Sialic acids are a diverse family of naturally occurring 2-keto-3-deoxy-nononic acids that are involved in a wide range of biological processes. Sialic acids normally appear at terminal positions of oligosaccharides of glycoproteins and glycolipids where they are $\alpha(2,3)$ or $\alpha(2,6)$ linked to galactosides or $\alpha(2,6)$ linked to 2-acetamido-galactosides. The disialosyl structures Neu5Ac$\alpha(2\rightarrow8)$Neu5Ac and Neu5Ac$\alpha(2\rightarrow9)$Neu5Ac have also been found as constituents of oligosaccharides of glycoproteins and lipids. While relatively efficient methods have been developed for the introduction of Neu5Ac$\alpha(2\rightarrow3)$Gal and Neu5Ac$\alpha(2\rightarrow6)$Gal glycosidic linkages, the synthesis of oligosaccharides that contain $\alpha(2\rightarrow8)$-linked fragments is complicated by the low reactivity of the C-8 hydroxyl of Neu5Ac. The latter glycosides have been successfully synthesized by indirect sialylation approaches, whereas direct sialylation often leads to either low yields or formation of unnatural $\beta$-sialosides.

It is obvious that a versatile sialyl donor needs to be developed that gives excellent yields and high $\alpha$-anomeric selectivities in direct glycosylations with a wide range of acceptors of different reactivities. Such a donor would allow efficient synthesis of oligosaccharides of biological or medical importance that contain multiple sialic acids of different linkage type.

As a part of the program to develop novel synthetic approaches a new method for the stereoselective $\alpha$-sialylation has been developed. It has been shown that the readily available sialyl donor, methyl(methyl 4,7,8,9-tetra-O-acetyl-3,5-dIDEOXY-2-thio-5-trifluoroacetamido-D-glycero-$\beta$-D-galacto-nonulopyranosid) onate (1), gives good yields
and excellent $\alpha$-anomeric selectivities in direct glycosylations with a variety of glycosyl acceptors ranging from sterically hindered C-8 hydroxyls of a sialic acid (e.g. 2) to reactive primary alcohols such as C-6 hydroxyls of galactosides. It has been shown that the new sialylation approach allows efficient synthesis of Neu(2-3)Gal derivatives especially when sterically hindered alcohols are used as glycosyl acceptors.

The versatility of the donor allowed a highly efficient synthesis of a range of biologically important compounds. For example, the human melanoma associated antigen GD$_3$ derivative (5), which has multiple Neu5Ac residues.

INDEX WORDS: Sialic Acids, Novel Synthetic Methodology, Glycosylation, Carbohydrates,
A STEREOSELECTIVE APPROACH FOR THE SYNTHESIS OF ALPHA-SIALOSIDES

by

CRISTINA DE MEO

Master Degree from Universita’ degli studi di Catania, Italy, 1997

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2001
A STEREOSELECTIVE APPROACH FOR THE SYNTHESIS OF ALPHA-SIALOSIDES

by

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December 2001
To respect, understanding and love, without which life would be worthless.

To Silio, Maria, Marzia and Alexei, who taught me so.
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LIST OF ABBREVIATIONS

Å ........................................................... Angstrom
Ac .......................................................... Acetyl
AIBN ...................................................... 2,2’-Azobisisobutyronitrile
Ala .......................................................... Alanine
Arg .......................................................... Arginine
Bn ............................................................ Benzyl
Boc ........................................................... tert-Butyloxycarbonyl
Bu ............................................................. Butyl
Bz ............................................................. Benzoyl
CAD ........................................................ Coronary artery disease
CBz ........................................................ Benzyloxycarbonyl
CMP ......................................................... Cytidine monophospho
CRD ........................................................ Calcium dependent recognition domain
CSA ........................................................... (±)10-Camphorsulfonic acid
d ............................................................... Duplet
DBU ......................................................... 1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE .......................................................... 1,2-Dichloroethane
DCM ........................................................ Methylene chloride
dd ............................................................. Double duplet
DDP .......................................................... Dibenzyl N,N-diethylphosphoro amidite
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tr>
<td>DMAP</td>
<td>N,N-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMTST</td>
<td>Dimethyl(methylthio) sulfonium trifluoromethanesulfonate</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor domain</td>
</tr>
<tr>
<td>ESL-1</td>
<td>E-Selectin Ligand</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>ETEC</td>
<td>Enterotoxigenic E. coli</td>
</tr>
<tr>
<td>FAB-MS</td>
<td>Fast atom bombardment mass spectroscopy</td>
</tr>
<tr>
<td>Gal</td>
<td>Galactose</td>
</tr>
<tr>
<td>Gc</td>
<td>Glycolyl</td>
</tr>
<tr>
<td>gCOSY</td>
<td>gradient Correlation Omonuclear Spectroscopy</td>
</tr>
<tr>
<td>gHSQC</td>
<td>gradient Correlation Heteronuclear Spectroscopy</td>
</tr>
<tr>
<td>Glc</td>
<td>Glucose</td>
</tr>
<tr>
<td>GlcNAc</td>
<td>N-Acetyl glucosamine</td>
</tr>
<tr>
<td>GLS</td>
<td>Glycosphingolipids</td>
</tr>
<tr>
<td>GlyCAM-1</td>
<td>Glycosylation-Dependent Cell Adhesion Molecule</td>
</tr>
<tr>
<td>HA</td>
<td>Hemmagglutinin</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IDCP</td>
<td>Iodonium dicollidine perchlorate</td>
</tr>
<tr>
<td>Cer</td>
<td>Ceramide</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoproteins</td>
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</table>
Heat-labile
Lysine
Multiple
Mass to charge ratio
Monoclonal antibodies
Mucosal Addressin Cell Adhesion Molecule
Myelin-associated glycoprotein
Mass assisted laser desorption ionization time-of-flight
Methyl
Molecular sieves
Neural Cell Adhesion Molecules
Neuraminic Acid
Neuramidase
N-iodosuccinimide
Nuclear magnetic resonance
PodoCalyxin-Like Protein
Polyethylene glycol
Phenyl
Phthalimido
Parts per million
Polysialic acid
P-Selectin Glycoprotein Ligand-1
Quartet
RBC................................................................................................................Red blood cell
Rf .......................................................................................................................Retention factor
s ......................................................................................................................... Singlet
Ser................................................................. Serine
SLe\(^a\) .................................................................Sialyl Lewis a
SLe\(^x\) .................................................................Sialyl Lewis x
SMP........................................................................................ Schwann cell myelin protein
Sn..............................................................................................................Sialoadhesin
ST.........................................................................................................Heat-stable
t.................................................................................................................. Triplet
TE ........................................................................................................2-(Trimethylsilyl)ethyl
TFA.................................................................................................. Trifluoroacetyl
Tf..........................................................................................Trifluoromethanesulfonylethyl triflate)
THF .................................................................................................Tetrahydrofuran
TLC ..................................................................................................Thin layer chromatography
TMS..................................................................................................Trimethylsilyl
Ts...................................................................................................p-Toluenesulfonyl
Z ...................................................................................................Benzylxycarbonyl
CHAPTER 1

INTRODUCTION

1.1 General features, occurrence and functions of sialic acids

Sialic acids are a family of about 40 naturally occurring 2-keto-3-deoxy-nononic acids involved in a wide range of biological processes.\(^1\)\(^2\) The C-5-amino derivative represents the long-known neuraminic acid and its amino function can either be acetylated (Neu5Ac) or glycolylated (Neu5Gc, Figure 1.1.1), while at all non-glycosidic hydroxyl residues one or several acetyl groups may be present: generally, there is only one O-acetyl group present at O-9, but di- and tri-O-acetylated sialic acids are also found, most often in mucins, from bovine submandibular gland and human colon. The sugar ring of Neu5Ac has a \(^2\text{C}_5\) conformation in which the bulky side chain and C-5 acetamido moiety adopt equatorial orientations.

[Chemical structures of Neu5Ac, Neu5Gc, and KDN]

**Figure 1.1.1**: Sialic acid
Sialic acid is widely distributed throughout mammalian tissues. It is found in several human body fluids, including serum, cerebrospinal fluid, saliva, urine, amniotic fluid, and milk. It has also been detected in particularly high levels in rat brain, adrenal gland and heart, as well as in human brain. Thus, the coincidence of high amounts of sialic acid containing oligosaccharides in colostrum and early milk, and the high need for sialic acid during early brain development, is considered as indicative of the usefulness of milk dialooligosaccharides. Sialic acid is found in red blood cell (RBC) membranes, and sialic acid levels in RBCs are actually decreased in aging persons and alcoholics. Most sialic acids are glycosidically linked to other sugar residues of oligosaccharides, glycoproteins, gangliosides and glycolipids. In contrast, the 2,3-unsaturated sialic acids, usually Neu2en5Ac, lack a glycosidic hydroxyl residue and, therefore, exist only as free monosaccharides in very low concentrations in the body fluids of man and animals. Another neuraminic acid incapable of binding glycosidically to other sugars is 2,7-anhydro-\(N\)-acetylneuraminic acid (Neu2,7-an5Ac). \(N\)-Acetylneuraminic acid (Neu5Ac), \(N\)-glycolylneuraminic acid (Neu5Gc) and \(N\)-acetyl-9-O-acetylneuraminic acid (Neu5,9Ac2) are the three most frequently occurring sialic acids. Only Neu5Ac is ubiquitous, while the others are not found in all species. The best investigated example after Neu5Ac is Neu5Gc, which occurs often in the animal kingdom, but not in healthy human tissue and not in bacteria. In N-linked glycoproteins, sialic acids appear essentially as terminal sugars \(\alpha(2\rightarrow3)\) or \(\alpha(2\rightarrow6)\)-linked to galactosides or \(\alpha(2\rightarrow6)\)-linked to N-acetyl-galactosaminides {e.a. Neu5Ac\(\alpha(2\rightarrow3)\)Gal, Neu5Ac\(\alpha(2\rightarrow6)\)Gal and Neu5Ac\(\alpha(2\rightarrow6)\)GalNAc} whereas in O-linked glycoproteins, often terminal Neu5Ac\(\alpha(2\rightarrow6)\)GalNAc moieties can be found (Figure 1.1.2). The disialosyl structures
Neu5Acα(2→8)Neu5Ac and Neu5Acα(2→9)Neu5Ac have also been found as constituents of glycoproteins and glycolipids. Neu5Ac or Neu5Gc also occur in linear homopolymers where they are usually linked internally by α(2→8), α(2→9) or alternating α(2→8)/α(2→9) glycosidic linkages. These polysialic acids are found in glycoproteins of embryonic neural membranes, where they play a role as neural cell adhesion molecules. They are also found in fish eggs and in the capsule of bacteria such as Neisseria meningitidis group B.  

![Figure 1.1.2: Main occurrence of sialic acid in glycoproteins and glycolipids](image)

As components of sugar-protein and sugar-lipid compounds (glycoconjugates), sialic acids cover all cells of higher animals and man with an negatively charged coat and exert a variety of different biological functions. In fact, in this exposed position sialic acids are important regulators of cellular and molecular interactions, where they play a
dual role. They can either mask recognition sites or serve as recognition determinants. As a masking agent, sialic acid can prevent biological interaction with hormone receptors or antigens, cooperating with other members of the glycan chain, as well as offering a more specific protection of subterminal galactose or N-acetylgalactosamine residues. Enzymatic removal of a sialic acid residue in the first case may induce better access of molecules to cell surfaces or increase antigenicity, while in the second case the exposed galactose can lead to the interaction with galactose-recognizing receptors. Indeed, acetylation of the C-9 hydroxyl of sialic acid prevents the attachment of influenza A and B viruses, whereby O-acetylation or N-acetyl hydroxylation hinders the action of sialidases leading to longer life times of rat erythrocytes. For its ability to modulate cellular aggregation and attachment, membrane sialic acids prevent aggregation due to electrostatic repulsion in blood platelets, erythrocytes, and carcinoma cells in culture. Sialic acid appears to function in immune modulation as well. It inhibits bronchial anaphylaxis in the guinea pig model, and desialylation of peripheral blood mononuclear cells promotes growth of HIV-1 in vitro. Another interesting aspect of the biological proprieties of sialic acid is its alteration in certain diseases, suggesting a functional role for the sugar and/or its glycoconjugates. For example, sialic acid is markedly decreased in upper airway epithelial cells in severely ill patients, which could be important in the development of opportunistic respiratory infections in these patients, since sialic acid glycoconjugates normally prevent cell aggregation (i.e. attachment of bacterial cells). The quantity and quality of sialic acid is tissue- and organ-specific. A change in the amount of sialic acids on the cell surface, or in organs and tissue, or a disturbance of sialic acid metabolism is very dangerous to the health of individuals. For example, a reduction of
sialic acids in red blood cells leads to their entrapment in liver and spleen and thus to anemia. On the other hand, sialic acid conjugates may be increased in cancerous tissue, which can stimulate tumor growth and facilitate the formation of metastases.\(^3\)

1.2 Sialic acid as determinant cell-cell recognition

Selectins and Siglecs

For many years evidence has accumulated that sialic acids function in cellular interactions either in masking or as recognition sites for pathogen’s host cells. However, just a decade ago, a new class of carbohydrate binding glycoproteins, termed selectin, was found on the surface of certain cells and designated E-(endothelium)\(^{16}\), P-(platelet)\(^{17}\) and L-selectin (lymphocyte)\(^{18,19}\). Selectins\(^{20}\) are members of the C-type (Ca\(^{++}\) dependent) lectin family, expressed on the surface of leukocytes and activated endothelial cells. Each selectin comprises five domains: a cytosolic tail that may play a role in signal transduction, a transmembrane domain, a series of complement-like molecules (CR), an epidermal growth factor domain (EGF), and an N-terminal calcium dependent recognition domain (CRD). L-selectin, the smallest of the vascular selectins, is present on most leukocytes. P-selectin is the largest selectin, expressed on activated platelets and endothelial cells. E-selectin is expressed on activated endothelium with chemically or cytokine-induced inflammation. During an inflammation, selectins are involved in the attachment of leukocytes from the blood stream.\(^{21}\) The migration of circulating leukocytes from the blood into inflamed tissues is a "three step" process comprising: (a) rolling of leukocytes along the endothelial wall, followed by (b) activation of both neutrophils and endothelial cells leading then to (c) extravasation (crawling along the
endothelium and migration into tissue) in response to a chemoattractant gradient (Figure 1.2.1).

**Figure 1.2.1:** Migration of circulating leukocytes from the blood into inflamed tissue

The first contact of a leukocyte with the activated endothelium is known as capture or tethering. Capture occurs after margination, which allows leukocytes to move in a position close to the endothelium, away from the central blood stream. During the inflammatory response, endothelial activation is required to initiate the capture. P-selectin on endothelial cells, is the primary adhesion molecule for capture and the initiation of rolling. The main leukocyte ligand for P-selectin is PSGL-1 (P-Selectin Glycoprotein Ligand-1). In addition, many studies in vivo suggest that L-selectin on leukocytes plays an important role in the capture as well. Once leukocytes are captured, they may transiently adhere to the venular endothelium and begin to roll. Each of the three selectins can mediate leukocyte rolling given the appropriate conditions. For example, when P-selectin is absent, trauma-induced rolling becomes L-selectin dependent, but the average
leukocyte rolling velocity is three to five times faster in this case. This suggests that L-selectin is much less efficient than P-selectin in mediating the rolling process. However, L-selectin is necessary for the normal inflammatory response in capturing leukocytes and initiating rolling. An apparent redundancy exists between P- and E-selectin in mediating leukocyte rolling on cytokine-activated endothelium. E-Selectin is thought to be responsible for slow rolling interactions below 5μm/s and possibly the initiation of firm adhesion.

As described above, the adhesion cascade of leukocytes requires bond formations between the selectins and their ligands. PSGL-1 (P-Selectin Glycoprotein Ligand) has been characterized as a ligand for P-selectin. For L-selectin, four ligands have been identified: GlyCAM-1 (Glycosylation-Dependent Cell Adhesion Molecule), CD34, MAdCAM-1 (Mucosal Addressin Cell Adhesion Molecule), and PCLP (PodoCalyxin-Like Protein). Evidence suggests that both the presences of sulfate groups and sialic acid bound through α(2,3) linkage are essential to L-selectin ligand activity. E-selectin ligands are expressed by neutrophilis, monocytes, eosinophilis, memory-effector T-like lymphocytes, and natural killer cells. Three different glycoproteins have been suggested as E-selectin ligand candidates: PSGL-1, ESL-1 (E-Selectin Ligand), and a third protein whose structure and other functional proprieties unknown.

All three selectins can recognize glycoproteins and/or glycolipids containing the tetrasaccharide sialyl-Lewis α (SLeα, Figure 1.2.2). This tetrasaccharide is found on all circulating myeloid cells and is composed of sialic acid, galactose, fucose, and N-acetyl-glucosamine.
Table 1.2.1

<table>
<thead>
<tr>
<th>Selectin</th>
<th>Expressed</th>
<th>GP-Ligands determinant</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>Activated endothelia in response to an injury signal</td>
<td>ESL-1, PSGL-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SLex, SLex, SLexSO3, SLexSO3)</td>
</tr>
<tr>
<td>L</td>
<td>Leukocytes, always</td>
<td>GlyCAM-1, CD34, MadCAM-1, PCLP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SLex, SLex, SLexSO3, SLexSO3, SLexSO3)</td>
</tr>
<tr>
<td>P</td>
<td>Activated platelets and endothelia in response to a signal, i.e. thrombin</td>
<td>PSGL-1,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SLex, SLex)</td>
</tr>
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</table>

The affinity of the selectins toward sialyl-Lewis\textsuperscript{x} is stronger for E-selectin and weaker for P-selectin. This result proves a different role of the selectins for different stages in the inflammatory process. In particular, for SLex interaction with E- and L-selectins, it has been shown that the three hydroxyls of fucose\textsuperscript{23,24} together with the hydroxy groups of galactose in position 2 and 6,\textsuperscript{25} and the carboxylate of sialic acid\textsuperscript{23} are required for the binding. The GlcNAc residue does not play a critical role in these interactions, but is believed to be important for reorganizing the residues of the tetrasaccharide.\textsuperscript{25-27} For P-selectin, the 2- or 4- hydroxy group of fucose seems to not be essential, while sulfation at the 6-position improves binding (Figure 1.2.2).\textsuperscript{23} Modifications of sialyl-Lewis\textsuperscript{x} also influence the binding: for example, L-selectin’s affinity increases when a sulfate group is on the 6-position of Gal or perhaps more likely on the 6-position of the GlcNAc residue.\textsuperscript{21}
With respect to the sialic acid residue, it is not clear which amino acid is essential for the binding: the basic amino acid Lys111 (P-selectin), Lys113 (P- and E-selectin) and Arg97 (E-selectin) have been considered as relevant from some experiment, but not from others. In addition, it has been reported that E- and P-selectin were converted from sialo- to mannoside binding proteins by substitution of Ala 77 to Lys. 28

More recently, the Siglec (Sialic acid binding Immunoglobulin-like lectin) family was defined within the superfamily of immunoglobulin-like molecules. Sialoadhesin (Sn, siglec-1), CD22 (siglec-2), CD33 (siglec-3), the myelin-associated glycoprotein (MAG, siglec-4a) and the Schwann cell myelin protein (SMP, siglec-4b) belong to this family. Recently, siglec-5, 29 has been identified by random sequencing of cDNA libraries. Up to date, four other siglecs have been reported, raising the members of the family to ten. 30-35

In contrast to the selectins, which are involved only in the initiation of leukocyte binding to specific endothelia, these proteins are associated with diverse biological processes, i.e. hemopoiesis, neuronal development and immunity. In addition, while the selectins accept considerable structural modifications of the sialic acid molecule, like shortening of the glycerol side chain, 23,36,37 sialoadhesins are truly sialic acid-dependent adhesion
receptors. Apart for MAG and SMP, found exclusively in the nervous system, all siglecs are expressed within the hemopoietic and immune systems (Table 1.2.2). In particular, Sn is expressed by a subset of macrophages; CD22 is found on B cells, CD33 on immature myeloid cells, while siglec-5 on mature myeloid cells. One of the most common features of all the members of the sialoadhesin family is the high distribution of cysteine residues in the first two N-terminal domains. Furthermore, it is likely that the latter contain the complete binding site for specific sialylated glycans. In fact modified proteins of CD22 or Sn, containing only the N-terminal two domains, showed the same specificity for sialylated glycans as the native protein.

| Table 1.2.2 |
|------------------|------------------|------------------|------------------|------------------|
| Sialoadhesin     | Distribution     | Sialic acid linkage and type | Target cells (proposed function) |
| Sn               | Macrophage subpopulation | 2,3>2,6 Neu5Ac>> Neu5Gc, Neu5,9Ac2 | Myeloid cells (macrophage cell interaction molecule) |
| CD22             | B cells          | 2,6>>2,3 Neu5Ac= Neu5Gc>>Neu5,9Ac2 | Lymphocytes (negative regulator of B cell activation) |
| CD33             | Myelomnocytic cells | 2,3>2,6          | Myelomnocytic cells |
| MAG              | Myelin of oligodendrocytes and Swann cells | 2,3>2,6 Neu5Ac>> Neu5Gc, Neu5,9Ac2 | Neurons, oligodendrocytes (mylin-axon interactions, regulation of neurite growth |
| Siglec-5         | Neutrophils, monocytes | 2,3=2,6          | Neutrophil cellular interaction |

The mode of carbohydrate recognition by Sn occurs through interactions between the protein and the substituents of sialic acid, in particular the carboxylate, the acyl group
and the glycerol side chain.\textsuperscript{40} Although the most complete information is available for Sn, some studies suggest that MAG and CD22 have very similar binding sites for sialic acid. However, the interactions with the substituent at C-5 of sialic acid seem to be different among the individual siglecs. In fact, while an aromatic group (Trp\textsuperscript{2}) of Sn interacts with the methyl of the N-acetyl group, in case of MAG and CD22 other amino acids are likely to contribute to the binding with the nitrogen amide instead of its substituents. This is proved by an increased binding for MAG and CD22 after hydroxylation of the N-acetyl (Neu5Gc) or its replacement with a hydroxyl group, while both modifications interfere with Sn binding. In addition, the increased H-donor capacity of the nitrogen after halogenation is followed by an improvement of the binding proprieties of MAG.\textsuperscript{40}

The discovery and molecular characterization of siglec proves sialic acid as a recognition molecule in a variety of cellular activities, ranging from myelin-axon interactions to regulation of B cell activation to macrophage adhesive proprieties.

\textit{Polysialic acid and N-CAM}

Polysialic acids (PSA) are a structurally diverse family of linear carbohydrate chains that consist of Neu5Ac or NeuGc residues, usually joined internally by $\alpha$2,8, $\alpha$2,9, or alternating $\alpha$2.8/$\alpha$2.9 ketosidic linkages.\textsuperscript{12} The most thoroughly characterized polysialylated glycoproteins are the N-CAM’s (Neural Cell Adhesion Molecules). N-CAM is a member of the immunoglobulin gene superfamily. Functionally, NCAM mediates cell-cell adhesion both in neural cells and a variety of other cell types through both a homophilic mechanism (NCAM to NCAM) and a heterophilic mechanism in which NCAM binds to heparin sulfate proteoglycans. A unique structural characteristic
of the embryonic form of N-CAM is the presence of α2,8-linked PSA chains that cap N-linked oligosaccharide on the NCAM polypeptide. (Figure 1.2.3)

![PSA and NCAM Diagram]

**Figure 1.2.3:** Polysialic acids and Neural cell adhesion molecules

Maximum expression of the PSA glycotope on N-CAM is usually found on embryonic tissues, when cells are migratory, but is greatly reduced in adult tissue, limiting its expression in the retina, optic nerve, brain and spinal cord. Thus, PSA expression influences the adhesiveness of one cell toward another.\(^\text{43}\) Cells with a relatively low amount of sialic acid on their N-CAM aggregate four times more readily than those with the high levels of sialic acid.\(^\text{44}\) As the embryo gets older, most of the N-CAM proteins progress from the high-sialic acid to the low-sialic acid forms,\(^\text{45,46}\) which may contribute to stabilize mature tissues. In this manner, PSA in N-CAM may play a role both in stimulating two adjacent cells to form regions of contact and in inhibiting such contact.\(^\text{47}\) Overexpression of PSA in tumor cells will be described further.
1.3. Sialic acid as recognition determinant for pathogens

Sialic acid-specific adhesion of bacteria and viruses is a phenomenon drawing an increasing level of interest, as a critical step in infectious diseases. It has been known for many years that microbial pathogens, i.e., viruses, mycoplasma, bacteria, and protozoa, take advantage of cell surface sialic acids to adhere to their respective host cell6 (Table 1.3.1).

Table 1.3.1

<table>
<thead>
<tr>
<th>MICROORGANISM</th>
<th>SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VIRUSES</strong></td>
<td></td>
</tr>
<tr>
<td>Influenza A and B</td>
<td>Neu5Ac</td>
</tr>
<tr>
<td>Influenza C</td>
<td>Neu5,9Ac2</td>
</tr>
<tr>
<td>Corona virus</td>
<td>Neu5,9Ac2</td>
</tr>
<tr>
<td>Sendai virus</td>
<td>Neu5Ac</td>
</tr>
<tr>
<td>Polyoma virus</td>
<td>Neu5Ac,α2,3Galβ1,3GalNAc</td>
</tr>
<tr>
<td>Rotavirus group C</td>
<td>Neu5Ac</td>
</tr>
<tr>
<td><strong>BACTERIA</strong></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em>, K99</td>
<td>Neu5Gc</td>
</tr>
<tr>
<td><em>Escherichia coli</em>, S-fimbrie</td>
<td>Neu5Ac,α2,3Gal</td>
</tr>
<tr>
<td>New born human meningitis</td>
<td>Neu5Gc-GM3, GD3, GD1b</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Neu5Ac</td>
</tr>
<tr>
<td><em>Streptococcus suis</em></td>
<td>Neu5Ac,α2,3Galβ1,4GlcNAcβ1,3Gal</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Neu5Ac,α2,3Lac&gt; Neu5Ac,α2,6Lac</td>
</tr>
<tr>
<td><strong>TOXINS</strong></td>
<td></td>
</tr>
<tr>
<td>Pertussis toxin</td>
<td>Neu5Ac</td>
</tr>
<tr>
<td>Tetanus toxin</td>
<td>Sialoglycolipids</td>
</tr>
<tr>
<td><strong>PROTOZOA</strong></td>
<td></td>
</tr>
<tr>
<td>Malaria (MSA-1)</td>
<td>Neu5Ac</td>
</tr>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td></td>
</tr>
<tr>
<td>Chagas disease</td>
<td>Neu5Ac</td>
</tr>
<tr>
<td><em>Trypanosoma cruzi</em></td>
<td></td>
</tr>
</tbody>
</table>
**Bacteria and Protozoa**

Binding of pathogenic bacteria and bacterial toxins to host cell surfaces is an essential step in establishing infection in tissues and producing a toxic effect. Since cell surfaces are covered with glycoconjugates, it is not surprising that an increasing number of sialic acid-specific bacterial adhesins have been discovered. For example, *Helicobacter pylori* is a human pathogen associated with chronic type B gastritis and peptic ulcer. The bacterial colonization is due to the presence of sialic acid–binding surface proteins specific for a 2-3-linked sialic acid of the cell surface. *Streptococcus suis*, common cause of sepsis, meningitis, and other serious infections in young piglets and meningitis in humans, recognizes the sequence Neu5Acα2-3Galβ1-4GlcNAc. As a pathogen, *Escherichia coli* is known for its ability to cause intestinal diseases. Of the five virotypes, Enterotoxigenic *E.coli* (ETEC) are major causative agents of traveler’s diarrhea as well as often fatal diarrheal diseases in infant and young children. Enterotoxins produced by ETEC include the LT (heat-labile) and ST (heat-stable) toxins. The adherence of LT to ganglioside GM1 (Figure. 1.3.1) present on the surface of the epithelial cells lining is the first step in the induction of diarrhea.

![Figure 1.3.1: GM1 Gangloside](image)
Little is known about the roles of sialic acid in the pathobiology of protozoa. The most prominent example is *Plasmodium falciparum* protozoan parasite causing malaria, which replicates in the host's erythrocytes. Sialic acid on glycophorin A and possibly other glycoproteins are the primary attachment sites for the invasion. Other protozoa of particular interest are Trypanosomes, since some species, like *Trypanosoma cruzi*, *T. brucei* and *T. congolense*, express unusual enzymes, the trans-sialidases. These enzymes hydrolyze sialic acid from glycoconjugates like regular sialidases, but at a much higher rate they transfer these sialic acid residues to the terminal Gal moieties of glycans on the protozoan cell surface or to exogenously added substrates.

**Viruses**

The world has been aware of the disease and symptoms of influenza for centuries with periodic epidemics recorded in the 19th and 20th centuries. The flu pandemic of 1918-1919 led to an estimated 20 million deaths around the world—more than the number killed in the battles of World War I.\(^{51}\) Influenza and other viruses, the myxoviruses, were the first to be shown to use sialic acid as recognition determinants for interactions with their host cells. Historically, the interaction of influenza A virus with cell surface sialic acid has been the first study of sialic acid function reported. In fact, these experiments were performed before the structure of sialic acid had been elucidated. Influenza type A and B viruses have two surface proteins, hemagglutinin (HA) and neuramidase (NA). The first binds cell surface sialic acid to initiate infection, and also mediates fusion of host and viral membranes, while NA removes sialic acid from progeny virions as they bud from infected cells to halt viral self-agglutination. (Figure 1.3.2) The influenza C receptor
binding activity recognizes 9-O-acetylated sialic acids, and the receptor destroying activity is sialate 9-O-acetylesterase. Receptor binding, destruction, and fusion functions are provided by a single glycoprotein, HEF.

![Figure 1.3.2: Influenza A and B life cycle](image)

**Figure 1.3.2:** Influenza A and B life cycle

Influenza A and B viruses preferentially bind to N-acetylneuraminic acid, though some strains may additionally recognize N-glycoloylneuraminic acid or N-acetyl-9-O-acetylated sialic acid. Apart from the type of sialic acid, the binding of influenza virus is also dependent on the linkage that connects the sialic acid residue with the oligosaccharides of the receptor molecule. In general, strains of influenza viruses have a preference for Neu5Acα(2→3)Gal and Neu5Acα(2→6)Gal. For the interaction of sialic
acid with the hemagglutinin, the N-acetyl group and the orientation of the carboxy group are critical, whereby in the glycerol side chain, the hydroxy groups at C-7 and C-8 appear to be important for binding through hydrogen bonds. (Figure 1.3.3)

**Figure 1.3.3:** Model for the binding site of the influenza A hemagglutinin

In addition, sialic acid binds NA mainly through its glycerol chain and the carboxylate group. Recently, several potent inhibitors of NA have been synthesized based on the mechanism and structure of this enzyme, while the development of high-affinity inhibitors of HA is related to the use of sialic acid-containing polymers as multivalent inhibitors.⁵²
1.4 Role of sialic acid in acquired glycosylation changes in human diseases

Arteriosclerosis and Asthma

As mentioned previously, selectin-leukocyte interactions are an essential step during the inflammatory reaction. However, when too many neutrophils are recruited, there is the danger of damage to normal cells. Thus, the partial inhibition of the adhesion step can be a strategy to avoid reactions such as in stroke, asthma, psoriasis and arthritis. For example, deposition of low density lipoproteins (LDL) in the arterial wall to form atherosclerotic plaques or the recruitments of eosinophils and basophils in the lungs during an asthma attack, are all consequences of selectin-glycoproteins interactions. Arteriosclerosis is due to deposition of lipids, especially cholesterol, in the arterial wall intimal layer both intra- and extra cellular. Cholesterol in the atherosclerotic plaques is mainly derived from circulating lipoproteins, especially LDL (low density lipoprotein), whose high level in blood are associated with coronary artery disease (CAD). The early phase of the development of arteriosclerosis lesion involves the expression of P-selectin, which recognizes PSGL-1 on circulating monocytes, causing their entry into the subendothelial regions of the blood vessels.

Asthma is a disease responsible for airway wall inflammation, and luminal obstruction of airways by inflammatory exudates. Eosinophils may contribute to the disease by stimulating histamine release from mast cells and basophils, releasing major basic protein, a granule-derived protein that has toxic effect on the respiratory epithelium. Recent studies indicate that selectins are responsible for the recruitment of eosinophils and basophils in the lungs, suggesting a future use of inhibitors for selectins to treat the early stages of an asthmatic attack.
Cancer

Like normal cells during embryogenesis, tumor cells undergo activation and rapid growth, adhere to a variety of other cells types and cell matrices, and invade tissues. The changes that occur in malignant cells can take a variety of forms, i.e. alteration in branching of N-glycans, or in glycosaminoglycan expression, changes in the amount and linkage of sialic acid, etc. For example, an increase in sialylation is often manifested as increases in α2-6 linked sialic acids attached to outer lactosamine (Galβ1-4GlcNAc unit) or to inner GalNAc-O-Ser/Thr units on O-glycans. The latter epitope Neu5Acα2,6GalNAc1,0 Ser/Thr (Sialyl Tn, Figure 1.4.1) is currently a target for attempts at immunotherapy in some cancers. Some tumor cell types have been reported to express sialic acid modified in their substituents, like the ganglioside epitope 9-O-acetylated CD3, increased in melanoma cells, or even de-N-acetyl gangliosides.

Another peculiarity of some tumor cells is the overexpression of Neu5Gc. As mentioned previously, N-Glycolylneuraminic acid (Neu5Gc) is an abundant sialic acid, occurring in the glycoconjugates of most animals. Homo sapiens is a notable exception, since normal human tissues don’t express significant levels of Neu5Gc. This is due to a deletion in the human gene coding for CMP-Neu5Ac hydroxylase, the enzyme usually responsible for Neu5Gc biosynthesis. Despite this mutation, persistent reports in the literature suggest that Neu5Gc occurs in the glycoconjugates of many human tumors, where it may be responsible for the formation of the so-called Hanganutziu-Deicher antibodies that are directed against Neu5Gc gangliosides.

Recently, it has been proved that human multiple myeloma cells express the embryonic form of N-CAM. The presence of polysialylated N-CAM on neoplastic
plasma cells and not on normal plasma cells suggest that this epitope may play a critical role in myeloma tumorigenicity. The presence of polysialylated N-CAM has also been detected in malignant lymphoma and acute nonlymphocytic leukemia.

A lot of progress has been made to identify several glycosphingolipids (GLS) as tumor associated antigens, but it seems that all of them are also found on normal cells. In tumors, however, they are overexpressed or display abnormal glycosylation patterns. In addition, at the cell surface membrane GLS are not distributed randomly or homogeneously, but rather form various-size clusters through self-assembly. This may explain why many of the antibodies show preferential or ‘specific’ reactivity with tumors. Typical examples of tumor associated gangliosides antigens are shown in Table 1.4.1.54 The mechanisms by which GSL’s are involved in the tumor cell-invasion and metastasis are not clear: GSL itself may be an adhesion molecule for selectins in the target cells, or be recognized by another GSL on the metastatic cell. In addition, GSL may act indirectly, by modulating an adhesion or motility receptor to promote tumor cell invasiveness. Thus, GSLs, particularly gangliosides and their degradation product, may directly trigger transmembrane signaling that enhances tumor cell mobility. Furthermore, current research trends are focused on functional roles of ceramide, sphingosine, and their derivatives as second messengers in signal transduction.
Table 1.4.1

<table>
<thead>
<tr>
<th>Ganglioside</th>
<th>Structure</th>
<th>Tumor expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD3</td>
<td>Neu5Ac8Neu5Acα2,3Galβ1,4GlcCer 9-O-Ac-Neu5Ac8Neu5Acα2,3Galβ1,4GlcCer</td>
<td>melanoma</td>
</tr>
<tr>
<td>GD2</td>
<td>Neu5Ac8Neu5Acα2,3Galβ1,4GlcCer 4GalNAcβ</td>
<td>melanoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>neuroblastoma</td>
</tr>
<tr>
<td>GM2</td>
<td>Neu5Acα2,3Galβ1,4GlcCer 4GalNAcβ</td>
<td>melanoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gastric cancer</td>
</tr>
<tr>
<td>Extended</td>
<td>Neu5Acα2,3Galβ1,4GlcNAcβ1,3Galβ1,4Glc Cer 4GalNAcβ</td>
<td>gastric cancer</td>
</tr>
<tr>
<td>GM2</td>
<td></td>
<td>melanoma</td>
</tr>
<tr>
<td>GM3</td>
<td>Neu5Acα2,3Galβ1,4GlcCer</td>
<td>melanoma</td>
</tr>
<tr>
<td>Sialosyl-Le&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Neu5Acα2,3Galβ1,3GlcNAcβ1,3Galβ1,4Glc Cer</td>
<td>Lung small cell carcinoma, mucin of pancreatic and ovarian cancer</td>
</tr>
<tr>
<td>Sialosyl-Le&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Neu5Acα2,3Galβ1,3GlcNAcβ1,3Galβ1,4Glc Cer 4Fuc α</td>
<td>mucin of pancreatic, colorectal and lung cancer</td>
</tr>
<tr>
<td>SLe&lt;sup&gt;x&lt;/sup&gt;</td>
<td>Neu5Acα2,3Galβ1,4GlcNAcβ1,3Galβ1,4Glc Cer 3Fuc α</td>
<td>Gastrointestinal, colorectal, breast and lung cancer</td>
</tr>
</tbody>
</table>

In particular, some GSL antigens have been identified as adhesion molecules that promote tumor-cell metastasis. One of them, GM<sub>3</sub> (Figure 1.4.1), overexpressed in melanoma, binds to LacCer or Gg3Cer through GSL-GSL interactions, increasing the mobility of melanoma cells. The overexpression of SLe<sup>x</sup>, SLe<sup>x</sup>- SLe<sup>x</sup>, and SLe<sup>a</sup> in some tumor cells, together with the knowledge that SLe<sup>x</sup> is recognized by E- and P-selectin and to some extent by L-selectin, strongly suggest that the interaction between selectin (or a selectin-related molecule) and carbohydrate plays a role in tumor progression and
metastasis. Many studies clarified that tumor cells expressing SLe\(^x\) bind to E-selectin, while leukemic leukocytes show clear binding to P-selectin through PSGL-1. In general, patients with primary tumors expressing SLe\(^x\), SLe\(^a\), or their analogue had significantly shorter survival than patients whose tumors did not express these epitopes. Thus, assuming that tumor cell metastasis and invasion are initiated by adhesion of tumor cells to basement membranes, endothelial cells, platelets and parenchymatous cells in certain organs, blocking of such adhesion may abrogate metastasis and invasion.

**Figure 1.4.1**: SialylT, GD3 and GM3 as examples of tumor associated antigens
1.5. Sialic acid in drug design

*Vaccines*

Antibodies are the primary mechanism for the elimination of pathogens from the blood stream. They are ideally suited for elimination of circulating tumor cells and micrometastases. On the basis of studies of bacterial infections, the mechanism of protection by antibodies is probably complement-mediated attack and lysis, and possibly antibody-dependent cell-mediated cytotoxicity of tumor cells, with cell surface antigens as targets. In some cases, antibodies may also have a direct effect, for example by inhibiting tumor cells attachment or growth hormone receptor. Of the many well-defined bacterial antigens studied as target for vaccine therapy, carbohydrate antigens have proven to be the most clinically relevant. Antibodies against capsular polysaccharides on *Neisseria meningitis, Streptococcus pneumonia* and *Haemophilus influenza B* have been shown to correlate with protection from subsequent bacterial challenge and vaccines containing these purified carbohydrate antigens have been shown to be protective. Regarding human cancer antigens, carbohydrates have also proven to be potent targets for immune recognition and attack, because of their abundance and immunogenity. In particular, gangliosides have been shown to be effective targets for passive immunotherapy with monoclonal antibodies (mAb). Major clinical responses have been seen following treatment of patients with mAbs against GM2, GD2 and GD3. There are a number of advantages to using cell surface carbohydrate antigens as targets. Their abundance at the tumor surface, their role in intracellular interactions as targets for selectins and siglecs, combined with the possibility to synthesize the carbohydrate antigens offer a crucial possibility to develop carbohydrate-based vaccines against tumor
and metastatic process. However, the use of carbohydrate antigens as targets for active immunotherapy of cancer with vaccine presents some disadvantages. These problems are related to the so-called T-cell independence of pure polysaccharides. For most immunogens, including polysaccharides, antibody production is based on the cooperative interactions of two types of lymphocytes (white blood cells), B-cells and T-cells. Unfortunately, pure polysaccharides have a restricted immunogenicity because they are incapable of activating T-cells. Thus, even if T-lymphocytes with γδ receptors can recognize non-peptide antigens, while the ones with αβ receptors recognize carbohydrates on short peptides, it has not yet been possible to design vaccines against carbohydrate cancer antigens, because it is not known to which aminoacids on which peptides any cancer epitope is linked. Conjugate vaccines, in which the carbohydrate antigen is covalently attached to an immunogenic carrier protein, are able to overcome the lack of T-lymphocyte help. Indeed, the latter are activated by the protein carrier and secrete a variety of cytokines, required to B-lymphocytes for optimal activation.

**Drugs**

Several disadvantages are associated with the use of complex oligosaccharides (e.g. SLe\(^5\)) as therapeutic agents. In many cases, they display unfavorable pharmacokinetics and have often a poor metabolic stability and poor oral adsorption. Furthermore, many carbohydrates bind with low affinity to proteins (mmol or umol range), which complicates their use as drugs. These disadvantages have stimulated the development of therapeutic carbohydrate-analogues. As a typical example, the development of SLe\(^5\) analogues,\(^{55}\) required in the first instance synthetic studies targeting
SLex itself, whereby, several successful syntheses of SLex have been reported. The identification of the functional groups critical for SLex binding to selectin represented the next step in the design of its mimics. The main goal is to find a compound having the same (or even more) binding affinity of the original carbohydrate, that is easy to synthesize, stable, inexpensive. Several oligosaccharides have been prepared in which one or more of the sugars from SLex have been replaced with a variety of more stable linkers.

Much recent effort has been focused towards preparing multimers of SLex, by incorporation of SLex into polymeric displays to enhance its binding affinities.

The above mentioned developments will make it possible to design glycomimetics which may find applications in areas where selectin mediated mechanisms are thought to be important (e.g. reperfusion injury, psoriasis, septic shock, rheumatoid arthritis, asthma, cancer and inflammatory bowel disease).

It is noteworthy to mention the recent progresses that have been made in the research related to anti-influenza drugs. An important discovery in this area was a deep, central dent, or cleft in viral neuraminidase (NA): given that influenza viruses cannot spread readily from cell to cell without help from NA, the new discovery implied that a drug able to occupy, and jam, the active site would inhibit NA in all versions of the influenza virus. Next step was the identification of the amino acids in the cleft of NA responsible for the binding: for example, there are three positively charged amino acids that bind sialic acid through its carboxylate group. In addition, the bottom of the cleft presents a small pocket containing two negatively charged amino acids. These amino acids—glutamates—have no contact with sialic acid but are nonetheless present in all
influenza neuraminidases. Furthermore, the hydroxy group at C-4 of sialic acid points down toward that extra pocket without reaching it. These features suggested that replacing this OH with a large, positively charged atomic grouping might yield a tight-binding derivative. The positive group would presumably nestle into the extra pocket at the bottom of the active site and would lock itself there by binding to the previously unused, negatively charged glutamates in the pocket. In 1993 Mark von Itzstein and his colleagues at Monash University in Melbourne found that introducing a guanidino group as replacement for the hydroxy group C-4 produced an extraordinarily potent inhibitor of influenza neuraminidases.\textsuperscript{60} Furthermore, the inhibitor had little effect on related enzymes made by bacteria and mammals, a sign that the compound probably would not disrupt human cells. This substance-Zamavir-proved not only to prevent flu symptoms in individuals subsequently infected with influenza but also to reduce the severity of symptoms in those who took the drug after being infected. However, the compound cannot be taken orally, due to the difficulty of the positively charged guanidino group to cross cell membranes from the blood stream. New efforts has been made to find a new NA inhibitor that can be taken as a tablet. The new drug, GS 4071\textsuperscript{61}, retains one major neuraminidase-binding group also found in Zanamivir: a negatively charged carboxylate group that can bind to positively charged amino acids in the enzyme’s active site. In addition, the interaction of GS 4071 with the active site somehow causes one of the amino acids in the cleft to rotate and create a new hydrophobic pocket which binds hydrophobic components of the drug. Unfortunately, also in this case, the charged carboxylate group of the drug inhibits its crossing out of the gut into the bloodstream in animals. For this reason, the GS4071 prodrug, GS 4104,\textsuperscript{62} has been synthesized to allow
easily entry into the bloodstream, where after it is converted back to its original (GS 4071) form (Figure 1.5.1).

![Figure 1.5.1: Zamavir, Gs-4071 an Oseltamir Phosphate (A) and their binding sites (B)](image)

Exploration of the role of sialic acids will thus lead to the early diagnosis of diseases and their successful treatment. Furthermore, development of new methods for stereospecific sialylation is especially important since only chemical methods would provide enough material for biological testing of these glycoconjugates.
1.6. Glycosylation Methods for oligosaccharide synthesis

The chemical synthesis of oligosaccharides in general is more complicated than the synthesis of other biopolymers such as peptides and nucleic acids. The difficulties in the preparation of complex oligosaccharides are a result of a greater number of linkage possibilities for the combination of monomeric units to form oligosaccharides. In addition, the glycosidic linkage has to be introduced stereospecifically (α/β selectivity).

Glycosidic bond formation is generally achieved by condensing a fully protected glycosyl donor, which bears a potential leaving group at its anomeric center, with a suitably protected glycosyl acceptor that contains often only one free hydroxyl group. The glycosylation can result in an α- and/or β-anomer and the stereocontrol of this condensation reaction is one of the most challenging aspects in oligosaccharide chemistry. The nature of the protecting group at C-2 of the glycosyl donor is a major determinant of the anomeric selectivity. An ester protecting group at C-2, which can perform neighboring group participation during glycosylation, will favor the formation of a 1,2-trans-glycosidic linkage. On the other hand, when a non-assisting functionality is present at C-2, the reaction conditions (e.g. solvent, temperature, and promoter) can determine the anomeric selectivity. Also the constitution of the glycosyl donor and acceptor (e.g. type of saccharide, leaving group at the anomeric center, protection and substitution pattern) can have a major effect on the α/β selectivity. As described in Scheme 1.6.1, activation of the anomeric center of A and participation of the 2-O-acetyl protecting group yields a more stable acyloxonium-ion B. Attack of the acceptor at the anomeric center results in a 1,2-trans-glycosidic linkage. On the other hand, when a non-participating functionality (e.g. Bn ether) is present at C-2, then the reaction conditions,
such as solvent, temperature, and promoter will determine the anomeric selectivity and properly selected conditions may result in an excellent 1,2-cis-stereoselectivity.\(^8\)

\[
\begin{align*}
\text{L} = \text{Leaving group: halide, SR', } & \text{OC(=NH)CCl}_3, \text{O(CH}_2)_2\text{CH}=\text{CH}_2, \text{ect.} \\
\text{P} = \text{Me, Ph ect.}
\end{align*}
\]

\[
\text{R} = \text{non-participating group Me, Bn, ect.}
\]

**Scheme 1.6.1**

### 1.7. Chemical approaches to the synthesis of \(\alpha\)-sialosides

The lack of efficient technology to accomplish glycosylations with sialic acid is one of the long-standing deficiencies in carbohydrate chemistry. Many of the early methods for the synthesis of sialic acid glycosides are generally plagued with side reactions, low yields and poor stereoselectivity. There are several reasons that make sialic acid chemistry more complicated than every other monosaccharide’s chemistry. The electron-withdrawing carboxylate at the anomeric center disfavors the formation of the oxocarbenium ion C (Scheme 1.7.1). The latter is also somewhat sterically hindered, so
the attack of hydroxyl nucleophiles is slower. Therefore, an elimination to provide D can be a significant competitive pathway. In addition, the little steric biasing of one face of C over the other and the lack of a C-3 functionality to direct the stereoisomeric outcome contribute to poor stereoselectivity of glycosylations. As result of these factors, glycosyl reactions often provide low yields of the more desired thermodynamically but less stable \( \alpha \)-anomer.

**Scheme 1.7.1**

Glycosides of N-acetyl neuraminic acid can be introduced by direct or indirect approaches. For the direct approach the synthetic challenge of an efficient glycosylation with sialic acid involves finding the best reaction conditions such as leaving group, solvent, temperature. On the other hand, trying to improve the reactivity and stereoselectivity of the donor, bearing auxiliaries at C-3 is important for indirect methods.
Direct Sialylations

Direct O-sialylations include those methods which lead to the formation of O-sialosides in one synthetic step involving the coupling of a glycosyl acceptor having a free hydroxyl with a glycosyl donor with an appropriate leaving group at C-2. (Scheme 1.7.2) Since the course and outcome of glycosylation reactions is greatly influenced by different factors (i.e. leaving group, promoter, solvent, temperature, protecting groups), the goal of many research groups is to optimize them for the synthesis of sialosides with high yield and stereoselectivity.

\[
\begin{align*}
\text{AcO} & \quad \text{OAc} & \quad \text{CO}_2\text{Me} & \quad \text{X} + \text{ROH} \quad \text{Promoter} \quad \rightarrow \\
\text{AcHN} & \quad \text{AcO} & \quad \text{OAc} & \quad \text{OR} \\
\end{align*}
\]

\(X = \text{Br, Cl, F, SMMe, SPh, SC(=S)OEt, OP(OR')_2, OCOR'}\)

Scheme 1.7.2

Up to date, the choice of leaving group and/or promoter is strictly depending on the nature of the glycosidic bond. A summary of the most common leaving group and promoters is shown in the following table:
<table>
<thead>
<tr>
<th>Leaving Group</th>
<th>Promoters</th>
<th>Characteristic (Advantages and Disadvantages)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>AgOTf, ( \text{Ag}_2\text{CO}_3 ), ( \text{Hg(CN)}_2/\text{HgBr}_2 ), Silver salicylate,</td>
<td>High stereoselectivity for primary alcohols Expense and toxicity of promoters; not practical for large scale; poor stereoselectivity for hindered alcohols: some of them are not stable</td>
</tr>
<tr>
<td></td>
<td>2-Thio DMSTM, NIS/TfOH, MeOTf, lDGP PhSeOTf</td>
<td>Chemical stability; activated in mild conditions, at low temperatures, high stereoselectivity and yields for polyols</td>
</tr>
<tr>
<td>2-Phosphite</td>
<td>TMSOTf</td>
<td>High stereoselectivity for hindered alcohols</td>
</tr>
</tbody>
</table>

*Optimizing the leaving group and activation method*

Among the **halides** 2-chloro derivatives of Neu5Ac were the first compounds to be used for the glycosidation reaction involving sialic acid. This approach was the main tool for the synthesis of compounds containing N-acetyleneuraminic acid from the 60’s through the 80’s. Nowadays, the use of these derivatives is limited to the glycosylation of simple alcohols. Thus, 2-Chloro derivatives of Neu5Ac have a reasonable shelf-life when handled with care and stored in a freezer. Several methods have been reported for their synthesis. For example, they can be prepared from the corresponding C-2 acetate by the treatment with HCl/AcCl,\textsuperscript{70} TiCl\textsubscript{4} \textsuperscript{71} or AcCl/MeOH in CHCl\textsubscript{3}.\textsuperscript{72} The first glycosides of N-acetyleneuraminic acid were obtained by coupling simple alcohols (e.g. methanol, benzyl alcohol, substituted benzyl alcohols etc.) with the free acid of an acetochloro derivative of Neu5Ac using \( \text{Ag}_2\text{CO}_3 \) (classical Koenigs-Knorr) or \( \text{Hg(CN)}_2/\text{HgBr}_2 \) (Helferich modification) as promoter.\textsuperscript{73} The corresponding \( \alpha \)-ketosides were obtained in modest yields (30-50%). The methyl ester of acetochloroneuraminate is a much more
stable derivative (compound 2, Scheme 1.7.3) and in the presence of the promoter \( \text{Ag}_2\text{CO}_3 \),\textsuperscript{70} proved to be a far superior glycosyl donor. Many variation of the metal promoters have been examined to probe the effect of the activator on the Koenigs-Knorr glycosylation and stereoselectivity. The order of reactivity among promoters with glycosyl halides was determined experimentally to be \( \text{AgOTf} > \text{Ag}_2\text{CO}_3 > \text{Silver salicylate} > \text{HgBr}_2 > \text{Hg(CN)}_2 \). Generally, the more reactive promoter gives higher stereoselectivity but lower yields. (Scheme 1.7.3)

\[
\begin{align*}
\text{1} & \quad \text{TiCl}_4 \quad \text{or TiBr}_4 \\
\text{2} & \quad \text{Ag}_2\text{CO}_3 \quad 67\% \\
\text{3} & \quad \text{2+} \\
\text{4} & \quad \text{Ag}_2\text{CO}_3 \\
\text{5} & \quad \text{Ag}_2\text{CO}_3 \quad 20\% \alpha \text{ only} \\
\text{6} & \quad \text{Hg(CN)}_2/\text{HgBr}_2 (3/1) \quad 84\%, \alpha/\beta 3/4
\end{align*}
\]

Scheme 1.7.3

Methods derived from the Koenigs-Knorr approach are useful for glycosylation involving reactive alcohols, such as primary alcohols. Several Neu5Ac(2→6), (2→3)Glc and Neu5Ac→Gal derivatives were synthesized by a \( \text{Ag}_2\text{CO}_3 \) promoted glycosylation of glycosyl donor 2 in CHCl\textsubscript{3} (Scheme 1.7.3). Unfortunately, when applied to less reactive
secondary or sterically hindered primary hydroxyl groups (e.g. 5) silver-promoted glycosylations led to lower stereoselectivities and yields, obtaining in that case glycal (2,3-dehydro derivative) as main product.\textsuperscript{74} Higher yield can be obtained by employing Hg(CN)\textsubscript{2}/HgBr\textsubscript{2} as the promoter.\textsuperscript{74} For example, a Neu5Ac(2→6)Gal derivative was obtained in an excellent yield of 84\% although as a 3/4 mixture of α/β-anomers.

It can be concluded that the application of 2-chloro derivatives of Neu5Ac gives in general good yields when coupled with simple or primary sugar alcohols.\textsuperscript{75} As a matter of fact, these methods offer the most reliable approach for the synthesis of simple glycosides of Neu5Ac.\textsuperscript{76} 2-Bromo derivatives have found limited application in chemical O-sialylation most likely due to their high reactivity and, therefore, low chemical stability, while 2-fluoro derivatives give unnatural β-glycosides in the presence of BF\textsubscript{3}-OEt\textsubscript{2}. Many of the methods of activation using Koenigs-Knorr conditions require heterogeneous promoters and long reaction times at room temperature. In addition, the expense and the toxicity of silver and mercury make these methods impractical for large-scale synthesis. Consequently, much research has focused on the development of glycosyl donors that can be activated at low temperature, under mild conditions and without the need for heavy metal promoters.

Sialic acid thioglycosides, although initially synthesized for use as biological probes, have been found to be excellent glycosyl donors. Due to their excellent chemical stability, anomeric alkyl(aryl) thio groups offer efficient protection of anomeric centers and are compatible with many reaction conditions often employed in carbohydrate chemistry. Furthermore, in the presence of at least an equimolar amount of a thiophilic promoter,\textsuperscript{77} thioglycosides can be activated and used in direct glycosylations. The most
commonly used activating reagents include methyl trifluoromethanesulfonate (MeOTf), dimethyl(methylthio) sulfonium trifluoromethanesulfonate (DMTST), N-iodosuccinimide (NIS)-trifluoromethanesulfonic acid (triflic acid, TfOH), iodonium dicollidine perchlorate (IDCP) and phenyl selenyl trifluoromethanesulfonate (PhSeOTf). The most important 2-thio derivatives for glycosidation of Neu5Ac include S-alkyl (methyl, ethyl), S-aryl (phenyl and substituted phenyl) glycosides and glycosyl xanthates (ethoxydithiocarbonate).

Among the S(Alkyl) as leaving group, S-methyl and S-ethyl derivatives give very similar yields and anomic selectivities. Originally 2-thiomethyl derivatives were synthesized by methylation of a sodium salt of 2-thiol derivatives of Neu5Ac (7, Scheme 1.7.4) with MeI in DMF. This method was substituted by a very efficient procedure, which involves the direct conversion of a C-2 anomeric acetate into 2-thiomethyl derivatives by the treatment with methylthiotrimethylsilane (TMSSMe) in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-triflate, TMSOTf) in 1,2-dichloroethane (DCE). This approach gives a fully acetylated thioglycoside, in almost quantitative yield (98%) as a 1:1 mixture of α/β-anomers. In glycosylations, the α- and β-anomers have very comparable glycosyl donor properties and therefore do not need to be separated. Thioethyl glycosides of Neu5Ac are readily available by treatment of the corresponding 2-O-acetyl derivative with ethanethiol in the presence of BF₃·Et₂O (75% yield, α/β 1:24). However, it is possible to obtain the anomerically pure α-glycoside from the 2-chloro derivative using phase transfer catalysis conditions (EtSH/Bu₄NHSO₃/CH₂Cl₂/ 1M aq. Na₂CO₃). The synthesis of 2-thiomethyl glycosides of Neu5Ac and in the application as sialyl donors was thoroughly developed by
Hasegawa and co-workers.\textsuperscript{83,88,89,90} For example, (2→6)-linked disaccharides 11 can be obtained by glycosylation of 2-thiomethyl glycoside 9 with 6-hydroxy derivatives of galactose in the presence of DMTST (68\%, Scheme 1.7.4).\textsuperscript{91-93} The best $\alpha$-anomeric selectivities were obtained when the participating solvent acetonitrile (MeCN) was employed at low temperature.\textsuperscript{83}

Scheme 1.7.4

Good yields (~50\%) and high $\alpha$-anomeric selectivities are achieved also for the synthesis of $\alpha$-2,3-linked sialosides when glycosyl acceptors are partially protected (glycosylations of diol and triols, Schemes 1.7.5 and 1.7.6). In these cases, steric hindrance influences the glycosylation outcome. In general, higher yields and stereoselectivities are obtained with galactosyl acceptors as triols instead than diols.\textsuperscript{91-93}
Although the glycoside acceptor presents more than one free hydroxyl, only one regioisomer is obtained. The regioselectivity of this glycosylation is due to the greater reactivity of the equatorial alcohol compared to the axial C-4 hydroxyl. Furthermore, the C-2 hydroxyl has a lower nucleophilicity due to the electron withdrawing effect of the adjacent anomeric center. When a similar glycosylation is performed with a galactosyl acceptor having only a free C-3 hydroxyl group, the yield and anomeric stereoselectivity is significantly reduced. Further improvement in the glycosylation outcome can be obtained with the application of the highly reactive promoters NIS/catalytic TfOH, especially when applied to glycosylations of sterically more hindered hydroxyls.\textsuperscript{94} For example, NIS/TfOH mediated glycosylation of 3',4'-diol of a lactoside gives a much higher yield of the (2→3)-linked product (69\%) and improved anomeric stereoselectivity (\(\alpha/\beta\) 6:1), compared to a similar glycosylation promoted by DMTST (Scheme 1.7.6). The promoter system NBS/\(\text{Bu}_4\text{N}OTf\) has been proposed as an alternative for NIS/TfOH and it has been found to be equally effective.\textsuperscript{95} An alternative promoter is PhSeOTf\textsuperscript{96} Good yields and anomeric stereoselectivities were achieved when performed in MeCN at low temperature.\textsuperscript{85} This promoter was applied for an orthogonal glycosylation strategy whereby a tetrasaccharide was obtained in good yield by coupling a 2-thiomethyl sialosyl donor with a glycosyl fluoride trisaccharide acceptor.
In general, 2-thioaryl glycosyl donors are activated by using similar promoter systems as employed for S-alkyl derivatives. In some cases, 2-thiophenyl glycosides proved to be more efficient than corresponding 2-thiomethyl derivatives.\(^\text{97}\) 2-thiophenyl glycosides are readily available by reaction of thiophenol with 2-O-acetyl, 2-chloro, or 2-fluoro derivatives of Neu5Ac.\(^\text{86,98}\) The most valuable use of thiophenyl glycosides for O-sialylation was for the formation of (2→8)-linked oligomeric sialyl units.\(^\text{97,99-107}\)

High stereoselectivity in general is observed for these glycosylations when performed in acetonitrile, even though the thioglycoside starting material is already an anomeric mixture, suggesting a control of the reaction conditions on the stereochemistry outcome. Reaction mechanisms of activation of thioglycosides of Neu5Ac by either DMTST or NIS/TfOH in MeCN have been proposed\(^\text{84}\) (Scheme 1.7.7) and involve the generation of an electrophilic species (\(^{+}\)SMe or \(^{+}\)I\(^{+}\)), which reacts with the lone pair of sulfur resulting in the formation of a sulfonium intermediate. The sulfonium moiety is an excellent leaving group and can be displaced by a hydroxyl of a glycosyl acceptor or
alternatively by nitrogen of acetonitrile to give a nitrilium ion. The latter adopts a preferred axial (β) configuration. Nucleophilic substitution of the nitrilium ion with an alcohol gives predominantly equatorial α-glycosides. It has been observed that less reactive (secondary) alcohols give much higher α-selectivity than primary alcohols.

Scheme 1.7.7

2-(Ethoxy)dithiocarbonate, or 2-xantho, derivative of Neu5Ac can be synthesized from the 2-chloride by the reaction with potassium ethoxydithiocarbonate in EtOH (Scheme 1.7.8). Sialyl xanthate is a stable crystalline material, which has a good shelf-life. A DMTST promoted glycosylation of the 6-hydroxyl of a galactosyl acceptor afforded a (2→6)-linked disaccharide in a 64% yield as a 3/1 mixture of α/β-anomer. A 2-thioalkyl glycosyl donor gave a lower yield (32%) of disaccharide when reacted under similar conditions illustrating the advantageous properties of 2-xanthates.
An attractive feature of this leaving group is that sialyl xanthates can be selectively activated in the presence of thioglycosides using methylsulfenyl trifluoromethanesulfonate (MeSOTf) as the promoter. This highly reactive thiophilic reagent can be generated \textit{in situ} by reaction of methylsulfenyl bromide (MeSBr) with AgOTf. It activates 2-xanthates at low temperature (−70°C) and the best results are obtained when a mixture of MeCN/CH₂Cl₂ (3/2 v/v) was used as the reaction solvent.¹⁰⁹

Sialyl phosphites, which were independently introduced by both Schmidt¹¹⁰ and Wong,¹¹¹ have found wide application in chemical O-sialylations.⁶⁹,⁸⁰,⁸¹,¹¹² Sialyl phosphites are very reactive glycosyl donors and only require a catalytic amount of TMSOTf (usually 10-20 mol %) for their activation.

Diethyl β-sialyl phosphites ²² can be prepared in high yields (97%) by reacting of the 2-hydroxyl derivative of Neu5Ac ²¹ with ClP(OEt)₂ in the presence of the hindered base, i-Pr₂NEt (Scheme 1.7.9).¹¹⁰,¹¹³ Glycosylation of the 6-hydroxyl derivative of
glucoside 24 in the presence of TMSOTf (0.1 equiv.) in MeCN at –40°C afforded a (2→6)-linked disaccharide 25 as a 4:1 mixture of α/β-anomers (70%).

![Scheme 1.7.9](image)

**Scheme 1.7.9**

Although the application of a di-benzyl phosphite derivative 23 gives a higher yield and improved anomeric selectivity (80%, α/β 5:1, Scheme 1.7.9)\textsuperscript{111,114} α/β 6:1,\textsuperscript{115} the use of this leaving group has not yet found wide application probably due to the fact the reagent di-benzyl N,N-diethylphosphoramidite (DDP) required for its introduction is not commercially available. Moreover, the reaction of a 2-hydroxy-sialyl derivative with DDP in the presence of tetrazole provided the desired phosphite in a yield of 70%
whereas a yield of 97% was obtained for the diethyl analog. The glycosyl donor properties of these two anomeric phosphites were directly compared in a synthesis of the tetrasaccharide sialyl Lewis\textsuperscript{x}, in this case it was found that the diethyl phosphite 22 was more efficient (36%) than the di-benzyl analogue 23 (20%).\textsuperscript{116,117} It is noteworthy, that the C-1 benzyl ester of Neu5Ac gave a higher $\alpha$-selectivity than those protected as a methyl ester.\textsuperscript{115}

Summarizing, the anomeric chloride of Neu5Ac offers the most reliable glycosyl donor for the preparation of glycosides of simple alcohols. Sometimes, they give lower yields compared to 2-thiomethyl donors but are still the glycosyl donor of choice because of higher anomeric selectivities.\textsuperscript{118} 2-Thioalkyl, 2-thiophenyl, 2-xanthate and 2-(di-benzyl) or 2-(di-ethyl)phosphites are the leaving groups of choice when more complex hindered sugar alcohols need to be sialylated. The best results are obtained when saccharide acceptors have a free diol or triol and in many of these cases the sialylations proceed with excellent regioselectivities in combination with high yields and anomeric selectivities. In some cases, a 2-(diethyl)phosphite gives higher yields than the use of a similar 2-xanthate\textsuperscript{80,119} or 2-thiomethyl derivatives.\textsuperscript{80} In another case, the 2-thiomethyl glycosyl donor proves to be more efficient\textsuperscript{120} In general, for glycosylations of secondary hydroxyls, diethyl or dibenzyl phosphites are preferred over the 2-chloro derivative\textsuperscript{75} however, controversial observations have been reported.\textsuperscript{121} The comparison data clearly show that there is no method that gives satisfactory results for a wide range of glycosyl acceptors. Certainly, more active phosphtite donors produce better results when applied to the glycosylation of sterically hindered alcohols, whereas thio-derivatives can be successfully applied for the regio/stereoselective glycosylation of polyols, such as a
2,3,4-tri-hydroxy derivative of galactosides. The orthogonal glycosylation strategy exploits the finding that a thioglycoside can be activated in presence of an anomeric fluoride as well as xanthates.\textsuperscript{121,122} This feature is attractive since the methodology allows complex oligosaccharides to be assembled without extensive manipulations at the anomeric center.

**Optimization of the protecting groups**

The glycosylation outcome can be also influenced by seemingly insignificant modifications of the protecting groups in the donor or acceptor. For example, employing as donor a sialyl chloride having a benzyl ester rather than a methyl ester to protect the carboxylate group has profound consequences on the stereoselectivity of the glycosylation.\textsuperscript{123}

Protection of the amino group at C-5 with tert-butyloxycarbonyl (Boc),\textsuperscript{124} benzyloxycarbonyl (CBz, Z),\textsuperscript{125} azido,\textsuperscript{125,126} or phthalimido (Phth) groups\textsuperscript{127} allowed the access to a free C-5 amine upon deprotection. This amine can then be subsequently derivatized with glycolyl or other moieties. However, no report indicates that the use of these protecting groups results in a change of reactivity or stereoselectivity, except that the application of 5-azido derivatized 2-thiomethyl substrate gave lower yields of coupling product (26\%) compared to the use of a similar donor that has an N-acetamido moiety (51\%).\textsuperscript{126}

Recently, a significantly more reactive 2-thioglycosyl donor \textbf{26} was introduced which bears a di-N-acetyl (N-acetylacetamido) functionality at C-5.\textsuperscript{128} It was observed that the additional N-acetyl moiety of the glycosyl donor dramatically increases its
reactivity resulting in improved yields of glycosylation products (Scheme 1.7.10). For example, an NIS/TfOH-promoted coupling of 2-(trimethylsilyl)ethyl 6-O-benzoyl-β-D-galactopyranoside 12 with mono-N-acetylated glycosyl donor 9 proceeded with high regioselectivity to give after a reaction time of 2 h, an α(2→3)-linked disaccharide in a yield of 61%. The application of the novel di-N-acetylated glycosyl donor, under similar reaction conditions, afforded a similar disaccharide within 5 min in an improved yield of 72%. Moreover, only a small excess of donor was required to achieve the high yield (1.1 mol equiv. of the donor instead of the conventional 1.7-2.0 mol equiv.). Excellent yields were also obtained when a 4,6-O-benzylidene protected galactoside was used as glycosyl acceptor. Furthermore, when a 1,2-cyanoethylidene derivative was applied as glycosyl acceptor, the corresponding disaccharide was obtained in high yield (85%), however, the anomeric stereoselectivity was compromised (α/β 1:1). The additional N-acetyl function can be easily cleaved under Zemplen deacetylation conditions with concomitant O-acetyl group removal. The highly reactive glycosyl donor was also applied for the direct synthesis of (2→8) and (2→9)-linked dimers of N-acetylneuraminic acid. 129
Scheme 1.7.10

**Indirect Chemical Methods**

An alternative approach for the synthesis of sialosides involves modified sialyl donors that have an auxiliary at C-3. The presence of a participating group at the C-3 position can control the anomeric selectivity of a glycosylation via the neighboring group participation effect, as well as prevent 2,3-eliminations, which are often a major side reaction of direct O-sialylations. Thus, \(\alpha\)-glycosides will be obtained in case of equatorial auxiliaries whereas \(\beta\)-glycosides will be favored when the auxiliary is in the axial position. Apart from these properties, an auxiliary should be easily introduced, and easily removed after the glycosylation.\(^{130}\)

Usually, auxiliaries are introduced by a chemical modification of a 2,3-dehydro derivative of Neu5Ac either through a 2,3-oxirane derivative or by an addition reaction to the double bond of 2-ene derivatives.(Scheme 1.7.11) The 2,3-dehydro derivative is easily accessible in high yield (81%) by 2,3-elimination of methyl
acetochloroneuraminate by the treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). \(^{131}\)

\[
\begin{align*}
\text{X} &= \text{halide, SR', phosphite} \\
\text{Y} &= \text{halide, O-Acyl, SR'', SePh}
\end{align*}
\]

**Scheme 1.7.11**

Several auxiliaries groups have been reported for the synthesis of 3-O-derivatives of Neu5Ac. For example, synthesis of a 3-hydroxy substituted compound in combination with a halide leaving group at C-2 (Cl, Br, F) can be accomplished by a three step reaction sequence starting from the glycal \(^{27}\)^\(^{131,132}\). Thus, coupling of the 2-bromo derivative \(^{28}\) with the 6-hydroxyl of a glucoside \(^{24}\) in the presence of AgOTf/NaHPO\(_4\) in an aprotic solvent at –10°C gives a (2→6)-linked disaccharide as a mixture of anomers (79%, \(\alpha/\beta\) 4:1, Scheme 1.7.12).\(^{132,133}\) No glycosylation of the C-3’ hydroxyl was observed. The auxiliary can be removed by phenoxythiocarbonylation in the presence of DMAP (95%) followed by reduction with Bu\(_3\)SnH in the presence of 2,2’-azobisisobutyronitrile (AIBN, 97%) to give a (2→6)-linked derivative of Neu5Ac. Although the 2-hydroxy auxiliary approach was efficiently applied for the first synthesis of complex (2→8)-linked dimeric structures as well as (2→9)-linked derivatives\(^{134,135}\), it
is not the best choice when applied to the synthesis of Neu5Ac(2→3)Gal derivatives, resulting in this case in a complex mixture of α/β-anomers.

Scheme 1.7.12

A 3-thio- or 3-seleno-groups provide one of the most reliable auxiliaries and have, therefore, been studied extensively.\textsuperscript{136-139} The first glycosylations with 3-S-phenyl and 3-Se-phenyl substituted derivatives of Neu5Ac were performed by Ito and Ogawa.\textsuperscript{140-142} Electrophilic addition of PhSeOAc to the double bond of the 2,3-dehydro derivative of Neu5Ac afforded mainly a 2-O-acetyl-3-Se-phenyl derivative 29 (3ax/3eq=11:1, Scheme 1.7.13).\textsuperscript{140,142} However, in order to obtain naturally occurring α-sialosides, the glycosyl donor requires an equatorially substituted auxiliary at C-3. This results in an additional step to separate the C-3 equatorial isomer from its epimer. Thus, glycosylation of 1,2:3,4-di-O-isopropylidene galactose 3 in the presence of AgOTf/SnCl\textsubscript{2} in CCl\textsubscript{4} affords a α(2→6)-linked product in a 46\% yield (Scheme 1.7.13), the only by-product being the 2,3-dehydro derivative. However, a different promoter such as AgOTf /n-Bu\textsubscript{3}SnOTf
causes epimerization of C-3 resulting in the formation of a substantial amount of the β-linked product.

Scheme 1.7.13

The 2-fluoro-3-thiophenyl derivative seems to be a more efficient glycosyl donor than the corresponding 3-seleno compound.\textsuperscript{141,142} In fact, glycosylation of the 2-fluoro-3-thiophenyl derivative with the same acceptor under similar reaction conditions gives the α(2→6)-linked disaccharide in a 72% yield (46% for 3-seleno analog). Hg(CN)\textsubscript{2}/HgBr\textsubscript{2} promoted glycosylation of the 2-bromo-3-thiophenyl derivative is equally effective (72%), whereas an AgOTf promoted glycosylation of a 2-chloro analog gives a modest yield of 52%. It has been proposed that these glycosylations proceed through an
episulfonium (or episelemium) intermediate 31 (Scheme 1.7.14), which provides excellent α-stereoselectivity.\(^\text{142}\)

![Scheme 1.7.14](image)

### Scheme 1.7.14

Sialyl donors having an S-alkyl leaving group proved to be highly efficient for the formation of glycosidic linkages. Not surprisingly, extensive efforts have been directed towards the design and synthesis of 3-S-phenyl-substituted donors bearing S-alkyl leaving group at C-2. These derivatives can be effectively synthesized from glycal as shown in Scheme 1.7.15. The 2-chloro function can be replaced by a thioethyl moiety by the treatment with Hg(OAc)\(_2\) to give an anomeric acetyl moiety which was substituted by ethane thiol in the presence of BF\(_3\)Et\(_2\)O. The resulting compound 33 was synthesized in a 50% over-all yield starting from glycal 27 and proved to be a highly efficient glycosyl donor.\(^\text{143,144}\) For example, MeSBr/AgOTf-mediated glycosylation of a sterically hindered 3’-hydroxyl of a lactoside 34 at –40°C afforded the desired α-linked trisaccharide in 67% yield. In the same conditions, direct glycosylations of either 2-xanthate or S-methyl
derivatives of N-acetylneuraminic with the lactosyl acceptor give a trisaccharide as a mixture of anomers in yields of 40% (α/β 9:1) and 33% (α/β 7:1), respectively (Scheme 1.7.15). 143,144

Scheme 1.7.15

2,3-Di-thiosubstituted derivative was also attached to the soluble polymer polyethylene glycol (PEG) via a succinyl linker to be used for the polymer-supported synthesis. 145 Thus, glycosylation of 24 in the presence of DMTST, followed by cleavage
from the polymer support by base treatment gave the target disaccharide in 70% yield (Scheme 1.7.16). The 3-thiophenyl moiety can be conveniently removed to furnish a (2→6)-linked O-sialoside. To date, this is the only example whereby a glycosyl donor of a modified Neu5Ac was attached to a polymeric support and successfully glycosylated illustrating the synthetic potential of these pseudo sialosyl donors.

Scheme 1.7.16

In conclusion, among the indirect methods, glycosyl donors which possess a S-phenyl participating auxiliary at C-3 and thioalkyl leaving group at C-2 generally give the best yields and anomeric selectivities especially when applied to the glycosylation of sterically hindered alcohols. A 3-O-phenylthiocarbonyl auxiliary also gives excellent yields and stereoselectivities.

However, the major drawbacks of the indirect methods are the additional chemical steps required for introduction and removal of the auxiliary at C-3. Furthermore, stereoselective introduction of a C-3 auxiliary is problematic in many cases and forms mixtures of diastereoisomers, which often require separation and/or epimerisation.
1.8. Synthesis and Introduction of Dimers

Neu5Ac or Neu5Gc also occur in linear homopolymers where they are usually linked internally by $\alpha(2\rightarrow8)$, $\alpha(2\rightarrow9)$ or alternating $\alpha(2\rightarrow8)/\alpha(2\rightarrow9)$ glycosidic linkages. Among the glycosidic linkages characterizing natural sialosides, $\alpha(2\rightarrow8)$ is maybe the most difficult to be obtained by chemical methods. In fact, in addition to the problems related to sialic acid as a glycosyl donor, the synthesis of oligosaccharides that contain $(2\rightarrow8)$-linked fragments is complicated by the low reactivity of the C-8 hydroxyl of Neu5Ac. The 8-hydroxyl of Neu5Ac has low nucleophilicity due to a combination of obvious steric effects, interactions with the acetamido group at C-5, and/or the presence of an internal hydrogen bond between the C-8 hydroxyl and C-1 carboxyl or 2-OR ($R = \text{Me, Ac, H etc.}$) moieties$^{148}$ (Figure 1.8.1).

![Figure 1.8.1](image)

To date, several successful syntheses of this dimer have been reported. Most of these approaches are based on the use of indirect glycosylation methods. Among these, the use of a 2-bromo-3-hydroxy substituted glycosyl donor (Scheme 1.8.1)$^{134,135}$ in the presence of AgOTf and Na$_2$HPO$_4$ affords the dimer in 34% yield as 3:1 mixture of $\alpha/\beta$-anomers. These results were significantly improved by applying the 2-bromo-3-ephenylthio substituted donor.$^{149,150}$ In this case, the dimer was obtained as only the $\alpha$-
anomer in a good yield of 64%. Further improvements came by the application of glycosyl donors that have a 2-phosphite leaving group and a 3-O-phenylthiocarbonyl participating functionality.\textsuperscript{148} By employing this donor, the $\alpha$-anomer was also formed in a stereoselective manner but in an improved yield of 83%. Surprisingly, under similar reaction conditions 3eq-bromo-2-phosphite donor gave only $\beta$-linked product in 58% yield.\textsuperscript{148} These results suggest that the 3-O-phenylthiocarbonyl group is a more efficient participating functionality than the 3-bromo moiety. A 2-thioethyl leaving group in combination with a 3-thiophenyl participating auxiliary afforded $\alpha(2\rightarrow8)$-linked product in a disappointing yield of 28%.\textsuperscript{144,151} This result, however, was significantly improved by using a glycosyl donor that has an additional activating N-acetyl function at the acetamido group of C-5.\textsuperscript{128,129} In this case, the $\alpha$-linked dimer was obtained in an improved yield of 44%.\textsuperscript{152}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Scheme_1.8.1.png}
\caption{Scheme 1.8.1}
\end{figure}

It is noteworthy, that all of these approaches allow easy access to complex oligosaccharides, since the 2,3-double bond of the coupling product could be converted
into a glycosyl donor for a subsequent glycosylation. Unfortunately, as was noted above, the major drawbacks of the indirect approaches are the additional synthetic steps required for the introduction and removal of the participating auxiliary at C-3, reducing, therefore, the over-all efficiency of the process.

However, early attempts to prepare (2→8)-linked dimers by direct methods gave very disappointing results. For example, direct glycosylation of a 8-hydroxyl sialyl acceptor 35 with a 2-thiomethyl neuraminyl donor 9 in the presence of DMTST in MeCN at –40°C affords only trace amount (5%) of the desired α-linked dimer (Scheme 1.8.2). 83

Scheme 1.8.2

Glycosylphosphites proved to be more effective for direct sialylation. Thus, (2→8)-linked derivatives were obtained in good yields (22-68%) (Scheme 1.8.3), 153,154 in this case, however, the reactivity of the acceptor can be increased by conformational changes in the glycosyl acceptor to minimize steric and electronic interactions around the 8-hydroxyl of Neu5Ac. Some of these interactions can also be removed by the introduction of the 2,3-dehydro moiety in the acceptor. In this case, the C-1 carbonyl is remote from C-8 hydroxyl and no hydrogen bonding can take place. The improvement of
the glycosyl acceptor properties resulted in good yields of coupling products but unfortunately mainly β-anomeric selectivity was achieved (Scheme 1.8.3).

**Scheme 1.8.3**

Recently, another way to address the difficulties of the direct synthesis of the Neu5Acα(2→8)Neu5Ac dimer was reported. As already discussed above for different glicosidic linkages, an additional N-acetyl moiety at C-5 of a glycosyl donor dramatically increases its reactivity and gives higher yields in glycosylations. In addition, this highly reactive donor can be successfully applied to the synthesis of Neu5Acα(2→8)Neu5Ac dimers. For example, coupling of a 2-thiomethyl-5-acetylamido glycosyl donor with the C-8 hydroxyl of a mono-N-acetylated acceptor in the presence of NIS/TfOH in MeCN at -40°C gives a dimer as a mixture of
anomers in a modest yield (16%, α/β 1:1, Scheme 1.8.4). The application of a di-N-acetylated glycosyl acceptor under identical reaction conditions, gives the dimer in a much improved yield and stereoselectivity (50%, α/β 2:1). These results illustrate that high yields of the coupling products can be obtained when the 5-acetamido moieties of both the neuraminyl glycosyl donor and acceptor are derivatized into N-acetylacetamido functionalities. An attempt to reduce steric hindrance around O-8 by applying a 7,8-diol as an acceptor resulted in an excellent yield (61%) of the coupling product but in this case mainly β-anomer was formed (α/β 1:2). These findings are remarkable since reduction of steric hindrance around C-3 of a galactosyl acceptor results in higher α-stereoselectivities.

Scheme 1.8.4

A completely different strategy for obtaining oligosaccharides that have Neu5Acα(2→8)Neu5Ac moiety involves mild acid treatment of colominic acid,¹⁵⁵ which is a homopolymer consisting of the repeating Neu5Acα(2→8)Neu5Ac moieties. The dimer can be separated from higher oligomers and monomers and then be converted into
a glycosyl donor by chemical manipulations. Unfortunately, such a donor gives lower yields of coupling products compared to monomeric glycosyl donors, especially when applied for the glycosylation of secondary sugar alcohols. For example, a NIS/TfOH mediated coupling of a 2-thiophenyl dimeric derivative 36 with the C-3 hydroxyl of the lactoside 37 afforded a (2→3)-linked product in a yield of 31% (Scheme 1.8.5). Trimeric as well as tetrameric glycosyl donors have also been coupled with a lactosyl acceptor to give oligosaccharides in reasonable yields of 49% and 35%, respectively.

![Scheme 1.8.5](image)

2-Thiomethyl and 2-chloro dimeric derivatives have also been employed. It was, however, observed that these compounds (especially halides) give lower yields compared to similar 2-thiophenyl donors.

The synthesis of (2→9)-linked oligomers is relatively straightforward due to the high reactivity of the primary C-9 hydroxyl group. It is not surprising that the first synthesis of a dimeric structure was accomplished by the coupling a 9-hydroxyl and a sialyl chloride 2 to give a (2→9)-linked dimer in reasonable yield as a mixture of
anomers (α/β-anomers 2:1, 41%, Scheme 1.8.6).\textsuperscript{160} The yield was significantly improved (83%, α/β 2:1) by employing a partially protected acceptor that has less steric hindrance around C-9.\textsuperscript{161} In a similar fashion, a 2-thiomethyl sialyl donor was successfully applied for the glycosylation of a 7,8,9-triol glycosyl acceptor.\textsuperscript{162} The (2→9)-linked dimer was obtained with high regioselectively in a yield of 67% as 2:1 mixture of α/β anomers. Again, the N-acetylacetamido approach\textsuperscript{129} gives (2→9)-linked derivatives in improved yields of 67-98% (α/β 2.0-2.5:1).

![Diagram of glycosylation reaction](image)

**Scheme 1.8.6**

In the above-discussed cases, the anomeric stereoselectivities are far from being satisfactory. Therefore, indirect methods, using a participating auxiliary at C-3, were employed. Thus, the 2-bromo-3-hydroxy glycosyl donor was coupled with an acceptor in the presence of AgOTf/Na₂HPO₄.\textsuperscript{132,135} Unfortunately, even in this case the (2→9)-linked dimer was isolated in 63% yield as a 2:1 mixture of α/β-anomers. It is to be expected that other indirect methods would allow more efficient anomeric control in the glycosylation of a C-9 hydroxyl.
1.9. NMR Rules for the Anomeric Assignments

NMR spectroscopy offers the most efficient method to determine the anomeric configuration of sialosides, although other physical methods have been used.¹ For example, reliable anomeric assignments based on $[\alpha]_D$ determination have been reported but this method requires both $\alpha$- and $\beta$- anomers. For normal pyranosides, assignment of the anomeric configuration is based on measuring the following coupling constants: $^3J_{H_1,H_2}$ and $^1J_{C-1,H-1}$. This approach is not applicable for sialic acid due to the absence of an anomeric hydrogen at C-2. Therefore, empirical rules have been developed based on chemical shift data and coupling constants. Five different parameters can be used which include $\delta H-3eq$, $^\delta H-4$, $^J_{H-7,H-8}$, $\Delta\delta \{H-9a - H-9b\}$, and $^J_{C-1,H-3ax}$. Thus, it was observed that the H-3eq signals of $\alpha$-linked compounds are shifted downfield ($\delta = 2.67\text{-}2.72$ ppm) compared to those of $\beta$-anomers ($\delta = 2.25\text{-}2.40$ ppm).¹⁶³ This rule is especially useful for unprotected sialosides and does not always apply to O-acetylated derivatives. Another rule, which states that the signal of H-4 of an $\alpha$-anomer appears at higher field ($\delta = 4.89\text{-}4.93$ ppm) than that of the corresponding $\beta$-anomer ($\delta = 5.68\text{-}5.81$ ppm), can also be applied for unprotected sialosides ($\delta = 3.6\text{-}3.8$ ppm is characteristic for an $\alpha$-glycosidic linkage, whereas $\delta = 3.9\text{-}4.2$ ppm for $\beta$-anomers).¹⁶⁴,¹⁶⁷ The $^J_{H-7,H-8}$ coupling constants of $\alpha$-glycosides are larger (6.2\text{-}8.5 Hz) than those of $\beta$-anomers (1.5\text{-}2.6 Hz), which suggests different conformations of the side chain.¹⁶⁴,¹⁶⁵,¹⁶⁸ The anomeric configuration of the sialosides can also be determined from the chemical shift difference of two vicinal protons at C-9, e. a. the $\Delta\delta \{H-9a - H-9b\}$ value, is smaller than 0.5 ppm for $\alpha$-glycosides, whereas being around 1.0 ppm for the corresponding $\beta$-anomers.¹⁶⁵ A $^{13}$C NMR technique and 2D-methods can be employed for the determination of the
anomeric configuration by measuring the long-range $J_{C,H}$ coupling constants. It was demonstrated that sialic acid residues having an axial carboxylic function ($\alpha$-anomers) show a larger $J_{C-1,H-3ax}$ coupling constant (5.8-7.5 Hz) than the corresponding equatorial carboxyl derivative ($\beta$-anomers, 1.0-1.7 Hz).\textsuperscript{166,169,170} Moreover, the $J_{C-2,H-3ax}$ values were found to be $\sim$8.0 Hz for the $\alpha$-anomers and $\sim$4.0 Hz for the $\beta$-anomers (Table 1.9.1).\textsuperscript{171}

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CHAPTER 2

A STEREOSELECTIVE APPROACH FOR THE SYNTHESIS OF \( \alpha \)-SIALOSIDES

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\(^1\)De Meo, C.; Demchenko, A. V.; Boons, G-J. *J. Org. Chem.*, **2001**, 66, 5490-5497

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Abstract

A highly efficient synthesis of the human melanoma associated antigen GD₃ derivative has been described. A key feature of the synthetic approach was the use of sialyl donors that were protected with a C-5 trifluoroacetamide moiety. These sialyl donors gave high yields and excellent α-anomeric selectivities in direct glycosylations with a wide variety of glycosyl acceptors ranging from C-8 hydroxyls of sialic acids, C-3 hydroxyls of galactosides to reactive primary alcohols.

Introduction

Sialic acids are a diverse family of naturally occurring 2-keto-3-deoxy-nononic acids that are involved in a wide range of biological processes.¹ ² The most abundant sialic acid derivative is N-acetylneuraminic acid (Neu5Ac), however, compounds that have a glycolyl moiety at the C-5 amino group (Neu5Gc) or acetyl esters at one or more hydroxyls are also frequently encountered in nature.¹ ¹⁰ Sialic acids normally appear at terminal positions of oligosaccharides of glycoproteins and glycolipids where they are α(2,3) or α(2,6) linked to galactosides or α(2,6) linked to 2-acetamido-galactosides. The disialosyl structures Neu5Acα(2-8)Neu5Ac and Neu5Acα(2-9)Neu5Ac have also been found as constituents of oligosaccharides of glycoproteins and lipids. These sub-structures are receptors for viruses and bacteria and constitute the immunodominant epitope of tumor-associated antigens. Neu5Ac or Neu5Gc also occur in linear homopolymers where they are usually linked internally by α(2,8), α(2,9) or alternating α(2,8)/α(2,9) glycosidic linkages. These polysialic acids play important roles as neural cell adhesion molecules.⁷
While relatively efficient methods have been developed for the introduction of Neu5Acα(2-3)Gal and Neu5Acα(2-6)Gal glycosidic linkages, the synthesis of oligosaccharides that contain α(2→8)-linked fragments is complicated by the low reactivity of the C-8 hydroxyl of Neu5Ac\textsuperscript{130,172,173} The latter glycosides have been successfully synthesized by indirect chemical approaches whereby modified sialyl donors are employed that have a participating auxiliary at C-3.\textsuperscript{144,148,151,152} These highly elaborated donors require, however, laborious procedures for their preparation and after a glycosylation, additional steps are needed for the removal of the auxiliary. Alternative direct glycosylation approaches have been reported but these lead to either low yielding glycosylations or formation of unnatural β-sialosides or mixtures of anomers.\textsuperscript{129,153,154}

It is obvious that a versatile sialyl donor needs to be developed that gives excellent yields and high α-anomeric selectivities in direct glycosylations with a wide range of acceptors of different reactivities.\textsuperscript{130} Such a donor would allow efficient synthesis of oligosaccharides of biological or medical importance that contain multiple sialic acids of different linkage type.

Here, we report that the readily available sialyl donor methyl(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-5-trifluoroacetamido-D-glycero-β-D-galacto-nonulopyranosid)onate (2a) gives good yields and excellent α-anomeric selectivities in direct glycosylations with a wide variety of glycosyl acceptors ranging from sterically hindered C-8 hydroxyls of a sialic acid, C-3 hydroxyls of galactosides to reactive primary alcohols. The versatility of the donor allowed a highly efficient synthesis of the human melanoma associated antigen GD\textsubscript{3} derivative, which has multiple Neu5Ac residues.
**Results and discussion:**

The synthesis of the human melanoma associated antigen GD₃ derivative requires the introduction of a Neu5Acα(2-8)Neu5Ac and a Neu5Acα(2-3)Gal glycosidic linkages. In addition, the anomeric center of a sialyl acceptor needs temporary protection, which required glycosylation with a primary alcohol. Previous syntheses of the carbohydrate part of this biologically important glycosphingolipid could only be achieved by *indirect* sialylation protocols¹⁴⁹,¹⁷⁴,¹⁷⁵ or a strategy whereby an α(2-8)-linked fragment was obtained by controlled degradation of colominic acid.¹⁰⁰,¹⁵⁵,¹⁵⁷ Our attempts to synthesize (2-8)-linked dimers by direct sialylation employing conventional glycosyl donors (2-thiomethyl, 2-thiophenyl, 2-xanthate, and 2-phosphite) have failed and only traces of the desired products were obtained.

It was anticipated that sialyl donor 2a, which is protected with a 5-trifluoroacetamido (N-TFA) group, would have superior glycosyl donor properties compared to the traditional donor 2b that has an acetamido functionality at C-5 and therefore would allow an efficient synthesis of GD₃ by a *direct* glycosylation approach. This expectation was based on the recent finding that 2-thio-sialyl donor 2c, which has an electron withdrawing N-acetylacetamido moiety at C-5, is more reactive and gives significantly higher yields in glycosylations compared to donor 2b.¹²⁸,¹²⁹ In particular, this glycosyl donor allowed the synthesis of a Neu5Acα(2-8)Neu5Ac dimer in a good yield but as a mixture of anomers.¹²⁹ Probably, a strong electron withdrawing amino protecting group reduces the nucleophilicity and basicity of the acetamido functionality; properties that hamper successful glycosylations. Furthermore, an N-TFA protecting group has as an additional advantage that it can be easily introduced and removed under mild reaction
conditions. The latter feature makes it possible to functionalize the amino group of sialic acids as N-acetyl, N-glycolyl or other derivatives.

The TFA protected sialyl donor 2a and acceptor 5a were prepared in a highly convergent manner from the known precursor 1. Thus, selective N-trifluoracetylation of 1 with CF₃COOMe in the presence of Et₃N in MeOH followed by O-acetylation with Ac₂O/pyridine gave sialyl donor 2a in overall yield of 73%. Compound 1 was readily converted into glycosyl acceptor 5a. Thus, glycosylation of thioglycoside 2a with 2-(trimethylsilyl)ethyl alcohol (TEOH) in the presence of NIS/TfOH and molecular sieves in MeCN at −33°C furnished 3 in a good yield as mainly the α-anomer (75%, α/β =10/1). Separation of the anomers could be easily accomplished at a later stage of the synthesis. The glycoside 3 was converted into the selectively protected derivative 4 by subsequent deacetylation with MeONa in MeOH, regioselective protecting of the 8,9-diol as a benzylidene acetal by treatment with dimethoxytoluene in the presence of camphorsulfonic acid (CSA) in acetonitrile and O-acetylation of the remaining hydroxyls at C-4 and C-7 with Ac₂O/pyridine. The benzylidene acetal of 4 was regioselectively opened to a C-8 hydroxyl by the treatment with BH₃·NMe₃ complex in the presence of anhydrous AlCl₃ and molecular sieves 4Å in dry THF to give sialyl acceptor 5a.
The glycosyl donor properties of 2a were examined in glycosylations with the C-8 hydroxyl of sialyl acceptors 5a-c bearing a N-TFA, acetamido, or acetylacetamido moiety at C-5, respectively (Scheme 2.2). Thus, coupling of 2a with 5a in the presence of NIS/TfOH/MS3Å in acetonitrile at -35°C gave (2-8)-linked sialoside 6a in an excellent yield of 55%. After Sephadex LH-20 size exclusion column chromatography, HPLC and NMR analysis of the product showed the presence of only one anomer. Conventional empirical NMR rules for the assignment of anomeric configurations of sialosides may
not be applicable to TFA-protected derivatives. Therefore, dimer \(6a\) was converted into the known \(N\)-acetamido derivative \(7\) by concomitant \(N,O\)-deacylation with \(1\)M aq. NaOH-MeOH, followed by selective \(N\)-acytylation with \(\text{Ac}_2\text{O}\) in MeOH. The NMR data and optical rotation of \(7\) unambiguously confirmed the \(\alpha\)-anomeric configuration of the product. Thus, \(H-3'e\)q in \(^1\text{H}-\text{NMR}\) spectrum of \(7\) was shifted downfield (\(\delta = 2.60\) ppm) compared to that of the previously synthesized \(\beta\)-anomer (\(\delta = 2.37\) ppm),\(^{129}\) whereas \(H-4'\) of \(7\) appeared at higher field (\(\delta = 3.53\) ppm) than that of \(\beta\)-anomer (\(\delta = 4.05\) ppm).

Glycosylation of \(5b\) and \(5c\)\(^{129}\) with \(2a\) under similar reaction conditions (NIS/TfOH/MS3Å/MeCN, -35°C) provided stereoselective formation of \(\alpha\)-sialosides \(6b\) and \(6c\), respectively but yields were significantly lower (34 and 30\% yield, respectively). The modest yield of \(6c\) could be explained by a competing acetyl group migration from \(N-5\) to \(C-8\) of acceptor \(5c\), whereas the nucleophilicity and basicity of the acetamido moiety of \(5b\) probably caused the low yield of \(6b\). It is important to note that glycosylation of \(2b\)\(^{84}\) or \(2c\)\(^{128}\) with acceptor \(5a\) gave mixtures of anomers in poor or \(N-5\) to \(C-8\) of acceptor \(5c\), whereas the nucleophilicity and basicity of the acetamido moiety of \(5b\) probably caused the low yield of \(6b\). It is important to note that glycosylation of \(2b\)\(^{84}\) or \(2c\)\(^{128}\) with acceptor \(5a\) gave mixtures of anomers in poor or reasonable yield, respectively, demonstrating the superior glycosyl donating properties of \(2a\).
Encouraged by these results, attention was focused on the formation of sialosides of a C-3 hydroxyl of the galactosyl residue of a partially protected derivative of D-lactose (Scheme 2.3). Thus, NIS/TfOH-mediated coupling of 2a with partially protected lactosyl acceptor 8a\textsuperscript{128} gave trisaccharide 9 in an excellent yield of 84% as only the $\alpha$-anomer. This is a remarkable result because similar glycosylations with monosaccharide donor 2b,
which has a conventional NHAc at C-5, gave anomeric mixtures with yields ranging from 30-60\%. Furthermore, these traditional glycosylations require up to three equivalents of glycosyl donor 2a whereas for the preparation of 9 only two equivalents of 2a were used. The N-TFA protecting group of 9 was converted into N-acetyl derivative by two-step procedure to give 10 in an 85% over-all yield. In this case, the NMR data of 10 also unambiguously confirmed the presence of α-sialyl linkage (H-4' δ = 3.79 ppm, Δδ {H-9’a – H-9”b} = 0.12 ppm).

For the synthesis of GD3 derivative 14, dimer 6a was converted into the second-generation thioglycosyl donor 12. Cleavage of the anomeric 2-(trimethylsilyl)ethyl glycoside of 6a with TFA/DCM, followed by acetylation of the resulting lactol with Ac2O/pyridine gave anomeric acetate 11 as predominantly the β-anomer. Conversion of the anomeric acetate of 11 into a 2-thiophenyl sialoside 12 was easily accomplished by the treatment with thiophenol in the presence of BF3·Et2O. On the other hand, reaction of 11 with TMSSMe was very sluggish and gave the corresponding 2-thiomethyl sialoside in a low yield. NIS/TfOH mediated glycosylation of 12 with lactosyl acceptor 8b afforded tetrasaccharide 13 in an excellent yield of 42% as only the α-anomer. In this case, only one equivalent of sialyl donor 12 was used to achieve this result. Furthermore, the stereoselective preparation of disaccharides 6a-c, trisaccharide 9, and tetrasaccharide 13 indicates that regardless of the protecting group pattern of donor and acceptor, N-TFA protected sialyl donors give stereoselective formation of α-sialosides in good yields.
Scheme 2.3. Reactions and conditions: i: NIS/TfOH/MS3Å, MeCN, -35°C
ii: 1M aq. NaOH - MeOH; iii: Ac₂O/MeOH; iv: TFA, DCE
v: Ac₂O/C₆H₅N; vi: PhSH/BF₃-Et₂O/MS3Å, DCM
vii: H₂, Pd(OAc)₂, EtOH-EtOAc 1/1
The benzyl ethers of compound 13 were removed by catalytic hydrogenation over Pd(OAc)$_2$ and the N-TFA of the resulting partially deprotected intermediate was converted into the corresponding acetamido moiety by the treatment with sodium hydroxide followed by N-acetylation with acetic anhydride in methanol to give the requisite GD$_3$ derivative 14. The NMR data of 14 showed that only the required $\alpha$-linked sialosides were formed. Based on the NMR data of 2a, 6a-c, 9, and 11-13 the anomeric configuration of N-TFA-protected derivatives can be determined from the chemical shift data of $H$-4 ($\delta = 5.30 – 5.45$ ppm for $\beta$-anomers and $\delta = 4.92-5.11$ ppm).

**Conclusion**

It has been shown that modification of the C-5 amino group of 2-methyl and 2-thiophenyl sialosides into N-TFA derivatives provides glycosyl donors that give good yields and high $\alpha$-anomeric selectivities in direct glycosylation with a wide range of glycosyl acceptors of differing reactivities. These new donors allowed, for the first time, the stereoselective synthesis of $\alpha$-(2-8)-linked dimers in high yields. The best yields were obtained when the amino functionality of the sialyl acceptor was also protected as N-TFA derivative. Previous synthetic routes towards this dimer required laborious indirect sialylation protocols. The favorable properties of the new donors allowed a highly efficient synthesis of the human melanoma associated antigen GD$_3$ derivative, which has two Neu5Ac residues of different linkage type. The N-TFA-protecting group could be easily transformed into Neu5Ac derivatives and potentially they open an efficient route towards sialic acid containing oligosaccharides that have modified amino functionalities. The high yields of the glycosylations probably results from low nucleophilicity of the amino functionalities of sialosides. The origin of the high $\alpha$-selectivity of N-TFA
protected sialyl donors is unclear and theoretical studies are underway to shed light on this effect.

**Experimental section**

**General.** Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), size exclusion column chromatography was performed on Sephadex LH-20 (MeOH or MeOH-CH$_2$Cl$_2$, 1/1, v/v elution) or Sephadex G-25 (water elution). HPLC chromatography was performed on Prodigy 5µ Silica 100Å column (250 x 10 mm, CH$_2$Cl$_2$-ethyl acetate elution). Reactions were monitored by TLC on Kieselgel 60 F$_{254}$ (EM Science) and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40°C. CH$_2$Cl$_2$, (ClCH$_2$)$_2$, and MeCN were distilled from CaH$_2$ (twice) and stored over molecular sieves (3Å). THF was distilled from sodium directly prior to the application. Methanol was dried by refluxing with magnesium methoxide, distilled and stored under argon. Pyridine was dried by refluxing with CaH$_2$, then distilled and stored over molecular sieves (3Å). Molecular sieves (3Å and 4 Å), used for reactions, were crushed and activated in vacuo at 390°C during 8 h in the first instance and then for 2-3 h at 390°C directly prior to application. Optical rotations were measured with a ‘Jasco P-1020’ polarimeter. $^1$H NMR and $^{13}$C NMR spectra were recorded with a Varian Inova500 spectrometer and a Varian Inova600 spectrometer equipped with Sun workstations. Unless otherwise noted $^1$H NMR spectra were recorded in CDCl$_3$ and referenced to residual CHCl$_3$ at 7.24 ppm, and $^{13}$C NMR spectra to the central peak of CDCl$_3$ at 77.0 ppm. Assignments were made by standard gCOSY and gHSQC. High resolution mass
spectra were run in a JMS SX/SX102A tandem mass spectrometer, equipped with FAB source. The matrix used was thioglycerol and the internal standards ultramark 1621 and PEG.

Methyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-5-trifluoroacetamido-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (2a). Methyl trifluoroacetate (3.5 mL, 34 mmol) was added to a solution of 1 (1.07 g, 3.44 mmol) and triethylamine (0.96 mL, 6.9 mmol) in methanol (40 mL). After 1.5 h, the reaction mixture was concentrated under reduced pressure and dried *in vacuo*. Pyridine (20 mL) and Ac₂O (10 mL) were added to the residue. After 16h, the reaction was quenched with MeOH (15 mL), concentrated, co-evaporated with toluene (3 x 15mL) and dried *in vacuo*. The residue was purified by silica gel column chromatography (10% gradient ethyl acetate in hexane) to afford 2a as a white foam (1.44 g, 73%): FAB MS: m/z = 598.1 [M+Na]⁺; Rf 0.51 (acetone/toluene, 3/7, v/v); [α]²⁷_D = - 44.4 (c 0.6, CHCl₃); ¹H NMR: δ: 6.77 (d, 1H, J NH,5 = 10.1 Hz, NH), 5.37-5.44 (m, 2H, J₄,₅ = 11.5 Hz, J₇,₈ = 11.2 Hz, H-4, 7), 5.16-5.19 (m, 1H, J₈,₉_a = 2.5 Hz, J₈,₉_b = 7.8 Hz, H-8), 4.78 (dd, 1H, J₉_a,₉_b = 12.5 Hz, H-9a), 4.50 (dd, 1H, J₆,₇ = 2.5 Hz, H-6), 4.18 (dd, 1H, H-9b), 4.03 (dd, 1H, J₅,₆ = 10.5 Hz, H-5), 3.83 (s, 3H, OCH₃), 2.58 (dd, 1H, J₃_e,₄ = 5.0 Hz, J₃_e,₃_a = 13.4 Hz, H-3e), 2.16 (dd, 1H, H-3a), 2.14, 2.09, 2.05, 2.04, (4s, 12H, OCOCH₃), 2.03 (s, 3H, SMe). ¹³C NMR: δ: 171.4, 171.1, 170.6, 170.0, 167.8, 167.7, 85.0, 72.6, 71.3, 68.9, 68.5, 62.5, 53.2, 50.6, 37.2, 21.3, 21.1, 20.9, 11.73. HR-FAB MS [M+H]⁺ calcd for C₂₁H₂₉O₁₂NF₃S 576.1363, found 576.1360.
Methyl [2-(trimethylsilyl)ethyl 4,7,9-tri-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α,β-D-galacto-non-2-ulopyranosid]onate (3). A mixture of 2a (1 g, 1.74 mmol), 2-(trimethylsilyl)ethanol (0.5 mL, 3.5 mmol) and activated molecular sieves (3Å, 300 mg) in MeCN (35 mL) was stirred for 16 h under an atmosphere of argon at rt, then cooled to -35°C and NIS (783 mg, 3.48 mmol) and TfOH (31µL, 0.35 mmol) were added. The reaction mixture was stirred for 15 min until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (100 mL), the solids were filtered-off and the residue was washed with DCM (3 x 100 mL). The combined filtrate (400 mL) was washed with aqueous Na₂S₂O₃ (20 %, 150 mL) and H₂O (3 x 80 mL). The organic phase was dried (MgSO₄), filtered and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (10% gradient ethyl acetate in hexane) to afford 3 as 10/1 α/β-mixture (848 mg, 75%) as a white foam: Analytical data for α-3: FAB MS: m/z = 646.2 [M+H]⁺; \( R_f \) 0.35 (ethyl acetate/hexane, 2/3, v/v); [α]²⁷⁷ D = - 8.3 (c 0.6, CHCl₃); \(^1\)H NMR: δ; 6.24 (d, 1H, J NH,5 = 9.8 Hz, NH), 5.35-5.40 (m, 1H, J 8,9a = 2.4 Hz, J 8,9b = 4.9 Hz, H-8), 5.26 (dd, 1H, J 7,8 = 8.3 Hz, H-7), 4.92-4.98 (m, 1H, J 4,5 = 10.2 Hz, H-4) 4.27 (dd, 1H, J 9a,9b = 12.7 Hz, H-9a), 4.23 (dd, 1H, J 6,7 = 1.9 Hz, H-6), 4.11 (dd, 1H, H-9b), 3.94 (dd, 1H, H-9b), 3.84-3.90 (m, 1H, J 2 = 9.3Hz, OCH₂a) 3.79 (s, 3H, OCH₃), 3.28-3.34 (m, 1H, OCH₂b), 2.62 (dd, 1H, J 3e,4 = 4.4 Hz, J 3c,3a = 12.7 Hz, H-3e), 2.14, 2.11, 2.01, 1.99, (4s, 12H, OCOCH₃), 1.90 (s, 3H, TMS), 0.80-0.92 (m, 2H, CH₂TMS), 0.00 (s, 9H, TMS) \(^{13}\)C NMR: 71.5, 67.0, 62.8, 62.7, 62.0, 52.6, 50.4, 38.3, 20.8, 20.5, 20.4, 17.9, 10.9. HR-FAB MS [M+H]⁺ calcd for C₂₅H₃₉O₁₃NF₃Si 646.2143, found 646.2148.
Methyl [2-(trimethylsilyl)ethyl 4,7-di-O-acetyl-9-O-benzyl-3,5-dideoxy-5-trifluoroacetamido-
D-glycero-α-D-galacto-non-2-ulopyranosid]onate (5a). Sodium methoxide (7 mL, 1M solution in methanol) was added to the solution of 3 (848 mg, 1.31 mmol) in methanol (100 mL). After 2 h, the reaction mixture was neutralized with Dowex-50 H⁺ resin (pH = 7), which was removed by filtration. The filtrate was concentrated under reduced pressure to afford methyl [2-(trimethylsilyl)ethyl 3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-
D-galacto-non-2-ulopyranosid]onate (615 mg, 98%). The residue was dissolved in MeCN (15 mL) and benzaldehyde dimethyl acetal (0.39 mL, 2.57 mmol) and CSA (30 mg, 0.13 mmol) were added. After 1 h, the reaction mixture was neutralized with triethylamine (pH = 7), and the solvent was evaporated under reduced pressure. The residue was dissolved in DCM (70 mL) and washed with saturated aqueous NaHCO₃ (20 mL) and H₂O (3 x 25 mL). The organic phase was dried (MgSO₄), filtered and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (10% gradient ethyl acetate in toluene) to afford anomerically pure methyl [2-(trimethylsilyl)ethyl 8,9-O-benzylidene-3,5-dideoxy-5-trifluoroacetamido-D-
glycero-α-D-galacto-non-2-ulopyranosid]onate as a white foam (447 mg, 61%, exo/endo 1/1), which was dissolved in a mixture of Ac₂O (8 mL) and pyridine (16 mL). After 16 h, the reaction was quenched with MeOH (10 mL), concentrated in vacuo and residue was co-evaporated with toluene (3 x 20 mL) to give 4 as a white foam (492 mg, 96%), which was used without further purification; Rf 0.42 (acetone/hexane, 2/3 v/v). Comparison of the integral intensities of NH 6.31 (d, 1H) and 6.20 (d, 1H), or CHPh 5.87 (s, 1H) and 5.79 (s, 1H) signals in the ¹H NMR spectrum showed 1:1 mixture of exo/endo isomers. BH₃·NMe₃ (307 mg, 4.21 mmol), AlCl₃ (546 mg, 4.10 mmol) were added to a solution of
4 (441 mg, 0.68 mmol) and activated molecular sieves (4Å, 2.65 g) in THF (8 mL) at 0°C. After stirring for 2 h at 0°C and another 3 h at rt, the reaction mixture was diluted with Et₂O (50 mL), the solids were filtered-off and washed with Et₂O (3 x 50 mL). The combined filtrate (200 mL) was washed with saturated aqueous NaHCO₃ (2 x 70 mL) and H₂O (3 x 40 mL). The organic phase was dried (MgSO₄), filtered and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (10% gradient ethyl acetate in toluene) to afford 5a as a white foam (319 mg, 72%): FAB MS: m/z = 652.2 [M+H]⁺; Rf 0.55 (ethyl acetate/toluene 2/3, v/v); [α]²⁷D = -4.7 (c 1.2, CHCl₃); ¹H NMR: δ; 7.20-7.37 (m, 5H, aromatic), 6.54 (d, 1H, J₆₇,₅ = 7.8 Hz, NH), 5.12 (d, 1H, J₇₈ = 8.8 Hz, H-7) 4.92-5.00 (m, 1H, J₄₅ = 9.8 Hz H-4), 4.50-4.58 (m, 2H, CH₂Ph), 4.11-4.17 (m, 1H, J₈₉a = 3.4 Hz, J₈₉b = 6.0 Hz, H-8), 4.05-4.10 (m, 2H, H-5, 6), 3.89 (dd, 1H, J₂ = 8.5 Hz, OCH₂ᵃ), 3.86 (s, 3H, OCH₃), 3.76 (d, 1H, JOH₈ = 6.0 Hz, OH), 3.53 (dd, 1H, J₉₉a,₉₉b = 10.2 Hz, H-9a), 3.46 (dd, 1H, H-9b), 3.42 (dd, 1H, OCH₂ᵇ), 2.73 (dd, 1H, J₃c₃a = 4.9 Hz, J₃c₃a = 12.7 Hz, H-3e), 2.09, 2.02, (2s, 6H, OCOCH₃) 1.98 (t, 1H, H-3a), 0.84-0.95 (m, 2H, CH₂TMS), 0.00 (s, 3H, TMS). ¹³C NMR: δ, 170.8, 170.2, 169.5, 158.0, 138.0, 128.5, 128.2, 127.8, 98.8, 73.8, 72.2, 70.9, 69.6, 69.2, 62.77, 53.7, 50.3, 37.9, 21.1, 20.9, 18.3, -0.93. HR-FAB MS [M+H]⁺ calcd for C₂₈H₄₁O₁₁NF₃Si 652.2401, found 652.2410.

Methyl [2-(trimethylsilyl)ethyl 4,7-di-O-acetyl-9-O-benzyl-3,5-dideoxy-8-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-5-trifluoroacetamido-D-glycero-α-D-galacto-non-2-ulopyranosid]onate (6a). A mixture of donor 2a (85 mg, 0.147 mmol), 5a (32 mg, 0.049 mmol) and activated molecular sieves (3Å, 250 mg) in MeCN (1.5 mL) was stirred for 16h under an
atmosphere of argon at rt. The mixture was cooled to -35°C and NIS (0.294 mmol) and TfOH (0.03 mmol) were added and the reaction mixture was stirred for 5 min until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (10 mL), the solids were filtered-off and the residue was washed with DCM (3 x 10 mL). The combined filtrate (40 mL) was washed with aqueous Na₂S₂O₃ (20 %, 15 mL) and H₂O (3 x 20 mL). The organic phase was dried (MgSO₄), filtered and the filtrate was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford 6a as a white foam (32.0 mg, 55%): FAB MS: m/z = 1201.3 [M+Na]⁺; Rf 0.51 (ethyl acetate/hexane, 5/5, v/v); [α]²⁷_D = +2.7 (c 1.1, CHCl₃); ¹H NMR: δ; 7.67 (d, 1H, J₉,₅ = 9.8 Hz, NH), 7.25-7.37 (m, 5H, aromatic), 6.24 (d, 1H, J₉,₅' = 9.3 Hz, NH), 5.58-5.62 (m, 1H, J₆,7 = 11.7 Hz, H-6), 4.99-5.06 (m, 1H, J₄,₅ = 11.7 Hz, H-4), 4.89-4.96 (m, 1H, J₄,₅ = 11.7 Hz, H-4'), 4.38 (dd, 1H, J₃'a,₃'' = 12.7 Hz, H₃'a), 3.81 (m, 1H, J₃'' = 8.8 Hz, OCH₂), 3.54 (dd, 1H, H-9b), 3.31-3.37 (m, 1H, OCH₂), 2.78 (dd, 1H, J₃'' = 4.4 Hz, J₃'' = 12.7 Hz, H₃'e), 2.20, 2.06, 2.05, 2.02, 2.01, 2.00 (6s, 18H, OCOCH₃), 2.07-2.18 (m, 1H, H-3'a), 1.93 (dd, 1H, H-3a), 0.75-0.90 (m, 2H, CH₂TMS), -0.01 (s, 3H, TMS). ¹³C NMR: 172.3, 171.2, 170.8, 170.3, 169.9, 169.8, 168.9, 168.4, 157.6, 138.9, 128.5, 127.6, 127.4, 99.01, 96.9, 73.9, 73.8, 72.5, 71.8, 10.6, 69.9, 69.4, 68.7, 68.6, 66.9, 62.7,
Methyl [2-(trimethylsilyl)ethyl 5-acetamido-4,7-di-O-acetyl-9-O-benzyl-3,5-dideoxy-8-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-non-2-ulopyranosylonylate)-D-glycero-α-D-galacto-non-2-ulopyranosid]onate (6b). A mixture of donor 2a (81 mg, 0.140 mmol), 5b (28 mg, 0.047 mmol) and activated molecular sieves (3 Å, 300 mg) in MeCN (0.5 mL) was stirred for 16h under an atmosphere of argon. The mixture was cooled to -35°C and NIS (0.28 mmol) and TfOH (0.03 mmol) were added and the reaction mixture was stirred for 20 min until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (10 mL), the solids were filtered-off and the residue was washed with DCM (3 x 10 mL). The combined filtrate (40 mL) was washed with aqueous Na₂S₂O₃ (20 %, 15 mL) and H₂O (3 x 20 mL). The organic phase was dried (MgSO₄), filtered and the filtrate was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford 6b as a white foam (18.0 mg, 34%); FAB MS: m/z = 1125.4 [M+H]^+; Rf 0.51 (acetone / toluene, 5/5, v/v); [α]²⁷_D = +2.4 (c 0.3, CHCl₃); ¹H NMR: δ; 7.31-7.37 (m, 5H, aromatic), 6.37 (d, 1H, J_NH,S' = 9.6 Hz, NH), 6.17 (d, 1H, J_NH,S = 9.9 Hz, NH), 5.51-5.56 (m, 1H, J_8',9' = 4.3 Hz, H-8'), 5.29 (dd, 1H, J_7',8' = 9.8 Hz, H-7'), 5.24 (bs, 1H, H-7), 5.00-5.07 (m, 2H, J_4',5' = 10.2 Hz, J_9a,9b = 8.9 Hz, H-4', 8), 4.86-4.92 (m, 1H, J_4,5 = 11.7 Hz, H-4), 4.71 (dd, 2H, J_7 =11.6 Hz, CH₂Ph), 4.25-4.30 (m, 2H, J_9a,9b = 12.7 Hz, H-9') 4.17 (dd, 1H, J_6,9 =1.9 Hz, H-6), 4.02-4.08 (m, 2H, J_6,7 = 1.4 Hz, J_9a,9b = 11.9 Hz, H-6', 9a), 3.93-4.02 (m, 2H, J_5,6 = 9.7 Hz, J_5,6 = 10.6 Hz, H-5', 5), 3.86 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 3.78-3.84
(m, 1H, J^2 = 9.3 Hz, OCH\textsubscript{2}^a), 3.55 (dd, 1H, H-9b), 3.34-3.40 (m, 1H, OCH\textsubscript{2}^b), 2.76 (dd, 1H, J\textsubscript{3e,4} = 4.7 Hz, J\textsubscript{3e,3a} = 12.9 Hz, H-3'e), 2.65 (dd, 1H, J\textsubscript{3e,4} = 4.8 Hz, J\textsubscript{3e,3a} = 12.8 Hz, H-3e), 2.22 (s, 3H, NHCOCH\textsubscript{3}), 2.06, 2.05, 2.04, 2.02, 2.01, 1.90 (6s, 18H, OCOC\textsubscript{H}^3), 2.03 (m, 1H, H-3'a), 1.91 (dd, 1H, H-3a), 0.76-0.90 (m, 2H, CH\textsubscript{TMS}), 0.00 (s, 3H, TMS). \textsuperscript{13}C NMR: δ; 171.3, 171.1, 170.8, 170.6, 170.2, 170.0, 168.7, 168.5, 138.9, 128.5, 127.6, 127.4, 99.0, 97.4, 74.4, 74.0, 72.7, 71.7, 70.5, 70.0, 68.6, 67.1, 62.6, 62.4, 53.5, 52.8, 50.9, 49.3, 38.7, 38.1, 30.0, 23.4, 21.8, 21.3, 21.1, 21.0, 18.3, -0.89. HR-FAB MS [M+H]\textsuperscript{+} calcd for C\textsubscript{48}H\textsubscript{68}O\textsubscript{23}N\textsubscript{2}F\textsubscript{3}NaSi 1125.3934, found 1125.3900.

Methyl [2-(trimethylsilyl)ethyl 5-(N-acetylacetamido)-4,7-di-O-acetyl-9-O-benzyl-3,5-dideoxy-8-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-\alpha-D-galacto-non-2-ulopyranosyl)onate]-D-glycero-\alpha-D-galacto-non-2-ulopyranosid] onate (6c). A mixture of donor 2a (97 mg, 0.169 mmol), 5c (36 mg, 0.056 mmol) and activated molecular sieves (3Å, 340 mg) in MeCN (2.0 mL) was stirred for 16h under an atmosphere of argon. The mixture was cooled to -35°C and NIS (0.34 mmol) and TfOH (0.03 mmol) were added and the reaction mixture was stirred for 5 min until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (10 mL), the solids were filtered-off and the residue was washed with DCM (3 x 10 mL). The combined filtrate (40 mL) was washed with aqueous Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} (20 %, 15 mL) and H\textsubscript{2}O (3 x 20 mL). The organic phase was dried (MgSO\textsubscript{4}), filtered and the filtrate was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford 6c as a white foam (19.5 mg, 30%); FAB MS: m/z = 1189.4 [M+Na]\textsuperscript{+}; \textit{R}_f 0.53 (ethyl acetate/ hexane, 5/5, v/v); [\alpha]\textsuperscript{27}_D = -6.2 (c 0.7, CHCl\textsubscript{3}); \textsuperscript{1}H NMR: δ; 7.23-7.39 (m, 5H,
aromatic), 6.21 (d, 1H, J_{NH,5'} = 9.6 Hz, NH), 5.49-5.56 (m, 1H, J_{4,5} = 10.4 Hz, H-4), 5.29-5.33 (m, 1H, J_{8,9a} = 2.4 Hz, J_{8,9b} = 3.7 Hz, H-8'), 5.26 (dd, 1H, J_{7,8} = 9.2 Hz, H-7'), 5.04-5.11 (m, 2H, J_{4,5} = 10.4 Hz, H-4'), 4.96 (dd, 1H, J_{6,7} = 1.4 Hz, H-6), 4.59 (dd, 2H, J^2 = 12.0 Hz, CH_2Ph.), 4.41-4.45 (m, 1H, J_{8,9a} = 4.3 Hz, J_{8,9b} = 6.4 Hz, H-8), 4.24 (dd, 1H, J_{9a,9b} = 12.6 Hz, H-9'a), 4.03-4.12 (m, 4H, J_{5,6} = 9.9 Hz, J_{6,7'} = 1.8 Hz, J_{9a,9b} = 11.2 Hz H-5, 6', 9a, 9'b), 3.89 (m, 1H, J_{8,9} = 9.9 Hz, H-8'), 3.87 (m, 1H, J_{8,9b} = 11.2 Hz H-5, 6', 9a, 9'b), 3.85 (s, 3H, OC\_H\_3), 3.84 (s, 3H, OC\_H\_3), 3.85 (s, 3H, OC\_H\_3), 3.68 (dd, 1H, J_{8,9b} = 11.2 Hz H-5, 6', 9a, 9'b), 3.86 (s, 3H, OCH\_2\_a), 2.75 (dd,1H, J_{3a,3a} = 5.5 Hz, J_{3e,3a} = 13 Hz, H-3e), 2.67 (dd,1H, J_{3e,3a} = 4.8 Hz, J_{3e,3a} = 13 Hz, H-3e), 2.30, 2.28 (2s, 6H, N(COCH\_3)_2), 2.16, 2.03, 2.01, 1.99, 1.98, (5s, 18H, OCOCH\_3), 2.01 (m, 1H, H-3'a), 1.84 (dd, 1H, H-3'a), 0.84-0.88 (m, 2H, CH\_2TMS), 0.00 (s, 3H, TMS). 13C NMR: 174.2, 174.0, 170.7, 170.2, 170.1, 169.7, 169.6, 168.2, 168.1, 157.4, 139.0, 128.3, 127.3, 127.4, 99.4, 98.9, 75.3, 73.6, 71.7, 71.1, 70.8, 70.3, 68.7, 68.4, 67.1, 66.9, 62.4, 62.1, 60.7, 57.9, 53.4, 52.9, 50.7, 39.1, 38.5, 30.0, 28.2, 26.1, 21.4, 21.3, 21.1, 21.0, 20.9, 18.4, 14.6, -0.88. HR-FAB MS [M+Na]^+ calcd for C_{50}H_{69}O_{24}N_{2}F_{3}NaSi 1189.3859, found 1189.3880.

2-(Trimethylsilyl)ethyl 5-acetamido-8-O-(5-acetamido-3,5-dideoxy-D-glycero-\alpha-D-galacto-non-2-ulopyranosylonic acid)-9-O-benzyl-3,5-dideoxy-D-glycero-\alpha-D-galacto-non-2-ulopyranosonic acid disodium salt (7). From 6a. 1M aqueous NaOH (0.5 mL) was added dropwise to a solution of 6a (20 mg, 16.9 µmol) in MeOH (1 mL). After 72 h (control TLC, methanol/n-buthanol/water/triethylamine, 20/60/15/5, v/v/v/v) the reaction mixture was concentrated in vacuo and the residue was subjected to freeze drying for 16 h. MeOH (0.8 mL) and Ac\_2O (0.2 mL) were added and the reaction mixture was kept for 16 h and then concentrated under the reduced pressure. The residue was purified by size exclusion
column chromatography Sephadex G25 (35 cm$^3$, water elution) to afford 7 (11 mg, 78%) as a white film: FAB MS: m/z = 857.3 [M+H]$^+$; $R_f$ 0.42; $[\alpha]^{27}_D$ = +7.8 (c 0.375, H$_2$O); $^1$H NMR (D$_2$O): $\delta$; 7.21-7.30 (m, 5H, arom), 4.51 (dd, 2H, $J^2$ =11.8 Hz, CH$_2$Ph), 4.35-4.38 (m, 1H, $J_{8',9'a} = 2.4$ Hz, H-8'), 4.11 (dd, 1H, $J_{9'a,9'b} = 11.7$ Hz, H-9'a), 3.64-3.80 (m, 8H, $J_{7',8'} = 7.8$ Hz, H-5, 5', 6', 7', 9a, 9'b, OCH$_2$$_a$), 3.50-3.58 (m, 2H, H-4', 8), 3.35-3.47 (m, 4H, H-4, 7, 9b, OCH$_2$$_b$), 2.60 (dd, 1H, $J_{3'e,3'a} = 12.6$ Hz, $J_{3'e,4'} = 4.7$, H-3'e), 2.49 (dd, 1H, $J_{3e,3a} = 12.6$ Hz, $J_{3e,4} = 4.1$, H-3e), 1.92, 1.88 (2s, 6H, NHCOC$_3$) 1.60 (t, 1H, H-3'a), 1.41 (dd, 1H, H-3a), 0.67-0.77 (m, 2H, CH$_2$TMS), -0.18 (s, 3H, TMS). $^{13}$C NMR (D$_2$O): 175.2, 173.9, 173.5, 137.8, 128.8, 128.3, 101.3, 100.4, 76.1, 74.4, 73.4, 72.7, 72.2, 71.8, 71.0, 70.2, 68.8, 68.4, 68.2, 63.2, 62.7, 52.7, 51.9, 41.2, 40.6, 22.5, 22.2, 18.0, -3.0. The compound 7 obtained from 6b, 6c, and isolated in a similar way was completely identical (comparison of the NMR data, h.p.l.c. retention times and $[\alpha]_D$ values) to the sample obtained from 6a.

3-Azidopropyl $O$-[methyl 4,7,8,9-tetra-$O$-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-$\alpha$-D-galacto-non-2-ulopyranosylonate]-(2$\rightarrow$3)-$O$-(2,6-di-$O$-benzyl-$\beta$-D-galactopyranosyl)-(1$\rightarrow$4)-2,3,6-tri-$O$-benzyl-$\beta$-D-glucopyranoside (9). A mixture of donor 2a (70 mg, 0.122 mmol), 8a (53 mg, 0.060 mmol) and activated molecular sieves (3Å, 320 mg) in MeCN (1.0 mL) was stirred for 16h under an atmosphere of argon. The mixture was cooled to -35°C and NIS (0.24 mmol) and TfOH (0.02 mmol) were added and the reaction mixture was stirred for 5 min until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (10 mL), the solids were filtered-off and the residue was washed with DCM (3 x 10 mL). The combined filtrate (40 mL) was washed with aqueous Na$_2$S$_2$O$_3$ (20 %, 15 mL) and H$_2$O (3 x 20 mL).
The organic phase was dried (MgSO₄), filtered and the filtrate was concentrated in vacuo.

The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford 9 as a white foam (71mg, 84%); FAB MS: m/z = 1425.5 [M+Na]⁺; \( R_f \) 0.51 (acetone / toluene, 3/7, v/v); [\( \alpha \)]\(^{27}\)D = +3.7 (c 0.7, CHCl₃);

\(^1\)H NMR: \( \delta \); 7.20-7.42 (m, 25H, aromatic), 6.28 (d, 1H, J\( _{NH,5''} \) = 9.8 Hz, NH), 5.44-5.50 (m, 1H, J\(_{8'',9'a} = 2.4\) Hz, J\(_{8'',9'b} = 5.4\) Hz, H-8''), 5.28 (dd, 1H, J\(_{7'',8''} = 8.3\) Hz, H-7''), 4.95-5.01 (m, 1H, J\(_{4'',5''} = 10.2\) Hz, H-4''), 4.86 (dd, 2H, J\(^2\) = 10.7 Hz, CH\(_2\)Ph), 4.77 (dd, 2H, J\(^2\) = 11.2 Hz, CH\(_2\)Ph), 4.73 (dd, 2H, J\(^2\) = 11.7 Hz, CH\(_2\)Ph), 4.59 (d, 1H, J\(_{1,2} = 7.3\) Hz, H-1'), 4.49 (dd, 2H, J\(^2\) = 12.2 Hz, CH\(_2\)Ph), 4.40 (dd, 2H, J\(^2\) = 11.7 Hz, CH\(_2\)Ph), 4.34 (d, 1H, J\(_{1,2} = 7.8\) Hz, H-1), 4.27 (dd, 1H, J\(_{9'a,9'b} = 12.2\) Hz, H-9'a), 4.17 (dd, 1H, J\(_{6'',7''} = 2.0\) Hz, H-6'', 4.05 (dd, 1H, J\(_{3',4'} = 3.4\) Hz, H-3'), 3.90-4.03 (m, 4H, H-4, 5'', 9''b, OCH\(_2\)\(^a\)), 3.81 (bt, 1H, H-4'), 3.77 (s, 3H, OCH\(_3\)), 3.73 (d, 2H, H-6), 3.67 (dd, 1H, J\(_{6'a,6'b} = 11.2\) Hz, H-6'a), 3.58-3.62 (m, 2H, H-3, OCH\(_2\)\(^b\)), 3.46-3.57 (m, 3H, J\(_{2,3} = 9.3\) Hz, J\(_{5,6'a} = 8.8\) Hz, H-2', 5', 6'b), 3.34-3.41 (m, 4H, J\(_{5,6} = 2.9\) Hz, H-2', 5, CH\(_2\)N), 2.70 (d, 1H, J\(_{OH,4'} = 3.4\) Hz, OH), 2.56 (dd, 1H, J\(_{3'e,3''a} = 13.1\) Hz, J\(_{3'e,4'} = 4.8\) Hz, H-3''e), 2.01 (m, 1H, 3''a), 2.10, 2.01, 1.98, 1.89 (4s, 12H, OCOCH\(_3\)), 1.87 (m, 2H, CCH\(_2\)C). \(^{13}\)C NMR: 170.8, 170.7, 170.3, 169.8, 168.2, 158.0, 157.5, 139.2, 139.0, 138.7, 138.6, 138.5, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 103.1, 102.5 83.2, 82.1, 78.7, 76.7, 75.6, 75.4, 75.3, 73.6, 73.4, 72.7, 72.2, 69.3, 68.8, 68.7, 68.2, 67.3, 66.7, 62.4, 53.4, 50.3, 48.7, 36.7, 29.6, 21.4, 21.3, 21.0, 20.7. HR-FAB MS [M+Na]⁺ calcd for C\(_{70}H_{81}O_{23}N_{4}F_{3}Na\) 1425.5141, found 1425.5109.
3-Azidopropyl O-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl acid)-(2→3)-O-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside sodium salt (10). 1M aqueous NaOH (0.4 mL) was added dropwise to a solution of 9 (6 mg, 4.27 µmol) in MeOH (1 mL). After 5 h (control TLC, methanol/n-propanol/water/triethylamine, 20/60/15/5, v/v/v/v) the reaction mixture was concentrated in vacuo and the residue was subjected to freeze drying for 16 h. MeOH (0.6 mL) and Ac₂O (0.2 mL) were added and the reaction mixture was kept for 16 h and then concentrated under the reduced pressure. After purification by size exclusion column chromatography on Sephadex LH-20 (35 cm³, MeOH elution) compound 10 was isolated as a white foam (4.3 mg, 85%): FAB MS: m/z = 1211.6 [M+Na]+; Rf 0.70; ¹H NMR (CD₃OD): δ; 7.60-7.20 (m, 25H, aromatic), 4.86 (dd, 2H, J² = 10.7 Hz, CH₂Ph), 4.79 (dd, 2H, J² = 11.2 Hz, CH₂Ph), 4.72 (dd, 2H, J² = 11.7 Hz, CH₂Ph), 4.48 (d, 1H, J₁₂ = 7.8, H-1'), 4.42 (dd, 2H, J² = 11.7 Hz, CH₂Ph), 4.38 (dd, 2H, J² = 11.7 Hz, CH₂Ph), 4.37 (d, 1H, J₁₂ = 7.8, H-1), 4.15 (dd, 1H, J₃₄ = 3.3 Hz, H-3'), 4.01 (d, 1H, H-4'), 3.93-3.98 (m, 1H, J₈₉ = 2.8 Hz, J₈₉b = 5.0 Hz, H-8'), 3.88-3.92 (m, 2H, J₄₅ = 1.9 Hz, H-4, OCH₂), 3.83 (dd, 1H, J₆₆b = 11.2 Hz, H-6a), 3.79-3.81 (m, 2H, H-4", 5"), 3.74 (dd, 1H, J₉₉ = 11.2 Hz, J₉₉b = 11.2 Hz, H-9"a), 3.46-3.68 (m, 10H, J₇₈ = 8.7 Hz, H-6'a, 9'b, 6b, 6'b, 2', 3, 7", 5", 6" ,OCH₂), 3.38 (t, 2H, CH₂N), 3.34 (dd, 1H, J₅₆a = 4.2 Hz, H-5), 3.25 (dd, 1H, J₂₃ = 9.3 Hz, H-2), 2.86 (dd, 1H, J₃₄ = 12.6, J₃₄ = 4.4 Hz, H-3")e, 2.02 (s,3H, NHCOC₂H₅), 1.84 (dd, 1H, J₃₄ = 4.1 Hz, H-3"a), 1.83 (m, 2H, CCH₂C). ¹³C NMR (CD₃OD): δ; 191.4, 178.5, 174.2, 139.3, 138.8, 138.5, 138.3, 128.4, 128.2, 128.0, 127.9, 127.7, 127.5, 127.0, 103.4, 102.8, 82.8, 81.8, 78.6, 76.7, 76.0, 75.3, 75.1, 74.9, 74.8, 74.0, 73.8, 73.2, 73.0, 71.8,
69.8, 68.8, 68.7, 68.1, 66.5, 63.4, 53.0, 48.5, 40.3, 29.7, 21.5. HR-FAB MS [M+Na]^+ calcd for C_{61}H_{73}O_{19}N_{4}Na_{2} 1211.4664, found 1211.4636.

Methyl [2,4,7-tri-O-acetyl-9-O-benzyl-3,5-dideoxy-8-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-5-trifluoroacetamido-D-glycero-ωD-galacto-non-2-ulopyranosidy]onate (11). Trifluoroacetic acid (3 mL) was added dropwise to a solution of 6a (210 mg, 0.18 mmol) in 1,2-dichloroethane (6 mL). After 2 h, the reaction mixture was concentrated under reduced pressure and dried in vacuo. Pyridine (6 mL), Ac₂O (3 mL) and DMAP (catalytic amount) were added to the residue. The reaction mixture was kept for 3 days at rt, then quenched with MeOH (6 mL) and concentrated in vacuo. The residue was co-evaporated with toluene (3 x 6 mL) and dried in vacuo to give 11 as 1/3.5 α/β-mixture (186 mg, 93%) as white foam, which was separated by silica gel column chromatography (10% gradient ethyl acetate in hexane). Selected analytical data for α-11: FAB MS: m/z = 1143.3 [M+Na]^+; Rf 0.35 (ethyl acetate/hexane 3/2, v/v); [α]_{25}^D = + 3.0 (c 0.6, CHCl₃) ¹H NMR: δ; 7.95 (d, 1H, J_{NH,5} = 10.2 Hz, NH), 7.25-7.42 (m, 5H, aromatic), 6.23 (d, 1H, J_{NH,5'} = 9.8 Hz, NH), 5.67-5.71 (m, 1H, J, J_{8',9'a} = 2.5 Hz H-8'), 5.31 (dd, 1H, J_{7',8'} = 9.1 Hz, H-7'), 5.24 (bs, 1H, H-7), 5.00-5.12 (m, 3H, J_{8,9b} = 8.9 Hz, J_{4',5'} = 9.7 Hz, H-4, 4', 8), 4.51 (dd, 2H, J² = 11.7 Hz, CH₂Ph), 3.83 (s, 3H, OCH₃), 3.78 (s, 3H, OCH₃), 3.54 (dd, 1H, J_{9a,9b} = 11.6 Hz, H-9b), 2.78 (dd,1H, J_{3'e,4'} = 4.8 Hz, J_{3'e,3a'} = 12.9 Hz, H-3'e), 2.65 (dd,1H, J_{3e,4} = 5.0 Hz, J_{3e,3a} = 13.6 Hz, H-3e), 2.20, 2.10, 2.06, 2.05, 2.02, 2.01, 1.94 (7s, 21H, OCOCH₃), 2.11-2.21 (m, 1H, H-3' a), 1.98.04 (dd, 1H, H-3a).
Analytical data for \( \text{β-11} \): \( R_f \) 0.31 (ethyl acetate/hexane 3/2, v/v); \( [\alpha]_{D}^{25} = -5.0 \) (c 1.3, CHCl\( _3 \))

\( ^1H \) NMR: \( \delta \); 7.29-7.40 (m, 6H, NH, aromatic), 6.30 (d, 1H, \( J_{\text{NH,5}} = 9.2 \) Hz, NH), 5.34-5.40 (m, 2H), \( J_{8',9'a} = 2.5 \) Hz H-8’, 4), 5.22 (dd, 1H, \( J_{7',8'} = 9.1 \) Hz, H-7’), 5.20 (bs, 1H, H-7), 4.96-5.02 (m, 1H, \( J_{4',5'} = 9.7 \) Hz, H-4’), 4.59-4.64 (m, 1H, \( J_{8,9b} = 7.9 \) Hz, H-8), 4.53 (dd, 2H, \( J = 12.1 \) Hz, CH\( _2 \)Ph), 4.33 (dd, 1H, \( J_{6,7} = 2.2 \) Hz, H-6), 4.25 (dd, 1H, \( J_{9'a,9'b} = 12.4 \) Hz, H-9’a), 3.89-4.07 (m, 5H, \( J_{5,6} = 10.9 \) Hz, \( J_{6',7'} = 1.6 \) Hz, \( J_{9a,9'b} = 11.5 \) Hz, H-5, 5’, 6’, 9a,9’b), 3.79 (s, 3H, OCH\( _3 \)), 3.74 (s, 3H, OCH\( _3 \)), 3.56 (dd, 1H, \( J_{1, H-9b} = 6.9 \) Hz, \( J_{3c,3a} = 13.5 \) Hz, H-3e), 2.18, 2.16, 2.03, 2.02, 1.98, 1.97, 1.96 (7s, 21H, OCOC\( _3 \)), 1.94-2.00 (m, 2H, H-3’ a, 3a). \( ^{13}C \) NMR: 172.3, 171.2, 170.8, 170.3, 169.9, 169.8, 168.9, 168.4, 157.4, 138.9, 128.5, 127.6, 127.4, 99.01, 96.9, 73.9, 73.8, 72.5, 71.8, 70.6, 69.9, 69.4, 68.7, 68.6, 66.9, 62.7, 62.5, 53.6, 52.9, 50.8, 50.0, 38.9, 38.2, 31.2, 30.0, 21.5, 20.9, 20.8, 20.7, 18.2, -0.92

Methyl [phenyl 4,7-tetra-O-acetyl-9-O-benzyl-3,5-dideoxy-8-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-\( \alpha\)-D-galacto-non-2-ulopyranosylonate)-2-thio-5-trifluoroacetamido-D-glycero-\( \alpha\)-D-galacto-non-2-ulopyranosid]onate (12). To a solution of 11 (82.5 mg, 0.07 mmol) in 1,2-dichloroethane (1 mL), benzenethiol (30 \( \mu \)L, 0.3 mmol) and BF\( _3 \)-Et\( _2 \)O (37 mL, 0.3 mmol) were added. After 30 min., the reaction mixture was diluted with DCM (6 mL), then washed with aqueous NaHCO\( _3 \) (15 %, 2 mL) and H\( _2 \)O (3 x 6 mL). The organic phase was dried (MgSO\( _4 \)), filtered and the filtrate was concentrated \textit{in vacuo}. The residue was purified by silica gel column chromatography (10% gradient acetone in toluene) to afford anomerically pure 12 as white foam (72 mg, 84%); FAB MS: \( m/z = 1172.0 \) [M+H]+; \( R_f \) 0.60 (acetone/toluene 3/7, v/v); \( [\alpha]_{D}^{25} = +5.7 \) (c 0.1, CHCl\( _3 \)) \( ^1H \) NMR: \( \delta \); 8.03 (d, 1H, \( J_{\text{NH,5}} = 10.5 \) Hz, NH ), 7.18-7.54 (m, 10H,
aromatic), 6.16 (d, 1H, J_{NH,5'} = 9.4 Hz, NH), 5.60-5.66 (m, 1H, J_{8',9'a} = 2.3 Hz, J_{8',9'b} = 4.9 Hz, H-8'), 5.33-5.40 (m, 2H, H-4, 7), 5.27 (d, 1H, J_{7',8'} = 10.0 Hz, H-7'), 5.09 (dd, 1H, J_{6,7} = 1.8 Hz, H-6), 4.99-5.06 (m, 1H, H-4',8), 4.49 (dd, 2H, J^2 = 12.2 Hz, CH2Ph), 4.15 (dd, 1H, J_{9'a,9'b} = 12.6 Hz, H-9'a), 4.12 (dd, 1H, J_{9a,9b} = 12.3 Hz, H-9a), 3.98-4.10 (m, 3H, J_{5,6} = 10.4 Hz, J_{6',7'} = 1.2 Hz, H-5, 5', 6'), 3.95 (dd, 1H, H-9'b), 3.87 (s, 3H, OCH3), 3.44 (dd, 1H, H-9b), 3.41 (s, 3H, OCH3), 2.86 (dd, 1H, J_{3'e,4'} = 8.5 Hz, J_{3'e,3'a} = 12.9 Hz, H-3'e), 2.24, 2.05, 2.03, 2.02, 2.00 (6s, 18H, OCOCH3), 2.17 (dd, 1H, H-3a), 2.05 (dd, 1H, H-3'a). \(^{13}\)C NMR (\(^{1}H\)): 75.0, 72.9, 72.7, 71.6, 69.9, 69.0, 68.5, 68.1, 66.3, 61.8, 53.5, 52.2, 49.9, 50.5, 38.9, 38.3

Methyl \(O\)-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-\(\alpha\)-D-galacto-non-2-ulopyranosylonate)-(2\(\rightarrow\)8)-\(O\)-(methyl 4,7-di-O-acetyl-9-O-benzyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-\(\alpha\)-D-galacto-non-2-ulopyranosylonate)-(2\(\rightarrow\)3)-\(O\)-(2,6-di-O-benzyl-\(\beta\)-D-galactopyranosyl)-(1\(\rightarrow\)4)-2,3,6-tri-O-benzyl-\(\beta\)-D-glucopyranoside (13). A mixture of donor 12 (37 mg, 0.03 mmol) and 8b (25.5 mg, 0.03 mmol) and activated molecular sieves (3 Å, 200 mg) in MeCN (1.5 mL) was stirred for 16h under an atmosphere of argon. The mixture was cooled to -35°C and NIS (0.06 mmol) and TfOH (6.3 µmol) were added and the reaction mixture was stirred for 30 min until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (5 mL), the solids were filtered-off and the residue was washed with DCM (3 x 5 mL). The combined filtrate (20 mL) was washed with aqueous Na2S2O3 (20 %, 7 mL) and H2O (3 x 10 mL). The organic phase was dried (MgSO4), filtered and the filtrate was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to 13 as a
white foam (23.5 mg, 42%); FAB MS: m/z = 1889.6 [M+Na]+; Rf 0.69 (ethyl acetate/hexane, 3/2, v/v); [α]_D^{27} = +4.6 (c 0.6, CHCl_3); ^1H NMR: δ; 7.61 (d, 1H, J_{NH,5''} = 9.8 Hz, NH), 7.14-7.37 (m, 30H, aromatic), 6.16 (d, 1H, J_{NH,5''} = 9.2 Hz, NH), 5.50-5.56 (m, 1H, J_{8''-9''a} = 2.4 Hz, J_{8''-9''b} = 4.9 Hz, H-8''), 5.27 (dd, 1H, J_{7''-8''} = 9.8 Hz, H-7''), 5.20 (s, 1H, H-7''), 5.06 (d, 1H, H-8''), 4.92-5.03 (m, 2H, J_{4''-5''} = 10.7 Hz, J_{4''-5''} = 10.3 Hz, H-4'', 4''), 4.81 (dd, 2H, J^2 = 10.7 Hz, CH_2Ph), 4.75 (dd, 2H, J^2 = 10.7 Hz, CH_2Ph), 4.70 (dd, 2H, J^2 = 11.7 Hz, CH_2Ph), 4.30-4.56 (m, 6H, CH_2Ph) 4.39 (d, 1H, J_{1',2'} = 7.8 Hz, H-1'), 4.34 (dd, 1H, J_{6''-7''} = 1.9 Hz, H-6''), 4.28 (dd, 1H, J_{9''a,9''b} = 12.7 Hz, H-9''a), 4.26 (d, 1H, J_{1,2} = 7.3 Hz, H-1), 4.22 (dd, 1H, J_{9''a,9''b} = 11.7 Hz, H-9''a), 4.20 (dd, 1H, H-9''b), 4.04 (dd, 1H, J_{5',6'} = 10.7 Hz, H-5'', 4.00 (dd, 1H, J_{6'-7''} = 1.5 Hz, H-6''), 3.97 (dd, 1H, J_{5',4'} = 3.4 Hz, H-3'), 3.92 (t, 1H, J_{5'',6''} = 10.3 Hz, H-5''), 3.65-3.86 (m, 4H, H-4, 4', 6), 3.81 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.54 (s, 3H, OCH_3), 3.42-3.60 (m, 4H, J_{2,3} = 9.3 Hz, H-2', 3, 6'a, 9''b), 3.30-3.40 (m, 3H, H-2, 5, 6'b), 3.12 (t, J_{5',6'a} = 6.3 Hz, H-5'), 2.75 (dd, 1H, J_{3''c,3''a} = 13.2 Hz, J_{3''c,4''} = 4.9 Hz, H-3''), 2.41 (s, 1H, OH), 2.01 (m, 1H, 3''a), 1.98 (m, 1H, 3''a), 2.17, 2.03, 1.98, 1.97, 1.90, 1.86 (6s, 18H, OCOCH_3). ^13C NMR: 170.0, 168.8, 168.3, 138.9, 138.7, 138.5, 138.3, 128.6, 128.4, 128.3, 128.1, 127.9, 127.6, 127.4, 104.8, 102.5, 98.9, 96.8, 83.0, 82.1, 76.7, 7.4, 75.6, 75.1, 74.8, 74.2, 73.6, 73.5, 73.3, 72.5, 71.6, 70.6, 69.4, 69.2, 68.7, 68.5, 68.4, 67.9, 66.8, 62.4, 57.3, 53.7, 53.4, 50.1, 50.0, 39.1, 36.7, 21.6, 20.9, 20.8, 20.6. 

**Methyl O-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyonic acid)-(2→8)-O-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyonic acid)-(1→3)-O-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside disodium salt** (14). Compound 13 (13 mg, 7 µmol) was dissolved in ethanol/ethyl acetate (2mL, 1/1,
and Pd(OAc)$_2$ (13mg) was added. The mixture was stirred under the atmosphere of H$_2$ for 18 h. The catalyst was removed by filtration and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in MeOH (1 mL) and 1M aqueous NaOH (0.3 mL) was added dropwise. After 72 h (control TLC, methanol/\(n\)-butanol/water/triethylamine, 20/60/15/5, v/v/v/v) the reaction mixture was concentrated in vacuo and the residue was subjected to freeze drying for 16 h. MeOH (0.5 mL) and Ac$_2$O (0.1 mL) were added and the reaction mixture was kept for 6 h and then concentrated under the reduced pressure. The residue was purified by size exclusion column chromatography on Sephadex G25 (35 cm$^3$, water elution) to afford 14 as white foam (4.4 mg, 71%): FAB MS: m/z = 983.3 [M+H]$^+$; $R_f$ 0.40; $[\alpha]_{D}^{27} = +3.4$ (c 0.5, H$_2$O); $^1$H NMR (D$_2$O): $\delta$: 4.40 (d, 1H, J$_{1',2'} = 8.3$ Hz, H-1'), 4.20 (d, 1H, J$_{1,2} = 8.3$ Hz, H-1), 4.06 (dd, 1H, J$_{9''a,9''b} = 12.2$ Hz, H-9''a), 4.00-4.03 (m, 1H, J$_{8''',9''''a} = 2.4$ Hz, J$_{8''',9''''b} = 5.4$ Hz, H-8'''), 3.97 (dd, 1H, J$_{3',4'} = 3.4$ Hz, H-3''), 3.91 (dd, 1H, J$_{9''a,9''b} = 12.2$ Hz, H-9''a), 3.84 (d, 1H, H-4'), 3.42-3.82 (m, 20 H, H-2', 3, 4, 4', 4'', 5, 5', 5'', 6, 6', 6'', 7'', 7''', 8'', 9''b, 9'''b), 3.45 (s, 3H, OCH$_3$), 3.19 (t, 1H, J$_{2,3} = 8.8$ Hz, H-2), 2.66 (dd, 1H, J$_{3''e,3''''a} = 12.2$ Hz, J$_{3''e,4''} = 4.4$ Hz, H-3'''), 2.56 (dd, 1H, J$_{3''e,3''''a} = 12.2$ Hz, J$_{3''e,4''} = 4.4$ Hz, H-3''e), 1.95, 1.91 (2s, 6H, NHCOCH$_3$), 1.58-1.66 (m, 2H, H-3''a,3''''a). $^{13}$C NMR (D$_2$O): 181.5, 175.1, 162.8, 103.3, 102.9, 100.7, 100.4, 78.4, 75.7, 75.4, 75.0, 74.2, 73.1, 72.9, 71.9, 69.6, 68.7, 68.4, 62.8, 61.8, 61.3, 60.2, 57.5, 52.5, 52.0, 40.7, 40.0, 22.6, 22.3.
CHAPTER 3

N-TRIFLUOROACETAMIDO SUBSTITUENT AT C-5: SCOPE AND LIMITATION
FOR THE SYNTHESIS OF SIALYL-GALACTOSIDES

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1De Meo, C.; Demchenko, A. V.; Boons, G-J. To be submitted to European Journal of Organic Chemistry

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**Abstract:** As a part of a program targeted to the development of a general method for the regio- and stereoselective synthesis of α-sialosides, glycosylation of sialyl donor 1 bearing a trifluoroacetamido group at C-5 with partially protected galactose derivatives 2a-d was attempted to afford (2-3)-linked disaccharides 3a-d with high yields and excellent stereoselectivity. It has been shown that the new sialylation approach allows efficient synthesis of Neu(2-3)Gal derivatives even when sterically hindered alcohols are used as glycosyl acceptors.

**Keywords:** sialic acid, direct synthesis, trifluoroacetamido group, thioglycosides, gangliosides

Sialic acids are a family of about 40 naturally occurring 2-keto-3-deoxy-nononic acids involved in a wide range of biological processes.\(^1\) The C-5-amino derivative represents the long-known neuraminic acid and its amino function can either be acetylated (Neu5Ac) or glycolylated (Neu5Gc). Sialic acids occur in body fluids, especially in blood, in mucous secretions and in cell membranes. As terminal components of glycopolysaccharides, they cover all cells of higher animals and man with an electro-negatively charged coat and exert a variety of different biological functions. Some of these may act as tumor-associated antigen, and play important roles in cell-cell and cell-surface interactions.\(^{181}\) Thus, one of the most important class of neuraminic acid derivatives are gangliosides that influence the cell growth by interaction with the epidermal growth factor and they are involved in apoptosis, the molecular regulation of cell death.
Introduction of sialic acid residues with a high regio- and stereoselectivity is a challenging problem of a contemporary oligosaccharide synthesis.\textsuperscript{130} The use of sialyl donors is complicated by the lack of a participating substituent at C-3 and, in addition, the presence of the deoxy moiety in combination with the electron withdrawing carboxylic acid at the anomeric center makes these derivatives prone to elimination to give 2,3-dehydro derivatives. Therefore, direct sialylations with the conventional sialyl donors (thioglycosides, phosphates, etc.) often lead to the formation of anomeric mixtures in moderate yields, especially when applied to the glycosylation of sterically hindered alcohols. Indirect methods, which use glycosyl donors that are substituted at C-3, generally provide higher yields and stereoselectivity. However, such donors are more laborious to prepare, and the removal of an auxiliary after the glycosylation step may be difficult. It is obvious that a versatile and easily accessible sialyl donor that gives excellent yields and high $\alpha$-anomeric selectivities in direct glycosylations with a wide range of acceptors of different reactivities needs to be developed.

Recently, we have established an efficient method for the first direct synthesis of Neu5Ac$\alpha$(2-8) Neu5Ac using the glycosyl donor 1 bearing a trifluoroacetamido group.\textsuperscript{182} It has been shown that modification of the C-5 amino group of 2-thiomethyl and 2-thiophenyl sialosides into N-TFA derivatives improves their reactivities. Furthermore, an N-TFA protecting group has the additional advantage that it can be easily introduced as well as removed under mild reaction conditions, which makes it possible to functionalize the amino group of sialic acids as N-acetyl, N-glycolyl or other derivatives. The higher reactivity of the novel donor was rationalized as follows: substitution of the 5-acetamido functionality with a strong electron withdrawing protecting group reduces the nucleophilicity and basicity of the amine, therefore, minimizing possible side reactions,
such as glycosylation at the $N$-position of the acetamido group at C-5. Thus, a glycosyl donor bearing a trifluoroacetamido group would have superior glycosyl donor properties compared to a similar donor having a $N$-acetylacetamido moiety at C-5, \textit{1a}, or to the traditional donor bearing a $N$-acetamido group \textit{1b}. (Figure 3.1)

![Chemical Structure](image)

\textbf{Figure 3.1}

In an effort to develop a general approach that gives high yields and stereoselectivity for a wide range of glycosidic linkages, we now report that the new sialylation approach gives good yields and excellent $\alpha$-anomeric selectivities in the direct synthesis of Neu5Ac$\alpha$(2 $\rightarrow$ 3)Gal derivatives, especially when sterically hindered alcohols are used as glycosyl acceptors. The glycosyl donor properties of \textit{1} were evaluated by coupling with the C-3 hydroxyl of a series of galactosyl acceptors \textit{2a-d} having different protecting-group patterns (Scheme 3.1).
Scheme 3.1

First, we investigated the coupling of 2a and 2b with 1 equivalent of the glycosyl donor 1 in the presence of N-iodosuccinimide (NIS, 2 equiv. to donor), trifluoromethanesulfonic acid (TfOH, 10 mol% to NIS) and molecular sieves (3Å) in MeCN at –40ºC. Similar glycosylations using mono- and di-N-acetyl neuraminyl derivatives stereoselectively afforded the corresponding α-linked disaccharides in yields 43-60% in case of the N-acetamido derivative 1b,91,183 and 73% for the N-acetylacetamido derivative 1a.128

Glycosylation of acceptor 2a (2,3,4-triol)91,129 with donor 1 proceeded with complete stereoselectivity and disaccharide 3a was isolated as α-anomer in a good yield of 61%. Apart from 3a, a small amount of a (2-2)-linked regioisomer was isolated (12%), the
formation of which can be rationalized by the high reactivity of the sialyl donor. Mass spectrometry analysis of the crude reaction mixture revealed trace amounts of a trisaccharide (<1%), product of 2,3-disialylation of 2a. Coupling of donor 1 with glycosyl acceptor 2b (2,3–diol) was performed under identical reaction conditions to afford 3b with a yield of 68%. This result is in agreement with the synthesis previously reported by Kiso. The sialylation reaction proceeded with complete stereoselectivity, however a small amount of 3,2-tricyclic lactone derivative and the (2-2)-linked regioisomer were isolated in a combined yield of 11%. Glycosylation of the 3,4 diol 2c gave also the desired product 3c as the α-anomer in a yield of 63% This result is a significant improvement in comparison with the previously reported attempts using a donor bearing an N-acetamido group for the glycosylation of a similar acceptor where only 17% of the desired α anomer was obtained. In addition, the (2-4)-linked regioisomer was also identified and isolated with a yield of 14%.

To exclude the formation of regioisomers, the attention was turned to the glycosylation of sterically hindered 2,4,6-substituted acceptor 2d. It is noteworthy, that even though the glycosylation of this derivative was not technically possible to date (low yields and poor stereoselectivity), these types of derivatives are of high strategic importance for the multi-step synthetic sequences. For example, subjecting the disaccharide derivatives 3a,b to further synthetic manipulations would normally require protection of the remaining hydroxy group. Glycosylation of 2d with 1 afforded 3d in an exceptional yield of 84% and, remarkably, this reaction also proceeded with complete stereoselectivity. To date, this is the only example whereby a glycosyl donor of a modified Neu5Ac successfully glycosylated a sterically hindered galactosyl acceptor.
At this point, the glycosyl donor properties of 1 were tested by coupling with the C-6 hydroxyl of a series of galactosyl acceptors 4a-c (Scheme 3.2). In general, it is quite challenging to obtain good stereoselectivity involving primary hydroxyls as acceptors. Despite that, a range of glycosyl acceptors were tested under a variety of reaction conditions, at this point it seems to be that only mixtures of α/β-anomers can be obtained. Thus, sialylation of 4a-c with donor 1 under standard conditions afforded the corresponding 2-6-linked disaccharides 5a-c with high yields (82-96%) as 2-3:1 mixtures of α/β-anomers (Scheme 3.2).

Scheme 3.2

Reaction Conditions: Donor (1.3 equiv.), Acceptor (1 equiv.), NIS/TfOH, MS 3Å, MeCN
In conclusion, we found that 5-N-trifluoroacetyl neuraminyl derivative 1 is significantly more reactive and gives higher yields in glycosylations than the corresponding mono- and di-N-acetylated derivatives. Although this improvement in the reactivity can lead to side reactions when reactive galactosides are used as glycosyl acceptors, the introduction of a trifluoroacetamide moiety allows for the first time glycosylations with unreactive acceptors, opening a new route towards sialic acid containing oligosaccharides. The usefulness of the developed strategy for the synthesis of Neu(2-6)Gal derivatives remains to be uncertain.

Experimental section

**General.** Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), size exclusion column chromatography was performed on Sephadex LH-20 (MeOH or MeOH-CH₂Cl₂, 1/1, v/v elution) or Sephadex G-25 (water elution). Reactions were monitored by TLC on Kieselgel 60 F₂₅₄ (EM Science) and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40°C. CH₂Cl₂, (ClCH₂)₂, and MeCN were distilled from CaH₂ (twice) and stored over molecular sieves (3Å). Molecular sieves (3Å and 4 Å), used for reactions, were crushed and activated in vacuo at 390°C during 8 h in the first instance and then for 2-3 h at 390°C directly prior to application. ¹H NMR and ¹³C NMR spectra were recorded with a Varian Inova500 spectrometer and a Varian Inova600 spectrometer equipped with Sun workstations. Unless otherwise noted ¹H NMR spectra were recorded in CDCl₃ and referenced to
residual CHCl₃ at 7.24 ppm, the assignments were made by standard gCOSY and gHSQC.

2- (Trimethylsilyl) ethyl 6- O- benzoyl-3- O-[ methyl 4,7,8,9- tetra- O-acetyl - 5- (N-trifluoroacetamido)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate]-β-D-galactopyranoside (3a). A suspension of 1 (53 mg, 0.09 mmol), 2a (35.2 mg, 0.09 mmol) and powdered 3Å molecular sieves (200 mg) in dry acetonitrile (1 mL) was stirred at room temperature for 16 h. The mixture was cooled to -40 ºC and NIS (41 mg, 0.18 mmol) and TfOH (1.6 µL, 0.018 mmol) were added. After a reaction time of 20 minutes, TLC analysis showed conversion of the starting materials into a product. The reaction mixture was diluted with dichloromethane (5 mL) and filtered. The filtrate was washed successively with aqueous sodium thiosulfate (15%, 7 mL) and water (3 x 10mL). The combined organic extracts were dried (MgSO₄), filtered and concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford a mixture of regioisomers which was separated by silica gel column chromatography (5% gradient of acetone in hexanes) to give 3a as white foam (50 mg, 61%): MALDI MS: m/z = 934.3 [M+Na]+; ¹H NMR: δ;

7.43-7.51 (m, 5H, aromatic), 6.29 (d, 1H, J₉₈',₅ = 8.9 Hz, NH), 5.44-5.48 (m, 1H, J₈',₉ₐ = 2.4 Hz, J₈',₉ₕ = 4.9 Hz, H-8'), 5.29 (dd, 1H, J₇',₈ = 8.6 Hz, H-7'), 5.07-5.10 (m, 1H, J₄',₅ = 11.7 Hz, H-4'), 4.62-4.64 (dd, 1H, J₆ₐ,₆₄ = 11.5 Hz, H-6a) 4.51-4.55 (dd, 1H, J₆ₐ,₆₉ = 14.4 Hz, H-9'a), 4.09-4.22 (m, 2H, H-3, 9'b), 4.00-4.06 (m, 1H, OCH₃), 3.91 (dd, 1H, J₅₆',₉ = 9.9 Hz, H-5'), 3.84 (t, 1H, J₅₆',₅₆ = 5.68 Hz, J₅₆,₅ = 7.1 Hz, H-5), 3.78 (s, 3H, OCH₃), 3.66-
3.71 (m, 3H, H-2, 4, OCH$_2$H$_3$), 2.78 (dd, 1H, J$_{3\gamma,3\alpha}$ = 12.9 Hz, J$_{3\gamma,4}$ = 4.8 Hz, H-3'e), 2.57 (s, 1H, OH), 2.41(s, 1H, OH), 2.13, 2.04 (2s, 12H, OCOCH$_3$), 1.10 (m, 2H, CCH$_2$H) 2.01 (m, 1H, 3'a), 0.05 (s, 3H, TMS).

2-(Trimethylsilyl)ethyl 4,6-O-benzylidene-3-O-[methyl 4,7,8,9-tetra-O-acetyl-5-(N-trifluoroacetamido)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate]-β-D-galactopyranoside (3b). A suspension of 1 (59 mg, 0.10 mmol), 2b (37.9 mg, 0.10 mmol) and powdered 3Å molecular sieves (200 mg) in dry acetonitrile (1 mL) was stirred at room temperature for 16 h. The mixture was cooled to -40 °C and NIS (46 mg, 0.20 mmol) and TfOH (2 µL, 0.02 mmol) were added. After a reaction time of 10 minutes, TLC analysis showed conversion of the starting materials into a product. The reaction mixture was diluted with dichloromethane (5 mL) and filtered. The filtrate was washed successively with aqueous sodium thiosulfate (15%, 7 mL) and water (3 x 10mL). The combined organic extracts were dried (MgSO$_4$), filtered and concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford a mixture of regioisomers which was separated by silica gel column chromatography (5% gradient of acetone in toluene) to give 3b as white foam (61 mg, 68%): MALDI MS: m/z = 918.4 [M+Na]$^+$; $^1$H NMR: δ; 7.32-7.51 (m, 5H, aromatic), 6.42 (d, 1H, J$_{NH,5'}$ = 9.7 Hz, NH), 5.43-5.48 (m, 1H, J$_{8',9'a}$ = 2.4 Hz, J$_{8',9'b}$ = 4.3 Hz, H-8'), 4.86 (s, 2H, CH$_2$Ph), 5.29 (dd, 1H, J$_{7',8'}$ = 8.8 Hz, H-7'), 4.98-5.05 (m, 1H, J$_{5',5'}$ = 12.3 Hz, H-4'), 4.47 (d, 1H, J$_{1,2}$ = 8.0 Hz, H-1), 4.32 (dd, 1H, J$_{9'a,9'b}$ = 11.7 Hz, H-9'a), 4.29 (dd, 1H, J$_{6a,6b}$ = 12.9 Hz, H-6a), 4.24 (dd, 1H, J$_{6',7'}$ = 1.7 Hz, H-6'), 4.22 (dd, 1H, H-3), 4.04-4.13 (m, 3H, H-6b, 9'b, OCH$_2$H$_3$), 3.96 (s, 1H, H-4), 3.92-3.98 (m, 1H, J$_{5',6'}$ = 10.0 Hz, H-5'), 3.88 (t, 1H, H-2), 3.62-3.67 (m, 1H, OCH$_2$H$_3$), 3.78 (s,
3H, OCH$_3$), 3.47 (s, 1H, H-5), 2.80 (dd, 1H, J$_{3c,3a} = 12.4$ Hz, J$_{3c,4'} = 3.4$ Hz, H-3'e), 2.55 (s, 1H, OH), 2.21, 2.17, 2.16, 2.04, 2.02 (4s, 12H, OCOCH$_3$), 1.00-1.13 (m, 2H, CCH$_2$C), 2.03 (m, 1H, 3'a), 0.03 (s, 3H, TMS).

**Methyl 2,6-di-O-benzyl-3-O-[methyl 4,7,8,9-tetra-O-acetyl-5-(N-trifluoroacetamido)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate]-β-D-galactopyranoside (3c).** A suspension of 1 (54 mg, 0.09 mmol), 2c (35.1 mg, 0.09 mmol) and powdered 3Å molecular sieves (200 mg) in dry acetonitrile (1 mL) was stirred at room temperature for 16 h. The mixture was cooled to -40 °C and NIS (42.2 mg, 0.18 mmol) and TfOH (1.5 µL, 0.018 mmol) were added. After a reaction time of 10 minutes, TLC analysis showed conversion of the starting materials into a product. The reaction mixture was diluted with dichloromethane (5 mL) and filtered. The filtrate was washed successively with aqueous sodium thiosulfate (15%, 7 mL) and water (3 x 10mL). The combined organic extracts were dried (MgSO$_4$), filtered and concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford a mixture of regioisomers which was separated by silica gel column chromatography (5% gradient of acetone in toluene) to give 3c as white foam (51 mg, 63%): MALDI MS: m/z = 924.2 [M+Na]$^+$; $^1$H NMR: δ; 7.21-7.41 (m, 10H, aromatic), 6.44 (d, 1H, J$_{NH,5'} = 9.8$ Hz, NH), 5.39-5.42 (m, 1H, J$_{8',9'a} = 2.4$ Hz, J$_{8',9'b} = 4.4$ Hz, H-8'), 5.29 (dd, 1H, J$_{7',8'} = 7.8$ Hz, H-7'), 4.95-5.02 (m, 1H, J$_{4',5'} = 11.8$ Hz, H-4'), 4.85 (dd, 2H, J$^2 = 11.6$ Hz, CH$_2$Ph), 4.70 (dd, 2H, J$^2=11.7$ Hz, CH$_2$Ph) 4.59 (s, 2H, CH$_2$Ph) 4.37 (d, 1H, J$_{1,2} = 7.8$ Hz, H-1), 4.33 (dd, 1H, J$_{9'a,9'b} = 13.5$ Hz, H-9'a), 4.19 (dd, 1H, J$_{6',7} = 1.4$ Hz, H-6'), 4.20 (dd, 1H, H-3), 3.98-4.04 (m, 2H, J$_{5',6'} = 10.8$ Hz, H-5', 9'b), 3.73-3.83 (m, 6H, H-6, 4, OCH$_3$), 3.66 (t, 1H, H-5), 3.58 (s, 3H, OCH$_3$), 3.54 (t, 1H, H-2), 2.55 (s, 1H, OH),
2.59 (dd, 1H, J_{3\text{e},3\text{a}} = 12.8 Hz, J_{3\text{e},4\text{e}} = 5.0 Hz, H-3'e), 2.03, 2.00, 1.98 (3s, 12H, OCOCH$_3$), 2.04-1.98 (m, 1H, 3'a).

**Methyl 2,4,6-tri-O-benzyl-3-O-[methyl 4,7,8,9-tetra-O-acetyl-5-(N-trifluoroacetamido)-3,5-dideoxy-D-glycero-\alpha-D-galacto-non-2-ulopyranosylonate]-\beta-D-galactopyranoside (3d).** A suspension of 1 (56 mg, 0.10 mmol), 2d (41.7 mg, 0.10 mmol) and powdered 3Å molecular sieves (200 mg) in dry acetonitrile (1 mL) was stirred at room temperature for 16 h. The mixture was cooled to -40 °C and NIS (44 mg, 0.19 mmol) and TfOH (1.7 µL, 0.019 mmol) were added. After less than 5 minutes, TLC analysis showed conversion of the starting materials into a product. The reaction mixture was diluted with dichloromethane (5 mL) and filtered. The filtrate was washed successively with aqueous sodium thiosulfate (15%, 7 mL) and water (3 x 10mL). The combined organic extracts were dried (MgSO$_4$), filtered and concentrated *in vacuo*. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford to give 3d as white foam (81 mg, 84%): MALDI MS: m/z = 1014.4 [M+Na]$^+$; $^1$H NMR: δ; 7.24-7.43 (m, 15H, aromatic), 6.48 (d, 1H, J$_{NH,5'}$ = 9.7 Hz, NH), 5.42-5.46 (m, 1H, J$_{8',9a}$ = 2.5 Hz, J$_{8',9b}$ = 5.2 Hz, H-8'), 5.28 (dd, 1H, J$_{7',8'}$ = 7.8 Hz, H-7'), 5.02-5.08 (m, 1H, J$_{4',5'}$ = 11.8 Hz, H-4'), 4.78 (dd, 2H, J$^2$ = 11.4 Hz, CH$_2$Ph), 4.68 (dd, 2H, J$^2$=12.3 Hz, CH$_2$Ph), 4.50 (2H, J$^2$=12.3 Hz, CH$_2$Ph), 4.37 (dd, 1H, J$_{9a,9b}$ = 13.3 Hz, H-9'a), 4.36 (d, 1H, J$_{1,2}$ = 7.6 Hz, H-1), 4.13 (dd, 1H, J$_{6',7'}$ = 1.5 Hz, H-6'), 4.09 (dd, 1H, H-3), 4.00 (dd, 1H, H-9'b), 3.99 (m, 1H, H-5'), 3.52-3.55 (m, 7H, H-6, 4, 2, 5, OCH$_3$), 3.53 (s, 3H, OCH$_3$), 2.73 (dd, 1H, J$_{3\text{e},3\text{a}}$ = 12.8 Hz, J$_{3\text{e},4\text{e}}$ = 5.0 Hz, H-3'e), 2.18, 2.04, 2.02, 1.98 (4s, 12H, OCOCH$_3$), 1.83 (m, 1H, 3'a).
Methyl 2,3,4-tri-O-benzoyl-6-O-[methyl 4,7,8,9-tetra-O-acetyl-5-(N-trifluoroacetamido)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyloxy]-β-D-galactopyranoside (5a). A suspension of 1 (49 mg, 0.08 mmol), 4a (33.3 mg, 0.06 mmol) and powdered 3Å molecular sieves (200 mg) in dry acetonitrile (1 mL) was stirred at room temperature for 16 h. The mixture was cooled to -40 °C and NIS (38 mg, 0.17 mmol) and TfOH (1.5 µL, 0.0017 mmol) were added. After a reaction time of 5 minutes, TLC analysis showed conversion of the starting materials into a product. The reaction mixture was diluted with dichloromethane (5 mL) and filtered. The filtrate was washed successively with aqueous sodium thiosulfate (15%, 7 mL) and water (3 x 10mL). The combined organic extracts were dried (MgSO₄), filtered and concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford 5a as anomic mixture (α/β 3:1, 52.4 mg, 85%): MALDI MS: m/z = 1058.0 [M+Na]+; Selected data ¹H NMR for α−5a : δ; 6.52 (d, 1H, J NH ,5' = 10.3 Hz ,NH), 5.99 (dd, 1H, H-4), 5.71 (d, 1H, J2,3 = 10.4 Hz, H-2)-5.61 (dd, 1H, J3,4 = 4.02 Hz H-3), 5.39-5.43 (m, 1H, J8',9'a = 2.5 Hz, J8',9'b = 5.9 Hz, H-8'), 5.27 (dd, 1H, J7',8' = 8.3 Hz, H-7'), 4.89-4.96 (m, 1H, H-4'), 4.78 (d, 1H, J4',5' = 11.0 Hz H-4'), 4.74 (d, 1H, J1,2 = 8.3 Hz H-1), 4.40 (dd, J9'a,9'b = 12.9 Hz 1H, H-9'a), 4.26 (dd, J6,7 = 1.9 Hz 1H, H-6'), 4.12-4.16 (m, 1H, H-4'), 5.07 (dd, 1H, J7,8' = 8.3 Hz, H-7'), 4.74 (d, 1H, J1,2 = 8.3 Hz H-1).
1,2:3,4-di-O-(iso-propyldene)-6-O-[methyl4,7,8,9-tetra-O-acetyl-5-(N-trifluoroacetamido)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate]-α-D-galactopyranose (5b). A suspension of 1 (101 mg, 0.19 mmol), 4b (35.3 mg, 0.13 mmol) and powdered 3Å molecular sieves (150 mg) in dry acetonitrile (1 mL) was stirred at room temperature for 16 h. The mixture was cooled to -40 °C and NIS (79 mg, 0.35 mmol) and TfOH (3 µL, 0.03 mmol) were added. After a reaction time of less than 5 minutes, TLC analysis showed conversion of the starting materials into a product. The reaction mixture was diluted with dichloromethane (5 mL) and filtered. The filtrate was washed successively with aqueous sodium thiosulfate (15%, 20 mL) and water (3 x 20mL). The combined organic extracts were dried (MgSO₄), filtered and concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford 5b as white foam (α/β 2:1, 84 mg, 82%): Selected data ¹H NMR for α−5a: 6.90 (d, 1H, J NH,5 = 10.7 Hz, NH), 5.47 (d, 1H, H-1), 5.32-5.40 (m, 1H, J₈₉ₐ,₉₉₉₂ = 2.8 Hz, J₈₉₉₂,₉₉₉₃ = 5.1 Hz, H-8'), 5.28 (dd, 1H, J₇₈,₇₈ = 7.3 Hz, H-7'), 4.94-5.04 (m, 1H, H-4'), 4.74 (dd, 1H, J₉₉ₐ,₉₉₉₂ = 12.7 Hz, H-9'a) 4.63 (dd, J₆7 = 1.7 Hz 1H, H-6'), 2.63 (dd, 1H, J₃₃₃₄,₃₃₃₄ = 13.0 Hz, J₃₃₃₄,₃₃₄₅ = 4.7 Hz, H-3'e); β−5a: δ; 6.77 (d, 1H, JNH,5 = 10.2 Hz, NH), 5.31-5.41 (m, 2H, H-4', 7'), 5.13-5.20 (m, 1H, H-8), 2.51 (dd, 1H, J₃₃₃₄,₃₃₃₄ = 13.0 Hz, J₃₃₃₄,₃₃₄₅ = 4.5 Hz, H-3'eq).

Methyl 2,3,4-tri-O-benzoyl-6-O-[methyl 4,7,8,9-tetra-O-acetyl-5-(N-trifluoroacetamido)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylate]-α-D-galactopyranoside (5c). A suspension of 1 (31 mg, 0.05 mmol), 4c (19 mg, 0.04 mmol) and powdered 3Å molecular sieves (100 mg) in dry acetonitrile (0.8 mL) was stirred at room temperature for 16 h. The mixture was cooled to -40 °C and NIS (24 mg, 0.11 mmol) and TfOH (1
µL, 0.011 mmol) were added. After less than 5 minutes, TLC analysis showed conversion of the starting materials into a product. The reaction mixture was diluted with dichloromethane (5 mL) and filtered. The filtrate was washed successively with aqueous sodium thiosulfate (15%, 7 mL) and water (3 x 10mL). The combined organic extracts were dried (MgSO₄), filtered and concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v) to afford 5c as white foam (α/β 3:1, 39 mg, 96%): Selected data ¹H NMR for α−5a:

4.99-5.07 (m, 1H, H-4'), 5.35-5.39 (m, 1H, J₈',₉ₐ = 2.8 Hz, J₈',₉₉ = 5.2 Hz, H-8'), 5.28 (dd, 1H, J₇',₈' = 7.8 Hz, H-7'), 4.69 (d, 1H, H-1), 4.28 (dd, J₉ₐ,₉₉ = 12.7 Hz 1H, H-9'a), 4.23 (dd, J₆,₇ = 1.7 Hz 1H, H-6'), 4.13 (dd, 1H, H-9'a), 4.0 (s, 1H, H-5), 2.63 (dd, 1H, J₃',₃ₐ = 13.2 Hz, J₃',₄' = 5.1 Hz, H-3'e), 1.95 (m, 1H, H-3'a); β−5a: δ; 4.99-5.07 (m, 1H, H-4'), 3.99 (t, 1H, H-5'), 2.43 (dd, 1H, J₃',₃ₐ = 12.7 Hz, J₃',₄' = 4.8 Hz, H-3'eq), 1.85 (m, 1H, H-3'a).
CHAPTER 4
SYNTHESIS OF C-5 ANALOGS OF N5Ac

As reported previously, it seems clear that modification at the C-5 position of Neu5Ac can influence the glycosylation outcome in terms of both reactivity and anomeric selectivity. The increased reactivity of the donor can be related to a decreased nucleophilicity of the amido group, avoiding side reactions with the promoter or the acceptor. However, the influence on the anomeric selectivity remains unclear; presumably it is related to the conformational changes in the glycosylation intermediate. However, no practical evidence has yet been acquired. It is to be expected that the analysis of the reactivity of 5NR derivatives in combination with molecular modeling studies, may give sufficient evidence on the kind of intramolecular stereoelectronic effects that take place in this system.

For this purpose, a library of N-acetylneuraminic acid modified at C-5 was designed (Figure 4.1).

The choice of a substituent at the N-5 position is related to the attempt to increase/decrease steric hindrance and/or electronwithdrawing proprieties in comparison to that in 1 bearing the common acetamido group. In this chapter the syntheses of compounds 3-6 are described, whereas 7127 have already been reported.
The introduction of an additional group such as benzoate (3) or methyl (4) is quite straightforward. In the former case, N-benzylation of 1 in the presence of benzyl chloride (BzCl) in pyridine gave sialyl donor 3 in 86% yield (Scheme 4.1), whereas in the latter case, an attempt of N-methylation in the presence of MeOTf afforded as the only product the imino ether 4a in 50% yield. This is in agreement with a similar result previously reported by Danishefsky. On the other hand, the synthesis of 5 and 6 required additional steps: in this case 9 was used as the common precursor.
Thus, $N$ and $O$ benzylation of \( \textbf{9} \) in the presence of benzyl chloride (BzCl) in pyridine gave the intermediate \( \textbf{10} \). The latter was de-$O$ benzylated in the presence of MeONa in MeOH and then converted in \( \textbf{5} \) by the treatment with Ac\(_2\)O in pyridine with the overall yield of 89%. (Scheme 4.2)
Scheme 4.2

The synthesis of derivative 6 was performed by selective introduction of dichloroacetyl group at the N-position of 9 with methyldichloroacetate ester (Cl₂CHCOOMe) in the presence of triethylamine in methanol. However, along with the main product, a small amount of O-dichlorooacetylated product was detected, therefore, a subsequent de-O-acetylation was required (MeONa in MeOH) to afford 11, which was then O-acetylated with Ac₂O in pyridine to give 6 with 68% overall yield (Scheme 4.3).

Scheme 4.3

i: Cl₂COOMe, Et₃N, MeOH
ii: MeONa, MeOH
iii: Ac₂O, Py
In conclusion, a library of different C-5 derivatives of Neu5Ac has been synthesized: as it can be envisaged, these derivatives will serve as excellent intermediates for the future mechanistic as well as computational studies due to their various glycosyl donor properties.

**Experimental section**

**General.** Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), Reactions were monitored by TLC on Kieselgel 60 F254 (EM Science) and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40°C. Methanol was dried by refluxing with magnesium methoxide, distilled and stored under argon. Pyridine was dried by refluxing with CaH2, then distilled and stored over molecular sieves (3Å). Molecular sieves (3Å and 4 Å), used for reactions, were crushed and activated *in vacuo* at 390°C during 8 h in the first instance and then for 2-3 h at 390°C directly prior to application. 1H NMR and 13C NMR spectra were recorded with a Varian Inova500 spectrometer and a Varian Inova600 spectrometer equipped with Sun workstations. Unless otherwise noted 1H NMR spectra were recorded in CDCl3 and referenced to residual CHCl3 at 7.24 ppm, and 13C NMR spectra to the central peak of CDCl3 at 77.0 ppm. Assignments were made by standard gCOSY and gHSQC, and MALDI-TOF experiments.

**Methyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-5-benzylacetamido-D-glycero-β-D-galacto-non-2-ulopyranosid)onate** (3). Benzyl chloride (0.72 mL, 6.18
mmol) was added to a solution of 1 (0.93g, 1.78 mmol) in pyridine (10 mL). After 16h, the reaction was quenched with MeOH (15 mL), concentrated, co-evaporated with toluene (3 x 15mL) and dried in vacuo. The residue was purified by silica gel column chromatography (10% gradient acetone in hexane) to afford 3 as a white foam (0.95 g, 86%). MALDI-TOF: m/z = 648.1 [M+Na]⁺; ¹H NMR at 70°C: δ; 7.22-7.79 (m, 5H, aromatic), 5.92-5.98 (m, 1H, H-4), 5.22-5.34 (m, 3H, H-6, 7, 8), 4.6-4.74 (m, 2H, J₉₉,J₉₈ = 12.5 Hz, H-9a, 5), 4.20 (dd, 1H, H-9b), 3.82 (s, 3H, OCH₃), 2.78 (dd, 1H, J₃₃,J₃₄ = 5.0 Hz, J₃₃,J₃₅ = 13.4 Hz, H-3e), 2.16 (dd, 1H, H-3a), 2.16, 2.14, 2.08, 2.06, 2.05, (5s, 15H, NCOCH₃, OCOCH₃), 2.03 (s, 3H, SMe).

Methyl (methyl 4,7,8,9-tetra-O-acetyl- 3,5- dideoxy-2 -thio -5-dimethoxyimine –D -glycero-β-D-galacto-non-2- ulopyranosid)onate (4a) To a solution of 1 (0.65g, 1.25 mmol) and MS 3Å in DCM (2 mL) stirred under argon atmosphere for 16 h, methyl triflate (0.42 mL, 3.74 mmol) and triethylamine (5 mL) were added. After 2h, the reaction was diluted with DCM and filtered. The filtrate was washed successively with aqueous sodium bicarbonate (15%, 20 mL) and water (3 x 20mL). The combined organic extracts were dried (MgSO₄), filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (10% gradient acetone in hexane) to afford 4 as a white foam (334 mg, 50%): MALDI-TOF: m/z = 537.8 [M+H]⁺; ¹H NMR: δ; 5.35-5.37 (m, 1H, H-7), 5.25-5.17 (m, 1H, H-4), 5.12-5.16 (m, 1H, J₈₉,J₈₁ = 2.4 Hz, J₈₉,J₈₁ = 7.5 Hz, H-8), 4.67 (dd, 1H, J₉₉,J₉₈ = 12.4 Hz, H-9a), 4.38 (dd, 1H, J₉₆,J₉₇ = 2.3 Hz, H-6), 4.21 (dd, 1H, H-9b), 3.79, 3.59 (2s, 6H, OCH₃), 3.14 (t, 1H, J₅₆,J₅₇ = 9.4 Hz, H-5), 2.59 (dd, 1H, J₃₃,J₃₄ = 4.8 Hz, J₃₃,J₃₅ = 13.7 Hz, H-3e), 2.10 (dd, 1H, H-3a), 2.10, 2.05, 2.03, 2.02, 1.99 (5s, 15H, NCOCH₃, OCOCH₃), 1.80 (s, 3H, SMe).
Methyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-5-benzamido-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (5). Benzyl chloride (17.8 mL, 21.5 mmol) was added at 0°C to a solution of 9 (6.78 g, 1.78 mmol) in pyridine (5 mL). After stirring at room temperature for 2 h, TLC analysis showed the completion of the reaction. The reaction was quenched with MeOH (50 mL), concentrated, co-evaporated with toluene (3 x 50 mL) and dried in vacuo. The residue was purified by silica gel column chromatography (10% gradient acetone in hexane) to afford 10. MeONa (6 mL, 1M in MeOH), was added to a solution of 10 (1.47 g, 1.18 mmol) in MeOH (60 mL). After 2 h, the reaction mixture was neutralized with Dowex-50 H⁺ resin (pH = 7), which was removed by filtration. The filtrate was concentrated under reduced pressure to afford methyl (methyl 3,5-dideoxy-2-thio-5-benzamido-D-glycero-β-D-galacto-non-2-ulopyranosid)onate. Ac₂O (2 mL) was added to the residue in pyridine (15 mL). After 16 h, the reaction was quenched with MeOH (6 mL), concentrated in vacuo and residue was co-evaporated with toluene (3 x 20 mL) to give 5 as a white foam (0.91 g, 89%), MALDI-TOF: m/z = 604.9 [M+Na]⁺; ¹H NMR: δ; 7.26-7.64 (m, 5H, aromatic), 6.26 (d, 1H, J NH, 5 = 9.9 Hz, NH), 5.41-5.49 (m, 2H, J 4,5 = 10.2 Hz, H-4, 7), 5.17-5.21 (m, 1H, J 8,9a = 2.2 Hz, J 8,9b = 8.0 Hz, H-8), 4.81 (dd, 1H, J 9a,9b = 12.4 Hz, H-9a), 4.47 (dd, 1H, J 6,7 = 2.2 Hz, H-6), 4.31 (t, 1H, J 5,6 = 10.2 Hz, H-5), 4.18 (dd, 1H, H-9b), 3.82 (s, 3H, OCH₃), 2.56 (dd, 1H, J 3e,4 = 4.9 Hz, J 3e,3a = 14.0 Hz, H-3e), 2.22 (dd, 1H, H-3a), 2.18, 2.11, 2.04, 2.00, (5s, 15H, OCOCH₃, SMe).

Methyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-5-dichloroacetamido-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (6). Methyl dichloroacetate (2.5 mL, 24 mmol) was added to a solution of 9 (0.76 g, 2.43 mmol) and triethylamine (0.68 mL, 4.86
mmol) in methanol (30 mL). After 1.5 h, the reaction mixture was concentrated under reduced pressure and dried in vacuo. MeONa (2 mL, 1M in methanol) and methanol (20 mL) were added to the residue. After 1 h, the reaction mixture was neutralized with Dowex-50 H⁺ resin (pH = 7), which was removed by filtration. The filtrate was concentrated under reduced pressure. Pyridine (20 mL) and Ac₂O (10 mL) were added to the residue. After 16h, the reaction was quenched with MeOH (15 mL), concentrated, co-evaporated with toluene (3 x 15mL) and dried in vacuo. The residue was purified by silica gel column chromatography (10% gradient ethyl acetate in hexane) to afford 6 as white foam (974 mg, 68%), MALDI-TOF: m/z = 590.0 [M+Na]⁺; ¹H NMR: δ; 6.79 (d, 1H, J NH,5 = 10.1 Hz, NH), 5.83 (s, 1H, Cl₂CHCO), 5.38-5.47 (m, 2H, J 4,5 = 10.5 Hz, H-4, 7), 5.16-5.19 (m, 1H, J₈,₉ₐ = 2.2 Hz, J₈,₉ᵇ = 7.5 Hz, H-8), 4.79 (dd, 1H, J₉ₐ,₉ᵇ = 12.7 Hz, H-9a), 4.49 (dd, 1H, J₆,₇ = 1.76 Hz, H-6), 4.17 (dd, 1H, H-9b), 3.99 (dd, 1H, J₅,₆ = 10.1 Hz, H-5), 3.81 (s, 3H, OCH₃), 2.58 (dd, 1H, J₃ₑ,₄ = 4.8 Hz, J₃ₑ,₃ₐ = 13.6 Hz, H-3e), 2.16 (dd, 1H, H-3a), 2.13, 2.08, 2.05, 2.02, (4s, 12H, OCOCH₃), 2.00 (s, 3H, SMe).
CHAPTER 5
CONCLUSIONS AND FUTURE WORK

Despite recent progress in the chemical and enzymatic sialylation of saccharides, no approach that allows glycosylation of a wide range of acceptors in high yields and stereoselectivities has yet emerged. Anomeric chlorides of Neu5Ac offer the most reliable glycosyl donor for the preparation of glycosides of simple alcohols. 2-Thioalkyl, 2-thiophenyl, 2-xanthate and 2-(di-benzyl) or 2-(di-ethyl)phosphites are the leaving groups of choice when more complex hindered sugar alcohols need to be sialylated. Thus, in many cases the sialylations proceed with excellent regioselectivities in combination with high yields and anomeric selectivities.

Several glycosyl donors of Neu5Ac have been prepared that have an auxiliary at C-3. These auxiliaries control the anomeric selectivity of a glycosylation by neighboring group participation. Glycosyl donors that possess an equatorial S-phenyl participating auxiliary at C-3 and thioalkyl leaving group at C-2 generally give the best yields and α-anomeric selectivities especially when applied for the glycosylation of sterically hindered alcohols. A 3-O-phenylthiocarbonyl auxiliary also gives excellent yields and stereoselectivities. However, the major drawbacks of indirect methods are the additional chemical steps required for introduction and removal of the auxiliary at C-3. Furthermore, the introduction of a C-3 auxiliary in many cases proceeds with poor stereoselectivity.
Recent progress allows chemical, enzymatic or chemoenzymatic synthesis of complex sialoglycoconjugates and oligosaccharides. Each synthetic target, however, should be regarded as a research project and several reaction conditions, methods or strategies may need to be examined to obtain an efficient synthetic approach. Most synthetic efforts have been directed to the glycosylation of Neu5Ac. Less effort has been spent on the preparation of sialosides that have an \textit{N}-glycolyl moiety (Neu5Gc) or have an acetyl, lactoyl, methyl or phosphate at one of the hydroxyls.

It has been shown that modification of the \textit{C}-5 amino group of 2-methyl and 2-thiophenyl sialosides into \textit{N}-TFA derivatives provides glycosyl donors that give good yields and high \(\alpha\)-anomeric selectivities in direct glycosylation with a wide range of glycosyl acceptors of different reactivities. These new donors allowed, for the first time, the stereoselective direct synthesis of \(\alpha\)-(2-8)-linked dimers in high yields. The best yields were obtained when the amino functionality of the sialyl acceptor was also protected as \textit{N}-TFA derivative. Previous synthetic routes towards this dimer required laborious indirect sialylation protocols. The favorable properties of the new approach allowed a highly efficient synthesis of the human melanoma associated antigen GD\(_3\) derivative, which has two Neu5Ac residues of different linkage type. The \textit{N}-TFA-protecting group could be easily transformed into Neu5Ac derivatives and potentially they open an efficient route towards sialic acid containing oligosaccharides that have modified amino functionalities (e.g. \textit{N}-glycolyl derivatives). The high yields of the glycosylations probably results from lower nucleophilicity of the trifluoroacetamido functionalities of sialosides in comparison to that of conventional acetamido derivatives. The origin of the high \(\alpha\)-selectivity of the \textit{N}-TFA protected sialyl donors at this point remains to be uncertain.
Although this improvement in the reactivity of the sialyl donors can lead to side reactions when reactive galactosides are used as glycosyl acceptors, the introduction of a trifluoroacetamide moiety allows for the first time glycosylations with unreactive galactosyl acceptors, opening a new route towards sialic acid containing oligosaccharides. It is to be expected that the application of the new class of the sialyl donors will provide an excellent approach for the synthesis of scarcely accessible polysialic acid derivatives, a major feature of which is extremely low reactivity.
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