C 8), 68.3 (1C, C-4), 67.0 (1C, C-7), 62.2 (1C, C-9), 53.2 (1C, OMe), 49.0 (1C, C-5), 37.8 (1C, C-3), 34.2 (1C, CH_3-N), 22.9, 20.7, 20.6, 20.6, 20.6 (5C, $\underline{C}H_3C(O)$); M.S. (C.I., ether) m/z: 588 (13%, $[M + H]^+$), 474 (3%, $[M - MeIm]^+$); Anal. calcd for $C_{24}H_{33}N_3O_{12}S$: C, 49.06; H, 5.66; N, 7.15; S, 5.46; Found: C, 49.26; H, 5.91; N, 6.91; S, 5.23.

Methyl (Phenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 40 - A mixture of penta-O-acetate **21** (0.19 mmol, 100 mg), methylene chloride (3 ml), thiophenol (102 µl, 110 mg, 0.95 mmol, 5 eq) and boron trifluoride etherate (61 µl, 71 mg, 0.5 mmol) was kept overnight at room temperature. The mixture was then diluted with methylene chloride. The organic layer was washed with saturated hydrogen carbonate (2 x 10 ml) and brine (10 ml), dried over anh. sodium sulfate and evaporated in vacuo to dryness. The oil obtained was purified by chromatography on a Chromatotron (1 mm silica gel plate) using pure ethyl acetate as eluent. Phenyl-β-thiosialoside **40** was obtained as an oil which was recrystallized from ethyl acetate/hexanes 79 mg; yield 71%; m.p. 180.1-182.0°C; $[\alpha]_D$ -133° (c1.0, chloroform); lit.[124] m.p. 181-182°C; $[\alpha]_D$ -132° (c1.0, chloroform); ^1H NMR (200 MHz, C_6D_6); δ (ppm) 7.65 (dd, 2H, $J_{2',3'}$ = 7.6 Hz, $J_{2',4'}$ = 1.6 Hz, H-2'), 7.07 (dd, 1H, $J_{3',4'}$ = 6.7 Hz, H-4'), 6.97 (dd, 2H, H-3'),, 5.75 (dd, 1H, $J_{6,7}$ = 2.5 Hz, $J_{7,8}$ = 2.0 Hz, H-7), 5.44 (ddd, 1H, $J_{8,9a}$ = 2.2 Hz, $J_{8,9b}$ = 8.7 Hz, H-8), 5.35 (ddd, 1H, $J_{3ax,4}$ = 11.6 Hz, $J_{3eq,4}$ = 4.9 Hz, $J_{4,5}$ = 10.2 Hz, H-4), 5.03 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.63 (m, 1H, H-6), 4.54-4.42 (m, 2H, H-5, NH), 4.39 (dd, 1H, H-9b), 3.27 (s, 3H, OMe), 2.83 (dd, 1H, $J_{3ax,3eq}$ = 13.9 Hz, H-3eq), 2.03 (dd, 1H, H-3ax), 1.91, 1.87, 1.64, 1.62, 1.59 (5s, 15H, 5 $CH_3C(O)$).; lit.[124] ^1H NMR (400 MHz, C_6D_6): δ (ppm) 7.64, 7.10, 6.99 (3m, 5H, Ph), 5.72 (dd, 1H, $J_{6,7}$ = 2.5 Hz, $J_{7,8}$ = 2.1 Hz, H-7), 5.44 (ddd, 1H, $J_{8,9a}$ = 2.1 Hz, $J_{8,9b}$ = 8.6 Hz, H-8), 5.35 (ddd, 1H, $J_{3ax,4}$ = 11.7 Hz, $J_{3eq,4}$ = 4.8 Hz, $J_{4,5}$ = 10.2 Hz, H-4), 5.00 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, H-9a), 4.61 (m, 1H, H-6), 4.52-4.43 (m, 2H, H-5, NH), 4.38 (dd,

1H, H-9b), 3.26 (s, 3H, OMe), 2.80 (dd, 1H, $J_{3ax, 3eq}$ = 14.0 Hz, H-3eq), 2.01 (dd, 1H, H-3ax), 1.92, 1.88, 1.67, 1.63, 1.60 (5s, 15H, 5 $CH_3C(O)$).

4.4.2 Application of PTC Methodology

4.4.2.1 Neoglycoprotein Precursor - 4-Formylphenyl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid 55

Methyl (4-Formylphenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 53 - A solution of p-hydroxybenzaldehyde (48 mg, 0.40 mmol, 1.3 eq) and tetrabutylammonium hydrogen sulfate (125 mg, 0.37 mmol, 1.2 eq) in 1M NaOH (2 ml) was stirred at room temperature with a freshly prepared solution of chloride **22** (158 mg, 0.31 mmol) in methylene chloride (2 ml) for ≈10 minutes. When TLC indicated complete transformation of the chloride, the reaction mixture was diluted with 10 ml of ethyl acetate and 10 ml of saturated aqueous sodium hydrogen carbonate. The organic layer was separated and washed with a saturated sodium bicarbonate solution (2 x 10 ml) and with a saturated sodium chloride solution (1 x 10 ml). The solution was dried (anhydrous sodium sulfate) and was evaporated to dryness in vacuo. The oily residue was purified by chromatography using a Chromatotron (1 mm plate) using 100% ethyl acetate as eluent. The pure product **53** was crystallized from benzene/hexanes and was obtained in 65% yield as white crystals (120 mg); m.p. 90.4-91.6°C; $[\alpha]_D$ +32.4° (c1.0, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 9.90 (s, CHO), 7.81 (d, 2H, $J_{m, o}$ = 8.7 Hz, H-meta), 7.15 (d, 2H, H-ortho), 5.36 (m, 1H, $J_{8, 9a}$ = 2.2 Hz, $J_{8, 9b}$ = 4.4 Hz, H-8), 5.35 (m, $J_{6, 7}$ = 1.4 Hz, H-7), 5.20 (d, 1H, $J_{NH, 5}$ = 10.0 Hz, NH), 4.96 (ddd, 1H, $J_{3ax, 4}$ = 12.2 Hz, $J_{3eq, 4}$ = 4.7 Hz , $J_{4, 5}$ = 10.3 Hz, H-4), 4.58 (dd, 1H, $J_{5, 6}$ = 10.8 Hz, H-6), 4.23 (dd, 1H, $J_{9a, 9b}$ = 10.3 Hz, H-9), 4.11 (ddd, 1H, H-5), 4.10 (dd, 1H, H-9b), 3.62 (s, 3H, OCH_3), 2.71 (dd, $J_{3ax, 3eq}$ = 13.1 Hz, H-3eq), 2.28 (dd, 1H, H-3ax), 2.17, 2.10, 2.04, 2.03, 1.91 (5s, $CH_3C(O)$); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ (ppm) 191.2 (1C, CHO), 171.0, 170.7, 170.4, 170.3, 170.1 (5C, $CH_3\underline{C}(O)$), 168.4 (1C, C-1), 159.1 (1C, C-ipso), 131.9 (1C, C-para), 131.7 (2C, C-meta), 118.8 (2C, C-ortho), 99.4 (1C, C-2), 73.5 (1C, C-6), 68.6 (1C, C-8), 68.2 (1C, C-4), 67.0 (1C, C-7), 61.9 (1C, C-9), 53.0 (1C, OMe), 49.1 (1C, C-5), 38.5 (1C, C-3), 23.0, 20.8, 20.6, 20.5, 20.5 (5C, $\underline{C}H_3C(O)$); M.S. (C.I., ether) m/z: 596 (35%, $[M + H]^+$), 536 (11%, $[M - COOMe]^+$), 474 (14%, $[M - OPhCHO]^+$); Anal. calcd for $C_{27}H_{33}NO_{14}$: C, 54.45; H, 5.59; N, 2.35; Found: C, 54.36; H, 5.73; N, 2.31.

Methyl (4-Formylphenyl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 54 - Aldehyde **53** (120 mg, 0.20 mmol) was dissolved in methanol (10 ml) and cooled to 0°C. The pH of the solution was adjusted to 9 using a 1 M sodium methoxide in methanol and the solution was stirred for 5 hours at 0°C. The solution was neutralized using H^+ resin and after filtration and evaporation a clear oil was obtained. The oil was dissolved in water and the solution was lyophilized. The methyl ester **54** was obtained as an amorphous white powder (93% yield, 71 mg); m.p. 109.2-110.1°C; $[\alpha]_D$ +56.3° (c1.0, water); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 9.84 (s, 1H, CHO), 7.94 (d, 2H, $J_{m,o}$ = 8.9 Hz, H-meta), 7.31 (d, 2H, H-ortho), 4.28 (dd, 1H, $J_{5,6}$ = 10.5 Hz, $J_{6,7}$ = 1.3 Hz, H-6 (tentative)), 4.00 (dd, 1H, $J_{4,5}$ = 10.1 Hz, H-5 (tentative)), 3.87-3.58 (m, H-4, 8, 9a, 9b), 3.76 (s, 3H, OMe), 3.59 (dd, 1H, $J_{7,8}$ = 9.0 Hz, H-7 (tentative)), 2.85 (dd, 1H, $J_{3ax,3eq}$ = 12.9 Hz, $J_{3eq,4}$ = 4.5 Hz, H-3eq), 2.13 (dd, 1H, $J_{3ax,4}$ = 11.9 Hz, H-3ax) , 2.06 (s, 3H, $CH_3C(O)$); ^{13}C NMR (75.4 MHz, D_2O): δ (ppm) 194.6 (1C, CHO), 174.6 (1C, $CH_3\underline{C}(O)ND$), 169.3 (1C, C-1), 158.6 (1C, C-ipso), 131.8 (3C, C-meta, C-para), 119.6 (2C, C-ortho), 100.1 (1C, C-2), 73.7 (1C, C-6), 70.1 (1C, C-8), 67.7 (1C, C-4), 66.2 (1C, C-7), 62.8 (1C, C-9), 53.1 (1C, OMe), 51.1 (1C, C-5), 39.6 (1C, C-3), 21.7 (1C, $\underline{C}H_3C(O)$); M.S. (C.I., ether) m/z: 428 (5%, $[M+H]^+$), 306 (9% $[M - OPhCHO]^+$); Anal. calcd for $C_{19}H_{25}NO_{10}H_2O$: C, 51.23; H, 6.11; N, 3.14; Found : C, 50.81; H, 6.09; N, 3.12.

4-Formylphenyl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid 55 - A solution of methyl ester **54** (50 mg, 0.13 mmol) in 0.1M NaOH was stirred at 0°C for 6 hours. The reaction was quenched using H^+ resin and after filtration the solution was neutralized to pH = 7 using 0.1M NaOH. The solution was lyophilized to afford the product **55** as an amorphous powder, in 90% yield (43 mg); m.p. 180-184°C (decomposed); $[\alpha]_D$ +72.4° (water); ^{1}H NMR (300 MHz, D_2O): δ (ppm) 9.83 (s, 1H, CHO), 7.92 (d, 2H, $J_{m,o}$ = 8.8 Hz, H-meta), 7.32 (d, 2H, H-ortho), 4.16 (dd, 1H, $J_{5,6}$ = 10.4 Hz, $J_{6,7}$ = 1.5 Hz, H-6 (tentative)), 3.97 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-5 (tentative)), 3.59-3.88 (m, 5H, H 4, 7, 8, 9a, 9b), 2.87 (dd, 1H, $J_{3ax,3eq}$ = 12.7 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3eq), 2.06 (s, 3H, $CH_3C(O)$), 2.03 (dd, 1H, $J_{3ax,4}$ = 11.9 Hz, H-3ax); ^{13}C NMR (75.4 MHz, D_2O): δ (ppm) 194.7 (1C, CHO), 174.6 (1C, $CH_3\underline{C}(O)ND$), 172.2 (1C, C-1), 159.7 (1C, C-ipso), 130.8 (3C, C-para, C-meta), 119.8 (1C, C-ortho), 101.9 (1C, C-2), 73.2 (1C, C-6), 71.1 (1C, C-8), 67.8 (1C, C-4), 67.3 (1C, C-7), 62.3 (1C, C-9), 51.3 (1C, C-5), 40.5 (1C, C-3), 21.6 (1C, $\underline{C}H_3C(O)$); M.S. (Negative FAB) m/z: 412 (8%, $[M]^-$), 311 (7%, $[M - OPhCHO]^-$).

4.4.2.2 Synthesis of Fluorogenic Substrate for Neuraminidase - 4-Methylcoumarin-7-yl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid 58

Methyl (4-Methylcoumarin-7-yl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 56 - A freshly prepared solution of chloride **22** (158 mg, 0.31 mmol) in methylene chloride (2 ml) was added to a solution of 7 hydroxy-4 methylcoumarin (70 mg, 0.40 mmol, 1.3 eq) and tetrabutylammonium hydrogen sulfate (135 mg, 0.40 mmol, 1.3 eq) in base (1M NaOH or saturated sodium bicarbonate, 2 ml) which had previously been stirred at room temperature for 30 minutes. The two phase system was stirred until complete disappearance of the chloride as detected by TLC (2 x 100% ethyl acetate). The mixture was diluted with ethyl acetate (100 ml) and with a saturated bicarbonate solution (10 ml) and the phases were separated. The organic layer was washed with a saturated sodium bicarbonate solution (2 x 10 ml) and saturated sodium chloride (1 x 10 ml) dried (anhydrous sodium sulfate) and evaporated to dryness in vacuo. The oil obtained was chromatographed on the Chromatotron on a 1 mm rotor using 1:4 ether:ethyl acetate as eluent. The pure product **56** was crystallized from benzene and was obtained as white crystals (171 mg, yield 85% ($NaHCO_3$), 156 mg, 78% (NaOH)); m.p. 108.6-110.8°C (100°C sintered); $[\alpha]_D$ +51.0° (c1.0, chloroform); lit.[57] m.p. 98-100°C; $[\alpha]_D$ +52° (c1.0, chloroform);^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.50 (d, 1H, $J_{5',6'}$ = 8.8 Hz, H-5' coumarin), 7.04 (dd, 1H, $J_{6',8'}$ = 2.4 Hz, H-6' coumarin), 6.98 (d, 1H, H-8' coumarin), 6.18 (d, 1H, J_{3',CH_3} = 1.2 Hz, H-3' coumarin), 5.36 (ddd, 1H, $J_{8,9a}$ = 2.0 Hz, $J_{8,9b}$ = 4.6 Hz, H-8), 5.35 (dd, 1H, $J_{6,7}$ = 1.0 Hz, H-7) , 5.24 (d, 1H, $J_{NH,5}$ = 10.0 Hz, NH), 4.97 (ddd, 1H, $J_{3ax,4}$ = 12.6 Hz, $J_{3eq,4}$ = 4.6 Hz, $J_{4,5}$ = 10.4 Hz, H-4), 4.49 (dd, 1H, $J_{5,6}$ = 10.1 Hz, H-6), 4.27 (dd, 1H, $J_{9a,9b}$ = 10.5 Hz, H-9a), 4.11 (dd, 1H, H-9b), 4.08 (ddd, 1H, H-5), 3.68 (s, 3H, OCH_3), 2.71 (dd, 1H, $J_{3ax,3eq}$ = 13.2 Hz, H-3eq), 2.40 (d, 3H, CH_3 coumarin), 2.24 (dd, 1H, H-3ax), 2.15, 2.12, 2.03, 2.02, 1.91 (s, 15H, $CH_3C(O)$); lit.[57] ^{1}H NMR (100 MHz, $CDCl_3$): δ (ppm) 7.57-7.02 (m, 3H, aromatic), 6.20 (d, 1H, H-3' coumarin), 5.90 (d, 1H, NH), 5.38 (m, 2H, H-7, 8), 5.16-4.86 (m, 1H, H-4), 4.60-4.08 (m, 4H, H-5, 6, 9a, 9b), 3.72 (s, 3H, OCH_3), 2.76 (dd, 1H, $J_{3ax,3eq}$ = 13.0 Hz, $J_{3eq,4}$ = 4.5 Hz, H-3eq), 2.44 (d, 3H, Me coumarin), 2.16, 2.16, 2.06, 2.06, 1.94 (s, 15H, $CH_3C(O)$); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) 171.0, 170.8, 170.5, 170.3, 170.2 (5C, $CH_3\underline{C}(O)ND$), 168.1 (1C, C-1), 161.0 (1C, C-2' coumarin), 156.7 (1C, C-8' coumarin), 154.4 (1C, C-10' coumarin), 152.4 (1C, C-4' coumarin), 125.8 (1C, C-6' coumarin), 116.1 (1C, C-5' coumarin), 115.5 (1C, C-7' coumarin), 113.4 (1C, C-9' coumarin), 107.6 (1C, C-3' coumarin), 99.8 (1C, C-2), 73.6 (1C, C-6), 68.9 (1C, C-8), 68.3 (1C, C-4), 67.1 (1C, C-7), 61.9 (1C, C-9), 53.1 (1C, OMe),

49.1 (1C, C-5), 38.0 (1C, C-3), 23.0, 20.8, 20.6, 20.5, 20.5 (5C, $\underline{C}H_3C(O)$), 18.5 (1C, CH_3 coumarin); M.S. (ether) m/z: 650 (51%, $[M + H]^+$), 590 (3%, $[M - COOMe]^+$), 474 (77%, $[M - Ocoumarin]^+$); Anal. calcd for $C_{30}H_{35}NO_{15}$: C, 55.47; H, 5.43; N, 2.16; Found:C, 55.24; H, 5.67; N, 2.09.

Methyl (4-Methylcoumarin-7-yl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 57 - The pH of a cooled solution of **56** (155 mg, 0.24 mmol) in methanol (10 ml) was adjusted to 9 with a 1M sodium methoxide solution in methanol and the clear solution was stirred at 0°C for 2 hours. Resin (H^+) was added to the cooled solution and after neutralization and filtration, the solvent was evaporated in vacuo. The solids obtained were crystallized from methanol/ether and white crystals of methyl ester **57** were obtained in 95% yield (100 mg); m.p. 111.1-112.9°C (sint'd 103.0°C); $[\alpha]_D$ +59° (c1.0, chloroform); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.60 (d, 1H, $J_{5',6'}$ = 8.9 Hz, H-5' coumarin), 7.12 (dd, 1H, $J_{6',8'}$ = 2.4 Hz, H-6' coumarin), 7.04 (d, 1H, H-8' coumarin), 6.18 (d, 1H, J_{3',CH_3} = 1.1 Hz, H-3' coumarin), 4.12-3.56 (m, 7H, H-4, 5, 6, 7, 8, 9a, 9b), 3.82 (s, 3H, OCH_3), 2.88 (dd, 1H, $J_{3ax,3eq}$ = 13.0 Hz, $J_{3eq,4}$ = 4.5 Hz, H-3eq), 2.32 (d, 3H, CH_3 coumarin), 2.09 (dd, 1H, $J_{3ax,4}$ = 12.3 Hz, H-3ax), 2.05 (s, 3H, $CH_3C(O)$); ^{13}C NMR (50.3 MHz, D_2O): δ (ppm) 174.6, (1C, $CH_3\underline{C}(O)$), 169.3 (1C, C-1), 164.2 (1C, C-2' coumarin) 157.2 (1C, C-8' coumarin), 154.9 (1C, C-10' coumarin), 153.1 (1C, C-4' coumarin), 126.2 (1C, C-6' coumarin), 117.7 (1C, C-5' coumarin), 115.9 (1C, C-7' coumarin), 112.5 (1C, C-9' coumarin), 108.2 (1C, C-3' coumarin), 100.5 (1C, C-2), 73.8 (1C, C-6), 70.4 (1C, C-8), 67.9 (1C, C-4), 66.7 (1C, C-7), 62.7 (1C, C-9), 53.1 (1C, OMe), 51.2 (1C, C-5), 39.6 (1C, C-3), 23.0 (1C, $\underline{C}H_3C(O)$), 18.5 (1C, CH_3 coumarin); M.S. (C.I., ether) m/z: 482 (5%, $[M + H]^+$), 306 (9%, $[M - O coumarin]^+$); Anal. calcd for $C_{22}H_{27}NO_{11}\cdot H_2O$: C, 52.90; H, 5.85; N, 2.80; Found: C, 52.77; H, 5.93; N, 2.92.

4-Methylcoumarin-7-yl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid 58 - A solution of methyl ester **57** (87 mg, 0.20 mmol) in 0.1 M NaOH solution was stirred at 0°C for 3 hours. The reaction was quenched using H^+ resin at 0°C and the reaction mixture was filtered and lyophilized to yield the acid **58** as an amorphous powder (80 mg, 95% yield); m.p. 160-171°C (dec.); $[\alpha]_D$ +69° (c1.0, water); lit.[57] 171°C (dec.); $[\alpha]_D$ +70° (c1.0, water); ^1H NMR (300 MHz, D_2O): δ (ppm) 7.53 (d, 1H, $J_{5',6'}$ = 8.6 Hz, H-5'), 7.10 (dd, 1H, $J_{6',8'}$ = 2.4 Hz, H-6'), 7.02 (d, 1H, H-8'), 6.07 (d, 1H, J_{3',CH_3} = 1.2 Hz, H-3'), 4.13-3.58 (m, 7H, H-4, 5, 6, 7, 8, 9a, 9b), 2.91 (dd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3eq), 2.28 (s, 3H, CH_3 coumarin), 2.05 (s, 3H, $C\underline{H}_3CO$), 2.00 (dd, 1H, $J_{3ax,4}$ = 12.8 Hz, H-3ax); lit.[57] ^1H NMR (100 MHz, D_2O): δ (ppm) 7.60-7.06 (m, 3H, aromatic), 6.09 (d, 1H, H-3' coumarin), 4.12-3.58 (m, 7H, H-4, 5, 6, 7, 8, 9a, 9b), 3.00-2.80 (m, 1H, H-3eq),

2.32 (d, 1H, CH_3, coumarin), 2.10 (s, 3H, $CH_3C(O)$); ^{13}C NMR (50.3 MHz, D_2O): δ (ppm) 175.6 (1C, $CH_3\underline{C}(O)$), 172.9 (1C, C-1), 164.5 (1C, C-2' coumarin), 157.2 (1C, C-8' coumarin), 155.6 (1C, C-10' coumarin), 153.5 (1C, C-4' coumarin), 126.4 (1C, C-6' coumarin), 118.2 (1C, C-5' coumarin), 116.1 (1C, C-7' coumarin), 112.1 (1C, C-9' coumarin), 108.4 (1C, C-3' coumarin), 102.3 (1C, C-2), 74.1 (1C, C-6), 71.9 (1C, C-8), 69.1 (1C, C-7), 68.1 (1C, C-4), 63.2 (1C, C-9), 51.7 (1C, C-5), 41.2 (1C, C-3), 22.9 (1C, $\underline{C}H_3C(O)$), 18.5 (1C, CH_3 coumarin); lit.[4] ^{13}C NMR (60 MHz, D_2O): δ (ppm) 175.9 (1C, $CH_3\underline{C}(O)$), 173.4 (1C, C-1), 164.8 (1C, C-2' coumarin), 158.2 (1C, C-8' coumarin), 156.6 (1C, C-10' coumarin), 153.8 (1C, C-4' coumarin), 126.8 (1C, C-6' coumarin), 118.4 (1C, C-5' coumarin), 116.3 (1C, C-7' coumarin), 112.1 (1C, C-9' coumarin), 108.4 (1C, C-3' coumarin), 102.5 (1C, C-2), 74.4 (1C, C-6), 72.4 (1C, C-8), 69.2 (1C, C-7), 68.7 (1C, C-4), 63.6 (1C, C-9), 52.6 (1C, C-5), 41.7 (1C, C-3), 22.9 (1C, $\underline{C}H_3C(O)$), 18.5 (1C, CH_3 coumarin); M.S. (Negative FAB) m/z: 466 (100%, $[M - H]^-$), 291 (16%, $[M - HO$ coumarin$]^-$).

4.2.2.3 Synthesis of Glycosyl Phosphotriester by Phase Transfer Catalysis

Dibenzyl (Methyl (5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate phosphate 59 - To a solution of dibenzyl phosphate (172 mg, 0.62 mmol, 2 eq) and tetrabutylammonium hydrogen sulfate (105 mg, 0.31 mmol, 1 eq) in base (1M NaOH or saturated sodium bicarbonate, 2 ml) was added a freshly prepared solution of chloride **22** (158 mg, 0.31 mmol) in methylene chloride (2 ml). The two-phase reaction mixture was stirred at room temperature for the period of time necessary to obtain the maximum possible yield of glycosyl phosphate **59**. Ethyl acetate (10 ml) and water (10 ml) were subsequently added to the biphasic mixture and the phases were separated. The organic layer was successively washed with saturated sodium bicarbonate (2 x 10 ml), water (1 x 10 ml) and brine (1 x 10 ml), dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude oil obtained was purified by silica gel chromatography using a Chromatotron (1 mm plates) and 100% ethyl acetate as eluent (in the absence or presence of triethylamine in the eluent). ^1H NMR (200 MHz, $CDCl_3$) of crude phosphate: δ (ppm) 7.27 (m, 10H, Ph), 5.50 (d, 1H, $J_{NH,\ 5}$ = 10.0 Hz, NH), 5.32 (dd, 1H, $J_{6,\ 7}$ = 1.4 Hz, $J_{7,\ 8}$ = 6.8 Hz, H-7), 5.26 (ddd, 1H, $J_{8,\ 9a}$ = 4.0 Hz, $J_{8,\ 9b}$ = 4.9 Hz, H-8),5.05 (ddd, 1H, $J_{3ax,\ 4}$ = 11.9 Hz, $J_{3eq,\ 4}$ = 4.7 Hz, $J_{4,\ 5}$ = 9.9 Hz, H-4), 5.03 (d, 1H, $J_{P,\ CH_2}$ = 4.7 Hz, $OC\underline{H}_2Ph$), 4.97 (d, 1H, $J_{P,\ CH_2}$ = 5.0 Hz, $OC\underline{H}_2Ph$), 4.60 (dd, 1H, $J_{5,\ 6}$ = 10.2 Hz, H-6), 4.24 (dd, 1H, $J_{9a,\ 9b}$ = 12.0 Hz, H-9a), 4.11 (dd, 1H, H-9b), 4.05 (ddd, 1H, H-5), 3.58 (s, 3H, OCH_3), 2.54 (dd, 1H, $J_{3ax,\ 3eq}$ = 12.9 Hz, H-3eq), 2.13 (dd, 1H, H-3ax), 2.06, 2.01, 2.00, 2.00, 1.90 (s, 15H, $CH_3C(O)$).

CHAPTER 5 Synthesis of Sialylglycerolipids using α-Thioglycosides as Sialosyl Donors.

5.1 Introduction

The search for new glycosylation reactions has recently become the subject of active research[63] especially since the biomedical potential of glycoconjugates has been recognized[1]. These molecules often carry complex glycan chains composed of a variety of sugar residues with diverse types of interglycosidic linkages. Because of such complexity, it seems to be extremely difficult to find a general solution, based on a single concept, which is applicable to all the situations encountered in oligosaccharide synthesis. However, recent investigations have revealed the promising features of novel glycosyl donors such as fluorides[65,201], trichloroacetimidates[66], and thioglycosides[64,99,100,101,202-205], among other compounds. These substances are relatively stable and can be prepared under mild conditions. Such properties make these methods clearly distinct from the conventional Koenigs-Knorr type process[2,3], which usually requires the preparation of sensitive halides under rather harsh conditions. Furthermore, improvement in yield and stereoselectivity has quite often been achieved using such modern techniques.

Among these novel glycosyl donors, thioglycosides possess an obvious advantage, particularly in view of synthetic strategy, because there are especially insensitive to usual protection-deprotection conditions except catalytic hydrogenolysis. In addition, the activation of thioglycosides is possible under specific neutral conditions which do not affect most other functional groups. Accordingly, an S-glycoside linkage can be regarded as a mass reducing end, which is concurrently set armed as an electrophilic entity. In consequence, thioglycoside methodology is an extraordinarily attractive and logical choice for the block synthesis of complex glycoconjugates.

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201 T. Matsumoto, H. Maeta, K. Suzuki and G. Tsuchihashi, Tetrahedron Lett., **29** (1988) 3567

202 S. Hanessian, C. Baquet and N. Lehong, Carbohydr. Res., **80** (1980) c17.

203 S. Sato, Y. Ito and T. Ogawa, Tetrahedron Lett., **29** (1988) 4759

204 V. Pozgay and H. Jennings, J. Org. Chem., **52** (1987) 4635

205 H. Lönn, Glycoconjugate J., **4** (1989) 117.

Considerable effort has been devoted towards developing an effective glycosidation for N-acetylneuraminic acid[87-90,117,118,206-208]. This considerable interest is no doubt due to the increasing number of reports concerning the involvement of sialic acid in various important biological functions[102-104]. Regarding the chemical synthesis of sialyl containing glycoconjugates, two main problems have been identified, the control of stereoselectivity to α-glycoside and the elimination reaction which gives the 2,3-dehydro glycal derivative instead of glycosides. To solve these problems, several new methods[55] have been suggested employing thioglycosides as glycosyl donors or using a participating 3-substituent such as OH-, Br-, PhS- and PhSe in sialosyl halide derivatives. The use of a 3-substituent gave highest α-selectivity of glycosides and prevented glycal formation, but lowered the total yield due to the extra steps required for introduction and removal operations of the 3- substituents. Thus, as a part of our continuing effort on the synthesis of α-sialylglycerolipids, the study of the glycosidation of N-acetylneuraminic acid using thiosialosides as glycosyl donors was investigated.

The investigation was performed in two stages. First, the efficiency of various thiophilic promoters with selected α-thiosialoside was compared. The second stage involved the application of the most efficient promoter on a series of α-thiosialosides in order to compare the influence of the nature of the thiosialated aglycon.

5.2 Discussion

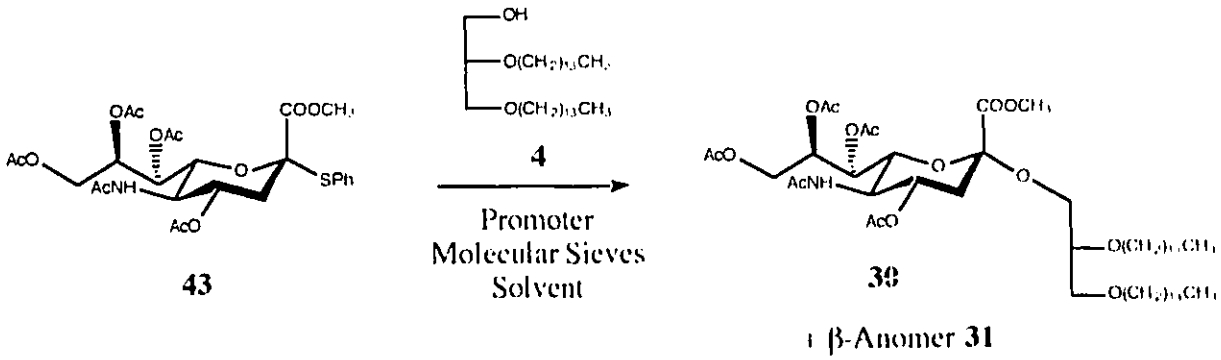
5.2.1 Study of Thiophilic Promoters using Phenyl α-Thiosialoside 43 as Glycosyl Donor

The study of the effect of various promoters was performed using phenyl α-thiosialoside **43** as glycosyl donor and glycerolipid **4** as acceptor. The selection of a thioaryl glycoside rather than of a thioalkyl glycoside was motivated by the belief that, in the glycosidation, the C-S cleavage step would be favored by the stabilized thiophenolate leaving group. All the glycosidation reactions were carried out under strictly anhydrous conditions in the presence of molecular sieves and all reagents were thoroughly dried. The results of the study are summarized in Table 14. The yields reported in Table 14 are the total yields of the anomeric mixtures of sialylglycerolipids **30**

206 T. Ogawa and M. Sugimoto, Carbohydr. Res., **135** (1985) c5.
207 H. Paulsen and U. von Deesen, Carbohydr. Res., **146** (1986) 147.
208 T. Kondo, H. Abe and T. Goto, Chem. Lett., (1988) 1657.

and **31** isolated from the reaction mixture. The α:β anomeric ratios were determined by ^{1}H NMR spectroscopy.

Table 14. Study of Glycosidation of Phenyl α-Thiosialoside **43**.



Promoter	Solvent	Temperature	Reaction Time	Yield of anomeric mixture of 30 and 31	α:β Anomeric Ratio
NBS	CH_2Cl_2	R.T.	> 3 days	0%	
NIS	CH_2Cl_2	R.T.	> 3 days	0%	
NIS/Triflic Acid	CH_2Cl_2	R.T.	> 3 days	0%	
IDCP	CH_2Cl_2	R.T.	> 3 days	0%	-
MeI	CH_2Cl_2	R.T.	> 3 days	0%	-
MeTf	CH_2Cl_2	R.T.	24 hours	37%	2:3
DMTST	CH_2Cl_2	R.T.	24 hours	81%	1:9
DMTST	CH_2Cl_2	-20°C	36 hours	72%	1:9
DMTST	$C_2H_4Cl_2$	R.T.	24 hours	75%	1:9
DMTST	CH_3CN	R.T.	> 3 days	0%	-
DMTST	CH_3CN	83°C	> 3 days	0%	-

The first thiophilic promoter used was N-bromosuccinimide (NBS). The NBS has previously been shown to promote the glycosidation of thioglycosides with alcohols[64,116]. Treatment of phenyl α-thiosialoside **43** with NBS at room temperature in methylene chloride did not result in the formation of any glycoside. Variation of solvent (toluene, nitromethane) or of the reaction temperature did not have any effect on the result of the reaction.

The use of iodonium ions was subsequently investigated. The sources of the ions employed were iodonium dicollidine perchlorate (IDCP)[209] or N-iodosuccinimide (NIS)[210,211] optionally in the presence of a catalytic amount of triflic acid. A mixture of phenyl α-thiosialoside **43** and of glycerolipid **4** in the presence of molecular sieves in methylene chloride was treated with IDCP (2eq) or NIS (1.2 eq) at room temperature. None of the reactions yielded any glycoside. No reaction was detected by TLC.

The use of halonium ions as thiophilic promoters did not successfully catalyze the glycosidation of phenyl α-thiosialosides **43**. In the published procedures[64,116,209-211], the halonium ions were never used on ketosic thioglycosyl donors such as α-thiosialosides. All examples found were performed with thioaldosides. The lack of reactivity observed for the α-thiosialoside can be explained by the increased steric hindrance of the ketosic thiosialoside and the presence of the carboxylic acid on the anomeric carbon which electronically disfavors oxonium formation. The thiophilic promoter has to be sufficiently thiophilic to overcome these factors.

The next type of thiophilic promoters examined were alkylating agents. More particularly, methyl iodide[212] and methyl triflate[213-215] were investigated. In the literature[212-215], these promoters have been shown to efficiently perform glycosidation using alkyl thioglycosides.

When methyl iodide was used as thiophilic promoter, the procedure typically involved exposing glycerolipid **4** and phenyl α-thiosialoside **43** to a solution of methyl iodide (1%, 3% or 10%) in methylene chloride. In none of the attempts was the formation of glycoside detected. Heating the reaction or changing the solvent (THF) did not produce any improvement.

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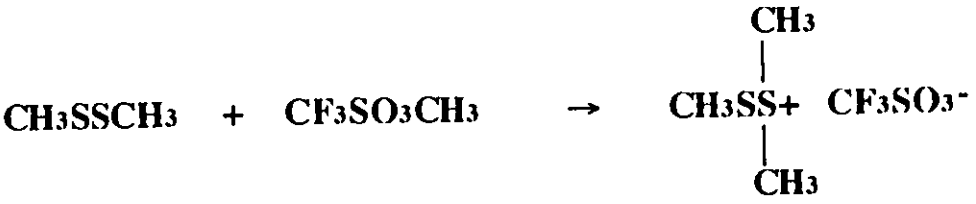
209 G.H. Veeneman and J.H. van Boom, Tetrahedron Lett., **31** (1990) 275.
210 G. H. Veeneman, S.H. van Leeuwen and J.H. van Boom, Tetrahedron Lett., **31** (1990) 1331.
211 P. Konradsson, U. E. Udodong and B. Fraser-Reid , Tetrahedron Lett., **31** (1990) 4313.
212 G. V. Reddy, V.R. Kulkarni and H.B. Mereyala, Tetrahedron Lett., **30** (1989) 4283.
213 H. Lönn, Carbohydr. Res., **139** (1985) 105.
214 H. Lönn, Carbohydr. Res., **139** (1985) 115.
215 H. Lönn, J. Carbohydr. Chem., **6** (1987) 301.

As seen from Table 14, the treatment of a mixture of phenyl α-thiosialoside **43** and of glycerolipid **4** in methylene chloride with methyl triflate yielded a mixture of α and β sialylglycerolipids **30** and **31** in 37% yield (α:β ratio 2:3). Unfortunately, the reaction was hindered by the simultaneous methylation of glycerolipid **4** resulting in the consumption of the aglycon. 3-O-Methyl-1,2-di-O-tetradecyl-*sn*- glycerol was isolated from the reaction mixture and identified by ^{1}H NMR and mass spectrometry. The use of various ratios of promoter:aglycon did not have any significant effect on the yield of the reaction.

The use of methylating agents as thiophilic promoters was met with more success than the use of halonium ions. Methyl triflate was found to be more efficient to promote the thioglycosidation of the aglycon than methyl iodide. The difference between the reactivity of the two methylating agents was attributed to the difference of leaving group ability of the iodide vs the triflate anion. Although the methyl triflate promoted reaction yielded a mixture of the α and β sialylglycerolipids **30** and **31**, the reaction was hindered by a side reaction, the methylation of the glycosyl acceptor.

The last thiophilic reagent considered as promoter for the glycosylation of glycerolipid **4** was dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST). This promoter was developed by Fügedi et al.[101] and was intended as a solution to the shortcomings of the promoter methyl triflate. The reasoning behind the development of that promoter was based on the knowledge that the regiospecific activation of a sulfur center should require the use of a soft Lewis acid. Alkylsulfenylation of the sulfur center would prevent O-alkylation, and the electrophilic intermediate would contain a leaving group better than the alkylsulfonium group. DMTST has successfully been used as a thiophilic promoter in the synthesis of sialosides using methyl thiosialosides in excellent yields[117,120].

The promoter DMTST was synthesized according to the method of Ravenscroft et al[216] depicted in Scheme 33.



Scheme 33. Synthesis of Dimethyl(methylthio)sulfonium Trifluoromethanesulfonate.

216 M. Ravenscroft, R.M.G. Roberts and J.G. Tillett, J. Chem. Soc. Trans. II, (1982) 1569.

The treatment of dimethyl disulfide in methylene chloride with methyl triflate (2eq) yielded, after 48 hours, the DMTST in 57% yield as white crystals (m.p. 50.2-54.5°C, lit.[216] m.p. 28-36°C).

Glycosidation of glycerolipid **4** with phenyl α-thiosialoside **43** in methylene chloride at room temperature in the presence of DMTST (4 eq.) and 4A molecular sieves afforded after 24 hours a mixture of the α and β anomers of sialylglycerolipid **30** and **31** in 81% yield. The anomeric ratio was 11: 9 in favor of the α anomer. Lowering the temperature had no significant impact on the anomeric ratio of sialosides but lowered slightly the yield and increased the reaction time.

The use of acetonitrile as reaction solvent, has been reported to favor the formation of α-sialosides[117,120]. This selectivity has been explained[217] by a mechanism involving the thermodynamically favored formation of a β-acetonitrium intermediate which undergoes S_N2 substitution at the anomeric center thus yielding the α-sialoside preferentially. These reactions were advantageously performed at low temperatures (-15°C). With this knowledge, the glycosidation of glycerolipid **4** was attempted in acetonitrile. Unfortunately, glycerolipid **4** was found to be absolutely insoluble in that solvent. Heating the reaction mixture in an attempt to improve the solubility of the glycerolipid failed. No sialosides were obtained from the reaction performed in acetonitrile.

By comparison with the methyl triflate catalyzed procedure, the method using DMTST as thiophilic promoter was vastly superior. The yields of DMTST catalyzed reactions were much higher since, as anticipated, no methylation of the glycerolipid was detected.

5.2.2 Study of the Glycosylation of Glycerolipid 4 with a Series of α-Thiosialosides.

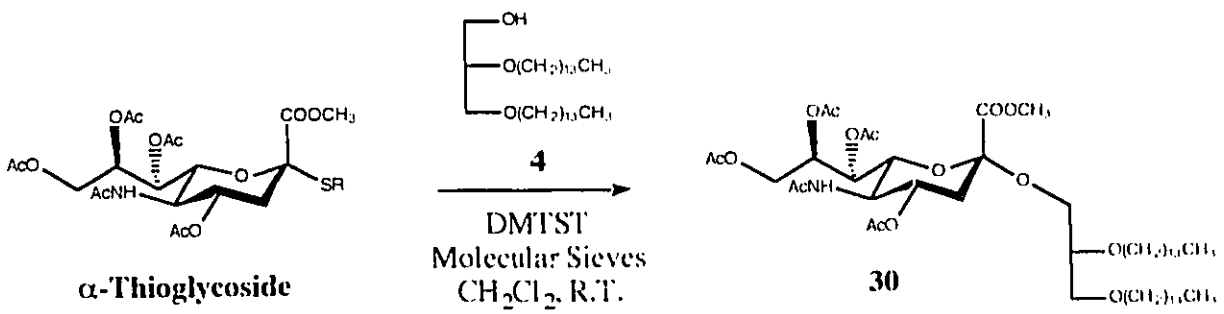
5.2.2.1 Study of the Glycosidation of a Series of α-Thiosialosides.

The study of the glycosidation of phenyl α-thiosialoside **43** indicated that DMTST was the most efficient catalyst to promote the glycosylation of glycerolipid **4**. Therefore, DMTST was

217 A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida and M. Kiso, J. Carbohydr. Chem., **10** (1991) 493.

selected as thiophilic promoter to study the glycosidation of a series of α-thiosialosides. The results of that study are summarized in Table 15.

Table 15. Glycosidation of the Series of α-Thiosialosides



Product #	R	Yield	α:β Anomeric Ratio	Reaction Time
43	Ph	81%	11:9	24 hours
46	Me	82%	3:2	1 hours
48	p-MePh	75%	1:1	16 hours
49	p-MeOPh	80%	1:1	8 hours
50	p-NO_2Ph	0%	-	> 3 days
51	2-Py	0%	-	> 3 days
52	1-Me-2-Im	0%	-	> 3 days

The glycosidations performed with the various α-thiosialosides gave very interesting results. Most of the α-thiosialosides were successfully glycosidated. In these reactions, the yields obtained were consistently between 75 and 82% and the α:β anomeric ratios of the sialylglycerolipids obtained varied from 3:2 for the glycosidation using methyl α-thiosialoside **46** to 1:1 for the p-methylphenyl and p-methoxyphenyl α-thioglycoside **48** and **49**. Furthermore, the glycosidation reaction using methyl α-thiosialoside **46** gave a 48% yield of the desired α-sialylglycerolipid **30**. This result represents an 11% improvement of the yield of α-

sialylglycerolipid **30** over the modified Koenigs-Knorr procedure[82]. The α:β anomeric ratio of the reaction was also slightly improved. The ratio obtained after the glycosidation using the thiosialyl donor **46** was 3:2 in favor of the α-anomer compared with 1:1 when chloride **22** was used as donor in the modified Koenigs-Knorr reaction conditions.

The first interesting result observed from the study concerns the reaction rate of the alkyl- vs the aryl α-thioglycosides. When the reaction was performed with methyl α-thiosialoside **46** as sialosyl donor, the reaction was observed to be very fast, as glycosides were obtained within 1 hour. The completion of the reaction for the methylphenyl-, methoxyphenyl- or phenyl α-thiosialosides **48**, **49** and **43** occurred respectively within 8, 16 and 24 hours. The difference of reaction rates between the alkyl and aryl thiosialosides can be attributed to the reduced electrophilicity of the anomeric sulfur atom of the aryl α-thioglycosides. Although it was originally believed that the formation of the activated cationic intermediate would be favored because of resonance stabilization of the cation by the aryl groups and that this would result in a quick reaction rate, it became apparent that the more important influence of the aryl groups was a resonance induced reduction of electrophilicity of the sulfur atom. By comparison with alkyl substituted α-thiosialosides which are not influenced by any resonance stabilization effect, the reaction rate of the glycosidation of aryl α-thiosialosides was reduced.

The second interesting result obtained from the study concerns the lack of reactivity of the nitrogen containing aromatic α-thiosialosides. Neither the pyridin-2-yl or the 1-methylimidazol-2-yl α-thiosialoside **51** and **52** were glycosidated under the DMTST catalyzed procedure. Although it was believed that the activation of these thiosialosides could have proceeded through direct activation of the anomeric sulfur atom or remote activation involving the nitrogen ring atom, no reaction occurred. The lack of reactivity of α sialosides **51** and **52** could be attributed to the low electrophilicity of the anomeric sulfur atom. Glycosidation of α-thiosialosides **51** and **52** was also attempted using methyl iodide as thiophilic promoter according to a procedure by Reddy et al.[212] but these attempts were not met with any success.

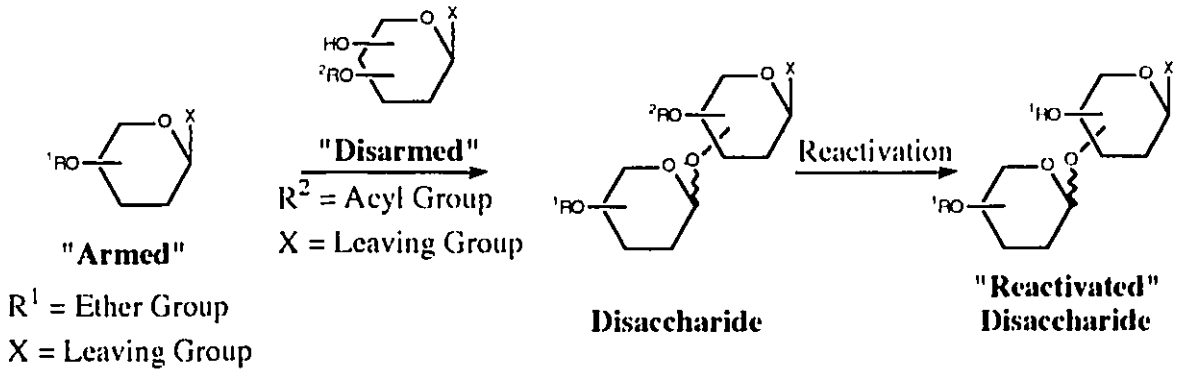
The last but most interesting result of the study was the outcome of the glycosidation of the substituted and unsubstituted aryl α-thiosialoside. Only the reactions using phenyl-, methylphenyl- and methoxyphenyl-α-thiosialoside **43**, **48** and **49** as sialosyl donors yielded glycosides. The nitrophenyl α-thiosialoside **50** was unreactive. Furthermore, the methyl and methoxy substituted phenyl α-thiosialosides reacted more quickly that the unsubstituted phenyl α-thiosialoside. From these observations, it was realized that the nature of the substituent on the arylthio moieties could be used to modulate the nucleophilicity of the sulfur atom toward thiophilic

promoters. Thus, when the phenyl group was substituted with an electron donating group, the thiophilicity of the sulfur atom was increased. The substitution of the phenyl group with an electron withdrawing group had the opposite effect. It reduced the thiophilicity of the anomeric sulfur atom rendering the thiosialoside inactive.

5.2.2.2 Introduction of the Concept of "Active" and "Latent" Thioglycosyl Donors.

The discovery of the controllable modulation of the anomeric sulfur nucleophilicities allowed the introduction of a conceptually new approach to oligosaccharide synthesis. The inspiration for this new approach originated from the remarkable recent contributions to oligosaccharide synthesis which rely on the concept of "armed" and "disarmed" glycosyl donors.

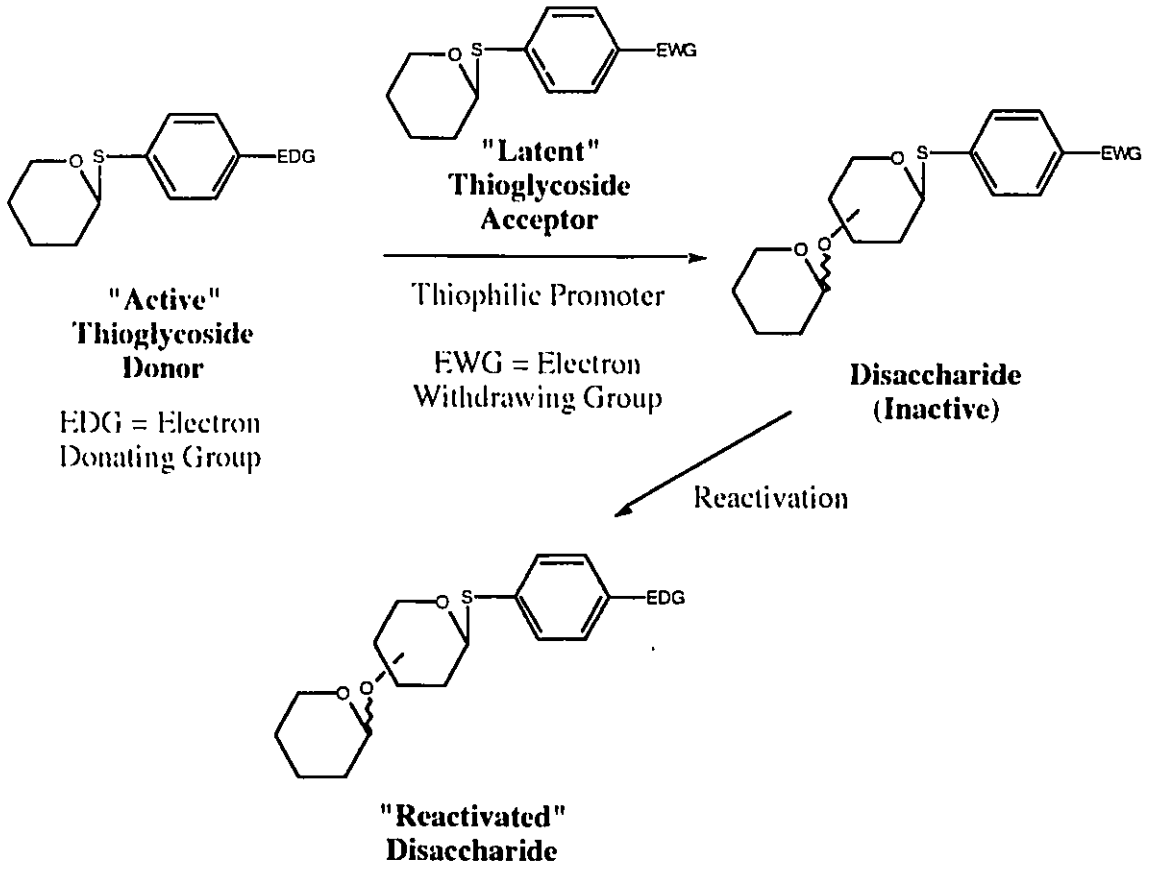
The concept of "armed" and "disarmed" glycosyl donors originated with the discovery that acylated glycosyl donors are glycosidated much more slowly than alkylated or benzylated glycosyl donors. This discovery suggested that glycosyl donors could be "armed" or "disarmed" by the type of protecting group placed on the free hydroxyl groups. Therefore, the synthesis of oligosaccharides was performed using an "armed" glycosyl donor which was glycosidated with a "disarmed" glycosyl acceptor bearing one free hydroxyl group. The disaccharide thus obtained could be "reactivated" by converting the acetyl protecting groups and used in further glycosidation reactions. This concept is illustrated in Scheme 34.



Scheme 34. "Armed" and "Disarmed" Glycoside Donors in Oligosaccharide Synthesis.

The concept of "armed" and "disarmed" glycosyl donors was found to be valid for various types of glycosyl donors such as n-pentenyl[218,219], thioglycosides[209,210] and glycals[67].

The realization that the nature of the substituent on the aryl α-thiosialosides could be used to modulate the thiophilicity of the anomeric sulfur atom was found to be analogous to the discovery that protecting groups could be used as "armed" or "disarmed" glycosyl donors. Thus an electron donating group placed in the para position of the aryl moiety of an aryl α-thioglycoside provided an "active" or "armed" glycosyl donor while electron withdrawing groups (like NO_2) afforded a "latent" or "disarmed" thioglycosyl donor. The "active" and "latent" thioglycoside donors could also be used as building blocks in blockwise oligosaccharide syntheses. This synthetic strategy is illustrated in Scheme 35.



Scheme 35. "Active" and "Latent" Thioglycosides in Oligosaccharide Synthesis.

218 D.R. Mootoo, P. Konradsson, U. Udodong and B. Fraser-Reid, J. Am. Chem. Soc., **110** (1988) 5583.
219 D.R. Mootoo, P. Konradsson and B. Fraser-Reid, J. Am. Chem. Soc., **111** (1989) 8540.

Similarly to the use of "armed" and "disarmed" glycosyl donors in oligosaccharide synthesis, the "latent" or "temporarily inactive" glycoside acceptor possessing one free hydroxyl group can be glycosylated with the "active" thioglycosyl donor yielding a disaccharide. The disaccharide can then be transformed into an "active" glycosyl donor by modification of the aryl substituent. The electron withdrawing group has to be converted into an electron donating group in order to be "reactivated". For example, an electron withdrawing group such as the nitro group can be transformed into an electron donating protected amino group (ex. NHAc: i- $SnCl_2$, EtOH, ii- Ac_2O, py). This transformation would in part "reactivate" the disaccharide which would be used as glycosyl donor in further glycosidation reactions. The transformation of the p-nitrophenyl α-thiosialoside **50** to the corresponding acetylated p-aminophenyl thiosialoside and the use of the latter as thiosialosyl donor was successfully accomplished by Roy et al[220]. This result confirmed the observation that aryl α-thiosialosides substituted with electron donating groups are "active" glycosyl donors useful in the synthesis of oligosaccharides.

These preliminary results confirm the concept of "active" and "latent" thioglycosyl donors as a complementary methodology to the "armed" and "disarmed" strategy and should add other controllable variables in blockwise oligosaccharide synthesis.

5.3 Conclusion

In conclusion, the usefulness of α-thiosialosides as glycosyl donors has been clearly demonstrated. Under carefully optimized reaction conditions, glycosidation of α-thiosialosides proceeded in high yields. Dimethyl(methylthio)sulfonium triflate was evaluated to be, by far, the best thiophilic promoter to catalyze the glycosidation of the thiosialosyl donors. The DMTST promoted glycosylation of glycerolipid **4** using methyl α-thiosialoside **46** gave a much improved yield of the desired α-sialylglycerolipid **30** over the previously used Koenigs-Knorr methodology.

The study of the glycosidation of substituted aryl α-thioglycosides resulted in the introduction of the concept of "active" and "latent" thioglycosyl donors. This concept constitutes an interesting tool for the blockwise synthesis of oligosaccharides.

220 R.Roy, F.O. Andersson and M. Letellier, Tetrahedron Lett., **33** (1992) 6053.

5.4 Experimental

5.4.0 General Methods

See Section **1.4.0** in Chapter 1.

5.4.1 General Thioglycosylation Procedures using Methyl (Phenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 43 as Glycosyl Donor.

Method A - Bromonium Ion as Promoter

A mixture of glycerolipid **4** (37 mg, 0.076 mmol, 1.5 eq), thioglycoside **43** (30 mg, 0.051 mmol) and 4Å powdered molecular sieves in a solvent (methylene chloride, toluene or nitromethane, 5 ml) was stirred under a nitrogen atmosphere at room temperature. The mixture was allowed to stir for 1 hour before recrystallized N-bromosuccinimide (9.9 mg, 0.056 mmol, 1.1 eq) was added. The reaction was monitored by TLC, eluent 2 x 100% ethyl acetate. The work-up of the reaction mixture involved dilution with methylene chloride (5 ml) followed by filtration through a Celite plug, successive washes of the organic layer with 10% W/V sodium bisulfite (2 x 10 ml), water (10 ml) and brine (10 ml), drying (anhydrous sodium sulfate), filtration and evaporation and furnished the crude reaction product.

Method B - Iodonium Ion as Promoter

A solution of of IDCP (47 mg, 0.10 mmol, 2eq) or of NIS (13.7 mg, 0.061 mmol, 1.2 eq) in a methylene chloride (2 ml) optionally containing a catalytic amount of triflic acid (0.12 eq) was added to a cooled, stirred mixture of thioglycoside **43** (30 mg, 0.051 mmol), glycerolipid **4** (30mg, 0.061 mmol, 1.2 eq) and 4Å powdered molecular sieves in the same solvent (4 ml). The mixture was sealed under nitrogen and was stirred at room temperature. The evolution of the reaction was followed by TLC (eluent 2 x 100% ethyl acetate). Dilution of the reaction mixture with methylene chloride (4 ml), filtration through Celite gave a solution which was washed with 10% aqueous sodium thiosulfate (10 ml), saturated sodium bicarbonate (10 ml) and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated in vacuo and the reaction products were obtained as a crude oil.

Method C - Methyl Iodide as Promoter

Thioglycoside **43** (29 mg, 0.050 mmol) and glycerolipid **4** (29 mg, 0.060 mmol, 1.2 eq) were dissolved in a 1%, 3% or 10% solution of methyl iodide in methylene chloride or ether (5 ml) containing powdered 4Å molecular sieves. The reaction mixture was stirred at room or reflux temperature and was monitored by TLC (eluent 2 x 100% ethyl acetate). The reaction was quenched by dilution with methylene chloride (5 ml) and filtration through Celite. The filtrate was washed with 0.1 M NaOH (2 x 10 ml), water (10 ml) and brine (10 ml). The solution was dried over anhydrous sodium sulfate, filtered and evaporated in vacuo. A crude oil was obtained.

Method D - Methyl Triflate as Promoter

Methyl triflate (28 µl, 0.25 mmol, 5 eq) was added to a solution of thioglycoside **43** (29 mg, 0.050 mmol) and glycerolipid **4** (29 mg, 0.060 mmol, 1.2 eq) in a solvent (toluene or methylene chloride, 5 ml) which contained powdered 4Å molecular sieves. The mixture was stirred at room temperature until completion of the reaction as detected by TLC (eluent: 2 x 100% ethyl acetate). Triethylamine was added to the mixture and the stirring continued for 10 minutes. The reaction mixture was diluted with the appropriate solvent (5 ml), filtered through Celite and the filtrate was washed with saturated sodium bicarbonate (2 x 10 ml), water (10 ml) and brine (10 ml). The solution was evaporated in vacuo and a crude oil was obtained.

Method E - Dimethyl(methylthio)sulfonium Triflate (DMTST) as Promoter

Synthesis of Dimethyl(methylthio)sulfonium Triflate

Dimethyl disulphide (0.23 ml, 2.54 mmol, 1.02 eq) in methylene chloride (5 ml) was added to a solution of methyl triflate (0.28 ml, 410 mg, 2.5 mmol) in methylene chloride with stirring at 25°C and the mixture was allowed to stand for 48 hours. The product was precipitated out of solution by the addition of dry ether to give white crystals of pure dimethyl (methylthio) sulfonium triflate (0.367 g, yield 57%); m.p. 50.2-54.5°C; lit.[216] 28-36°C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.29 (s, 6H, $(CH_3)_2S^+$), 2.68 (s, 3H, CH_3S); lit.[216] ^{1}H NMR (360 MHz, CH_2Cl_2): δ (ppm) 3.27 (s, 6H, $(CH_3)_2S^+$), 2.92 (s, 3H, CH_3S).

Thioglycosylation Procedure using Dimethyl(methylthio)sulfonium Triflate

Thioglycoside **43** (29 mg, 0.050 mmol) and glycerolipid **4** (29 mg, 0.060 mmol, 1.2 eq) were stirred in a solvent (methylene chloride, dichloroethane or acetonitrile, 5 ml) containing powdered 4Å molecular sieves under a nitrogen atmosphere. After stirring for 1 hour at room temperature, DMTST (58 mg, 0.225 mmol, 4.5 eq) was added. The reaction was performed at room temperature, at reflux or at -20°C (dry ice/CCl_4 bath). Thin Layer Chromatography was used to monitor the progress of the reaction (eluent 2 x 100% ethyl acetate). When the reaction was deemed complete the reaction mixture was brought to room temperature. Dilution of the mixture with the appropriate solvent followed by filtration through Celite resulted in a clear solution which was evaporated in vacuo, if the solvent was water soluble, and the oil obtained was solubilized in ethyl acetate. The solution obtained was washed with saturated sodium bicarbonate (2 x 10 ml), water (10 ml) and brine (10 ml), dried over anhydrous sodium sulfate and evaporated in vacuo. The crude oil obtained was flash chromatographed on silica gel (2% ethanol in methylene chloride) to isolate the glycoside mixture (α and ß anomeric mixture).

5.4.2 General Thioglycosylation Procedure using Methyl (Pyridin-2-yl or 1-Methylimidazol-2-yl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate 51 or 52 as Glycosyl Donor.

Method A - Methyl Iodide as Promoter

Thioglycoside **51** (30 mg, 0.050 mmol) or **52** (30 mg, 0.050 mmol) and glycerolipid **4** (29 mg, 0.060 mmol, 1.2 eq) were dissolved in methylene chloride (5 ml) containing powdered 4A molecular sieves. Methyl iodide was added to the solution in an amount such that a 3% or 10% solution resulted or so that there is 1.5 eq of methyl iodide to the thioglycoside. The reaction mixture was stirred at room temperature or at reflux for several days and was monitored by TLC (eluent 2 x 100% ethyl acetate). The reaction mixture was then diluted with methylene chloride (5 ml) and filtered through Celite. The filtrate was washed with saturated sodium bicarbonate (2 x 10 ml), water (10 ml) and brine (10 ml), dried over anhydrous sodium sulfate and evaporated. The crude products were obtained as an oil.

Method B - Dimethyl(methylthio)sulfonium Triflate as Promoter

Thioglycoside **51** (30 mg, 0.050 mmol) or **52** (30 mg, 0.050 mmol) and glycerolipid **4** (29 mg, 0.060 mmol, 1.2 eq) were stirred in methylene chloride (5 ml) containing powdered 4Å molecular sieves. After 1 hour of stirring at room temperature DMTST (58 mg, 0.225 mmol, 4.5 eq) was added and the mixture was stirred for several days while being followed by TLC (eluent 2 x 100% ethyl acetate). The mixture was then diluted and filtered through Celite. The clear solution obtained was washed with saturated sodium bicarbonate (2 x 10 ml), water (10 ml) and brine (10 ml), dried (anh. Na_2SO_4) and evaporated to yield a crude oil.

5.4.3 Thioglycosylations using a Series of Thiosialosides and Dimethyl(methylthio)sulfonium Triflate as Promoter

A thioglycoside (≈30 mg, 0.050 mmol), glycerolipid **4** (29 mg, 0.060 mmol, 1.2 eq) and 4Å powdered molecular sieves were suspended in methylene chloride. Stirring of the mixture at room temperature for 1 hour under a nitrogen atmosphere was followed by the addition of DMTST (58 mg, 0.225 mmol, 4.5 eq). The progress of the reaction was followed by Thin Layer Chromatography (eluent, 2 x 100% ethyl acetate). Upon completion of the reaction, the mixture was diluted with methylene chloride (5 ml) and filtered through Celite. The filtrate was successively washed with saturated sodium bicarbonate (2 X 10 ml), water (10 ml) and brine (10 ml), dried (anh sodium sulfate) and evaporated in vacuo. The crude oil obtained wad chromatographed on silica gel using a 2% ethanol in methylene chloride solution as eluent. The product was obtained as a crude oil and as a α:β mixture of anomers.

CONCLUSION

The synthesis of regio- and stereo-specifically labeled sialylglycerolipids **1a**, **1b** and **1c** was successfully accomplished.

The synthetic route designed to perform the synthesis was, at first glance very simple. It involved the coupling of the glycerolipid aglycon **4** with a protected sialosyl donor. Deprotection of the sialoside obtained yielded the desired glycoconjugate.

The aglycon, 1,2-di-O-tetradecyl-*sn*-glycerol **4** was synthesized according to a novel procedure which used 3,4-di-O-isopropylidene-D-mannitol as key intermediate instead of the more usual (R)-2,3-O-isopropylideneglyceraldehyde. In addition to having one less synthetic step, the procedure demonstrated several advantages over the previous procedure. The reaction intermediates and conditions were found to be safe against decomposition, polymerization, oxidation and racemization yielding a final product of high optical rotation. Furthermore, the synthetic route had the required flexibility to allow the introduction of a deuterium label in the last step of the procedure avoiding the needless repetition of numerous synthetic steps. The versatility of the novel procedure could easily be adapted to the synthesis of other glycerolipids bearing various lipophilic groups.

The glycosylation of glycerolipids **4** and **4a** was performed with sialosyl donors. Two types of donors were considered: acetochloroneuraminic acids and thiosialosides. Prior to the synthesis of the sialosyl donor, the stereoselective introduction of deuterons at the 3-position of sialic acid was accomplished through a base catalyzed enolization procedure which yielded selectively mono- and dideuteriated sialic acids **19a** and **19b**. Esterification of the unlabeled and labeled sialic acids yielded mono- and dilabeled sialic acid precursors which upon activation gave the necessary glycosyl donors.

Sialosyl chloride donors **22**, **22a** and **22b** were products of a new synthetic procedure involving the use of methanol in acetyl chloride solution. This highly efficient procedure simultaneously acetylated and activated the sialyl methyl esters to give the unlabeled and labeled glycosyl chlorides **22**, **22a** and **22b** in high yield.

Thiosialosyl donors were synthesized under phase transfer catalysis. PTC was found to be a mild, highly stereoselective and practical entry into useful α-thiosialosides. In addition to being

such an efficient method for the synthesis of the α-thiosialosides, the versatility of the phase transfer catalyzed procedure was convincingly demonstrated through the synthesis of important aryl- and heteroaryl-sialosides. A precursor to sialylated neoglycoproteins, a fluorogenic substrate and a sialosyl phosphotriester were easily synthesized using the phase transfer catalysis method.

The greatest challenge encountered in this research project was the glycosidation of the sialosyl donors. A search for reaction conditions which would favor the production of the α anomer and high reaction yields of the sialosides was made for both acetochloroneuraminic acid and α-thiosialoside donors. The glycosidations were hindered by several factors including the steric hindrance of the sialosyl donors and the competitive elimination reaction producing the 2,3-dehydro-derivative **26**. Sialosyl chlorides **22**, **22a** and **22b** were advantageously glycosidated under Koenigs-Knorr conditions using $Hg(CN)_2$:$HgBr_2$ as catalyst.

After careful optimization of reaction conditions, the DMTST catalyzed glycosidation of α thiosialosides proceeded in high yields and favored the production of the α-anomers of the sialylglycerolipid **30**. The glycosylation of glycerolipid **4** using α-thiosialosides gave improved yields of the desired α-sialylglycerolipid **30** over the previously used modified Koenigs-Knorr method. The study of the glycosidation of substituted aryl-α-thiosialosides resulted in the introduction of the concept of "active" and "latent" thioglycosyl donors. This concept constituted an interesting tool for the synthesis of oligosaccharides.

Deprotection of the labeled α-sialylglycerolipids **30a**, **30b** and **30c** completed the successful synthesis of the desired sialylconjugates **1a**, **1b** and **1c**.

^2H NMR study of the labeled sialylglycerolipids **1a**, **1b** and **1c** revealed information about molecular orientation and ordering of sialylglycerolipids in model membranes[97]. Deuterium nuclear magnetic resonance spectroscopy has become an excellent tool to probe membrane surfaces and to observe surface components directly. Recently[221], more attention has been paid to natural systems with all their increased complexity. Attempts are now made to correlate the various functions performed by membranes. Even closer collaborations between biologists and biophysicists will be necessary for this to succeed.

221 I.C.P. Smith, "NMR Principles and Applications to Biomedical Research", Ed. J.W. Pettigrew, (1990) pp 124-150.

CLAIMS

- **Novel Synthetic Methodology.**

Development of improved synthetic route for the synthesis of glycerolipids, in particular 1,2-di-O-tetradecyl-*sn*-glycerol **4** and **4a**.

- Development of an improved procedure for the synthesis of the important glycosyl donor methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate **22**.

- Development of a phase transfer catalyzed procedure for the efficient synthesis of α-thiosialosides, aryl and heteroaryl sialosides.

- Development of an improved procedure for the synthesis of α-sialylglycerolipids using methyl α-thiosialoside **46** as glycosyl donor anf DMTST as thiophilic promoter.

- Introduction of the concept of "Active" and "Latent" glycosyl donors for the blockwise synthesis of oligosaccharides.

- **The New Labeled Compounds:**

- Methyl 5-Acetamido-3,5-dideoxy-3a-deutero-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate **20a**.

- Methyl 5-Acetamido-3,5-dideoxy-3a,3e-dideutero-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate **20b**.

- Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-3a-deutero-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate **22a**.

- Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-3a,3e-dideutero-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate **22b**.

- O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3a-deutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol **30a**.

- O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3a,3e-dideutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol **30b**.

- O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol **30c**.

- O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3a-deutero-β-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol **31a**.

- O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3a,3e-dideutero-β-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol **31b**.

- O-[Methyl-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol **31c**.

- O-[Methyl-(5-acetamido-3,5-dideoxy-3a-deutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol **35a**.

- O-[Methyl-(5-acetamido-3,5-dideoxy-3a,3e-dideutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol **35b**.

- O-[Methyl-(5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol **35c**.

- Ammonium and Sodium O-[5-Acetamido-3,5-dideoxy-3a-deutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol **1a**.

- Ammonium and Sodium O-[5-Acetamido-3,5-dideoxy-3a,3e-dideutero-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-1,2-di-O-tetradecyl-*sn*-glycerol **1b**.

- Ammonium and Sodium O-[5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-3-deutero-1,2-di-O-tetradecyl-*sn*-glycerol **1c**.

- **Novel α-Thiosialosides:**

- Methyl (4-Methylphenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate **48**.

- Methyl (4-Methoxyphenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate **49**.

- Methyl (4-Nitrophenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate **50**.

- Methyl (Pyridin-2-yl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate **51**.

- Methyl (1-Methylimidazol-2-yl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate **52**.

- **The Novel Neoglycoprotein Precursors**

- Methyl (4-Formylphenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate **53**.

- Methyl (4-Formylphenyl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate **54**.

- 4-Formylphenyl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid **55**.

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