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Some New Methods in Sialic Acid Chemistry

Including studies towards a lactam analogue of [8,9]-lactonised G_{D3} and a novel promoter system for thioglycosides in *O*-glycoside synthesis

Teddy Ercegovic

Organic Chemistry 2
Lund Institute of Technology
Lund 2001

LUND UNIVERSITY

Some New Methods in Sialic Acid Chemistry

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Tekn. Lic., Fil. Kand.

Akademisk avhandling som för avläggande av filosofie doktorsexamen vid tekniska fakulteten vid Lunds Universitet kommer att offentligen försvaras å Kemicentrum, sal B, fredagen den 23 mars 2001, kl. 10.15.

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This thesis is dedicated to the memory of Professor Göran Magnusson

This thesis is based on a summary of the following papers **I-V**. Where required, each paper is reprinted with the kind permission of its publisher.

I Teddy Ercegovic, Göran Magnusson
A new *N*-acetylneuraminic acid donor for highly stereoselective α-sialylation
J. Chem. Soc., Chem. Comm. **1994**, 831-832

II Teddy Ercegovic, Göran Magnusson
Highly stereoselective α-sialylation. Synthesis of G_{M3}-saccharide and a *bis*-sialic acid unit
J. Org. Chem. **1995**, *60*, 3378-3384

III Teddy Ercegovic, Göran Magnusson
Synthesis of a bis(sialic acid) 8,9-lactam
J. Org. Chem. **1996**, *61*, 179-184

IV Teddy Ercegovic, Ulf Nilsson, Göran Magnusson
A study of the donor properties of sialyl phosphites having an auxiliary 3-(*S*)-phenylseleno group
Carbohydr. Res., accepted

V Teddy Ercegovic, Andreas Meijer, Göran Magnusson, Ulf Ellervik
Iodine monochloride/silver trifluoromethanesulfonate (ICl/AgOTf) as a convenient promoter system for *O*-glycoside synthesis
Org. Lett., accepted

List of abbreviations

1D	one-dimensional
2D	two-dimensional
Ac	acetyl
AgOTf	silver trifluoromethanesulfonate
AIBN	2,2'-azo-*bis*-isobutyronitrile
BF_3Et_2O	boron trifluoride ethyl etherate
Bn	benzyl
BSA	bovine serum albumin
Bz	benzoyl
Cer	ceramide
COSY	correlation spectroscopy
CSA	(±)-camphor-10-sulfonic acid
DAST	diethylaminosulfur trifluoride
DBU	1,8-azabicyclo[5.4.0]undec-7-ene
DMF	*N*,*N*-dimethylformamide
Et	ethyl
eq.	equivalents
FAB-MS	fast atom bombardment mass spectrometry
G	ganglioside
Gal	D-galactose
GalNAc	*N*-acetyl-D-galactosamine
Glc	D-glucose
GlcNAc	*N*-acetyl-D-glucosamine
GSL	glycosphingolipids
HETCOR	heteronuclear correlation spectroscopy
MAb	monoclonal antibody
Me	methyl
MS 3A	3Å molecular sieves
MSB	methylsulfenyl bromide
NBS	*N*-bromosuccinimide
Neu5Ac	*N*-acetylneuraminic acid, sialic acid
Neu5Gc	*N*-glycolylneuraminic acid
NIS	*N*-iodosuccinimide
NMNO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
NPhth	*N*-phthalimido
OTs	*O*-*p*-toluenesulfonyl
Ph	phenyl
PMB	*p*-methoxybenzyl
Q	tetra-*n*-butylammonium
THF	tetrahydrofuran
TfOH	trifluoromethanesulfonic acid
TLC	thin layer chromatography
TsCl	*p*-toluenesulfonyl chloride
TsOH	*p*-toluenesulfonic acid
TMSEt	2-(trimethylsilyl)ethyl
TMSOTf	trimethylsilyl trifluoro-methanesulfonate
UV	ultraviolet

Preface

As a result of my absence from active research 1996-1999, this thesis has a slightly unorthodox disposition. Some sections only disclose the state of the art before 1995, an arrangement which I believe makes the incentives behind the research performed 1992-1995 more clear to the reader. After the presentation of my earlier results, an updated survey of the pertinent fields is provided, and chapter 5 should meet this purpose. The state of the art as presented in said chapter was in turn decisive for initiating the research performed during the past year. I also advise the reader to at least glance through the papers **I-V** before reading this thesis.

The results in papers **I-III** are discussed in chapters 1-4, whereas work associated with paper **IV** is presented in chapter 6. Chapter 7 *inter alia* deals with preparation and study of a 3-(*S*)-(2-methoxyphenyl)thio analogue of the sialyl donor employed in papers **I-III**. This in turn generated the development of the ICl/AgOTf promoter system for thioglycosides (paper **V**), which is covered in section 7.4.

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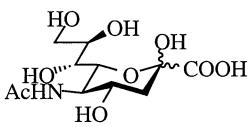
1 Background

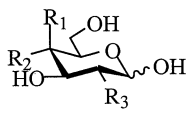
1.1 An Introduction to Glycosphingolipids

Glycosphingolipids were first isolated from brain tissue in 1874, and they are regarded as cell-bound components encompassing a large variety of carbohydrate compositions and structures which are usually characteristic for a given cell type[1,2]. In healthy individuals, they are found in either cell membranes or plasma lipoproteins and are present in virtually all vertebrate cells[3]. In *e.g.* red blood cells, it has been shown that glycosphingolipids are enriched in the plasma membrane, where their carbohydrate moieties are oriented toward the cell exterior[4,5].

All glycosphingolipids have two main features, and these are a hydrophilic and a hydrophobic moiety consisting of saccharide and lipid, respectively. Perhaps surprisingly, only about seven types of monosaccharides have been observed in the saccharide moiety of mammalian cell surface glycosphingolipids. These are D-glucose (Glc), D-galactose (Gal), L-fucose (Fuc)[6], D-xylose (Xyl)[7], *N*-acetyl-D-glucosamine (GlcNAc), *N*-acetyl-D-galactosamine (GalNAc) and sialic acid (*N*-acetylneuraminic acid, Neu5Ac) and derivatives of the latter (Neu5Gc, 4-*O*-acetyl-Neu5Ac and 9-*O*-acetyl-Neu5Ac). The structures of some of these monosaccharides are shown in Fig. 1.1 below.



1 Sialic acid (Neu5Ac)



2 R_1 = H, R_2 = R_3 = OH (Glc)
3 R_1 = R_3 = OH, R_2 = H (Gal)
4 R_1 = H, R_2 = OH, R_3 = NHAc (GlcNAc)
5 R_1 = OH, R_2 = H, R_3 = NHAc (GalNAc)

Fig. 1.1 Some monosaccharides present in glycosphingolipids in mammalian cells

Glycosphingolipids (GSL) can be subdivided into four major families: cerebrosides (one monosaccharide), neutral GSL, sulfato dito and gangliosides (G)[8]. Gangliosides are characterised by the presence of sialic acid, and their nomenclature is that devised by L. Svennerholm[9]. Sulfato GSL have sulfate ester groups on their carbohydrate moiety, whereas neutral GSL contain neither sialic acid nor sulfate ester groups. Neutral GSL are well exemplified by the blood group A glycolipids[10].

In mammalian cells, the saccharide directly attached to the lipid moiety is most often D-glucose, and the number of monosaccharide units in a glycosphingolipid typically ranges from one to eight (G_{Q1b})[6]. The lipid unit usually consists of ceramide, a 4-sphingenine (sphingosine) to which a $C_{14\text{-}26}$ fatty acid is linked via an amide bond, and it is β-linked to the reducing end saccharide (Fig. 1.2).

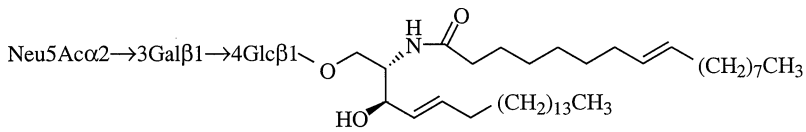


Fig. 1.2 Ceramide attached to a carbohydrate chain forming G_{M3}

1.2 The Biological Functions of Glycosphingolipids

Being rather complex molecules, it is not surprising that glycosphingolipids display a vast array of different biological functions, of which their most noteworthy in short are to:

i) contribute to the formation of the water permeability barrier in human skin[12];

ii) confer structural rigidity to biological membranes[13,14];

iii) serve as cell surface receptors for various substances, as exemplified by sialyl-Le^x on leucocytes, to which selectins adhere during inflammatory processes on human endothelial cells[15-18], and G_{M1}, to which cholera toxin can bind[19-20];

iv) serve as a Ca^{2+}-binding cofactor in synaptic transmission[21], and GSL changes in neuronal membranes is one of the characteristics of Alzheimer's disease[22];

v) together with membrane proteins regulate cell responses to growth factors[23,24];

vi) modulate T- and B-cell response[3,23];

vii) provide cell-to-cell recognition and communication, where they are distinguishing markers for cells from various organs of an individual[1,25];

viii) provide surface antigens, such as blood group determinants of various types[10,26].

Needless to say, the above functions have generated a solid general interest in glycosphingolipids.

1.3 Altered Ganglioside Expression in Malignant Cells

Since glycosphingolipids are secondary gene products, *i.e.* not directly encoded in the DNA, they are synthesised in a series of reactions catalysed by numerous enzymes commonly known as glycosyltransferases. As a consequence, disturbed activation/inhibition of promoter regions in DNA that control translation rate, or post-translational modifications, can affect the distribution, concentration and substrate specificity of a given glycosyltransferase in a most substantial manner. Several mechanisms control the cell composition of glycosphingolipids, and the sensitivity of the latter to subtle shifts in the environment is therefore expected to be considerable[1]. This has indeed proven to be the case.

In a pioneering work performed in 1967, cell lines from hamster kidney fibroblasts were treated with polyoma virus, after which the carbohydrate composition of the fibroblasts was examined and compared with normal cells originating from the same source[2]. The result was that the cultured malignant cells were richer in short glycosphingolipid homologues, in this

case lactosylceramide, a known precursor to G_{M3} (*vide supra*). It was thus suggested that the malignant cell transformation was accompanied by either an impairment in the sialyl transfer or enhanced neuraminidase activity, or a combination of both mechanisms. Following these results, numerous observations of glycosphingolipid and glycoprotein alterations associated with malignant cell transformation have been reported[6,27].

The carcinogen *N*-2-fluorenylacetamide gives rise to slowly developing malignant tumors in rat liver, and by monitoring variations in ganglioside composition in parallel with the tumor growth, it was confirmed *in vivo* that ganglioside alterations may be an early event in tumorigenesis[28]. It is now a general opinion that essentially all tumor cells display different profiles and structures of cell surface carbohydrates as compared to non-transformed progenitor cells[29]. The deviations in ganglioside levels were subsequently found to be caused by the increase or decrease of the activities of glycosyltransferases[23,29,30].

1.4 Lactonisation in Gangliosides

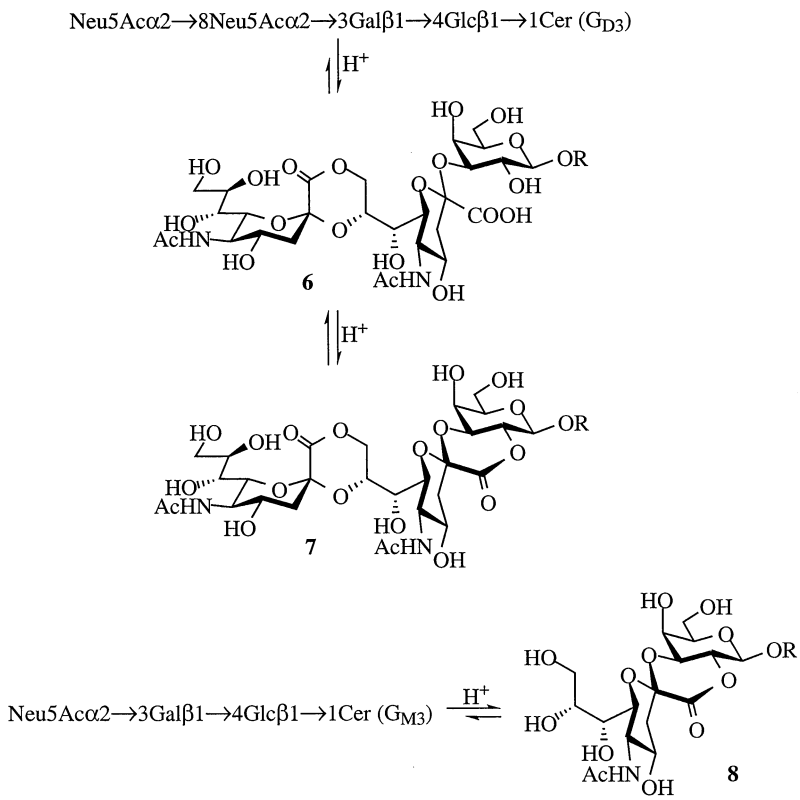
Due to hydroxyl groups in close proximity of a carboxylic acid functionality (pK_a ~2.5), it was suspected early that gangliosides could lactonise, *i.e.* form esters intramolecularly[31-33]. Then in 1966, lactones of Neu5Acα2→8Neu5Ac-residues (*bis*-sialic acid residues) in gangliosides were reported[34]. Subsequent work showed that lactone formation is catalysed *e.g.* by acetic acid, and various methods to accomplish lactonisation exist[35-37]. Ganglioside lactones are stable if stored at pH 6 in aqueous solution, and they are cleaved by aqueous Na_2CO_3 or $NaHCO_3$, albeit rather slowly with the latter reagent[36,38].

The most relevant lactones in this context are the [8,9]-lactone of *bis*-sialic acid residues, and the [2,3]-lactone of Neu5Acα2→3Gal-residues[39-41] depicted in Scheme 1 on the following page*. A combination of several lactones in one ganglioside molecule is possible[39,41,42].

It has been shown that in rodent brain tissue, approximately 10% of all sialic acid is present in some kind of esterified form, including 4- and 9-*O*-acetyl derivatives as well as various lactones[43]. Further work has indicated the *in vivo* existence of ganglioside lactones in human brain tissue[44]. In both investigations, special care was taken to exclude lactone formation during the isolation procedure, thereby minimising the possibility that ganglioside lactones are artefacts. The presence of the [2,3]-lactone of G_{M3} on the surface of murine malignant melanoma cells has also been evidenced[45].

---

* If IUPAC rules are strictly adhered to, the [8,9]- and [2,3]-lactone of *e.g.* G_{D3} should be referred to as [1d→9c]- and [1c→2b]-lactone, respectively. However, in order to increase the readability of the present text, I decided to use the former simplified type of notation.



Scheme 1. Lactonisation of G_{D3} and G_{M3} (R = 4Glcβ1→1Cer). For the sake of clarity, the lactonisation patterns are simplified.

1.5 Tumor-associated Antigens & Lactones

G_{M3} is highly abundant in syngeneic murine melanoma B16 cells[46]. When a monoclonal antibody (MAb) M2590 was prepared after immunisation with the B16 cell line in C57BL/6 mice, M2590 was found to be directed towards G_{M3}[46,47]. The puzzling matter in this case was that G_{M3} is present in essentially all types of cells *and* in a larger amount than any other ganglioside normally present in humans and animals[48,49]. In other words, M2590 had been raised against an indigenous substance. In view of the observations that G_{M3} is virtually non-immunogenic in mice, the reasonable possibility that G_{M3} did not constitute the immunogen *per se* was then suggested[48]. From here on, it was hypothesised that in the case of MAb M2590, the antigen G_{M3} was *not* identical to the immunogen[48]. This hypothesis of course opposes the general concept of structural antigen-immunogen identity. However, further investigations of M2590 revealed the following[48]:

i) irrespective of cell type and species, only cells having a relatively high concentration of G_{M3} (>0.2 μmol/g of packed cells) showed an immune response;

ii) incubation with sialidase lowered the amount of G_{M3} present on the cells, but no substantial decrease in the immune response resulted;

iii) lysis of G_{M3}-containing liposomes by M2590 showed a clear threshold effect around 8 mol% of G_{M3} in the liposome-forming mixture of lipids.

In conclusion, the immunogen formation appeared to be dependent on the density of G_{M3} on the cell surface. It was then assumed that the immunogen in question was some kind of lactone structure, and as hypothesised, the [2,3]-lactone of G_{M3} (**8**) displayed 20-30 times higher affinity to M2590 than G_{M3}[48]. Moreover, immunisation with **8** in mice gave several antibodies reacting with both **8** and G_{M3} together with a few antibodies reacting with **8** only. Clearly, G_{M3} is a substantially poorer immunogen than its [2,3]-lactone **8**.

A tumor-associated antigen should be abnormally frequent in malignant cells as compared to normal cells *and* give rise to tumor-specific antibodies. Hence, G_{M3} in syngeneic mice melanoma B16 cells can be regarded as a tumor-associated antigen (via its lactone immunogen **8**). Other examples of tumor-associated ganglioside antigens are G_{D3} in human malignant melanoma cells[50-53], sialyl-Le^x in adenocarcinomas[54,55] and isoglobotetraosylceramide in colonic cancer[56].

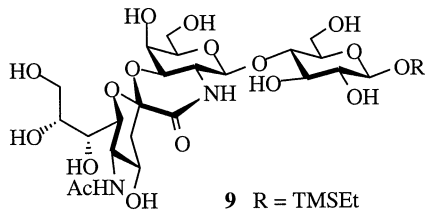
In subsequent work, an IgG_3 MAb named DH_2 was obtained after immunising mice with the lactone **8**[49]. Administration of DH_2 resulted in an inhibition of the growth of melanoma cells both *in vitro* and *in vivo*. The life span of DH_2-treated mice expressing melanoma was extended to ~35 days as compared to ~25 days for untreated mice[49]. It deserves mentioning that M2590, raised against B16 melanoma cells, turned out to be ineffective *in vivo*. Nevertheless, new possibilities for inhibition of tumor cell growth *in vivo* by using antibodies are now available by the discovery of lactones as the actual immunogens of the corresponding tumor-associated antigens.

1.6 Ganglioside Lactams as Immunogens

Although the MAb DH_2 effected inhibition of melanoma cell growth in mice, it was not considered satisfactory for broader therapeutic applications. A fully satisfactory result is obviously obtained only when complete regression of a tumor is accomplished in conjunction with antibody administration. A part of the explanation of the inability of M2590 and DH_2 to completely clear an organism from malignant tumor cells probably lies in the observed hydrolytic lability of ganglioside lactones[36,37,57,58]. In other words, a high concentration of a lactone immunogen can not be maintained *in vivo*, which leads to a weak immune response and thus few antibodies specific for the target structure.

A way of increasing the hydrolytic stability of an immunogenic lactone without altering the conformation significantly is to make its corresponding lactam ("inner amide"). Similarity

in the conformation between lactone and a corresponding lactam **9** (Fig. 1.3) has been demonstrated for G_{M3} by MM2 calculations as well as NMR spectroscopy[59]. In comparison with a lactone, the stability of its lactam analogue towards hydrolysis should lead to a prolonged life-span *in vivo* and thus a stronger immune response. Furthermore, since no acid-lactone equilibrium is present, the identity of the immunogen will be more well-defined in the lactam case.



9 R = TMSEt
10 R = $CH_2CH_2SCH_2CH_2CONH$-BSA

*Fig. 1.3 The [2,3]-lactam analogue (**9**) of G_{M3} and its corresponding spacer-BSA conjugate (**10**)*

Immunisation of a synthesised spacer-BSA conjugate **10** in Balb/c mice then gave the following results[60]:

i) a strong immune response providing more than 300 monoclonal hybridomas;

ii) eight monoclonal antibodies (IgG-type) were chosen randomly for specificity studies, and two of them (P5-1 and P5-3) recognised both the lactone **8** and the lactam **10**, where the binding to the latter was comparatively stronger;

iii) none of the selected eight antibodies displayed binding to G_{M3} itself.

These results were indeed promising, and the synthetic endeavours associated with the lactams **9** and **10** seemed worthwhile. However, the *in vivo* trials of P5-1 and P5-3 in mice expressing melanoma cells have not yet been performed, and the full potential of the lactam concept remains to be seen.

As a pure speculation at this stage, the medical applications of the lactam concept might provide tumor vaccines, active or passive immunotherapy, diagnostics in early tumor detection and/or tumor-directed drug carriers, the latter often referred to as "smart drugs".

1.7 G_{D3} and its Association with Human Malignant Melanoma

G_{M3}, G_{M2} and G_{D3} are all prominent gangliosides in human malignant melanomas constituting about 30, 15 and 50%, respectively, of the total ganglioside content[50,52]. As for G_{D3}, it is expressed in an at least tenfold higher amount than in normal tissues and major organ systems, excluding the brain, and is an established tumor-associated antigen for human malignant melanoma[50-53,61,62]. The presence of lactones of G_{D3} on the surface of various human melanoma cell lines has been indicated[63].

In view of the properties of G_{M3} discussed above, it is hardly surprising that G_{D3} is a poor immunogen in mice. Antibodies against G_{D3} have only in rare instances been detected in sera of human melanoma patients, and then always at a low titer[64,65]. Nevertheless, numerous monoclonal antibodies have been prepared against G_{D3}, and of these, a MAb denoted R_{24} is by far the most thoroughly studied and used[66-71]. MAb R_{24} was prepared in mice by immunisation with human SK-MEL-28 cells, the latter being a well-established melanoma cell line[69].

Epitope characterisation of R_{24} showed that the outer Neu5Ac residue is crucial for binding, whereas the inner Neu5Ac residue is significantly less important and can actually be either Neu5Ac or Neu5Gc[71]. Somewhat surprisingly though, R_{24} is reported to bind strongly to G_{D3}, weakly to the [8,9]-lactone **6** and not at all to the [8,9],[2,3]-*bis*-lactone **7**[39]. The relevancy of these data has been questioned[58], particularly in view of that the acid-lactone equilibrium of G_{D3} was not suppressed in the assays. In general, analytically pure lactones of G_{D3} are difficult to isolate[58], and my own results support this assumption (see paper **III**, where [8,9]-lactone opening with CD_3OD is evidenced). It deserves mentioning that in the report where the structure of the [8,9]-lactone **6** was elucidated, no 1D ^{1}H NMR spectrum of pure **6** was published[39]. In contrast thereto, the same authors have published the 1D ^{1}H NMR spectrum of the [2,3]-lactone **8** of G_{M3}[40].

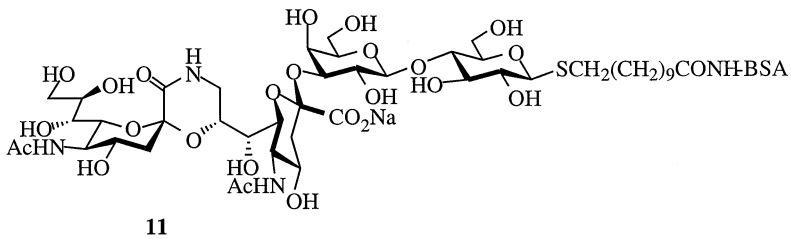
Further immunisation studies of G_{D3} in human melanoma patients have shown that the [8,9]-lactone **6** is more immunogenic than the *bis*-lactone **7**[64]. However, antibody responses in humans were short-lived with no memory B-cell induction, and the human anti-**6** antibodies obtained did *not* cross-react with G_{D3} and G_{D3}-expressing melanoma cells[64]. According to the authors, the potential cause(s) of these disencouraging results could be bad presentation of the immunogen **6**, suppressed immuno-defense in the human patients (normally the case for cancer patients) and/or inherent immunologic tolerance to G_{D3}.

Still, when the antibody R_{24} was administered to 12 human melanoma patients in an initial phase I trial, three of the patients experienced major tumor regression without any serious side effects[72,73]. The antibody R_{24} is currently in some clinical use, albeit in general only about 10% of human metastatic melanoma patients respond to treatment[74].

In summary, the above results have generated the need for stable analogues of the [8,9]-lactone **6** in order to gain a further understanding of the immunological importance, properties and usefulness of G_{D3}, where the ultimate goal is knowledge to devise efficient therapy against human malignant melanoma.

1.8 Objective of Thesis

It was decided that the target molecule for my initial work was the G_{D3} [8,9]-lactam-BSA conjugate **11** shown below.



11

Fig. 1.4 Target molecule

The reasons for choosing **11** were:

i) the [8,9]-lactone of G_{D3} is alleged to be a stronger immunogen than the [8,9],[2,3]-*bis*-lactone[64];

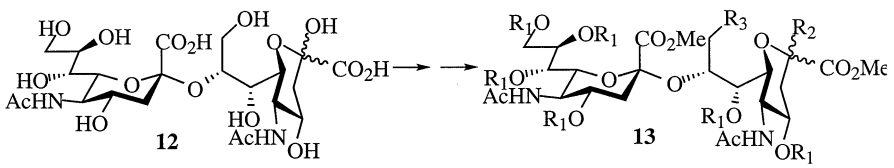
ii) the main epitope of MAb R_{24} is the outer sialic acid residue[71].

2 Approaching the Target *bis*-Sialic Acid [8,9]-Lactam

2.1 Retrosynthetic Analysis

Three possible routes to **11** were considered:

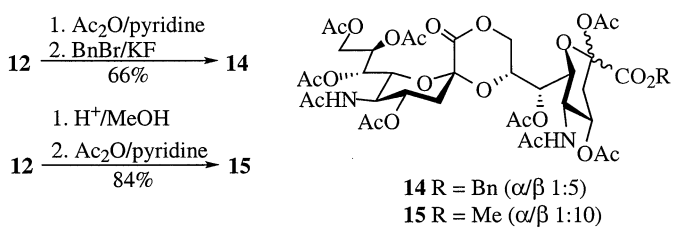
(i) Transformation of the commercially available *bis*-sialic acid (**12**) into a donor (**13**) where OH-9 has been substituted with a nitrogen functionality. Condensation of **13** with a lactoside acceptor would then provide the desired tetrasaccharide backbone (Scheme 2).



R_1 = Ac, Bz, Bn
R_2 = S-alkyl, S-aryl, SC(S)OEt
R_3 = N_3, NPhth

Scheme 2

The advantage with this route is that only one new *O*-glycoside bond needs to be formed in the synthesis of **11**. However, the disadvantages are severe. Firstly, the small difference in reactivity between OH-9 and OH-9' probably causes difficulties when a reaction aiming for regioselectivity between these hydroxyl groups is performed. Secondly, **12** readily forms a rather stable [8,9]-lactone under mild conditions (Scheme 3)[75,76], and it is by no means trivial to exploit this lactonisation synthetically for selective substitution of OH-9.

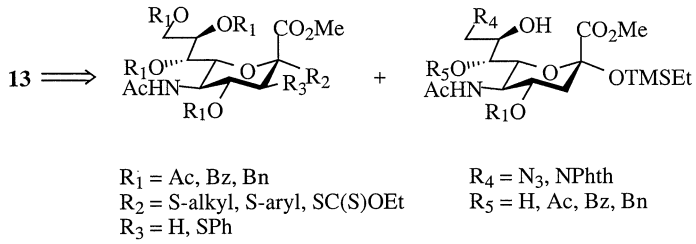


14 R = Bn (α/β 1:5)
15 R = Me (α/β 1:10)

Scheme 3

Thirdly, no kind of regioselective protective group chemistry on *bis*-sialic acid residues has yet been published.

(ii) Making the donor **13** by sialylation of a 9-protected acceptor followed by lactamisation (Scheme 4) either before or after coupling to a lactoside moiety.



R_1 = Ac, Bz, Bn
R_2 = S-alkyl, S-aryl, SC(S)OEt
R_3 = H, SPh

R_4 = N_3, NPhth
R_5 = H, Ac, Bz, Bn

Scheme 4

With only one primary hydroxyl group present, the introduction of a nitrogen functionality at *C*-9 in the acceptor moiety should be fairly straightforward. There exists a plethora of methods for substitutions and protective group manipulations in Neu5Ac[77-84]. The obvious drawback with this approach is the difficult α2→8 *bis*-sialo coupling. However, many useful sialyl donors were reported[85-90], and the α2→8 coupling did not at first seem to be a major obstacle*.

(iii) Sialylation of a 9-azido G_{M3}-like trisaccharide followed by lactamisation.

A serious inconvenience with route *(iii)* is that a trisaccharide is a rather complicated molecule to perform regioselective trials on. Hence, a suitable trisaccharide backbone would have to be synthesised first with carefully chosen protective groups before it is manipulated into a 9-azido acceptor. If route *(iii)* is to be chosen, it seems easier to first gain knowledge of the necessary reactions via simpler substates, and under all circumstances, this path also requires an α2→8 coupling.

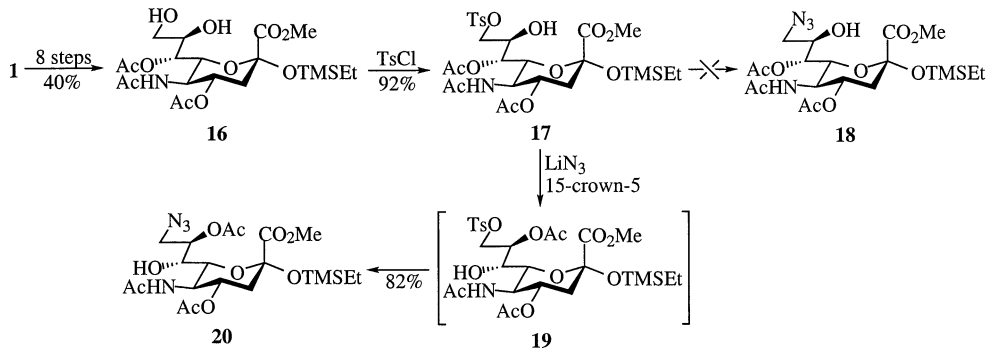
In view of the arguments set forth above, route *(ii)* was ultimately chosen as the first step towards the target molecule **11**.

2.2 Synthesis of 9-Azido Acceptors

In the first acceptor synthesis, Neu5Ac (**1**) was conveniently transformed into the known 4,7-di-*O*-acetylated diol **16** in a total yield of 40% after eight reaction steps[77,78,91,92]. Reaction of **16** with *p*-toluenesulfonyl chloride in pyridine selectively gave the 9-*O*-tosylated derivative **17** in 92% yield (Scheme 5). Only a trace of *bis*-*O*-tosylated compound was observed. However, treatment of **17** with LiN_3 in tetrahydrofuran using 15-crown-5 ether as catalyst did *not* give the desired 9-azido-7-*O*-acetyl derivative **18**. Instead, the 9-azido-8-*O*-acetyl derivative **20** was isolated in 82% yield. TLC-monitoring during the reaction revealed formation of a new strongly UV-absorbing intermediate which disappeared when the reaction was complete. The starting material **17** absorbs UV radiation much stronger than the product **20**. A a consequence,

* For obvious reasons (paper **I** published in 1994), only the most potent sialyl donors available in 1993 are referred to here.

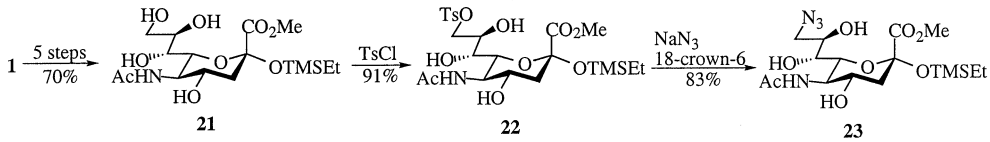
we suspected that the observed intermediate was the 8-*O*-acetyl-9-*O*-*p*-toluenesulfonyl derivative **19**, formed via a base catalysed acetyl migration. ^{1}H NMR analysis of a reaction mixture supported this assumption, since a (transient) H-8 proton signal from **19** could be observed at 5.18 ppm. As a comparison, the chemical shift of the H-8 proton of **17** and **20** is 4.17 and 5.28 ppm, respectively. Attempts to isolate **19** from the reaction mixture were unsuccessful.



Scheme 5

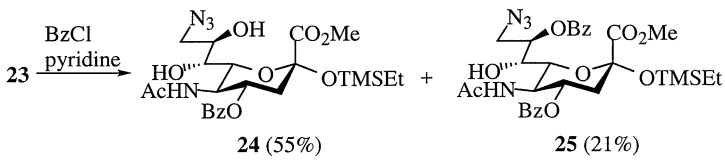
When **17** was treated with tetra-*n*-butylammonium azide in acetonitrile, no formation of **19** was observed, and the reaction was complete within less than 10 minutes at room temperature. TLC-monitoring showed that a weakly UV-absorbing compound different from **20** was formed, and this was probably the desired product **18**. However, rapid rearrangement into **20** during work-up of the reaction mixture occurred within less than an hour. Somewhat oddly, product **18** was stable for days if kept in the reaction mixture, but we still had to conclude that acyl protection of HO-7 should be avoided in the acceptor to be prepared.

As starting material in the next acceptor synthesis was chosen the tetrol **21**[77] prepared in five steps from **1** in 70% yield and also being an intermediate in the preparation of **16** *supra*. *p*-Toluenesulfonylation (tosylation) of **21** in dichloromethane/pyridine 2:1 at -80°C proceeded with very high regioselectivity and provided the desired 9-*O*-tosylate **22** in 91% yield (Scheme 6). The reaction was temperature sensitive and gave a complex product mixture above -40°C. Regioselective *p*-toluenesulfonylations of sialic acid derivatives have been performed by others, although comparatively lower yields were reported[81,93]. Azide substitution of **22** with NaN_3 in the presence of 18-crown-6 ether in *N*,*N*-dimethylformamide then gave the desired 9-azidotriol **23** in 83% yield, whereas the LiN_3 system provided **23** in only 69% yield. The structure of **23** was further substantiated by de-*O*-acetylation of **20** with sodium methoxide in methanol (89% yield of **23**).



Scheme 6

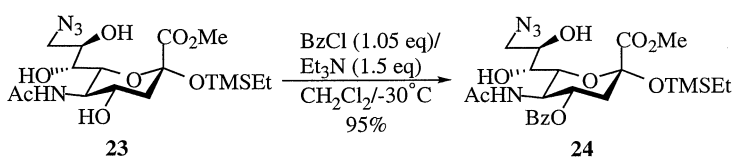
It was reported in 1982 that the reactivity order is HO-9>HO-4>HO-8>HO-7 when a methyl glycoside of Neu5Ac was treated with *t*-butylchlorodimethylsilane/imidazole[94]. Nevertheless, we decided to investigate the reactivity of the three hydroxyl groups of **23** in order to find the most suitable protective group pattern of a 9-azido acceptor. Benzoylation of **23** in dichloromethane/pyridine 2:1 at -80°C provided the 4-*O*-benzoylated compound **24** in 55% yield together with the 4,8-di-*O*-benzoylated derivative **25** in 21% yield (Scheme 7). Compound **24** was stable and appeared to be a suitable acceptor, but its preparation had to be improved.



Scheme 7

There are numerous methods of benzoylation[95], and benzoyl cyanide/triethylamine[96] and 1-(benzoyloxy)benzotriazole/triethylamine[97] are reported to be particularly useful in regioselective hydroxyl group protection. Still, a more convenient method was desired. Since benzoyl cyanide is reported to be unreactive in the absence of triethylamine[96], it seemed reasonable that the corresponding benzoyl triethylammonium cyanide intermediate was the actual species responsible for the selectivity. Hence, the system benzoyl chloride/triethylamine was expected to be a selective constellation in view of the similarity between cyanide and chloride ion. This was indeed the case.

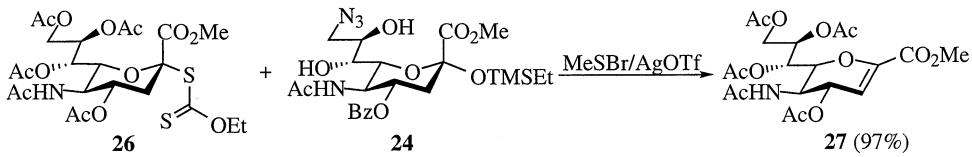
Treatment of **23** with benzoyl chloride/triethylamine in dichloromethane at -30°C provided **24** in an excellent 95% yield (Scheme 8). The order of reactivity between the three secondary hydroxyl groups of **23** was thus firmly established as HO-4>HO-8»HO-7.



Scheme 8

2.3 The First Sialylation of the 9-Azido Acceptor

Among the conventional sialyl donors available in 1993, the tetra-*O*-acetylated 2-xanthate **26**[85] appeared to be the best, and the optimum conditions for sialylation therewith had been elucidated. These consisted of performing the reaction in acetonitrile/dichloromethane 9:4 at -60°C using methylsulfenyl bromide/silver trifluoromethanesulfonate (MeSBr/AgOTf) as promoter system[98]. Highly successful (50-80% yield) sialylations of secondary hydroxyl groups of Gal and lactoside derivatives were reported under those conditions[59,98,99]. However, when **24** was subjected to sialylation with **26**, only the glycal **27**[100,101] was obtained in 97% yield together with the recovered acceptor (Scheme 9).



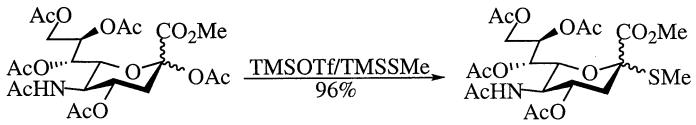
Scheme 9

The major factors behind this disappointing result were probably the expected low reactivity of HO-8 in combination with a sialyl donor of clearly insufficient capacity for this reaction. Whether the 9-azido group of **24** further decreased the reactivity of HO-8 was unclear at this stage, albeit it has been alleged that hydroxyl groups in the vicinity of azides actually have their nucleophilicity increased[102]. Since altering the structure of the acceptor **24** in order to increase the reactivity of HO-8 seemed unfeasible*, the attention was turned towards finding a sialyl donor of adequate capability.

2.4 Survey of Sialyl Donors Available in 1993

The methylthio glycoside **29**[86] can be prepared from the peracetylated methyl ester **28**[91,92] in 96% yield (Scheme 10). I have personally experienced that this reaction is very reliable. Compound **29** is a recognised potent sialyl donor, and it has accomplished an α2→8 coupling, albeit only 5% yield of an anomeric mixture of *bis*-sialosides was obtained[103]. However, a carefully designed comparative study (paper **I**) showed that **29** is slightly inferior to **26** regarding both the stereoselectivity and total yield. It therefore seemed improbable that the donor **29** would be capable of sialylating the 9-azido acceptor **24**.

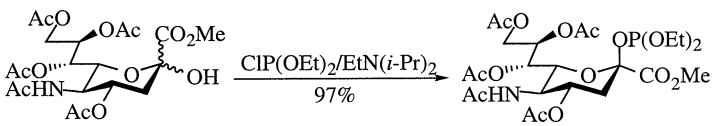
* In section **5.1**, some of the possible modifications of Neu5Ac derivatives made to increase the reactivity of HO-8 are reviewed.



28 **29** (α/β 1:1)

Scheme 10

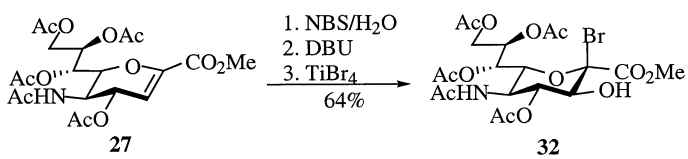
Another type of donor is the sialyl phosphite **31**, which is prepared from the corresponding hemiketal **30** by reaction with diethylphosphorochloridite in the presence of a sterically hindered base (Scheme 11)[87]. Many analogues of **31** have been reported, including a dibenzylphosphite[88]. The most attractive feature of sialyl phosphites is their convenient activation into useful sialyl donors by a catalytic amount of an acidic promoter, such as trimethylsilyl trifluoromethanesulfonate. Their widespread use is however impaired by the poor shelf life of phosphites, and the purification and handling of sialyl phosphites sometimes require precautionary measures. The reported donor capability of sialyl phosphites is comparable to that of the thioglycosides **26** and **29** *supra*, and the phosphite **31** was therefore not considered capable of sialylating the 9-azido acceptor **24**.



30 **31**

Scheme 11

For any sialyl donor, it is resonable to expect that its tendency to eliminate to a glycal such as **27** will be altered if a 3-substituent is introduced*. The 3-substituent is intended to be an auxiliary group which both suppresses elimination and directs the stereochemical outcome of the reaction. In the first investigation of such donors, a 3-(*S*)-hydroxyl group was introduced when the donor **32** was prepared from the glycal **27** (Scheme 12)[101].



27 **32**

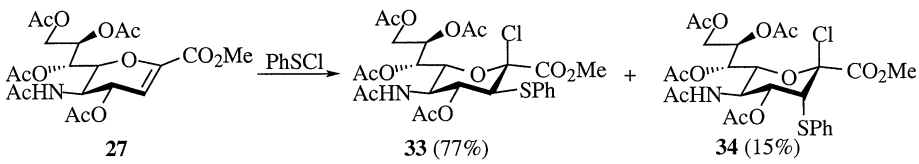
Scheme 12

Although **32** could provide a successful α2→8 *bis*-sialo coupling with a glycal acceptor in a total yield of 34% (α/β 3:1)[104], this donor is of limited practical value. The main reason is

* Since it provides a sialoside by indirect means, this type of sialyl donor is unconventional.

of course the auxiliary 3-(*S*)-hydroxyl group, which is only removable in good yield if all other hydroxyl groups in the product obtained are considerably less reactive. As is well known, it is a desirable strategy in oligosaccharide synthesis to keep several hydroxyl groups in a saccharide acceptor unprotected. Such strategy generally increases the yield of the glycosylations and usually also simplifies further protective group manipulation(s) and additional glycosylation(s).

In another approach, the donor **33** having a 3-(*S*)-phenylthio group was prepared in 77% yield by addition of benzenesulfenyl chloride to the glycal **27** in dichloromethane at 30°C (Scheme 13)[90].



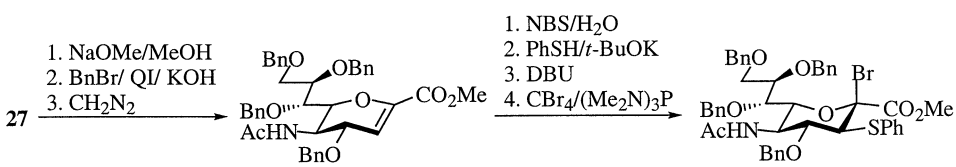
27 **33** (77%) **34** (15%)

Scheme 13

It seems justified to mention that no other but the authors appear to have managed to reproduce the above reaction entirely. In paper **I**, I disclose a yield of 57% of **33** in the addition step, whereas others have reported 58%[105] and 30%[106] yield, respectively.

The use of **33** as a sialyl donor has been rather limited, and this is probably a result of its high stability which may require sialylations to be carried out at elevated temperatures (80°C) even with silver trifluoromethanesulfonate as promoter[90]. The yields originally reported did not exceed those obtained with the more conventional donors **26**, **29** and **31**. My attempts to sialylate the 9-azido acceptor **24** with the donor **33** under the conditions given provided no *bis*-sialoside product. Nevertheless, a good yield (49%) in an α2→8 *bis*-sialo coupling using **33** as donor and a glycosyl fluoride as acceptor was reported in 1996[107]. However, observations by others indicate that **33** is not a sialyl donor with broad applicability[105]. A glycosyl bromide analogue of **33** has been reported, but it unexpectedly performed worse than **33**[108].

The tetra-*O*-benzylated glycosyl bromide **36** was probably the best unconventional sialyl donor available in 1993, and it is prepared in seven steps from the glycal **27** in 55% yield (Scheme 14)[89].



35 **36**

Scheme 14

The sialyl donor **36** has accomplished a yield of 64% in an α2→8 *bis*-sialo coupling with a glycal acceptor[109], and K.C. Nicolaou *et al.*[57] employed it in their synthesis of sialyl dimeric Le^x. However, in addition to the rather laborious seven reaction steps providing an unstable glycosyl bromide, the benzylation step in the preparation of **36** was reported to be poorly reproducable[57]. The latter difficulty was confirmed indirectly by the original authors in 1996[110].

With all the above taken into consideration, it was clear that a demand for a novel sialyl donor existed*.

2.5 Considerations in the Development of a Novel Sialyl Donor

As illustrated in the previous section, all reported sialyl donors with an auxiliary 3-(*S*)-substituent are glycosyl halides (halosugars). In general, halosugars are quite susceptible to hydrolysis upon contact with moisture either with or without the presence of a catalyst. Hence, halosugars are not considered long-term stable, and stability was a highly desirable characteristic of a novel sialyl donor. Thioglycosides are however considered stable in the absence of oxygen. Moreover, in all the conventional sialyl donors, the best results had been obtained with non-halosugars, *i.e.* thioglycosides and phosphites, and the desired structural features of the donor to be prepared were thus readily decided upon (Fig. 2.1).

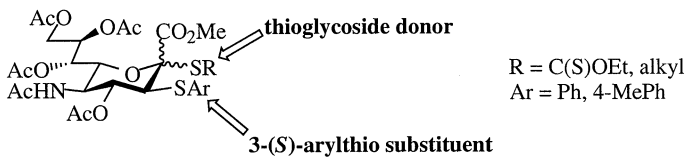


Fig. 2.1 Features of the novel sialyl donor

It seemed likely that chemoselective activation of only the alkylthio aglycon was possible. This assumption was based on the assumed difference between the *C*-2 and *C*-3 ring atoms in their capacity to stabilise a cationic species. The use of a thioglycoside would also give access to many powerful thiophilic promoters.

2.6 Preparation of a Novel Sialyl Donor

As starting material in the preparation of the novel sialyl donor was chosen the glycal **27**[100,101]. It was prepared in 92% yield by treating the peracetylated methyl ester **28**[91,92] with trimethylsilyl trifluoromethanesulfonate (2 eq.) in acetonitrile at 2°C (Scheme 15)[57,111].

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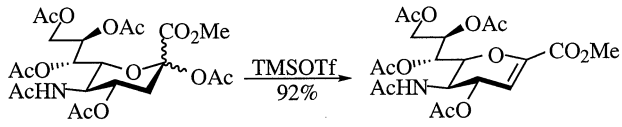
* Since most methods of enzymatic sialylation require non-artificial substrates, thereby possibly excluding the 9-azido acceptor **24**, I decided not to include such methodology in the above survey of sialyl donors.



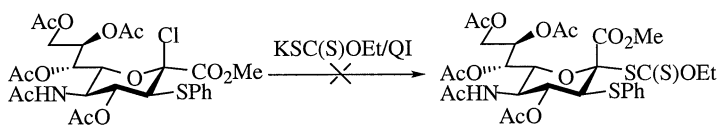
28 **27**

Scheme 15

In the original disclosure of this reaction, use of 0.2 eq. of TMSOTf was claimed to be sufficient[112], but those conditions led to an incomplete reaction. The latter observation has been reported also by others[57,111]. In order for this elimination to work properly, all reagents must be of excellent quality. Low grade (old) TMSOTf will lead to an incomplete reaction and extensive formation of a 4,5-oxazoline[80,113] analogue of **27**.

Addition of benzenesulfenyl chloride[114] to the glycal **27** in dichloromethane at 30°C for 2 days did not proceed nearly as well as reported[90]. As my best result, the known glycosyl chlorides **33** and **34** (Scheme 13 *supra*) were obtained in 57% and 19% yield, respectively, when the reaction was carried out in dichloromethane at room temperature for 7 days. I also recovered 10% of the glycal **27** in the reaction. As already indicated above, my results in this addition nevertheless appear to be representative.

Somewhat unexpectedly, the glycosyl chloride **33** could not be converted (no reaction) into the desired xanthate **37** by treatment with *O*-ethyl *S*-potassium dithiocarbonate/QI in dimethyl sulfoxide at 60°C (Scheme 16)*.

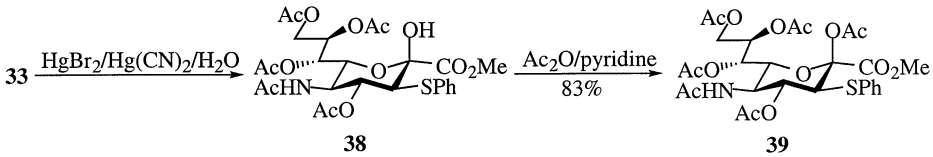


33 **37**

Scheme 16

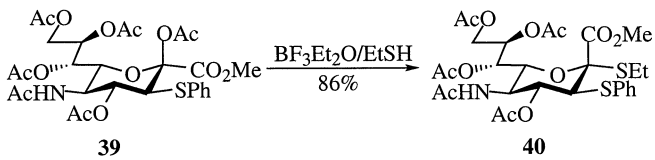
Compound **33** was instead hydrolysed into the known hemiketal **38** by refluxing with mercuric bromide/mercuric cyanide in 1,2-dichloroethane/H_2O 100:1 for 3.5 h (Scheme 17)[108]. After the removal of the mercury salts, the crude product was acetylated to give the desired penta-*O*-acetate **39** in a yield of 83% from **33**.

* In 1997, it was reported (ref. 105) that treatment of **33** with *O*-ethyl *S*-potassium dithiocarbonate in EtOH for 3 days at 50°C provided the xanthate **37** in 48% yield together with numerous by-products. The reaction was unsuccessful with acetonitrile as solvent.



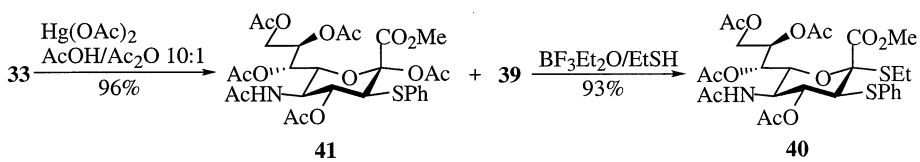
Scheme 17

Treatment of **39** with boron trifluoride etherate/ethanethiol in dichloromethane at room temperature over night gave the desired sialyl donor **40** in 86% yield as a stable and anomerically pure compound (Scheme 18). The absence of an anomeric mixture was both unexpected and fortunate.



Scheme 18

The synthetic pathway to the donor **40** from the glycal **27** now involved four reaction steps and a total yield of about 40%. A shortening of this sequence to three steps and a total yield of about 50% was possible by acetolysis of **33** with mercuric acetate in acetic acid/acetic anhydride 10:1 at 40°C over night, thereby providing the anomeric acetates **39** and **41** as a 1:5 mixture in 96% yield. Treatment of this mixture with boron trifluoride etherate/ethanethiol as above then gave the donor **40** in 93% yield (Scheme 19).



Scheme 19

A total yield of 50% of the donor **40** from the glycal **27** in three steps was deemed satisfactory, and the synthetic pathway was thus considered to be complete.

3 Studying the Novel Sialyl Donor

3.1 A Comparative Study (Paper I)

It was decided that a sterically hindered acceptor should be used in the investigation of the sialylating propeties of the donor **40**. Indeed, use of this type of unconventional donor is only motivated when unreactive alcohols are to be sialylated or when the separation of a resulting α/β mixture is particularly troublesome. The hexa-*O*-benzyl lactoside **42**[115] (Fig. 3.1) was chosen as a suitable acceptor.

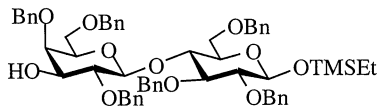
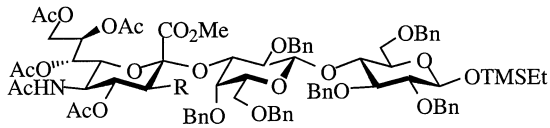


*Fig. 3.1 Lactoside model acceptor **42***

The conventional sialyl donors **26** and **29** were also included in the study, and great care was taken to keep the concentrations of the reactants as identical as possible in all the glycosylations. As promoters were used either methylsulfenyl bromide/silver trifluoromethanesulfonate (MeSBr/AgOTf)[116] or *N*-iodosuccinimide/trifluoromethanesulfonic acid (NIS/TfOH)[117,118]. The results are summarised in Table 1 in Paper **I**, and they show that **40** (compound **6** in paper **I**) is a powerful sialyl donor providing both good yield and superior stereoselectivity of the desired trisaccharide **43** (Scheme 20). The conventional xanthate donor **26** gave a slightly better total yield than the methylthio glycoside donor **29**, albeit both gave α/β mixtures of the trisaccharide **44**. In comparable reactions, the TLC's were substantially the same with both the promoters used. However, complete removal of the *N*-succinimide generated by NIS/TfOH normally required at least one extra chromatographic separation as compared to the work-up in the reactions promoted by MeSBr/AgOTf. Such separation endeavours of course contributed to the somewhat lower yields obtained with the NIS/TfOH system. The structures of the by-products related to **40** were not resolved, and their identity and distribution were for some reason not entirely reproducable. ^{1}H NMR analysis of various crude fractions showed *inter alia* the formation of the hemiketal **38**, the glycal **27** and traces of intramolecular *N*- or *O*-glycosides, where the latter (unstable) compounds were probably formed via the 5-acetamido group of **40**.

The removal of the auxiliary 3-(*S*)-phenylthio group of **43** was performed with triphenyltin hydride/AIBN in refluxing toluene over night, whereby **44** was obtained in 83% yield (Scheme 20). 12% of unreacted **43** was also recovered in the procedure. For some reason, the constellation tri-*n*-butyltin hydride/AIBN provided **44** in only 50% yield together with numerous by-products.



43 R = SPh

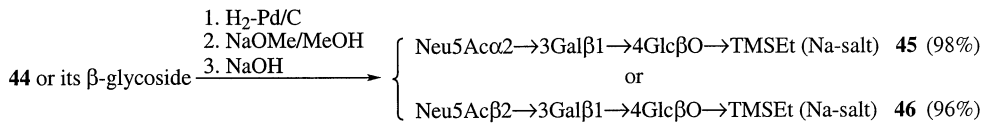
Ph_3SnH/AIBN

83%

44 R = H

Scheme 20

One-pot de-*O*-benzylation, de-*O*-acetylation and methyl ester hydrolysis of **44** and its corresponding β-glycoside then gave the unprotected trisaccharides **45** and **46** in 98% and 96% yield, respectively (Scheme 21).



Scheme 21

3.2 Determining the Anomeric Configuration of Neu5Ac Residues

Several purely empirical methods exist for determining the anomeric configuration of Neu5Ac residues, of which the most frequently used seem to be:

i) The chemical shift of H-3eq in the Neu5Ac residue is more downfield in the α-glycoside as compared to the β-glycoside[119]. Although the original authors hold this true only for unprotected sialosides, many researchers appear to use this method for anomeric assignment also of protected sialosides;

ii) $J\,H_{7,8}$ is claimed to assign α- or β-configuration irrespective of the nature of a 3-substituent, where $J\,H_{7,8}$ is ~2 Hz and >7 Hz for β- and α-glycosides, respectively[120];

iii) The chemical shift difference between the two H-9 protons is alleged to depend on the anomeric configuration, where Δδ H(9)-H(9') is ~1 ppm and <0.5 ppm for β- and α-glycosides, respectively[120].

There is however one method which is based on a recognised physical relationship. According to the well-known Karplus' equation, scalar *J* coupling between two vicinal protons is related to their dihedral angle (Φ), where the *J* coupling is at a maximum for Φ=0° and 180° and at a minimum when Φ=90°. These principles were empirically transferred to long-range *C-H* couplings by H. Hori *et al.*[121]. For Neu5Ac residues, it was found that $J_{C1\text{-}H3ax}$ was 4-6 Hz (Φ=180°) and <1 Hz (Φ=60°) for α- and β-glycosides, respectively.

The donor **40**, its precursors and the sialosides obtained in conjunction with the comparative study were investigated by high-field (125 MHz) ^{13}C NMR spectroscopy by weak irradiation of the methyl ester singlet of the Neu5Ac residue, as described by H. Hori *et al.* The results are shown in Table 2 in Paper **I**. As depicted there, all α-glycosides have a $J_{C1\text{-}H3ax}$ coupling of 5.8-7.5 Hz, whereas the β-glycosides show couplings of 1.0-1.7 Hz. It was found that the rule *(i) supra* is not valid for the protected trisaccharide **44** and its β-anomer, whereas it is so for the unprotected pair **45** and **46**. Moreover, the rules *(ii)* and *(iii)* do not apply to the glycosyl chloride **33** and the anomeric acetate **39**. In summary, it would seem that *only* the $J_{C1\text{-}H3ax}$ coupling is completely reliable when the anomeric configuration of Neu5Ac residues is to be determined. Nevertheless, the rules *i)-iii)* are still in use*.

3.3 The Mechanism of Sialylation in the Presence of a 3-(*S*)-Phenylthio Group

The reasons for the higher yields and stereoselectivities obtained with sialyl donors having a 3-(*S*)-phenylthio group are not obvious. It is plausible that the phenylthio group extends the life-span of a cationic species via an episulfonium ion, which would account for the higher total yield. However, the stereospecificity seems to be governed by a solvent effect. In a glycosylation study using a tetra-*O*-benzoylated analogue of **26** and nitriles as solvents, the stereodeterminancy was suggested to be caused mainly via an intermediate glycosyl β-nitrilium ion, and a low reaction temperature (-70°C) increased the α/β ratio of the sialosides obtained[122]. It deserves mentioning that a computational study indicated that episulfonium ions are probably not the principal stereodeterminants in glycosylations of 2-thioalkyl pyranosides[123]. In 1997, the mechanism set forth in Scheme 22 on the following page was suggested for sialylations in the presence of an auxiliary 3-(*S*)-phenylthio group[105]; note that the promoter is PhSOTf[124].

In the first step, the donor **47** reacts with phenylsulfenyl trifluoromethanesulfonate to give the oxonium ion **49** via the intermediate **48**. It was calculated that the oxonium ion **49** and the episulfonium ion **50** have approximately the same thermodynamic stability, and they are in equilibrium with each other[105]. The reaction of the episulfonium ion **50** with acetonitrile then provides the nitrilium ion **51**, where the latter according to calculations is 2.50 kcal/mol more stable than the actual sialylating species **52**[105]. A nucleophilic (axial) attack by the acceptor R'-OH on the nitrilium ion **51** would be hindered by the H-4 and H-6 protons and probably by the 3-(*S*)-phenylthio group as well, and this promotes formation of the desired *O*-glycoside **53**.

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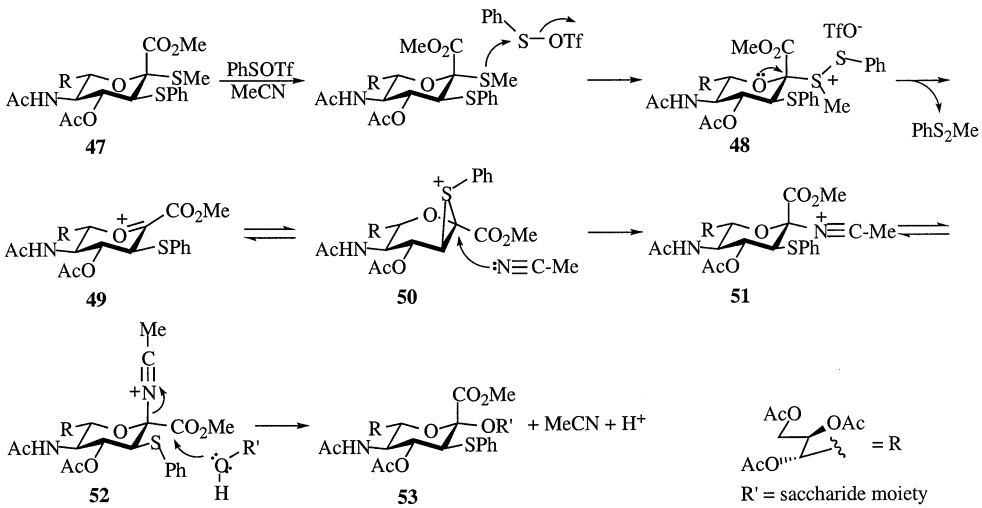
* See *e.g.* ref. 141, where only rule *i)* appears to have been used for the anomeric stereodetermination.



Scheme 22

3.4 An Expanded Study of the Novel Donor (Paper II)

In further trials, the donor **40** was evaluated with several other acceptors, namely the 2-azido galactoside **54**[99], the penta-*O*-benzylated lactoside **55**[125] and the 9-PMB-4-*O*-benzoylated sialoside **56** (Fig. 3.2). Comparisons with the conventional donors **26** and **29** were also made for some of these acceptors.

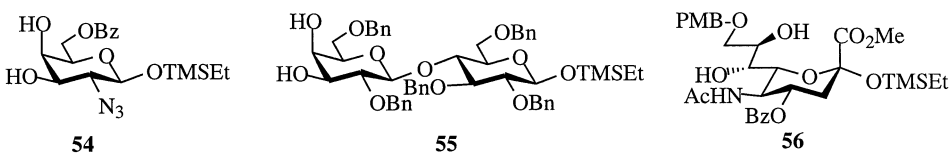
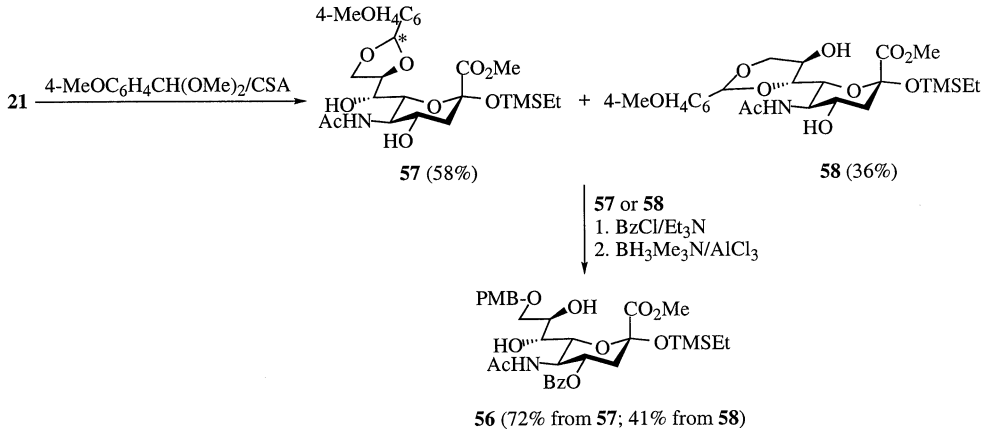


*Fig. 3.2 Acceptors used in the expanded study of **40***

The acceptor **56** is a 9-*O*-*p*-methoxybenzyl analogue of the 9-azido acceptor **24**, and it was prepared to examine if the reactivity of HO-8 would be significantly altered as a result of the different *C*-9 substituents (see also section **2.3**). By treating the tetrol **21** with *p*-methoxybenzaldehyde dimethyl acetal in acetronitrile at rt using CSA as catalyst, the *p*-methoxybenzylidene 8,9-acetal **57** and 7,9-acetal **58** were obtained in 58% and 36% yield, respectively (Scheme 23). Compound **57** was isolated as a 1:1 diastereoisomeric mixture, whereas **58** was a pure compound which appeared to epimerise slowly during storage. Regioselective benzoylation (*vide supra*) of **57** or **58** followed by regioselective acetal opening

by borane-trimethylamine/aluminium chloride in THF[126] then gave the desired acceptor **56** in 72% and 41% yield, respectively (Scheme 23).

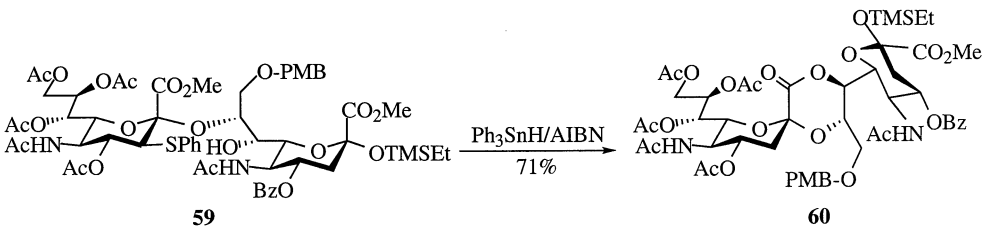


Scheme 23

As shown in Scheme 3 and Table 1 in Paper **II**, the yields and stereoselectivities in the sialylations with **40** (compound **6** in paper **II**) were generally very good when the reactions were performed with MeSBr/AgOTf as promoter in acetonitrile at -40°C. The desired *bis*-sialoside **59** (Scheme 24) was also obtained, albeit in a modest yield of 28% and about 40% of the acceptor **56** was recovered. According to ^{1}H NMR analysis (in $CDCl_3$) of various crude fractions obtained in the work-up of the *bis*-sialo coupling, the missing 20-30% did indeed correspond to another *bis*-sialoside. However, this unknown *bis*-sialoside was extremely unstable and decomposed to a complex mixture upon chromatography on silica gel and also during said ^{1}H NMR analysis. Use of NIS/TfOH instead of MeSBr/AgOTf in the coupling of **40** with **56** provided substantially the same products. Due to the observed acid-sensitivity of the unidentified *bis*-sialoside, I speculate that it could have been an ortho ester formed via the 4-*O*-acetyl group of the donor **40**. It does not seem unreasonable that an episulfonium ion, such as **50** (see Scheme 22), can be opened by a 4-*O*-acyl group and lead to an ortho ester in the presence of an unreactive acceptor, such an **56**. Nevertheless, the novel sialyl donor **40** appeared to be sufficiently potent for our purposes.

3.5 Isolation of a [7,8]-Lactone

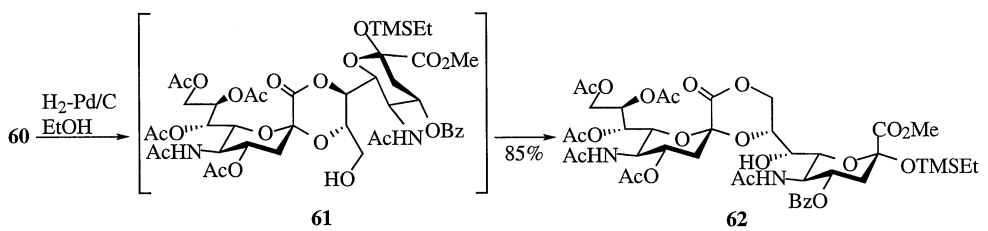
In 1994, only the [8,9]-lactone of *bis*-sialic acid residues had been reported[39,75,76]. However, we found that when the *bis*-sialoside **59** was treated with triphenyltin hydride/AIBN in refluxing toluene, the stable [7,8]-lactone **60** was obtained in 71% yield (Scheme 24).



59 **60**

Scheme 24

Hydrogenolysis of **60** in ethanol then provided the [8,9]-lactone **62** in 85% yield, presumably via the [7,8]-lactone **61** (Scheme 25). TLC-monitoring of the hydrogenolysis indicated the formation of **61**, where the latter disappeared when the reaction was complete. When the hydrogenolysis was performed in *N*,*N*-dimethylformamide, the [7,8]-lactone **61** appeared to rearrange into **62** rather slowly. Indeed, when pure [8,9]-lactone **62** was dissolved in *N*,*N*-dimethylformamide-d_7, a slow rearrangement into the [7,8]-lactone **61** could be evidenced by ^{1}H NMR, and an equilibrium (**62/61** ~2:1) was eventually attained after about 2 weeks at room temperature. These results also indicate that an open form ester intermediate is not an absolute requirement for lactonisations in *bis*-sialic acid residues.



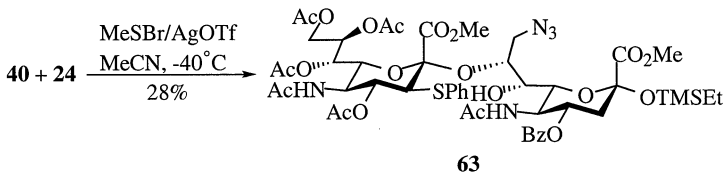
61 **62**

Scheme 25

4 Preparing a *bis*-Sialic Acid Lactam

4.1 Sialylating the 9-Azido Acceptor

When the 9-azido acceptor **24** was subjected to sialylation with an excess of the novel donor **40** in acetonitrile at -40°C using MeSBr/AgOTf as promoter, the desired *bis*-sialoside **63** was obtained in 28% yield (Scheme 26).



63

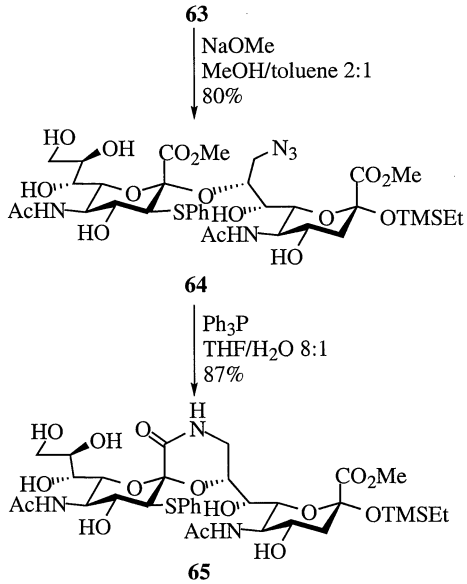
Scheme 26

Only about 40% of the acceptor could however be recovered, and an unstable *bis*-sialoside (not isolated pure) accounting for a yield of 25-30% was indicated also in this reaction. Oddly, when the concentrations of the reactants were halved, the yield of **63** was reduced to 23%, albeit 63% of the acceptor **24** could now be recovered, thereby raising the yield based on consumed **24** from 47% to 64%. It also deserves mentioning that the alternative of performing the sialylation with an excess of the acceptor **24** provided **63** in only 14% yield.

The reader might have noted that a yield of 28% was obtained also when the 9-*O*-PMB-acceptor **56** was sialylated with **40** (section **3.4**), and the reaction TLC's were in fact nearly identical. Since benzyl groups are not considered to decrease the nucleophilicity of vicinal hydroxyl groups, one can thereby conclude that the nucleophilicity of HO-8 of the acceptor **24** was not impaired by the 9-azido group, and nor did the 9-azido group *per se* seem to give any abnormal results.

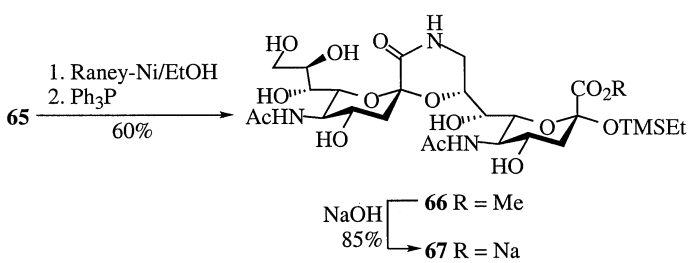
4.2 Lactamisation and Desulfurisation

Contrary to the expected[59,99], when the *bis*-sialoside **63** was treated with hydrogen sulfide in pyridine to reduce the azide functionality, only traces of a lactamised product could be observed. In order to minimise the suspected sterical interference in the lactamisation step, **63** was de-*O*-acylated with sodium methoxide in methanol/toluene 2:1 to provide **64** in 80% yield (Scheme 27). Subsequent reduction of the 9-azido group with triphenylphosphine in THF/water 8:1 at 40°C over night then gave the desired lactam **65** in 87% yield. TLC-monitoring with ninhydrine development revealed no 9-amine during the course of the reaction, albeit an amine may nevertheless have been formed as a short-lived intermediate. Use of hydrogen sulfide in the azide reduction of **64** provided **65** in only 65% yield together with numerous by-products.



Scheme 27

According to TLC-monitoring, the removal of the 3-(*S*)-phenylthio group of **65** proceeded smoothly to give **66** in nearly quantitative yield. However, the isolated yield of **66** never exceeded 20%, and this indicated that the product was strongly adsorbed on the Raney-Ni surface. By adding a rather large amount of triphenylphosphine (0.35 g/mL Raney-Ni) after the completion of the reaction, compound **66** could be liberated and isolated in an acceptable yield of 60% (Scheme 28)*. A sample of fully acetylated **65** was also treated with triphenyltin hydride/AIBN in refluxing toluene, but this provided a very complex product mixture. Methyl ester hydrolysis of **66** with sodium hydroxide then provided the sodium salt **67** in 85% yield, and it was thereby evidenced that *bis*-sialic acid [8,9]-lactams are hydrolytically stable.

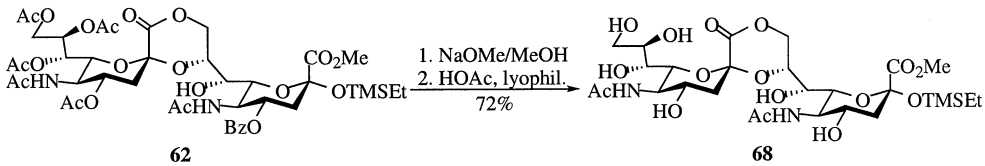


Scheme 28

* In later work (refs. 127 and 128) it was reported that sialosides can be liberated efficiently from Raney-Ni by washing repeatedly with methanol/toluene 1:1.

4.3 Conformational Lactam/Lactone Similarities (Paper III)

In order for a conformational comparison in solution to be made, the *bis*-sialoside **62** was de-*O*-acylated with sodium methoxide in methanol and then freeze-dried from an acetic acid solution to give the [8,9]-lactone **68** in 72% yield (Scheme 29).



Scheme 29

As set forth in Figure 2 and Table 1 in Paper **III**, the *J* couplings in methanol-d_4 are strikingly similar in **66** and **68**, and calculations [MM3(92)][129-131] further supported the nearly identical conformations of the two molecules. It is noteworthy that when dissolved in methanol-d_4, the lactone **68** immediately attained an equilibrium (~9:1) with the corresponding open form methyl ester, and FAB-MS analysis supported this observation. Since both **66** and **68** are poorly soluble in water, use of D_2O in the ^1H NMR analysis was excluded.

In summary, these data support the hypothesis that lactams in *bis*-sialic acid residues should be good substitutes, in an immunological perspective, for the naturally occurring lactones in *bis*-sialic acid residues of glycosphingolipids.

After the work set forth above had been done, I left the arena of active research, and the total synthesis of the target molecule **11** was continued by others.

5 Main Developments in Sialylations and Ganglioside Lactams 1996-1999

5.1 The Reactivity of HO-8 in Neu5Ac Residues

It is generally agreed upon that the low nucleophilicity of HO-8 in Neu5Ac residues has several causes, such as (Fig. 5.1):

i) steric hindrance by the surrounding *C*-7 and *C*-9 substituents;
ii) hydrogen bonding between HO-8 and the 5-acetamido group;
iii) hydrogen bonding between HO-8 and the carbonyl oxygen of *C*-1 (in β-position);
iv) hydrogen bonding between HO-8 and the oxygen of an aglycon (in β-position).

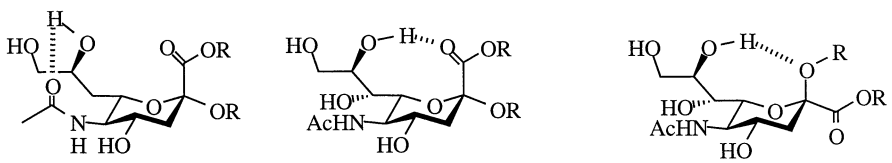


Fig. 5.1 Schematic representation of hydrogen bonding patterns for HO-8. For clarity reasons, HO-7 is omitted in structure ii).

In attempts to circumvent the conformations depicted above, several methods have been reported. These include preparation of an internal [1,7]-lactone or [1,5]-lactam of a Neu5Ac acceptor as well as using a glycal or 5-*N*-acetylacetamido Neu5Ac acceptor[132-135]. Although all of these approaches greatly increased the reactivity of HO-8 and consequently also the yields of *bis*-sialosides, the predominant products were in all instances the undesired β-glycosides. As a consequence of these results, more focus has been directed towards further improvement of the arsenal of sialyl donors.

5.2 Most Relevant Novel Sialyl Donors

In recent years, the sialyl donors **69**[136], **70**[110], **71**[137], **72**[138] and **73**[139] have been reported, and they are shown in Fig. 5.2 on the following page.

The donor **69** was prepared in an impressive 85% yield from the glycal **27** by addition of 2,4-dimethylbenzenesulfenyl chloride followed by treatment of the intermediate glycosyl chloride *exo* adduct with sodium methanethiolate. The performance of **69** has not been tested in a *bis*-sialo coupling, but its sialylating capability is probably very similar to that of **40**.

The donor **70** was prepared from the hemiketal precursor of **36** by acetylation followed by treatment with TMSOTf/TMSSMe. Model sialylations indicate that the donor **70** is slightly inferior to **40**.

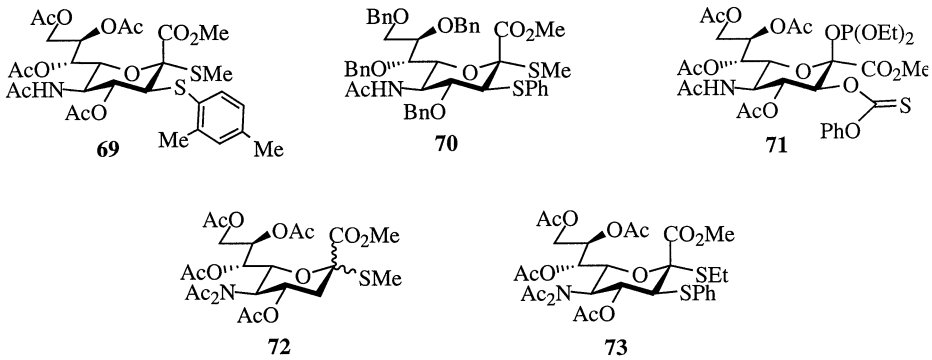


Fig. 5.2 Sialyl donors reported 1996-1999

The phosphite donor **71** is prepared in three steps from the glycal **27**. The latter is subjected to stereospecific dihydroxylation with OsO_4/NMNO, followed by regioselective phenoxythiocarbonylation with $PhC(Cl)=NMe_2^+Cl^-/H_2S$ and transformation into a phosphite with $ClP(OEt)_2/EtN(i\text{-}Pr)_2$ to provide **71** in 81% yield. The donor **71** has accomplished an α2→8 *bis*-sialo coupling in 83% yield, although a glycal acceptor was employed*, and it may be one of the best sialyl donors reported so far.

The 5-*N*-acetylacetamido donor **72** is prepared from the conventional donor **29** in 99% yield by treatment with *iso*-propenylacetate in the presence of a catalytic amount of *p*-toluenesulfonic acid. It is capable of performing 2→8 *bis*-sialo couplings in excellent yield (61%; α/β 1:2), albeit mainly β-glycosides are obtained. Nevertheless, *N*-acetylation of the 5-acetamido group does seem to have a profound effect on Neu5Ac residues, and the properties of **72** will most likely provide a useful starting point for many new types of sialyl donors.

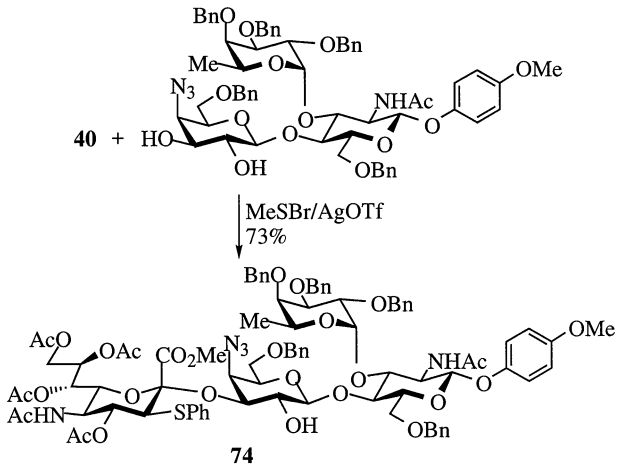
The donor **73** was prepared from the donor **40** in 89% yield in the same manner as in the preparation of **72**. It was capable of α-sialylating the acceptor **56** in 44% yield, and it is undoubtedly more powerful than the "old" donor **40**. Judging from its performance record (*vide infra*), **73** is probably the most powerful sialyl donor available today.

5.3 Ganglioside Lactams Prepared

Lactam analogues of the possible lactones of G_{M1}, G_{M2}, G_{M3} and G_{M4} were all prepared by employing the conventional sialyl donor **26**[99]. Said donor was utilised also in the synthesis of a sialyl-Le^x-1‴→2′-lactam[127]. However, in the preparation of a sialyl-Le^x-1‴→4′-lactam, the

* The glycal employed in the evaluation of **71** could be sialylated by the conventional phosphite **31** in an excellent 68% yield (α/β 1:4; ref. 133). The donor **31** is however reported uncapable of sialylating a Neu5Ac acceptor with a normal $^2C_5$ conformation (ref. 134).

sialylation was successful only when the donor **40** was used under the conditions set forth above, thereby giving the desired tetrasaccharide **74** in 73% yield (Scheme 30)[127]. The donor **26** was unable to provide even a trace of a tetrasaccharide.



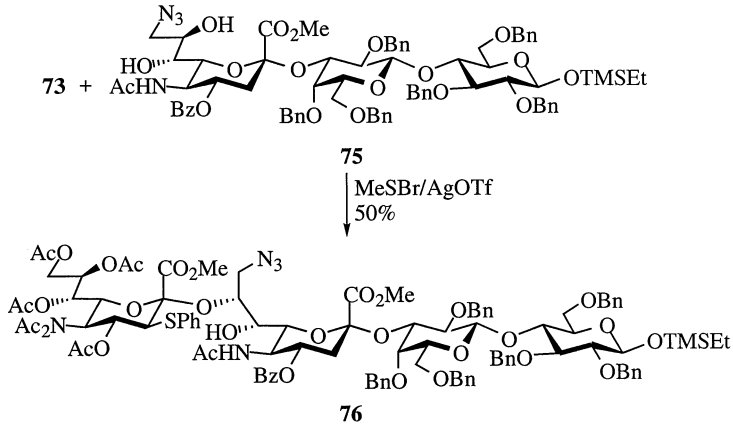
Scheme 30

After considerable experimentation, the removal of the 3-(*S*)-phenylthio group of **74** was accomplished with Raney-Ni*, and adsorbed product was liberated by washing repeatedly with toluene/methanol 1:1. Subsequent de-*O*-acetylation and hydrogenolysis gave the deprotected lactam tetrasaccharide in a total yield of 30% from **74**.

The synthesis of the main features of the target molecule **11** was eventually completed by sialylating the 9‴-azido trisaccharide acceptor **75** with the novel donor **73**, and the tetrasaccharide **76** was thereby obtained in 50% yield (Scheme 31)[140]. The donor **40** provided the desired tetrasaccharide in only ~20% yield, a result which necessitated the development of the donor **73**. Removal of the 3-(*S*)-phenylthio group on the lactamised tetrasaccharide was accomplished with Ph_3SnH/AIBN in 65% yield. The acceptor **75** was readily prepared from de-*O*-acetylated **44** by utilising the regioselective *O*-*p*-toluenesulfonylation and *O*-benzoylation methodology set forth in section **2.2** *supra*.

None of the lactams prepared have yet been tested *in vivo*. In a different approach towards similar goals in immunotherapy, an ether-bridged analogue of the [2,3]-lactone of G_{M3} has been prepared[141].

* Use of Ph_3SnH/AIBN gave a complex mixture.


73 +
N3
OH
CO2Me
OBn
HO
AcHN
BzO
BnO
OBn
OTMSEt
75
MeSBr/AgOTf
50%
AcO
OAc
Ac2N
SPh
76

Scheme 31

6 Study of 3-(*S*)-Phenylseleno Sialyl Donors

6.1 Background

In addition to our own observations, several groups have reported that the removal of the auxiliary 3-(*S*)-phenylthio group after a successful sialylation can be difficult[106]. Even in cases where the tin hydride reductions perform well, a significant amount of unreacted starting material is often recovered[57,89]. Taken all this into consideration, it seemed logical to further evaluate the performance of sialyl donors having an auxiliary 3-(*S*)-phenylseleno group. The weaker *C-Se* bond (243 kJ/mol) as compared to the *C-S* and *C-O* bonds (272 and 336 kJ/mol, respectively) should make an auxiliary phenylseleno group comparatively easier to remove from a saccharide.

Sialyl donors having an auxiliary 3-(*S*)-phenylseleno group have been evaluated before, but only with tetra-*O*-benzylated glycosyl halides of Neu5Ac[89]. Excellent stereoselectivities were obtained, although the yields were moderate. However, glycosyl halides of Neu5Ac are normally not very useful donors, and the properties of a sialyl donor having an auxiliary 3-(*S*)-phenylseleno group *and* an alkylthio or phosphite aglycon therefore seemed relevant to investigate.

6.2 Preparing the 3-(*S*)-Phenylseleno Donors (Paper IV)

The phenylseleno group was introduced by generating phenylselenenic acid ("PhSeOH") *in situ*. However, the original equilibrium method of reacting diphenyl diselenide with hydrogen peroxide[142] in the presence of the glycal **27** led to a very slow addition reaction, and weeks at room temperature seemed to be required for a complete reaction. It proved to be much more convenient to treat the glycal **27** with phenylselenenyl chloride, water and silver trifluoromethanesulfonate in tetrahydrofuran and then keep the reaction mixture at 0°C for seven days (Scheme 32). The desired *exo* adduct **77** could thereby be isolated in 51% yield, and it was easily separated from the *endo* adduct **78** obtained in 35% yield*. Attempts to epimerise the *endo* adduct **78** by treatment with trifluoromethanesulfonic acid provided **77** in low yield (~25%) together with numerous by-products, including the glycal **27**. No epimerisation could be observed under basic conditions (DBU).

Treatment of the hemiketal **77** with diethyl phosphorochloridite in the presence of *N*-ethyldiisopropylamine in acetonitrile then gave the phosphite sialyl donor **79** in 88% yield (Scheme 32). Contrary to the expected, **79** was stable if stored as an amorphous powder under argon at -30°C.

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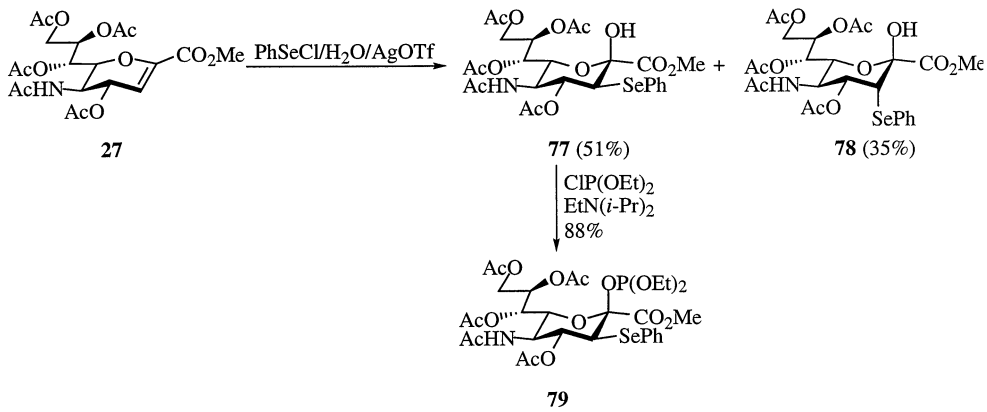
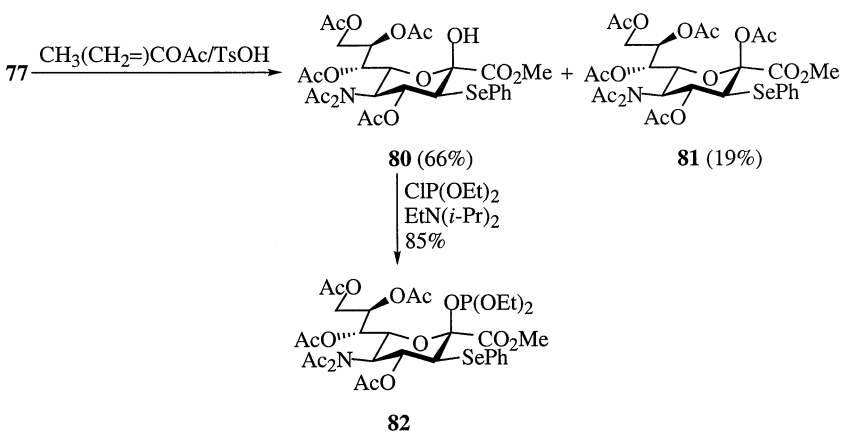
* The addition was complete over night if performed at room temperature, but this gave an *exo*/*endo* ratio of ~1:1 together with significant amounts of de-*O*-acetylated products.



Scheme 32

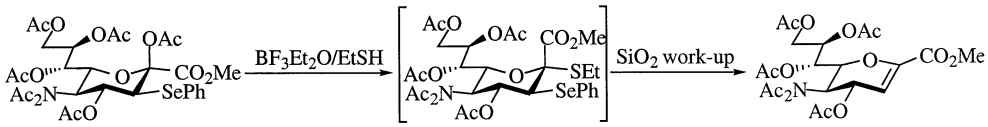
In an attempt to prepare a donor of supposedly greater sialylating capability than **79**, we observed that the 5-acetamido group of the hemiketal **77** could be selectively acetylated before its anomeric hydroxyl group. In order for this reaction to work, all components had to be kept at a fairly low concentration in the reaction mixture. Treating **77** with *iso*-propenylacetate in the presence of a catalytic amount of *p*-toluenesulfonic acid at 65°C over night thus gave the desired 5-*N*-acetylacetamido hemiketal **80** in 66% yield together with the peracetylated analogue **81** in 19% yield (Scheme 33). Careful selective hydrolysis of the anomeric *O*-acetyl group of **81** was unsuccessful in toluene/acetic acid/water 500:200:1 and primarily led to *N*-deacetylation. From this we concluded that the anomeric hydroxyl group of **77** is indeed very sterically hindered and hence quite unreactive under the acetylating conditions set forth above. It would be interesting to investigate if similar selective *N*-acetylations can be performed in other 3-(*S*)-substituted Neu5Ac derivatives.



Scheme 33

After some experimentation, the phosphite donor **82** could be isolated in 85% yield by treating **80** with diethyl phosphorochloridite as above, followed by work-up on silica gel, where the latter had been preconditioned with toluene/triethylamine 200:1 (Scheme 33). As indicated, compound **82** was found to be very acid-sensitive and generally unstable.

I also tried to prepare an anomeric alkylthio analogue of **82** by treating the peracetylated **81** with boron trifluoride etherate/ethanethiol in dichloromethane, and the reaction performed well according to TLC analysis. However, after work-up by conventional chromatography on silica gel, only the glycal **83** together with the glycal **27** and traces of other by-products were isolated (Scheme 34). It has been reported that 1,2-*trans-bis*-selenides readily decompose to the corresponding olefin and diselenide upon contact with silica gel, and that such compounds should preferably be distilled pure, if possible[143]. In view of said report as well, I concluded that an anomeric alkylthio analogue of **82** is probably extremely difficult to isolate and abandoned this route.



Scheme 34

The glycal **83** can also be readily prepared in 90% yield by treating the glycal **27** with *iso*-propenylacetate under the standard conditions given above.

6.3 Evaluating the Novel Donors

The acceptors **84**[144], **42**[115] and **85**[137] (Fig. 6.1) were used in the evaluation of the phosphite donors **79** and **82**.

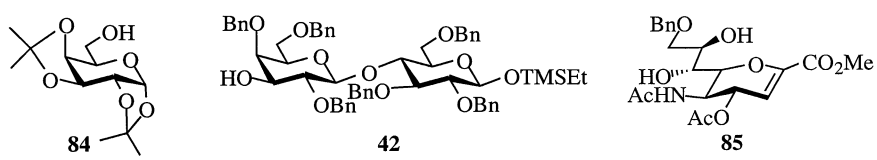
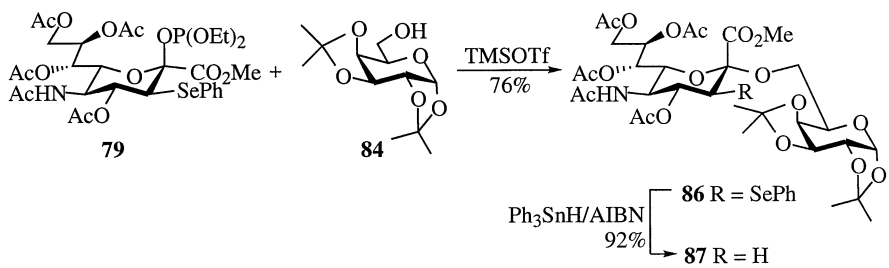


Fig. 6.1 Acceptors used in the study of 79 and 82

The diisopropylidene galactoside **84** was chosen due to its high reactivity, which should easily provide a sialylated product, and the full capacity of the novel phosphite donors should be unambiguous after trials with the less reactive hexa-*O*-benzyl lactoside **42** and the Neu5Ac glycal **85**. As indicated in section **5.1** *supra*, the glycal **85** is significantly more reactive than the corresponding Neu5Ac analogue having a normal $^2C_5$ conformation, although it is probably a rather unreactive acceptor in general*.

The optimum conditions for activating sialyl phosphites are well established, and they normally involve treatment with a catalytic amount of a Lewis acid, preferably trimethylsilyl trifluoromethanesulfonate or trifluoromethanesulfonic acid, in acetonitrile or dichloromethane at a temperature between -40 and -78°C[145]. Indeed, treatment of the donor **79** with the galactoside **84** in acetonitrile at -40°C afforded the desired sialoside **86** in an excellent 76% yield (Scheme 35)‡. The 3-(*S*)-phenylseleno group was easily removed by treatment of **86** with triphenyltin hydride/AIBN in refluxing toluene for 1 h, thereby providing the known α-sialoside **87**[146] in 92% yield.



Scheme 35

This result was promising, but when the donor **79** was reacted with the acceptors **42** and **85** under exactly the same conditions, not even a trace of a sialylated product could be obtained. Only the glycal **27**, recovered pure acceptor and a small amount (<5%) of trimethylsilyl-*O*-protected acceptor were isolated from the reaction. Despite many modifications in line with the optimum conditions referred to above, the donor **79** was consistently unable to sialylate the acceptors **42** and **85**. Eventually, we had to conclude that the

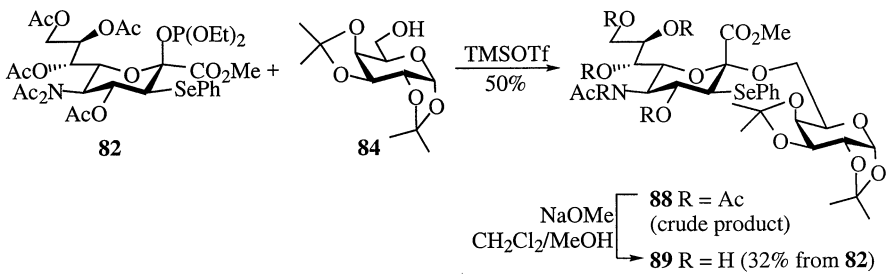
---

* The glycal **85** has been 2→8 sialylated by the conventional phosphite donor **31** in 68% yield (α/β 1:4; ref. 133). See also the footnote in section **5.2**.

‡ The sialoside **86** is in fact a β-glycoside, and this is a result of the 3-(*S*)-substituent (see Appendix). An α-glycoside is not obtained until the 3-(*S*)-substituent is removed. Numerous researchers, including myself, have fallen into this nomenclature trap. The α/β nomenclature used in papers **IV** and **V** is therefore correct, although it appears confusing at first. I obviously have no choice but to leave the nomenclature in papers **I-III** as it is.

donor **79** lacks the ability to sialylate less reactive acceptors. Needless to say, this result was a major disappointment.

When the 5-*N*-acetylacetamido donor **82** was reacted with the galactoside **84** under the same conditions as those used for the donor **79**, the desired sialoside **88** was obtained in about 50% yield (Scheme 36). However, ^{1}H NMR analysis revealed that **88** was no more than 80% pure, and conventional chromatography on silica gel was unable to remove the impurities. Eventually, by subjecting the crude product **88** to de-*O*-acetylation with sodium methoxide in dichloromethane/methanol 1:1, the unprotected sialoside **89** was obtained in 32% yield, as calculated from **82**.



Scheme 36

In view of the above result, it came as no surprise that the donor **82** proved to be incapable of sialylating the acceptors **42** and **85**, providing only the glycal **83** and recovered acceptor as main products. Hence, the 5-*N*-acetylacetamido group of **82** did not confer any improved sialylating capability as compared to the donor **79**.

6.4 Analysis of the Results

The main reason for the results obtained is probably the weak *C-Se* bond, but steric hindrance was most likely of some importance as well. The latter consideration is supported by the unexpected long range $J_{P,H}$ and $J_{H,H}$ couplings of 1.0-1.9 Hz observed in the ^{1}H NMR analysis of the 3-(*S*)-phenylseleno compounds **77**, **79**, **80** and **82** (see also Table 1 in paper **IV**). Such long range *J* couplings have been observed in other carbohydrates[147,148], where they are considered indicative of a so-called "W-conformation", in this case for the atom sequence H(3)-C(3)-C(2)-O-R (R=H or $P(OEt)_2$). There are no reports in the literature of such long-range *J* couplings in other Neu5Ac hemiketals or phosphites carrying a 3-(*S*)-bromine, iodine, phenylthio (*e.g.* hemiketal **38**) or thiobenzoyloxy (phosphite **71**) substituent[89,101,108,137]. However, a long-range *J* coupling of 1.5 Hz has been reported for a tetra-*O*-benzylated hemiketal (compound **21** in paper **IV**) having a 3-(*S*)-phenylseleno group, whereas the corresponding 3-(*S*)-phenylthio analogue displayed no such *J* coupling[89]. These NMR results show that a 3-(*S*)-

phenylseleno group confers a fairly rigid "W-conformation" in Neu5Ac derivatives. Hence, the rotation around the C(2)-OR bond is restricted, probably due to steric interference from the phenylseleno group*. For the hemiketal **77**, this steric effect could explain the selective *N*-acetylation. It appears likely that the phenylseleno group also sterically disfavors the formation of an (axial) α-nitrilium ion (see section **3.3**), and this would in turn further reduce the sialylating capability of the donors **79** and **82**. Judging from the high stereoselectivity nevertheless observed in the sialylation of the galactoside **84** with the donor **79**, I speculate that the sialoside **86** is formed via an *endo* attack on *C*-2 on a short-lived episelenonium ion intermediate.

Reactive acceptors tend to give lower α/β ratios than less reactive acceptors in sialylations with a donor lacking a 3-(*S*)-substituent[124]. In a scenario involving a reactive acceptor and an unusually troublesome separation of an α/β product mixture, the donor **79** might be useful, although I believe that those instances will be extremely rare, if any.

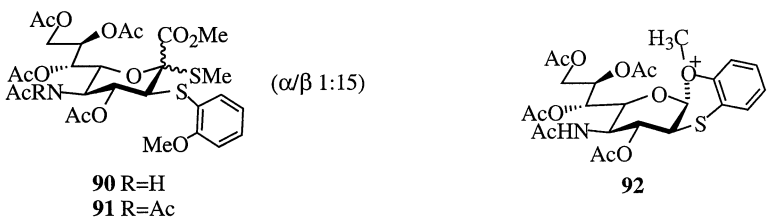
* However, no abnormal sterical interference was evident in preliminary calculations and physical models.

7 Study of 3-(*S*)-(2-methoxyphenyl)thio Sialyl Donors

7.1 Background

In view of the results presented in the previous sections, it seemed logical to now pursue a further improvement of the established donors **40** and **73**. In both these donors, the auxiliary group is a 3-(*S*)-phenylthio substituent, but other from use of a 3-(*S*)-(2,4-dimethylphenyl)thio substituent[136], no other substituted phenylthio group has been employed in Neu5Ac donors. However, use of an auxiliary (4-methoxyphenyl)thio group has been reported in Glc and Gal phosphoroamidate donors, and an improved stereoselectivity was obtained as compared to analogues having an auxiliary phenylthio group[149]. The (4-methoxyphenyl)thio group was introduced via addition of 4-methoxyphenylsulfenyl chloride to the corresponding glycal[149].

It has been reported that addition of 2,4-dimethylphenylsulfenyl chloride to the glycal **27** proceeds with much higher *exo*/*endo* selectivity (~20:1) as compared to phenylsulfenyl chloride (~3:1)[136], and the 2-methyl substituent is probably the main reason for that result[150]. Taken all the above into consideration, I decided to prepared the novel sialyl donors **90** and **91**, each having an auxiliary 3-(*S*)-(2-methoxyphenyl)thio substituent (Fig. 7.1). Simple models indicated that an intermediate "α-methoxonium ion" **92** was possible, and this could increase the sialylating capability beyond the expected on account of the electron-donating effect of the 2-methoxy ring substituent in an episulfonium species.



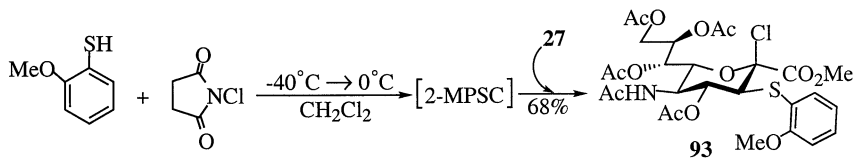
90 R=H
91 R=Ac

92

*Fig. 7.1 Novel Neu5Ac donors **90** and **91** and a schematic representation of the "α-methoxonium ion" **92** (C-1 omitted for clarity).*

7.2 Preparing the Donors

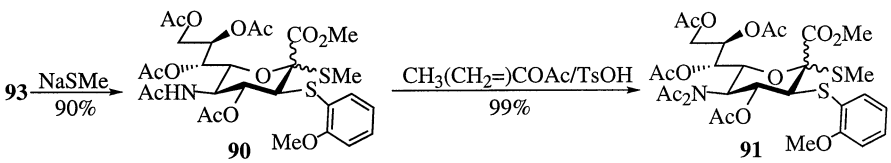
By addition of 2-methoxyphenylsulfenyl chloride (2-MPSC) to the glycal **27** in dichloromethane at room temperature for seven days, the desired *exo* adduct **93** was isolated in 68% yield (Scheme 37). The corresponding *endo* adduct was highly unstable and indicated only in ^{1}H NMR analysis (*exo*/*endo* ~3.5:1) of an aliquot of the reaction mixture.



Scheme 37

As indicated in Scheme 37, I avoided the somewhat messy isolation of 2-MPSC via destillation. Instead, 2-methoxybenzenethiol was added to a suspension of *N*-chlorosuccinimide in dichloromethane at -40°C, after which the *N*-succinimide was allowed to sediment over night at 0°C, thereby providing a ~1M solution of 2-MPSC* which was added to the solid glycal **27**. I believe that this protocol of preparing sulfenyl halides for glycal addition is applicable also for other thiols, and it may provide a practical means of preparing a series of different sulfenyl halides in order to investigate the neighboring group effect of various substituents.

Treatment of **93** with sodium methanethiolate in acetonitrile then provided the donor **90** in 90% yield as an α/β 1:15 mixture (Scheme 38). The donor **91** was obtained in 99% yield from **90** after acetylation with *iso*-propenylacetate under standard conditions.

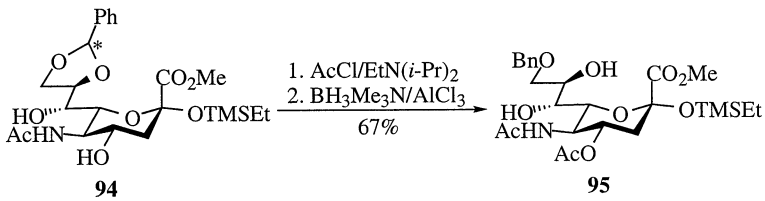


Scheme 38

7.3 Evaluation of the 5-Acetamido Donor

As model acceptor was chosen the Neu5Ac derivative **95** (Scheme 39). The Neu5Ac glycal **85** (Fig. 6.1) is an analogue of the acceptor **95**, and the latter was prepared to eventually allow a direct comparison between the two acceptors. The properties of the novel acceptor **95** are probably very similar to those of the 9-azido acceptor **24** and the 9-*O*-PMB acceptor **56**. Regioselective acetylation of the known benzylidene 8,9-acetal **94**[135] with acetyl chloride/*N*-ethyldiisopropylamine at -30°C followed by regioselective acetal opening by borane-trimethylamine/$AlCl_3$ in THF[126] readily provided **95** in 67% yield.

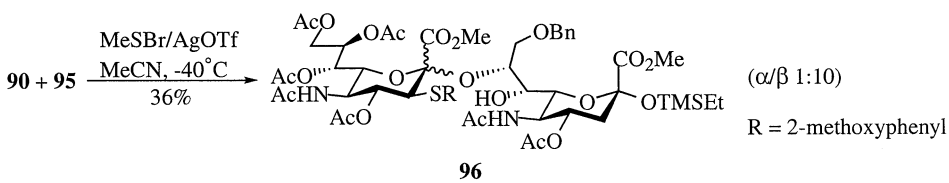
* A fresh solution of 2-MPSC has a red or deep orange color, whereas an outdated solution is substantially colorless. Oddly, the change/decay to colorless is very fast (over night) once it has started, and there is no risk of mistaking a fresh solution for an outdated dito. The solution is usually stable for ~4 weeks.



94 **95**

Scheme 39

When an excess of the acceptor **95** was sialylated with the donor **90** using MeSBr/AgOTf as promoter in acetonitrile at -40°C, the *bis*-sialoside **96** was obtained in 36% yield as an α/β 1:10 mixture (Scheme 40)*. The yield of **96** was 60% based on consumed acceptor, and some other *bis*-sialosides were clearly formed. Note that in the comparable reaction with the donor **40** and an excess of the 9-azido acceptor **24**, the yield of the *bis*-sialoside **63** was only 14% (section **4.1**), albeit the latter product was obtained anomerically pure. Hence, the donor **90** appeared to be rather powerful, but its stereoselectivity was a disappointment. Attempts to separate the α/β mixture with conventional chromatography on silica gel were unsuccessful, and all derivatisations (*O*-acylations, *N*-acetylation and de-*O*-acylation) were futile.



96

Scheme 40

7.4 Developing the ICl/AgOTf Promoter System (Paper V)

Use of the promoter system NIS/TfOH in the above reaction between **90** and **95** was considered, but the previous difficulties in removing the *N*-succinimide (section **3.1**) made me dismiss such a trial. Moreover, it has been reported recently that NIS/TfOH may give rise to *N*-succinimidyl glycosides if an unreactive acceptor is employed[151]. It is well known that MeSBr/AgOTf is somewhat laborious to use due to the required preparation of a methylsulfenyl bromide solution by the practitioner, an effort which probably prevents more widespread use of this otherwise excellent promoter system. For purely practical reasons, I therefore conceived the novel iodine monochloride/silver trifluoromethanesulfonate (ICl/AgOTf) promoter system and decided to first test it on this particular reaction. The decisive issue was the commercial availability of a dry 1.0 M solution of ICl in

* HR FAB-MS showed only one significant molecular ion *m/z* signal. A ^{1}H NMR temperature gradient analysis (20→80°C) in pyridine-d_5 excluded any conformational phenomena.

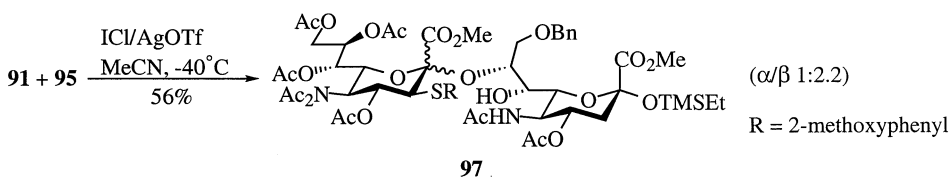
dichloromethane.

Indeed, when the coupling reaction between **90** and **95** was performed with ICl/AgOTf as promoter system under otherwise identical conditions as in the MeSBr/AgOTf-promoted reaction, the *bis*-sialoside **96** was obtained in 35% yield (α/β 1:10). The reaction TLC's were in fact near replicas. A broader evaluation of the ICl/AgOTf promoter system with various thioglycoside donors and acceptors clearly demonstrated its versatility, and I here refer the reader to Paper **V** in its entirety.

A more detailed study of the ICl/AgOTf promoter system, and closely related systems, is in progress by other researchers.

7.5 Evaluation of the 5-(*N*-Acetylacetamido) Donor

When the donor **91** was reacted with an excess of the acceptor **95** using ICl/AgOTf as promoter system in acetonitrile at -40°C, the *bis*-sialoside **97** was obtained in 56% yield as an α/β 1:2.2 mixture (Scheme 41)*. Repeating the reaction with an excess of the donor **91** provided **97** in 52% yield as an α/β 1:6 mixture. No acceptor was recovered in the latter reaction. Despite the report that the conventional 5-*N*-acetylacetamido donor **72** (Fig. 5.2) is prone to give more of the undesired β (axial) glycoside in *bis*-sialo couplings[135], the poor stereoselectivity with the donor **91** was a major disappointment. Also for the *bis*-sialoside **97**, efforts to separate the anomeric mixture were futile.



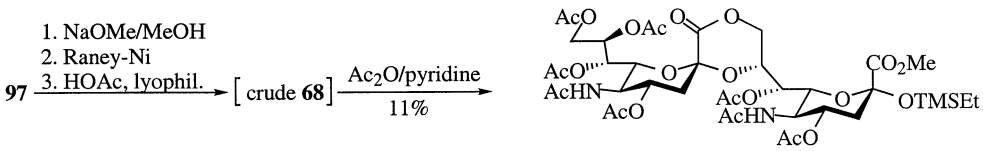
97

Scheme 41

The poor stereoselectivity with the donors **90** and **91** is of course disturbing and hard to explain, but it does not have to be disastrous *if* the α/β mixture is readily separated after a convenient removal of the 3-(*S*)-(2-methoxyphenyl)thio substituent. This was however not the case, as treatment of 7-*O*-acetylated **96** (to prevent [7,8]-lactonisation) with triphenyltin hydride/AIBN under standard conditions provided a complex mixture (>10 products formed). Eventually, **97** could be deprotected in a one-pot sequence (Scheme 42), where deacetylation, treatment with Raney-Ni in ethanol, freeze-drying from acetic acid to obtain the [8,9]-lactone **68** as a known (Scheme 29) intermediate crude product and finally *O*-acetylation provided the [8,9]-lactone **98** in 11% yield as an α/β 1.9:1 mixture. Most unexpectedly, several

* Also here, HR FAB-MS showed only one significant molecular ion *m/z* signal.

monosaccharides were isolated after the deprotection sequence, and it subsequently became clear that extensive cleavage of the 2→8 glycoside bond occurred during the Raney-Ni reduction. This of course explained the very low total yield of **98**.



98 (α/β 1.9:1)

Scheme 42

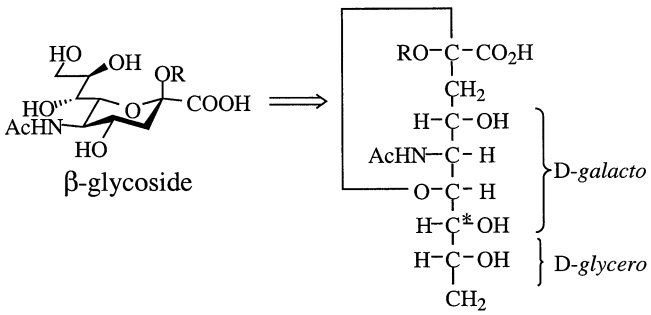
7.6 Concluding Remarks

It can only be concluded that the donors **90** and **91** will be of no use mainly due to the difficult removal of the 3-(*S*)-(2-methoxyphenyl)thio substituent. However, I believe that the protocol developed for the introduction of the auxiliary group may accelerate the search for the optimum 3-(*S*)-substituent by systematic scanning of available thiols.

Appendix

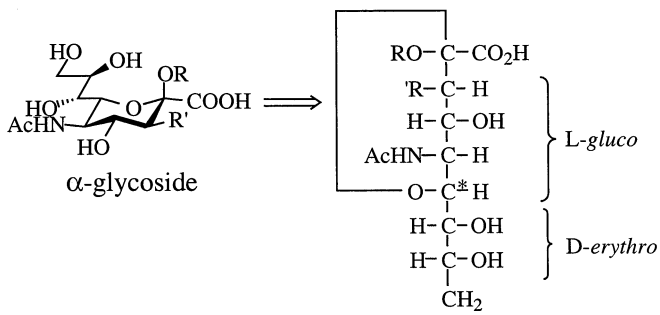
The nomenclature of Neu5Ac residues is deduced in accordance with the following Fischer projections:

Normal Neu5Ac:



* = reference carbon for the anomeric configuration

3-(*S*)-Substituted Neu5Ac:



Acknowledgements

Göran Magnusson for being a source of inspiration and a friend for as long as the circumstances allowed it to be so.

Ulf Nilsson, Olov Sterner, Andreas Meijer, Ulf Ellervik, Niklas Falk, Jörgen Ohlsson, Andreas Wållberg and Maria Levin as well as all other colleagues at the department for providing an enjoyable and creative working atmosphere.

My parents Marija and Niko, my sister Diana and Birgitta for your support.

Experimental

General. ^{1}H NMR spectra were recorded at 300, 400 or 500 MHz and assigned using 2D-methods (COSY, HETCOR). Optical rotations were measured at 22°C. Chemical shifts are expressed in ppm using residual $CHCl_3$ as reference. Reactions were monitored by TLC using alumina plates coated with silica gel 60 F254 (Merck) and visualised using either UV light or charring with H_3PO_4 (aqueous 5% dip solution). The Al_2O_3 used was of Activity II-III (Merck). Preparative chromatography was performed with Amicon silica gel (35-70 μm, 60Å). CH_2Cl_2 and toluene were dried over 4Å molecular sieves. MeCN was distilled over CaH_2 immediately before use. Compounds obtained as white powders were precipitated with *n*-hexane from a chloroform/diethyl ether (~1:2) solution. All reactions were carried out under an argon atmosphere. Anomeric configurations of Neu5Ac residues were determined in accordance with ref. 121.

Methyl [methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-thio-3-(2-methoxyphenyl)thio-3,5-dideoxy-D-*erythro*-β-L-*gluco*-2-nonulopyranosid]onate (90). To a stirred mixture of **93** (0.509 g, 0.785 mmol) and sodium methanethiolate (0.091 g, 1.3 mmol) cooled to 0°C was added acetonitrile (2.5 mL), and the mixture was stirred vigorously for 4 h at this temperature. Addition of acetic acid (0.2 mL), filtration through a short (<10 cm) column of silica gel, washing with toluene/acetone 1:1, concentration and chromatography on silica gel (toluene/acetone, 5:1 → 3:1, gradient) afforded **90** (0.464 g, 90%, α/β 1:15) as a white powder: $[\alpha]_D$ +94° (*c* 0.49, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51-6.85 (m, 4 H, Ph), 5.38 (ddd, 1 H, $J_{7,8}$ 8.2 Hz, $J_{8,9A}$ 2.6 Hz, $J_{8,9B}$ 5.2 Hz, H-8), 5.31 (dd, 1 H, $J_{6,7}$ 2.2 Hz, H-7), 5.27 (d, 1 H, $J_{5,NH}$ 10.2 Hz, NH), 5.18 (dd, 1 H, $J_{3,4}$ 11.1 Hz, $J_{4,5}$ 10.2 Hz, H-4), 4.33 (dd, 1 H, $J_{9A,9B}$ 12.5 Hz, H-9A), 4.14 (q, 1 H, *J* 10.4 Hz, H-5), 4.11 (dd, 1 H, H-9B), 3.91, 3.90 (s, 3 H each, CO_2Me, PhOCH_3), 3.88 (dd, 1 H, $J_{5,6}$ 11.0 Hz, H-6), 3.72 (d, 1 H, H-3), 2.20, 2.17, 2.13, 2.04, 1.85, 1.80 (s, 3 H each, 4OAc, NAc, SMe); ^{13}C NMR ($CDCl_3$): δ 171.2, 170.9, 170.4, 170.3, 170.2, 167.3 ($J_{C1,H3}$ 7.7 Hz, C-1), 158.5, 133.5, 129.4, 124.3, 121.1, 111.3, 87.3, 74.8, 74.3, 69.0, 67.5, 62.3, 55.8, 54.9, 53.2, 50.6, 23.3, 21.4, 21.0, 21.0, 20.8, 12.5. HR FAB-MS for $C_{28}H_{37}NO_{13}S_2Na$ (M + Na): Calcd 682.1604. Found 682.1616.

Methyl [methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamido)-2-thio-3-(2-methoxyphenyl)thio-3,5-dideoxy-D-*erythro*-β-L-*gluco*-2-nonulopyranosid]onate (91). To a stirred solution of **90** (0.458 g, 0.694 mmol) in *iso*-propenylacetate (5.0 mL) was added *p*-toluenesulfonic acid monohydrate (4.7 mg, 0.025 mmol) and the reaction was kept at 65°C for 14 h. Addition of triethylamine (0.2 mL), concentration with toluene and chromatography on silica gel (toluene/acetone, 15:1 → 8:1, gradient) afforded **91** (0.484 g, 99%, α/β 1:15) as a white powder: $[\alpha]_D$ +83° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56-6.83 (m, 4 H, $C_6H_4OCH_3$), 5.81 (dd, 1 H, $J_{3,4}$ 10.7 Hz, $J_{4,5}$ 9.7 Hz, H-4), 5.33 (ddd, 1 H, $J_{7,8}$ 8.6 Hz, $J_{8,9A}$ 2.9 Hz, $J_{8,9B}$ 4.8 Hz, H-8), 5.12 (dd, 1 H, $J_{6,7}$ 1.9 Hz, H-7), 4.85 (dd, 1 H, $J_{5,6}$ 10.5 Hz, H-6), 4.35

(dd, 1 H, H-5), 4.27 (dd, 1 H, $J_{9A,9B}$ 12.6 Hz, H-9A), 4.13 (dd, 1 H, H-9B), 3.95, 3.89 (s, 3 H each, $C_6H_4OCH_3$, CO_2Me), 3.64 (d, 1 H, H-3), 2.35, 2.27, 2.24, 2.17, 2.11, 2.02, 1.71 (s, 3 H each, 4OAc, Ac_2N, SMe); ^{13}C NMR ($CDCl_3$): δ 174.1, 174.0, 170.9, 170.4, 170.3, 170.2, 167.0, 158.3, 133.0, 128.9, 125.1, 121.2, 111.2, 86.9, 72.2, 71.9, 68.5, 67.2, 61.9, 57.2, 56.5, 55.8, 53.2, 28.3, 26.8, 21.3, 21.2, 21.0, 20.6, 12.5. HR FAB-MS for $C_{30}H_{39}NO_{14}S_2Na$ (M + Na): Calcd 724.1710. Found 724.1702.

Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-3-(2-methoxyphenyl)thio-3,5-dideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosonate (93). To a stirred suspension of *N*-chlorosuccinimide (1.222 g, 9.15 mmol) in dichloromethane (8.0 mL) cooled to -40°C was dropwise added 2-methoxybenzenethiol (1.120 mL, 9.18 mmol) over 5 min. After 1 h, the stirring was disrupted and the reaction mixture was kept 0°C over night, thereby allowing the *N*-succinimide formed to sediment. Of the obtained ~1.0 M solution of 2-MPSC, 4.0 mL (~4 mmol) was added to solid **27** (0.617 g, 1.30 mmol), and the reaction mixture was kept at room temperature for seven days in a dark place. Addition of aqueous 0.1 M $NaHCO_3$ (50 mL), transfer to a separatory funnel, extraction with CH_2Cl_2 (4 × 50 mL), concentration and chromatography (toluene/acetone, 6:1 → 3:1, gradient) on a silica gel column having a top layer (~1 cm) of Al_2O_3 afforded **93** (0.576 g, 68%) as a white powder: $[\alpha]_D$ ±0° (*c* 0.99, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.48-6.83 (m, 4 H, Ph), 5.45 (dd, 1 H, $J_{6,7}$ 2.2 Hz, $J_{7,8}$ 8.2 Hz, H-7), 5.43 (d, 1 H, $J_{5,NH}$ 10.5 Hz, NH), 5.39 (dd, 1 H, $J_{4,5}$ 10.1 Hz, $J_{3,4}$ 10.6 Hz, H-4), 5.13 (ddd, 1 H, $J_{8,9A}$ 2.7 Hz, $J_{8,9B}$ 5.4 Hz, H-8), 4.40 (dd, 1 H, $J_{5,6}$ 10.9 Hz, H-6), 4.35 (m, 1 H, H-5), 4.28 (dd, 1 H, $J_{9A,9B}$ 12.5 Hz, H-9A), 4.21 (d, 1 H, H-3), 4.01 (dd, 1 H, H-9B), 3.88 (s, 3 H, $PhOCH_3$), 3.83 (s, 3 H, CO_2Me), 2.13, 2.10, 2.05, 1.88, 1.72 (s, 3 H each, 4OAc, NAc); ^{13}C NMR ($CDCl_3$): δ 171.3, 170.8, 170.5, 170.1, 169.7, 164.4 ($J_{C1,H3}$ 1.5 Hz, C-1), 158.6, 133.8, 129.7, 121.4, 121.2, 111.2, 102.9, 74.7, 73.9, 69.3, 66.7, 62.2, 55.9, 54.3, 54.2, 49.2, 23.2, 21.2, 21.0, 20.9, 20.6. HR FAB-MS for $C_{27}H_{34}ClNO_{13}SNa$ (M + Na): Calcd 670.1337. Found 670.1337.

Methyl [2-(trimethylsilyl)ethyl 5-acetamido-4-*O*-acetyl-9-*O*-benzyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (95). To a stirred solution of **94** (0.331 g, 0.648 mmol) in dichloromethane (2.5 mL) was added *N*-ethyldiisopropylamine (0.23 mL, 1.3 mmol) and the temperature was lowered to -40°C. Acetyl chloride (0.050 mL, 0.70 mmol) was added dropwise, and the reaction mixture was kept at -30°C for 7 h, after which *t*-butanol (0.10 mL) was added. Filtration through a short column of silica gel, washing with toluene/acetone 2:1 and concentration afforded a residue which was dried *in vacuo* over night. To a vigorously stirred solution of the dried residue in tetrahydrofuran (3.5 mL) cooled to 0°C was then added borane trimethylamine (0.200 g, 2.75 mmol) and $AlCl_3$ (0.37 g, 2.78 mmol). After 25 min, dichloromethane (50 mL) and aqueous 0.1 M $NaHCO_3$ (50 mL) was added, and the mixture was transferred to a separatory funnel. Extraction with dichloromethane (3 × 100 mL), concentration and

chromatography on silica gel (toluene/acetone, 4:1 → 2:1, gradient) afforded **95** (0.242 g, 67%) as a white powder: $[\alpha]_D$ -32° (*c* 0.99, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.24 (m, 5 H, Ph), 5.90 (d, 1 H, $J_{5,NH}$ 7.8 Hz, NH), 4.92 (ddd, 1 H, $J_{3e,4}$ 4.9 Hz, $J_{4,5}$ 10.4 Hz, $J_{3a,4}$ 12.2 Hz, H-4), 4.64 (d, 1 H, $J_{HA,HB}$ 12.2 Hz, OCH_2Ph), 4.60 (d, 1 H, OCH_2Ph), 4.59 (d, 1 H, $J_{7,OH}$ 4.5 Hz, OH-7), 4.09 (m, 1 H, H-8), 4.03-3.90 (m, 2 H, H-5, OCH_2CH$_2$Si), 3.85 (m, 1 H, H-9A), 3.84 (s, 3 H, CO_2Me), 3.66 (dd, 1 H, $J_{8,9B}$ 6.0 Hz, $J_{9A,9B}$ 10.2 Hz, H-9B), 3.56 (m, 1 H, H-7), 3.55 (d, 1 H, $J_{8,OH}$ 4.1 Hz, OH-8), 3.51 (dd, 1 H, $J_{6,7}$ 1.7 Hz, $J_{5,6}$ 10.5 Hz, H-6), 3.40 (m, 1 H, OCH_2CH$_2$Si), 2.68 (dd, 1 H, $J_{3a,3e}$ 13.0 Hz, H-3e), 2.11 (s, 3 H, OAc), 2.01 (t, 1 H, *J* 12.7 Hz, H-3a), 1.97 (s, 3 H, NAc), 0.88 (t, 2 H, *J* 7.8 Hz, OCH$_2$CH_2Si), 0.00 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 172.9, 172.5, 169.5, 138.7, 128.5, 127.9, 127.7, 98.6, 74.2, 73.6, 71.8, 70.3, 69.3, 68.9, 62.3, 53.3, 52.0, 37.7, 23.3, 21.3, 18.1, -1.1. HR FAB-MS for $C_{26}H_{41}NO_{10}SiNa$ (M + Na): Calcd 578.2397. Found 578.2408.

Methyl [2-(trimethylsilyl)ethyl 5-acetamido-4-*O*-acetyl-9-*O*-benzyl-3,5-dideoxy-8-*O*-[methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3-(2-methoxyphenyl)thio-3,5-dideoxy-D-*erythro*-β-L-*gluco*-2-nonulopyranosid]onate]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (96). To a stirred mixture of **90** (0.095 g, 0.144 mmol), **95** (0.111 g, 0.200 mmol) and 3 Å molecular sieves (0.20 g) in acetonitrile (1.0 mL) was added a solution of AgOTf (0.043 g, 0.17 mmol) in acetonitrile (0.2 + 0.2 mL). After cooling to -40°C, a 2.8 M solution (0.060 mL, 0.17 mmol) of MeSBr in 1,2-dichloroethane was added dropwise over 10 min. After 1.5 h, diisopropylamine (0.060 mL) was added. Filtration (Celite), washing with chloroform/acetone 1:1, concentration and chromatography on silica gel (toluene/acetone, 5:1 → 2:1, gradient) afforded recovered **95** (0.065 g) and **96** (0.060 g, 36%, α/β 1:10), both as white powders. Data for **96**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49-6.76 (m, 9 H, Ph, $C_6H_4OCH_3$), 6.01 (d, 1 H, $J_{5,NH}$ 9.4 Hz, NH), 5.42 (t, 1 H, $J_{3',4'}$=$J_{4',5'}$ 9.7 Hz, H-4'), 5.36 (m, 2 H, NH', H-8'), 5.25 (dd, 1 H, $J_{6',7'}$ 1.8 Hz, $J_{7',8'}$ 8.3 Hz, H-7'), 4.99 (ddd, 1 H, $J_{3e,4}$ 5.0 Hz, $J_{3a,4}$ 11.3 Hz, $J_{4,5}$ 10.5 Hz, H-4), 4.81 (dt, 1 H, $J_{8,9A}$=$J_{7,8}$ 2.2 Hz, $J_{8,9B}$ 6.7 Hz, H-8), 4.50 (d, 1 H, *J* 12.0 Hz, OCH_2Ph), 4.40 (dd, 1 H, $J_{8',9'A}$ 2.9 Hz, $J_{9'A,9'B}$ 12.4 Hz, H-9'A), 4.40 (m, 1 H, H-5'), 4.35 (d, 1 H, OCH_2Ph), 4.19 (dd, 1 H, $J_{5',6'}$ 10.9 Hz, H-6'), 4.10 (q, 1 H, *J* 10.1 Hz, H-5), 4.05 (dd, 1 H, $J_{8',9'B}$ 7.4 Hz, H-9'B), 4.01 (dd, 1 H, $J_{8,9A}$ 2.0 Hz, $J_{9A,9B}$ 10.9 Hz, H-9A), 3.90 (m, 1 H, H-7), 3.81 (m, 1 H, OCH_2CH$_2$Si), 3.81 (s, 3 H, C'O_2Me), 3.76 (s, 3 H, CO_2Me), 3.76 (m, 2 H, H-6, H-3'), 3.70 (s, 3 H, $C_6H_4OCH_3$), 3.60 (m, 2 H, H-9B, OH-7), 3.32 (m, 1 H, OCH_2CH$_2$Si), 2.59 (dd, 1 H, $J_{3a,3e}$ 12.9 Hz, H-3e), 2.11, 2.09, 2.05, 2.04, 1.94, 1.91, 1.90 (s, 3 H each, 5OAc, 2NAc), 1.93 (m, 1 H, H-3a), 0.76 (t, 2 H, OCH$_2$CH_2Si), -0.07 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 172.0, 171.3, 171.2, 171.2, 170.7, 170.3, 170.1, 169.0 ($J_{C1',H3'}$ 5.0 Hz, C-1'), 168.5 ($J_{C1,H3a}$ 6.5 Hz, C-1), 158.6, 139.3, 134.0, 128.9, 128.4, 127.3, 123.1, 121.1, 111.0, 100.7, 98.8, 75.6, 75.5, 73.9, 72.9, 72.5, 71.3, 70.2, 69.3, 69.1, 68.0, 63.2, 62.0,

55.9, 55.0, 52.6, 52.5, 50.8, 50.0, 37.8, 23.4, 23.2, 21.2, 21.1, 20.9, 20.9, 20.8, 18.1, -1.1. HR FAB-MS for $C_{53}H_{74}N_2O_{23}SSiNa$ (M + Na): Calcd 1189.4070. Found 1189.4084.

Substantially identical reaction conditions were used in the preparation of **96** using ICl/AgOTf as promoter system. A 1.0 M solution (0.18 mL, 0.18 mmol) of ICl in dichloromethane was employed instead of the solution of MeSBr, thereby providing **96** (0.0595 g, 35%, α/β 1:10) and recovered **95** (0.077 g), both as white powders.

Methyl [2-(trimethylsilyl)ethyl 5-acetamido-4-*O*-acetyl-9-*O*-benzyl-3,5-dideoxy-8-*O*-[methyl [4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamido)-3-(2-methoxyphenyl)thio-3,5-dideoxy-D-*erythro*-β-L-*gluco*-2-nonulopyranosid]onate]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (97). To a stirred solution of **91** (0.108 g, 0.154 mmol), **95** (0.132 g, 0.238 mmol) and 3 Å molecular sieves (0.25 g) in acetonitrile (0.9 mL) was added a solution of AgOTf (0.054 g, 0.21 mmol) in acetonitrile (0.2 + 0.2 mL). After cooling to -40°C, a 1.0 M solution (0.21 mL, 0.21 mmol) of ICl in dichloromethane was added dropwise over 10 min. After 2 h, diisopropylamine (0.070 mL, 0.50 mmol) was added. Work-up as above and chromatography (toluene/acetone, 5:1 → 3:1, gradient) afforded recovered **95** (0.066 g) and **97** (0.103 g, 56%, α/β 1:2.2), both as white powders. Data for **97** (anomeric mixture): ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.90 (dd, 1 H, $J_{3',4'}$ 8.6 Hz, $J_{4',5'}$ 9.5 Hz, H-4'), 5.88 (d, 1 H, $J_{5,NH}$ 9.4 Hz, NH), 3.86, 3.81, 3.78 (s, 3 H each, $2CO_2Me$, $C_6H_4OCH_3$), 3.68 (d, 1 H, H-3'), 2.61 (dd, 1 H, $J_{3a,3e}$ 12.8 Hz, $J_{3e,4}$ 5.0 Hz, H-3e), 2.39, 2.20, 2.12, 2.05, 2.04, 2.04, 1.93, 1.88 (s, 3 H each, 5OAc, Ac_2N, NAc), -0.05 (s, 9 H, $Si(CH_3)_3$). HR FAB-MS for $C_{55}H_{76}N_2O_{24}SSiNa$ (M + Na): Calcd 1231.4176. Found 1231.4180.

Data for α-anomer of **97** (<1 mg ~80% pure material isolated by silica gel chromatography): ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.13 (d, 1 H, $J_{5,NH}$ 7.6 Hz, NH), 5.92 (dd, 1 H, $J_{3',4'}$ 8.2 Hz, $J_{4',5'}$ 9.5 Hz, H-4'), 3.89, 3.85, 3.83 (s, 3 H each, $2CO_2Me$, $C_6H_4OCH_3$), 2.70 (dd, 1 H, $J_{3a,3e}$ 12.6 Hz, $J_{3e,4}$ 4.8 Hz, H-3e), 2.40, 2.30, 2.12, 2.08, 2.02, 2.00, 1.89, 1.80 (s, 3 H each, 5OAc, Ac_2N, NAc), -0.03 (s, 9 H, $Si(CH_3)_3$).

Methyl {2-(trimethylsilyl)ethyl 5-acetamido-4,7-di-*O*-acetyl-3,5-dideoxy-8-*O*-[5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate 1'→9-Lactone (98). To a stirred solution of **97** (0.071 g, 0.059 mmol) in methanol (0.5 mL) was added a 1.0 M solution (0.050 mL, 0.050 mmol) of sodium methoxide in methanol. After 13 h at room temperature, acetic acid (0.1 mL) was added, and the mixture was concentrated with toluene/ethanol 1:1 and dried *in vacuo* over night. Raney-Ni (1 g) in ethanol (10 mL) was added, and the mixture was stirred vigorously for 24 h at room temperature. The Raney-Ni was then washed with toluene/methanol 1:1 (6 × 15 mL), where each washing involved stirring for at least 10 min. After filtration (Celite) and concentration of the combined wash solutions, the residue was dissolved in acetic acid (8 mL) and freeze-

dried, thereby providing a residue* to which acetic anhydride (3.0 mL) and pyridine (1.5 mL) was added. After 13 h, the reaction mixture was concentrated with toluene and the residue was chromatographed on silica gel (toluene/acetone, 3:1 → 3:2 → 1:2, gradient) to provide **98** (5.9 mg, 11%, α/β 1.9:1) as a white powder: ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.84 (s, 3 H, CO_2Me), 2.65 (dd, 1 H, $J_{3e,4}$ 4.9 Hz, $J_{3a,3e}$ 12.8 Hz, H-3e), 2.45 (dd, 1 H, $J_{3'e,4'}$ 5.5 Hz, $J_{3'a,3'e}$ 13.5 Hz, H-3'e), 2.20, 2.13, 2.08, 2.05, 2.04, 2.03, 1.93, 1.91 (s, 3 H each, 6OAc, 2NAc), 0.03 (s, 9 H, $Si(CH_3)_3$). HR FAB-MS for $C_{40}H_{60}N_2O_{22}SiNa$ (M + Na): Calcd 971.3305. Found 971.3278.

* When this residue was purified by silica gel chromatography, only various monosaccharides and crude **68** (α/β mixture and other impurities) were isolated.

References

1. Hakomori, S. *Sci. Amer.* **1986**, May, 32-41.
2. Hakomori, S.; Murakami, W.T. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *59*, 254-261.
3. Marcus, D.M. *Mol. Immun.* **1984**, *21*, 1083-1091.
4. Steck, G.L.; Dawson, G. *J. Biol. Chem.* **1974**, *249*, 2135-2142.
5. Gahmberg, C.G.; Hakomori, S. *J. Biol. Chem.* **1973**, *248*, 4311-4317.
6. Yogeeswaran, G. *Adv. Cancer Res.* **1983**, *38*, 289-350.
7. Freeze, H.H.; Sampath, D.; Varki, A. *J. Biol. Chem.* **1993**, *268*, 1618-1627.
8. Vankar, Y.D.; Schmidt, R.R. *Chem. Soc. Rev.* **2000**, *29*, 201-216.
9. Svennerholm, L. *J. Lipid Res.* **1964**, *5*, 145-155.
10. Hakomori, S.; Watanabe, K.; Laine, R.A. *Pure Appl. Chem.* **1977**, *49*, 1215-1227.
11. Kubo, H.; Irie, A.; Inagaki, F.; Hoshi, M. *J. Biochem.* **1990**, *108*, 185-192.
12. Wertz, P.W.; Van den Bergh, B. *Chem. Phys. Lipids* **1998**, *91*, 85-96.
13. Sharom, F.J.; Grant, C.W.M. *J. Supramol. Struct.* **1977**, *6*, 249-258.
14. Abrahamsson, S.; Dahlén, B.; Löfgren, H.; Pascher, I.; Sundell, S. in *Structure of Biological Membranes* (Eds.: Abrahamsson, S. and Pascher, I.), Plenum, New York, **1977**, pp. 1-23.
15. Philips, M.L.; Nudelman, E.; Gaeta, F.C.A.; Perez, M.; Singhal, A.K.; Hakomori, S.; Paulson, J.C. *Science* **1990**, *250*, 1130-1132
16. Waltz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132-1135.
17. Tiemeyer, M.; Swiedler, S.J.; Ishihara, M.; Moreland, M.; Schweingruber, H.; Hirtzer, P.; Brandley, B.K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1138-1142.
18. Berg, E.L.; Robinson, M.K.; Mansson, O.; Butcher, E.C.; Magnani, J.L. *J. Biol. Chem.* **1991**, *266*, 14869-14872.
19. Van Heyningen, W.E.; Carpenter, C.C.J.; Pierce, N.F.; Greenough, W.B. *J. Infect. Dis.* **1971**, *124*, 415-418.
20. Holmgren, J.; Lönnroth, I.; Svennerholm, L. *Infect. Immun.* **1973**, *8*, 208-214.
21. Svennerholm, L. *Adv. Exp. Med. Biol.* **1980**, *125*, 533-551.
22. Kopitz, J. in *Glycosciences* (Eds.: Gabius, H.-J. and Gabius, S.), Chapman & Hall, Weinheim, **1997**, pp. 153-189.
23. Radin, N.S.; Inokuchi, J. *Biochem. Pharm.* **1988**, *37*, 2879-2886.
24. Vaheri, A.A.; Ruoslahti, E.; Nodling, S. *Nature (London)* **1972**, *238*, 211-212.
25. Sharon, N.; Lis. H. *Sci. Amer.* **1993** (Jan), 74-81.
26. Koschielak, J.; Zdebska, E.; Wilczynska, Z.; Miller-Podraza, H.; Dzierzkowa-Borodej, W. *Eur. J. Biochem.* **1979**, *96*, 331-337.

27. Hakomori, S. *Chem. Phys. Lipids* **1986**, *42*, 209-233.
28. Merritt, W.D.; Richardson, C.L.; Keenan, T.W.; Morre, D.J. *J. Natl. Cancer Inst.* **1978**, *60*, 1313-1325.
29. Hakomori, S. *Cancer Res.* **1985**, *45*, 2405-2414.
30. Merritt, W.D.; Morre, D.J.; Keenan, T.W. *J. Natl. Cancer Inst.* **1978**, *60*, 1329-1337.
31. Bassi, R.; Riboni, L.; Sonnino, S.; Tettamanti, G. *Carbohydr. Res.* **1989**, *193*, 141-146.
32. Behr, J.P.; Lehn, J.M. *FEBS Lett.* **1972**, *22*, 178-180.
33. Kuhn, R.; Muldner, J. *Naturwissenschaften* **1964**, *51*, 635-636.
34. Wiegandt, H. *Ergeb. Physiol. Biol. Chem. Exp. Pharmacol.* **1966**, *57*, 190-222.
35. Evans, J.E.; McCluer, R.H. *Fed. Proc.* **1971**, *30*, 1133abs.
36. McCluer, R.H.; Evans, J.E. in *Sphingolipids, Sphingolipidoses and Allied Disorders* (Eds.: Volk, B.W. and Aronson, S.M.), Plenum, New York, **1972**, pp. 95-102.
37. Gross, S.K.; Evans, J.E.; Reinhold, V.N.; McCluer, R.H. *Carbohydr. Res.* **1975**, *41*, 344-350.
38. Nakahara, Y.; Iijima, H.; Ogawa, T. *Tetrahedron Lett.* **1994**, *35*, 3321-3324.
39. Ando, S.; Yu, R.K.; Scarsdale, J.N.; Kusunoki, S.; Prestegard, J.H. *J. Biol. Chem.* **1989**, *264*, 3478-3483.
40. Yu, R.K.; Koerner, T.A.W.; Ando, S.; Yohe, H.C.; Prestegard, J.H. *J. Biochem.* **1985**, *98*, 1367-1373.
41. Nakamura, T.; Bubb, W.A.; Saito, T.; Arai, I.; Urashima, T. *Carbohydr. Res.* **2000**, *329*, 471-476.
42. Goto, T.; Kondo, T. (MECT Corp.) Eur. Pat. Appl., **1989**, Nr. 89110635.3.
43. Gross, S.K.; Williams, M.A.; McCluer, R.H. *J. Neurochem.* **1980**, *34*, 1351-1361.
44. Riboni, L.; Sonnino, S.; Acquotti, D.; Malesci, A.; Ghidoni, R.; Egge, H.; Mingrino, S.; Tettamanti, G. *J. Biol. Chem.* **1986**, *261*, 8514-8519.
45. Magnusson, G.; Ding, K.; Nilsson, U.; Ray, A.K.; Rosén, A.; Sjögren, H.-A. in *Complex Carbohydrates in Drug Research* (Eds.: Bock, K. and Clausen, H.), Alfred Benzon Symposium 36, Munksgaard, Copenhagen, **1994**, pp. 89-100.
46. Hirabayashi, Y.; Hanaoka, A.; Matsumoto, M.; Matsubara, T.; Tagawa, M.; Wakabayashi, S.; Taniguchi, M. *J. Biol. Chem.* **1985**, *260*, 13328-13333.
47. Taniguchi, M.; Wakabayashi, S. *Gann* **1984**, *75*, 418-426.
48. Nores, G.A.; Dohi, T.; Taniguchi, M.; Hakomori, S. *J. Immunol.* **1987**, *139*, 3171-3176.
49. Dohi, T.; Nores, G.A.; Hakomori, S. *Cancer Res.* **1988**, *48*, 5680-5685.
50. Pukel, C.S.; Lloyd, K.O.; Travassos, L.R.; Dippold, W.G.; Oettgen, H.F.; Old, L.J. *J. Exp. Med.* **1982**, *155*, 1133-1147.
51. Portoukalian, J.; Zwingelstein, G.; Doré, J.F.; Borgoin, J.J. *Biochimie* (Paris), **1976**, *58*, 1285-1287.
52. Portoukalian, J.; Zwingelstein, G.; Abdul-Malak, N.; Doré, J.F. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 916-920.

53. Portoukalian, J.; Zwingelstein, G.; Doré, J.F. *Eur. J. Biochem.* **1979**, *94*, 19-23.
54. Fukushima, K.; Hirota, M.; Terasaki, P.I.; Wakisaka, A.; Togashi, H.; Chia, D.; Suyama, N.; Fukushi, Y.; Nudelman, E.; Hakomori, S. *Cancer Res.* **1984**, *44*, 5279-5285.
55. Fukushi, Y.; Kannagi, R.; Hakomori, S.; Shepard, T.; Kulander, B.; Singer, J.W. *Cancer Res.* **1985**, *45*, 3711-3717.
56. Hansson, G.C.; Karlsson, K.-A.; Larson, G.; Strömberg, N.; Teneberg, S.; Thurin, J.; Brodin, T.; Sjögren, H.; Hellström, I.; Hellström, K.-E. in *Proc. 7th, Intern. Symp. Glycoconjugates* (Eds.: Chester, M.A.; Heinegård, D.; Lundblad, A. and Svensson, S.) **1983**, 854-855.
57. Nicolaou, K.C.; Hummel, C.W.; Iwabuchi, Y. *J. Am. Chem. Soc.* **1992**, *114*, 3126-3128; including supplementary material.
58. Ritter, G.; Boosfeld, E.; Calves, M.J.; Oettgen, H.F.; Old, L.J.; Livingston, P.O. *Immunobiology* **1990**, *182*, 32-43.
59. Ray, A.K.; Nilsson, U.; Magnusson, G. *J. Am. Chem. Soc.* **1992**, *114*, 2256-2257; including personal communications with the authors.
60. Ding, K.; Rosén, A.; Ray, A.K.; Magnusson, G. *Glycoconj. J.* **1992**, *9*, 303-306.
61. Garin-Chesa, P.; Beresford, H.R.; Carrato-Mena, A.; Oettgen, H.F.; Old, L.J.; Melamed, M.M.; Rettig, W. *Amer. J. Pathol.* **1989**, *134*, 295-303.
62. Livingston, P.O.; Ritter, G.; Calves, M.J. *Cancer Immunol. Immunother.* **1989**, *29*, 179-184.
63. Kawashima, I.; Kotani, M.; Ozawa, H.; Suzuki, M.; Tai, T. *Int. J. Cancer* **1994**, *58*, 263-268.
64. Ritter, G.; Boosfeld, E.; Adluri, R.; Calves, M.J.; Oettgen, H.F.; Old, L.J.; Livingston, P.O. *Int. J. Cancer* **1991**, *48*, 379-385.
65. Yamaguchi, H.; Furukawa, K.; Fortunato, S.R.; Livingston, P.O.; Lloyd, K.O.; Oettgen, H.F.; Old, L.J. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2416-2420.
66. Tai, T.; Kawashima, I.; Tada, N.; Ikegami, S. *Biochim. Biophys. Acta* **1988**, *958*, 134-138.
67. Nudelman, E.; Hakomori, S.; Kannagi, R.; Levery, S.; Yeh, M.Y.; Hellström, K.E.; Hellström, I. *J. Biol. Chem.* **1982**, *257*, 12752-12756.
68. Yeh, M.Y.; Hellström, I.; Abe, K.; Hakomori, S.; Hellström, K.E. *Int. J. Cancer* **1982**, *29*, 269-275.
69. Dippold, W.G.; Lloyd, K.O.; Li, L.T.C.; Ikeda, H.; Oettgen, H.F.; Old, L.J.; *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6114-6118.
70. Ohta, S.; Honda, A.; Tokutake, Y.; Yoshida, H.; Hanai, N. *Cancer Immunol. Immunother.* **1993**, *36*, 260-266.
71. Tai, T.; Kawashima, I.; Furukawa, K.; Lloyd, K.O. *Arch. Biochem. Biophys.* **1988**, *260*, 51-55.
72. Houghton, A.N.; Mintzer, D.; Cordon-Cardo, C.; Welt, S.; Fliegel, B.; Vadhan, S.;

Carswell, E.; Melamed, M.R.; Oettgen, H.F.; Old, L.J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1242-1246.
73. Dippold, W.G.; Knuth, K.R.A.; Meyer zum Bueschenfelde, K.H. *Eur. J. Clin. Oncol.* **1985**, *21*, 907-912.
74. Nasi, M.L.; Meyers, M.; Livingston, P.O.; Houghton, A.N.; Chapman, P.B. *Melanoma Res.* **1997**, *7*, S155-S162, Suppl. 2.
75. Abbas, S.Z.; Sugiyama, S.; Diakur, J.; Pon, R.A.; Roy, R. *J. Carbohydr. Chem.* **1990**, *9*, 891-901.
76. Hasegawa, A.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1993**, *12*, 371-376.
77. Hasegawa, A.; Ito, Y.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1989**, *8*, 125-133.
78. Hasegawa, A.; Ito, Y.; Morita, M.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1989**, *8*, 135-144.
79. Hasegawa, A.; Morita, M.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1990**, *9*, 345-355.
80. Schreiner, E.; Zbiral, E.; Kleineidam, R.G.; Schauer, R. *Liebigs Ann. Chem.* **1991**, 129-134.
81. Warner, T.G. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 1323-1329.
82. Sharma, M.; Petrie, IIIrd, C.R.; Korytnyk, W. *Carbohydr. Res.* **1988**, *175*, 25-34.
83. von Itzstein, M.; Jin, B.; Wu, W.; Chandler, M. *Carbohydr. Res.* **1993**, *244*, 181-185.
84. von Itzstein, M.; Thomson, R.J. *Topics Curr. Chem.* **1997**, *186*, 119-170.
85. Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1990**, *195*, 303-308.
86. Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **1991**, *212*, 277-281.
87. Martin, T.J.; Brescello, R.; Toepfer, A.; Schmidt, R.R. *Glycoconj. J.* **1993**, *10*, 16-25.
88. Kondo, H.; Ichikawa, Y.; Wong, C.H. *J. Am. Chem. Soc.* **1992**, *33*, 6123-6126.
89. Ito, Y., Ogawa, T. *Tetrahedron* **1990**, *46*, 89-102.
90. Kondo, T.; Abe, H.; Goto, T. *Chem. Lett.* **1988**, 1657-1660.
91. Kuhn, R.; Lutz, P.; MacDonald, D.L. *Chem. Ber.* **1966**, *99*, 611-617.
92. Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *190*, 317-322.
93. Isecke, R.; Brossmer, R. *Tetrahedron* **1994**, *50*, 7445-7460.
94. Brandstetter, H.H.; Zbiral, E.; Schulz, G. *Liebigs Ann. Chem.* **1982**, 1-13.
95. Greene, T.W.; Wuts, P.G.M. in *Protective Groups in Organic Synthesis*, John Wiley & Sons Inc., 2nd ed. **1991**.
96. Abbas, S.A.; Haines, A.H. *Carbohydr. Res.* **1975**, *39*, 358-363.
97. Kim, S.; Chang, H.; Kim, W.J. *J. Org. Chem.* **1985**, *50*, 1751-1752.
98. Lönn, H.; Stenvall, K. *Tetrahedron Lett.* **1992**, *33*, 115-116.
99. Wilstermann, M.; Kononov, L.O.; Nilsson, U.; Ray, A.K.; Magnusson, G. *J. Am. Chem. Soc.* **1995**, *117*, 4742-4754*.

* For obvious reasons, the results of the sialylations reported in this paper were available to me in 1993.

100. Meindl, P.; Tuppy, H. *Monatsch. Chem.* **1969**, *100*, 1295-1306.
101. Okamoto, K.; Kondo, T.; Goto, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 631-636.
102. Peterson, M.A.; Polt, R. *J. Org. Chem.* **1993**, *58*, 4309-4314.
103. Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1988**, *7*, 501-506.
104. Okamoto, K.; Kondo, T.; Goto, T. *Tetrahedron Lett.* **1986**, *43*, 5229-5232.
105. Martichonok, V.; Whitesides, G.M. *Carbohydr. Res.* **1997**, *302*, 123-129.
106. Halkes, K.M.; St. Hilaire, P.M.; Jansson, A.M.; Gotfredsen, C.H.; Meldal, M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2127-2133.
107. Kondo, T.; Tomooo, T.; Abe, H.; Isobe, M.; Goto, T. *Chem. Lett.* **1996**, 337-338.
108. Ikeda, K.; Nagao, Y.; Achiwa, K. *Carbohydr. Res.* **1992**, *224*, 123-131.
109. Ito, Y.; Nunomura, S.; Shibayama, S.; Ogawa, T. *J. Org. Chem.* **1992**, *57*, 1821-1831.
110. Wang, Z-G.; Zhang, X-F.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Bioorg. Med. Chem.* **1996**, *4*, 1901-1908.
111. Schmid, W.; Christian, R.; Zbiral, E. *Tetrahedron Lett.* **1988**, *29*, 3643-3646.
112. Claesson, A.; Luthman, K. *Acta Chemica Scand. Ser. B* **1982**, *36*, 719-720.
113. Kumar, V.; Kessler, J.; Scott, M.E.; Pathwardan, B.H.; Tanenbaum, S.W.; Flaschner, M. *Carbohydr. Res.* **1981**, *94*, 123-130.
114. Fieser & Fieser in *Reagents for Organic Synthesis*, John Wiley & Sons Inc., *5*, 523, **1975**.
115. Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1989**, *193*, C1-C5.
116. Dasgupta, F.; Garegg, P.J. *Carbohydr. Res.* **1988**, *177*, C13-C17.
117. Veeneman, G.H.; Van Leeuwen, S.H.; Van Boom, J.H. *Tetrahedron Lett.* **1990**, *31*, 1331-1334.
118. Konradsson, P.; Mootoo, D.R.; McDevitt, R.E.; Fraser-Reid, B. *J. Chem. Soc., Chem. Comm.* **1990**, 270-272.
119. Dabrowski, U.; Brossmer, R.; Supp, M.; Friebolin, H. *Tetrahedron Lett.* **1979**, *48*, 4637-4640.
120. Okamoto, K.; Kondo, T.; Goto, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 637-643.
121. Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H.; Meguro, H. *Tetrahedron Lett.* **1988**, *29*, 6317-6320.
122. Birberg, W.; Lönn, H. *Tetrahedron Lett.* **1991**, *32*, 7453-7456.
123. Jones, D.K.; Liotta, D.C. *Tetrahedron Lett.* **1993**, *34*, 7209-7212.
124. Martichonok, V.; Whitesides, G.M. *J. Org. Chem.* **1996**, *61*, 1701-1706.
125. Hasegawa, A.; Nagahama, T.; Ohki, H.; Kiso, M. *J. Carbohydr. Chem.* **1992**, *11*, 699-714.
126. Ek, M.; Garegg, P.J.; Hultberg, H.; Oscarsson, S. *J. Carbohydr. Chem.* **1983**, *2*, 305-311.
127. Ellervik, U.; Grundberg, H.; Magnusson, G. *J. Org. Chem.* **1998**, *63*, 9323-9338.
128. Ellervik, U. Ph.D. Thesis, The Lund Institute of Technology, University of Lund, Lund, Sweden, **1998**; p.83.
129. Burkert, U.; Allinger, N.L. in *Molecular Mechanics*; American Chemical Society:

Washington, D.C., **1982**.
130. Allinger, N.L.; Yuh, Y.H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551-8566.
131. Minimisations were made with the MacMimic/MM3 package: InStar Software, Ideon Research Park, SE-22370 Lund, Sweden.
132. Tsvetkov, Y.E.; Schmidt, R.R. *Tetrahedron Lett.* **1994**, *35*, 8583-8586.
133. Castro-Palomino, J.C.; Tsvetkov, Y.E.; Schneider, R.; Schmidt, R.R. *Tetrahedron Lett.* **1997**, *38*, 6837-6840.
134. Schmidt, R.R.; Castro-Palomino, J.C.; Retz, O. *Pure Appl. Chem.* **1999**, *71*, 729-744.
135. Demchenko, A.V.; Boons, G.J. *Chem. Eur. J.* **1999**, *5*, 1278-1283.
136. Martichonok, V.; Whitesides, G.M. *J. Am. Chem. Soc.* **1996**, *118*, 8187-8191.
137. Castro-Palomino, J.C.; Tsvetkov, Y.E.; Schmidt, R.R. *J. Am. Chem. Soc.* **1998**, *120*, 5434-5440.
138. Demchenko, A.V.; Boons, G.J. *Tetrahedron Lett.* **1998**, *39*, 3065-3068.
139. Hossain, N.; Magnusson, G. *Tetrahedron Lett.* **1999**, *40*, 2217-2220.
140. Hossain, N.; Zapata, A.S.; Wilstermann, M.; Magnusson, G.; Nilsson, U.; submitted
141. Tietze, L.F.; Keim, H.; Janβen, C.O.; Tappertzhofen, C.; Olschimke, J. *Chem. Eur. J.* **2000**, *6*, 2801-2808.
142. Sharpless, K.B.; Lauer, R.F. *J. Am. Chem. Soc.* **1973**, *8*, 2697-2699.
143. Hermans, B.; Colard, N.; Hevesi, L. *Tetrahedron Lett.* **1992**, *33*, 4629-4632.
144. Schmidt, O.T. *Methods Carbohydr. Chem.* **1963**, *2*, 318-325.
145. Kondo, H.; Aoki, S.; Ichikawa, Y.; Halcomb, R.L.; Ritzen, H.; Wong, C.-H. *J. Org. Chem.* **1994**, *59*, 864-877.
146. Paulsen, H.; Tietz, H. *Carbohydr. Res.* **1984**, *125*, 47-64.
147. Jochims, J.C.; Taigel, G. *Tetrahedron Lett.* **1968**, 5483-5488.
148. Lee, Y.-H.; Picardt, F.; Pilkis, S.J. *Arch. Biochem. Biophys.* **1995**, *322*, 357-360.
149. Hashimoto, S.-I.; Yanagiya, Y.; Honda, T.; Ikegami, S. *Chem. Lett.* **1992**, 1511-1514.
150. Garratt, D.G.; Beaulieu, P.L. *Can. J. Chem.* **1980**, *58*, 2746-2753.
151. Andersson, M.; Konradsson, P.; Oscarsson, S.; Öhberg, L. *J. Carbohydr. Chem.*; in press

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A New *N*-Acetylneuraminic Acid Donor for Highly Stereoselective α-Sialylation

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The new sialyl donor **6** (prepared from *N*-acetylneuraminic acid in 44% yield over six steps) effects clean α-sialylation of 2-(trimethylsilyl)ethyl 2,3,6,2′,4′,6′-hexabenzyl-β-D-lactoside in 67% yield.

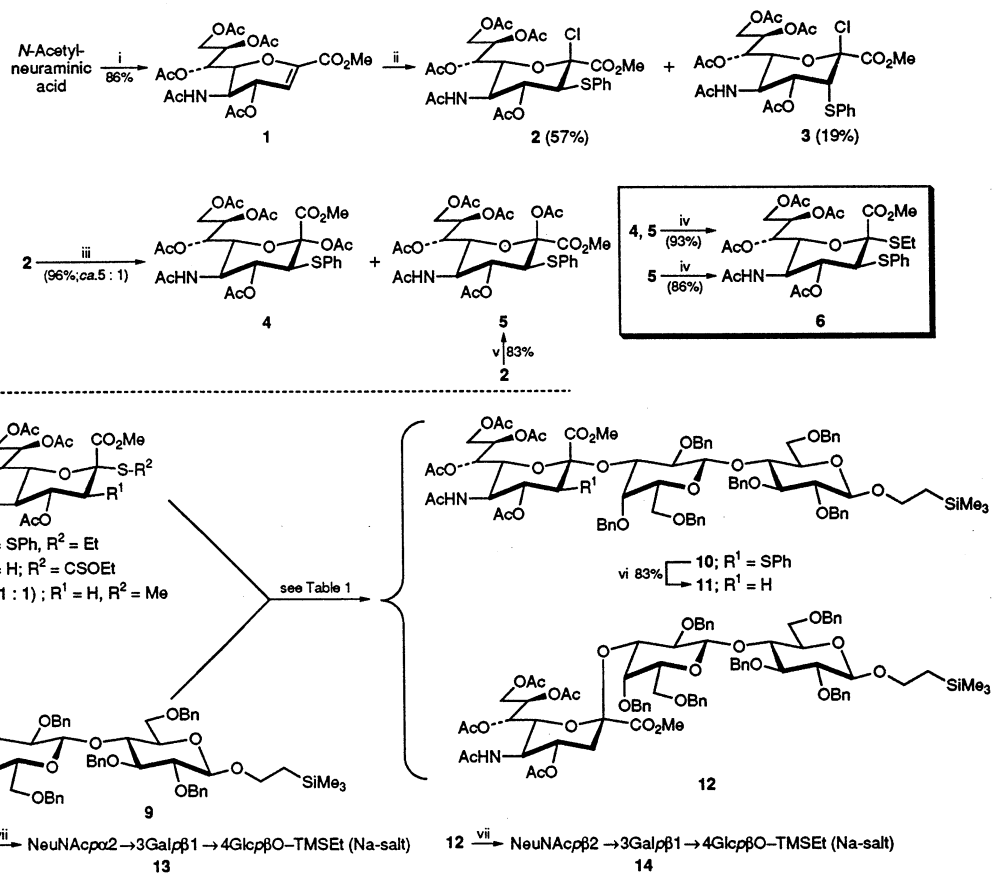
Sialylation in high yield and stereoselectivity is difficult.[1,2] Hindered glycosyl acceptors are particularly troublesome, causing a substantial fraction of the sialic acid-derived donor to undergo elimination to the corresponding glycal; yields obtained with such acceptors are consequently modest. However, yields may be increased with donors (*e.g.* **7**[3] and **8**[4]) carrying sulfur-containing leaving groups, and especially when the acceptors have several hydroxy groups unprotected (*e.g.* HO-2,3,4 in galactose residues). In order to improve the α:β-ratio in the sialylation reaction and also reduce glycal formation, an auxiliary participating 3-PhS-group has been introduced in 2-halogeno-sialic acid donors.[5] However, the most effective donors carry *O*-benzyl protecting groups and their syntheses require *ca.* six steps, which furnish the desired donor in 20–50% overall yield,[5,6] starting from the glycal **1**.

We now report the new sialyl donor **6**: (*i*) its synthesis

Table 1 Sialylation of lactoside acceptor **9** (0.15–0.50 mmol) with the sialic acid donors **6–8**

Donor	Mol. ratio[a]	P1,P2[a]	Reaction conditions[b]	Product	Yield[c] (%)	α:β
6	1.0:1.0:1.1:1.1	MSB–AgOTf	MeCN, −40 °C	**10**	54	>99:1
6	1.0:1.5:1.6:1.6	MSB–AgOTf	MeCN, −40 °C	**10**	67	>99:1
6	1.0:1.5:1.7:0.5	NIS–TfOH	MeCN, −40 °C	**10**	57	>99:1
7	1.0:1.5:1.3:1.2	MSB–AgOTf	MeCN, CH_2Cl_2, −60 °C	**11** + **12**	36 + 4	90:10
8	1.0:1.5:1.5:0.7	NIS–TfOH	MeCN, −40 °C	**11** + **12**	29 + 4	88:12

[a] Donor/acceptor/promotor 1 (P1)/promotor 2 (P2). [b] The concentration of **9** was ~0.10 mol dm^{-3}. [c] Based on the donor (**6–8**).



Scheme 1 *Reagents and conditions*: i, MeOH, Dowex-H^+, then Ac_2O, pyridine, then TMSOTf, MeCN, 0 °C, 6 h; ii, PhSCl, CH_2Cl_2, 20 °C, 7 days, Ar; iii, $Hg(OAc)_2$, AcOH–Ac_2O 10:1, 40 °C, 18 h. iv, EtSH, BF_3Et_2O, CH_2Cl_2, 20 °C, 18 h; v, $HgBr_2$, $Hg(CN)_2$, $ClCH_2CH_2Cl$–H_2O 100:1, reflux, 3.5 h, then Ac_2O, pyridine, DMAP, 20 °C, 1 h; vi, Ph_3SnH, AIBN, toluene, reflux, 14 h. vii, Pd/C, AcOH, 20 °C, overnight, then MeONa, MeOH, 20 °C, 2 h, then NaOH, H_2O, 20 °C, 0.5 h

Table 2 Anomeric configuration (NeuNAc residue) based on NMR analysis of compounds **2**, **4–6**, **10–14**

Compound	$JC(1)$–$H(3)_{ax}$/Hz[a]	$JH7,8$/Hz	$\delta H(3)_{eq}$/ppm	$\Delta\delta$ H(9)–H(9′)/ppm	Configuration[b]
2	1.7	8.0	—	0.28	β
4	5.9	6.5	—	0.32	α
5	1.4	4.0	—	0.38	β
6	7.5	7.9	—	0.21	α
10	6.3	8.1	—	0.34	α
11	7.4[c]	8.4	2.45	0.34	α
12	1.1	n.d.	2.71	~1.0[d]	β
13	5.8	n.d.	2.75	n.d.	α
14	1.0	n.d.	2.45	n.d.	β

[a] Identified by long-range HECTOR and measured according to ref. 21. [b] Anomeric (non-carboxyl) substituent. [c] $JC(1)$–$H(3)_{eq}$ = 1.1 Hz. [d] Estimated from COSY spectrum.

requires only three steps (*ca.* 50% overall yield) from glycal **1**; (*ii*) it is a stable, pure α-thioglycoside; (*iii*) it carries a 3-PhS auxiliary group; (*iv*) unreacted **1** and the potentially useful byproduct **3** can be rescued from the reaction mixture; (*v*) **6** is an efficient α-sialyl donor, even with sterically congested aceptors such as **9**[7] (Table 1).

The glycal **1**[8,9] was synthesised (Scheme 1) by treatment of fully acetylated neuraminic acid methyl ester[10,11] (9.3 mmol) with 2 equiv.[12,6] (not 0.2[13]) of fresh (to reduce 4,5-oxazoline[14,15] formation) trimethylsilyl trifluoromethanesulfonate.

Addition of fresh phenyl sulfenyl chloride (23 mmol) to **1** (8.55 mmol) in dichloromethane (30 ml) gave the diastereoisomers **2** (57%) and **3** (19%),[16] and unreacted **1** (10%) after chromatography (chloroform–acetone gradient 40 : 1 → 3 : 1).

Acetolysis of **2** (0.58 mmol) with $Hg(OAc)_2$ (0.71 mmol) in acetic acid–acetic anhydride (2.76 ml; 10 : 1) followed by chromatography (toluene–acetone gradient 4 : 1 → 3 : 1) gave pure **4** and a mixture of **4** and **5** in a total yield of 96% (**4** : **5** *ca.* 5 : 1). Hydrolysis of **2** (1.6 mmol) followed by acetylation of the intermediate β-hemiacetal[17] gave pure **5** (83%).

Treatment of the **4–5** mixture (0.53 mmol) with ethanethiol (1.05 mmol) and boron trifluoride etherate ($BF_3{\cdot}Et_2O$, 2.7 mmol) in dichloromethane (2.5 ml) gave, after chromatography (toluene–acetone 3 : 1) pure **6** in 93% yield; no β-anomer was detected. Similar treatment of **5** gave **6** in 86% yield.

A comparative glycosylation of the hexabenzyl lactoside **9**[7] was performed (Table 1) with donors **6**, **7**[3] and **8**[4] using either methyl sulfenyl bromide–silver trifluoromethanesulfonate[18] or *N*-iodosuccinimide–trifluoromethanesulfonic acid[4] as promoters.

The new donor **6** gave the GM_3-trisaccharide **10** in good yield and very high stereoselectivity, with both the methods used for anomeric activation. Note also the high yield obtained when **6** and **9** were used in a molar ratio of 1 : 1.

The donors **7**[3] and **8**[4] have been used extensively for sialylation of the 3-position of galactose residues; good yields (60–80%) have been reported with acceptors having two or three hydroxy groups unprotected.[4,19,20] However, sialylation of the sterically congested acceptor **9** with **7** and **8** proceeded in only 30–40% yield of GM_3-saccharide **11** (Table 1) and with concomitant formation of the corresponding β-glycoside **12**.

The auxiliary PhS-group was removed by treatment of **10** (0.12 mmol) with triphenyltin hydride (1.2 mmol)–AIBN (0.09 mmol), thus giving **11** (83%) and unreacted **10** (12%) after chromatography (toluene–MeCN gradient 4 : 1 → 2 : 1). We found that triphenyltin hydride is superior to tributyltin hydride, which gave **11** in low yield.

De-*O*-benzylation, de-*O*-acetylation, and hydrolysis of the methyl ester of **11** and **12** gave the TMSEt glycosides **13** and **14** (as the sodium salts) in 98 and 96% yields, respectively.

The anomeric configuration of the sialic acid residues of **2**, **4–6**, **10–14** were determined by measuring the long-range $JC(1)$–$H(3)_{ax}$ coupling constant.[21] As seen in Table 2, all sialic acid residues having an axial carboxyl (ester) group (as in α-glycosides) show couplings in the range 5.8–7.5 Hz, whereas the corresponding equatorial carboxyl compounds show couplings in the range 1.0–1.7 Hz.

Values of $\delta H(3)_{eq}$ have been suggested to be smaller for β- than for α-glycosides.[22] Data in Table 2 serve as a caveat in this respect, since the chemical shift order is reversed in the protected pair **11–12** as compared to the unprotected pair **13–14**.

$JH_{7,8}$ has also been used as an anomeric configuration probe (*ca.* 2 and >7 Hz indicating β and α configuration).[23] In addition, the chemical-shift difference between the two hydrogens at position 9 [$\Delta\delta$ H(9)–H(9′)] is reported to depend on the anomeric configuration, $\Delta\delta$ being *ca.* 1 for β glycosides and <0.5 for α glycosides.[23] However, these empirical rules do not apply for the 2-chloro and 2-*O*-acetyl compounds **2** and **5** (Table 2).

We are grateful to Ulf Nilsson for valuable participation in the NMR analyses. This work was supported by the Swedish Natural Science Research Council.

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References

1 K. Okamoto and T. Goto, *Tetrahedron*, 1990, **46**, 5835.
2 G. Magnusson, *Adv. Drug Deliv. Rev.*, 1993, in the press.
3 A. Marra and P. Sinaÿ, *Carbohydr. Res.*, 1990, **195**, 303.
4 A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida and M. Kiso, *Carbohydr. Res.*, 1991, **212**, 277.
5 Y. Ito and T. Ogawa, *Tetrahedron*, 1990, **46**, 89.
6 K. C. Nicolaou, C. W. Hummel and Y. Iwabuchi, *J. Am. Chem. Soc.*, 1992, **114**, 3126; supplementary material.
7 A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, 1989, **193**, C1.
8 P. Meindl and H. Tuppy, *Montash. Chem.*, 1969, **100**, 1295.
9 K. Okamoto, T. Kondo and T. Goto, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 631.
10 R. Kuhn, P. Lutz and D. L. MacDonald, *Chem. Ber.*, 1966, **99**, 611.
11 A. Marra and P. Sinaÿ, *Carbohydr. Res.*, 1989, **190**, 317.
12 W. Schmidt, R. Christian and E. Zbiral, *Tetrahedron Lett.*, 1988, **29**, 3643.
13 A. Claesson and K. Luthman, *Acta Chem. Scand. Ser. B*, 1982, **36**, 719.
14 V. Kumar, J. Kessler, M. E. Scott, B. H. Patwardhan, S. W. Tanenbaum and M. Flashner, *Carbohydr. Res.*, 1981, **94**, 123.
15 E. Schreiner, E. Zbiral, R. G. Kleineidam and R. Schauer, *Liebigs Ann. Chem.*, 1991, 129.
16 T. Kondo, H. Abe and T. Goto, *Chem. Lett.*, 1988, 1657.
17 K. Ikeda, Y. Nagao and K. Achiwa, *Carbohydr. Res.*, 1992, **224**, 123.
18 F. Dasgupta and P. J. Garegg, *Carbohydr. Res.*, 1988, **177**, C13.
19 H. Lönn and K. Stenvall, *Tetrahedron Lett.*, 1992, **33**, 115.
20 A. K. Ray, U. Nilsson and G. Magnusson, *J. Am. Chem. Soc.*, 1992, **114**, 2256.
21 H. Hori, T. Nakajima, Y. Nishida, H. Ohrui and H. Meguro, *Tetrahedron Lett.*, 1988, **29**, 6317.
22 U. Dabrowski, R. Brossmer, M. Supp and H. Friebolin, *Tetrahedron Lett.*, 1979, **48**, 4637.
23 K. Okamoto, T. Kondo and T. Goto, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 637.

II

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Highly Stereoselective α-Sialylation. Synthesis of GM_3-Saccharide and a Bis-Sialic Acid Unit

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The novel sialyl donor methyl [ethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-thio-3-(phenylthio)-2,3,5-trideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosid]onate (**6**) was synthesized in six steps from *N*-acetylneuraminic acid in an overall yield of 47%. Donor **6** was shown to be superior to conventional sialyl donors in that the sialylation yields were higher, even with sterically hindered and unreactive sialyl acceptors, and the α/β-selectivity was virtually complete.

Introduction

In carbohydrate chemistry, sialylation is considered to be difficult and it is a reaction that often proceeds in relatively low yield.[1] However, with the use of thioglycoside sialyl donors[2] and sterically nonhindered acceptor saccharides, the yields approach those of normal glycosylations (60–80%). Still, the desired products are in many cases contaminated with a small amount of the undesired anomer, and removal of the latter by chromatography is often difficult. Low yields in sialylations are always accompanied by extensive 2,3-elimination of the donor, giving the corresponding sialic acid glycal when unreactive acceptors are used.[1]

Introduction of an elimination-suppressing and stereodirecting 3-S-substituent in the donor has made it possible to sialylate even unreactive acceptors in good yield with high stereoselectivity (these sialylations have been suggested to proceed via a reactive episulfonium intermediate).[3] However, such donors are more laborious to prepare, and removal of an auxiliary 3-S-substituent after the glycosylation step might be difficult. As a consequence, these elaborated donors should be reserved for cases where low yields and difficult separation of diastereomeric products are anticipated.

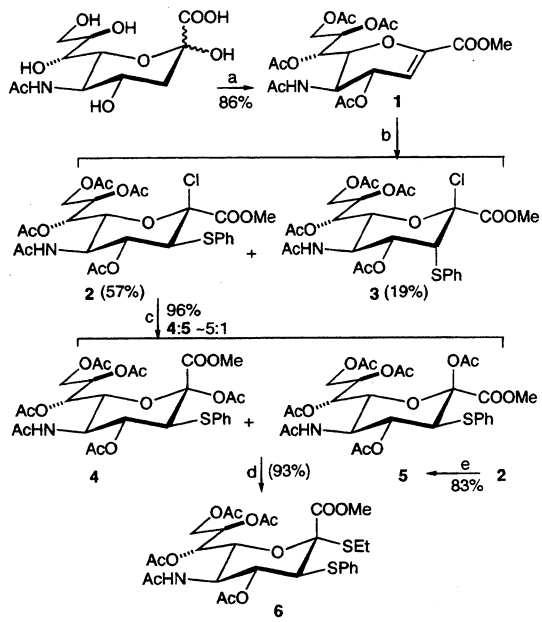
We now give a complete description of the synthesis of a novel sialyl donor (**6**) having several attractive features as stated in the preliminary report,[4] as well as its use for sialylation of some notoriously difficult acceptors, including a sialic acid acceptor (e.g. **10** and **11**).

Results and Discussion

1. Preparation of the Novel Sialyl Donor 6. Sialic acid was treated as described[5] with methanol and Dowex H^+-resin to give the corresponding sialic acid methyl ester, which in turn was acetylated to give the penta-*O*-acetate. Treatment of the latter with fresh trimethylsilyl trifluoromethanesulfonate (TMSOTf, 2 equiv; not 0.2[6]) gave the glycal **1** (92%; 86% from sialic acid, Scheme 1).[7] With lower grade TMSOTf, unwanted formation of a sialic acid 2,3-didehydro-4,5-oxazoline byproduct occurred to a significant extent.[8]

In a slight modification of the original procedure,[9] benzenesulfenyl chloride was added to **1** to give an easily separated mixture of the desired derivative **2** (57%), its

Scheme 1



[a] (a) MeOH, Dowex H^+, Ac_2O, pyridine, TMSOTf, MeCN, 0 °C, 6 h; (b) PhSCl, CH_2Cl_2, 20 °C, 7 d, Ar; (c) $Hg(OAc)_2$, $AcOH/Ac_2O$, 10:1, 40 °C, 18 h; (d) EtSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 20 °C, 18 h; (e) $HgBr_2$, $Hg(CN)_2$, $ClCH_2CH_2Cl/H_2O$, 100:1, reflux, 3.5 h, Ac_2O, pyridine, DMAP, 20 °C, 1 h.

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1995.

(1) (a) Okamoto, K.; Goto, T. *Tetrahedron* **1990**, *46*, 5835. (b) DeNinno, M. P. *Synthesis* **1991**, 583. (c) Kanie, O.; Hindsgaul, O. *Curr. Opin. Struct. Biol.* **1992**, *2*, 674.

(2) (a) Magnusson, G. *Adv. Drug Delivery Rev.* **1994**, *13*, 267. (b) Lönn, H.; Stenvall. K. *Tetrahedron Lett.* **1992**, *33*, 115.

(3) (a) Okamoto, K.; Kondo, T.; Goto, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 631. (b) Ito, Y.; Ogawa, T. *Tetrahedron* **1990**, *46*, 89.

(4) Ercégovic, T.; Magnusson, G. *J. Chem. Soc., Chem. Commun.* **1994**, 831.

(5) (a) Kuhn, R.; Lutz, P.; MacDonald, D. L. *Chem. Ber.* **1966**, *99*, 611. (b) Marra, A.; Sinay, P. *Carbohydr. Res.* **1989**, *190*, 317.

(6) Claesson, A.; Luthman, K. *Acta Chem. Scand., Ser. B* **1982**, *36*, 719.

(7) (a) Schmidt, W.; Christian, R.; Zbiral, E. *Tetrahedron Lett.* **1988**, *29*, 3643. (b) Nicolaou, K. C.; Hummel, C. W.; Iwabuchi, Y. *J. Am. Chem. Soc.* **1992**, *114*, 3126, supplementary material.

(8) (a) Kumar, V.; Kessler, J.; Scott, M. E.; Patwardhan, B. H.; Tanenbaum, S. W.; Flashner, M. *Carbohydr. Res.* **1981**, *94*, 123. (b) Schreiner, E.; Zbiral, E.; Kleineidam, R. G.; Schauer, R. *Liebigs Ann. Chem.* **1991**, 129.

(9) Kondo, T.; Abe, H.; Goto, T. *Chem. Lett.* **1988**, 1657.

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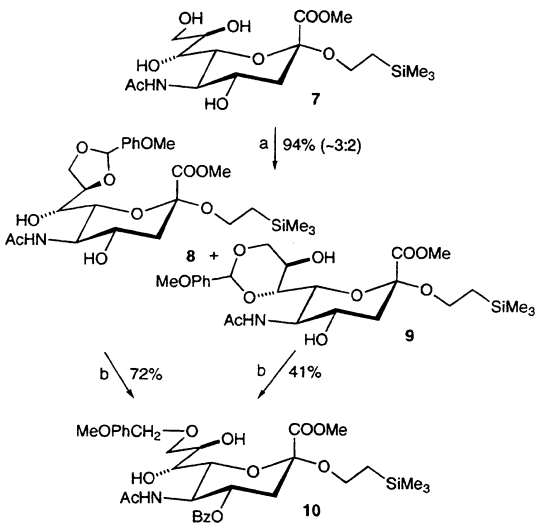
Scheme 2



[a] (a) $MeOPhCH(OMe)_2$, camphor−SO_3H, MeCN; (b) Et_3N, PhCOCl, −30 °C, CH_2Cl_2, BH_3Me_3N, $AlCl_3$, THF, 0 °C.

diastereomer **3** (19%), and unreacted **1** (10%). The somewhat higher **2/3** ratio claimed in the original procedure[9] was not realized in our study. The byproduct **3** might be a useful starting material for the preparation of a new sialic acid donor for β-sialylation.

Acetolysis of **2** with mercuric acetate in acetic acid/acetic anhydride, followed by chromatography of the crude reaction mixture, gave the α-acetate **4** and a mixture of **4** and the β-acetate **5** in a total yield of 96%. Hydrolysis of **2**, followed by acetylation of the intermediate hemiacetal, gave pure **5** (83%).

Treatment of the **4/5** mixture (α/β, ~5:1) with boron trifluoride etherate ($BF_3{\cdot}Et_2O$) and ethanethiol in dichloromethane gave the novel sialyl donor **6** (93%) as a white powder. Compound **6** was a pure α-glycoside; no β-anomer was detected in the reaction mixture. Similar treatment of pure **5** gave **6** in 86% yield. Attempts to obtain crystalline **6** have so far been unsuccessful.

To summarize, the novel sialyl donor **6** was synthesized from the known glycal **1** in ~50% yield over three steps. This should be compared with the synthesis of its benzyl-protected (chlorosugar) counterpart, which requires about six steps.[3] Furthermore, the benzylation step caused us (and others[7b]) problems, and we abandoned the route after several unsuccessful attempts.

Compound **6** is stable and well-suited for use as a sialyl donor, especially when sterically hindered or otherwise unreactive acceptors are used (see below).

2. Preparation of the Sialic Acid Acceptor 10. Disialic acids normally have a 2 → 8 intersaccharidic linkage. The order of reactivity of the four hydroxyl groups in **7** (Scheme 2) was determined to be HO-9 ≫ HO-4 > HO-8 ≫ HO-7.[10] Therefore, it was considered reasonable to anticipate selective sialylation of HO-8 over HO-7 in an acceptor such as **10**.

(10) Ercégovic, T.; Magnusson, G. unpublished work.

The known[11] tetrol **7** was treated with *p*-methoxybenzaldehyde dimethyl acetal and camphor-10-sulfonic acid to furnish the *p*-methoxybenzylidene 8,9-acetal (**8**) (58%) and 7,9-acetal (**9**) (36%). Compound **8** was isolated as a 1:1 diastereomeric mixture, whereas **9** was a pure compound, which slowly epimerized during storage.

Benzoylation of HO-4 in **8**, followed by borane-induced reductive opening of the benzylidene protecting group, gave the desired acceptor **10** (72%). The major byproducts resulted from hydrolysis of the 2-(trimethylsilyl)-ethyl (TMSEt) and/or benzylidene groups. Using the same reaction conditions with compound **9** also gave **10** (41%), showing that the mixture of **8** and **9** can be used for the preparation of **10** without previous separation.

3. Comparison between 6 and Other Sialyl Donors. Sialylation of the sterically congested lactosyl acceptor **11**[12] was considered to be a critical test of the efficiency of various sialyl donors. The most convenient and high-yielding procedures to date are based on sialic acid thioglycoside donors, and therefore, only the xanthate **13**[13] and the methyl α/β-methylthio glycoside **16**[14] were used in the comparison with our novel donor **6** (Scheme 3).

As seen in Table 1, treatment of the acceptor **11** with the donor **6** gave the best result when the acceptor and promotor were used in excess. However, the unexpectedly high yield (54%) of 3-(phenylthio)-α-GM_3-saccharide (**12**) in the experiment where the donor/acceptor ratio was 1:1 is worth noting. A slight preference for the promotor combination of methanesulfenyl bromide/silver trifluoromethanesulfonate (MSB/AgOTf) as compared with *N*-iodosuccinimide/trifluoromethanesulfonic acid (NIS/TfOH) was found. Not only did the use of **6** give an unprecedented high yield with the hindered acceptor **11**, but also as a bonus, the α/β-stereoselectivity was virtually complete. This is in sharp contrast to the case with donors **13** and **16**, which gave mixtures of the α- and β-GM_3 saccharides **14** and **15**. Determination of the anomeric configuration of the sialic acid residue in the sialylation products shown in Scheme 3 was based on the J_{C1-H3a} value[17] as described in the comparative study in our preliminary communication.[4]

The less sterically hindered acceptors **17**, **20**, and **22** were sialylated with the donors **6** and **13**. The products (**18**, **19**, **21**, and **23**) were obtained in approximately the same yields (>70%), but again, the new donor **6** gave pure α-glycosides whereas the xanthate donor **13** gave α/β-mixtures.

The ultimate challenge in sialylation chemistry is the formation of bis-sialic acids, where the glycosidic linkage is present between the 2- and the 8-position of the two monosaccharide units. The donor **6** gave with the acceptor **10** the bis-sialic acid derivative **24** in 28% yield ($J_{C1'-H3'a}$ = 5.9 Hz), and ~40% of the acceptor **10** was recovered; the corresponding β-glycoside was not detected in the reaction mixture. A labile, unidentified bis-

(11) Hasegawa, A.; Ito, Y.; Morita, M.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1989**, *8*, 125.
(12) Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1989**, *193*, C1.
(13) Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1990**, *195*, 303.
(14) Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **1991**, *212*, 277.
(15) Wilstermann, M.; Magnusson, G. Unpublished work.
(16) Ray, A. K.; Nilsson, U.; Magnusson, G. *J. Am. Chem. Soc.* **1992**, *114*, 2256.
(17) Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H.; Meguro, H. *Tetrahedron Lett.* **1988**, *29*, 6317.

Scheme 3

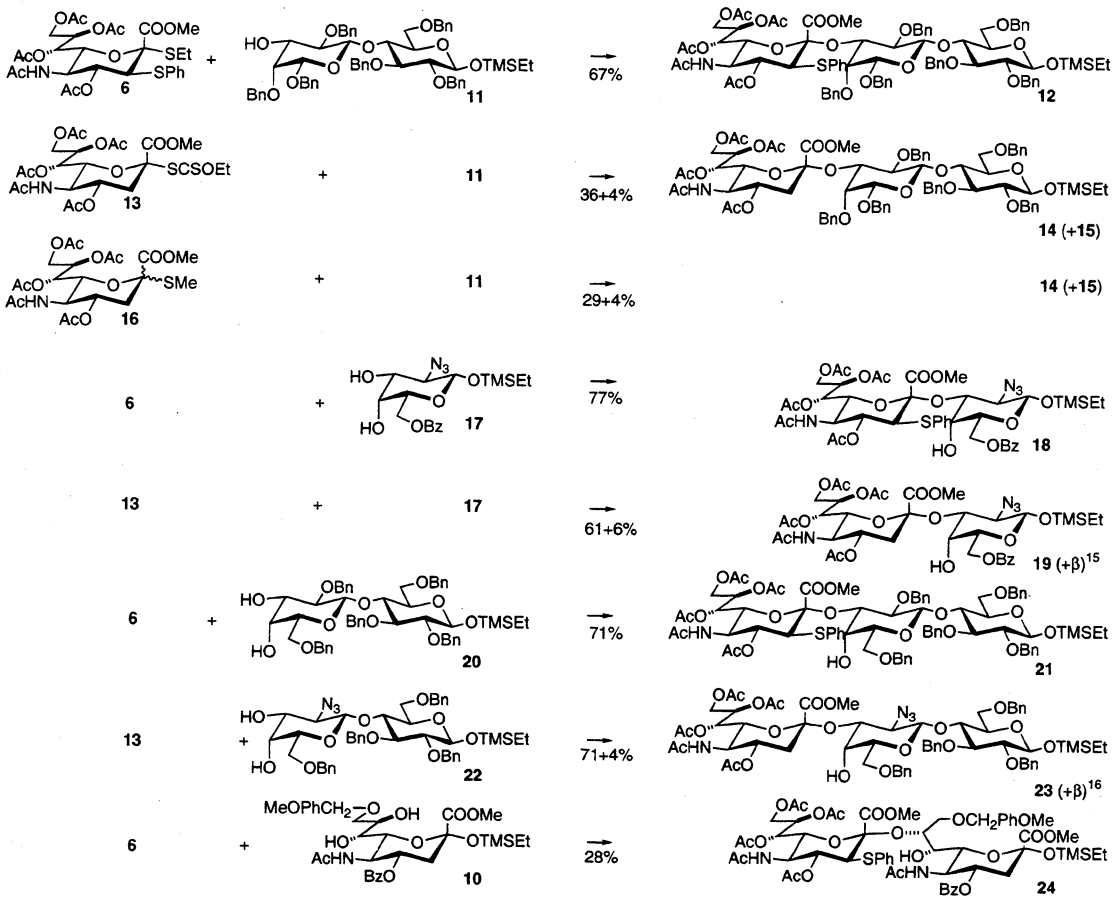


Table 1. Sialylation with the Sialic Acid Donors 6, 13, and 16

donor	acceptor	mole ratio[a]	P1/P2[a]	reaction conditions[b]	product	yield[c] (%)	α/β
6	**11**	1.0/1.0/1.1/1.1	MSB/AgOTf	MeCN/−40 °C	**12**	54	>99/1
6	**11**	1.0/1.5/1.6/1.6	MSB/AgOTf	MeCN/−40 °C	**12**	67	>99/1
6	**11**	1.0/1.5/1.7/0.5	NIS/TfOH	MeCN/−40 °C	**12**	57	>99/1
13	**11**	1.0/1.5/1.3/1.2	MSB/AgOTf	MeCN, CH_2Cl_2/−60 °C	**14** + **15**	36 + 4	90/10
16	**11**	1.0/1.5/1.5/0.7	NIS/TfOH	MeCN/−40 °C	**14** + **15**	29 + 4	88/12
6	**17**	1.0/1.0/1.3/1.3	MSB/AgOTf	MeCN/−40 °C	**18**	77	>99/1
13	**17**	1.0/0.7/1.0/1.0	MSB/AgOTf	MeCN, CH_2Cl_2/−78 °C	**19**[d]	61 + 6	90/10
6	**20**	1.0/1.5/1.4/1.4	MSB/AgOTf	MeCN/−40 °C	**21**	71	>99/1
13	**22**	1.0/0.5/1.0/1.0	MSB/AgOTf	MeCN, CH_2Cl_2/−78 °C	**23**[e]	71 + 4	95/5
6	**10**	1.0/0.6/1.4/1.4	MSB/AgOTf	MeCN/−40 °C	**24**	28	>99/1

[a] Donor/acceptor/promotor 1 (P1)/promotor 2 (P2). [b] The concentration of the acceptor **11** was ~0.10 M. [c] Based on the starting material (donor or acceptor) present in the smallest amount. [d] **19** was acetylated before isolation; this experiment was described in ref 15. [e] This experiment was described in ref 16.

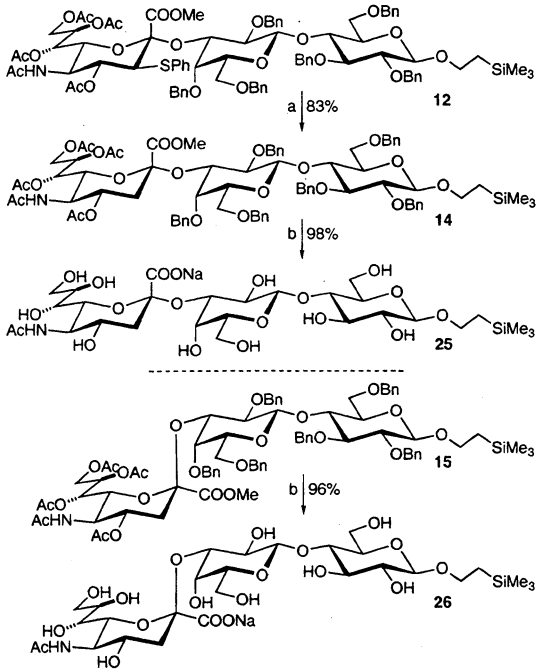
sialoside was also formed in an amount corresponding to the missing 20−30% of **10**. When the sialylation of **10** was attempted with **13** as the donor, the desired bis-sialic acid product was not formed; only elimination occurred to give the glycal **1** (85%). Such elimination reactions normally occur on attempted sialylation of highly hindered acceptors.[1a,b] Previous attempts to sialylate the 8-position in sialic acid acceptors either gave very low yields (~5%) or produced the β-anomer.[18,19]

4. Preparation of the GM_3-Trisaccharide. The auxiliary 3-phenylthio group in **12** was removed by treatment with triphenyltin hydride/azoisobutyronitrile in refluxing toluene, which gave the protected GM_3-trisaccharide **14** (83%) and recovered **12** (12%). When tributyltin hydride was used instead of triphenyltin hydride, the yield dropped to ~50%, with extensive formation of byproducts (Scheme 4).

The protecting groups of **14** were removed in a one-pot, three-step procedure consisting of hydrogenolysis of the benzyl groups, methanolysis of the acetate groups, and hydrolysis of the methyl ester. The GM_3-trisaccha-

(18) Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1988**, *7*, 501.
(19) Tsvektov, Y. E.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, 35, *8583*.

Scheme 4



[a] (a) AIBN, Ph_3SnH, toluene, 110 °C, Ar; (b) H_2, Pd/C, HOAc, rt, MeONa, MeOH, rt, Ar, NaOH, H_2O.

ride **25** was thus obtained as the sodium salt in 98% yield. Using the same reaction conditions with the β-anomer **15** gave the sodium salt of β-GM_3 (**26**) in 96% yield.

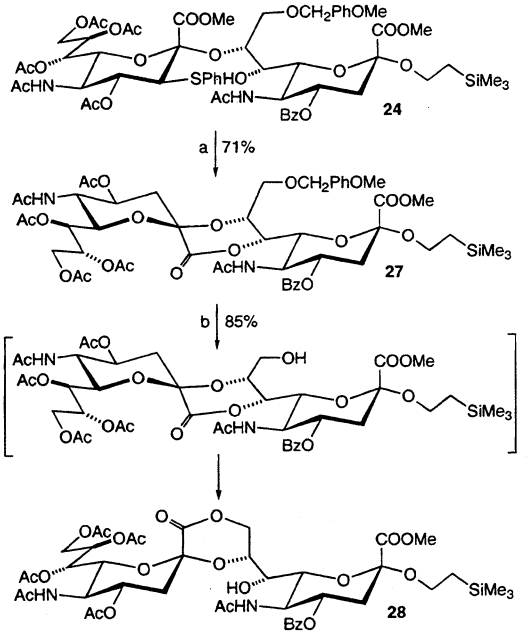
5. Lactonization of Bis-Sialic Acid Derivatives. The bis-sialic acid moiety present in gangliosides such as GD_3 and GQ_{1b} is known to undergo [8,9]-lactonization upon acid treatment.[20] We found that the bis-sialoside **24** formed the stable [8,7]-lactone **27** (71%) during the desulfurization reaction (Scheme 5). During hydrogenolysis of the 9-*O*-*p*-methoxybenzyl group of **27** in ethanol, the [8,7]-lactone isomerized to the more stable [8,9]-lactone **28** (85%), presumably via the corresponding unprotected [8,7]-lactone as indicated by TLC monitoring. Since ethanol was used as solvent, it might attack the lactone ring of the [8,7]-lactone; the intermediacy of an ethyl ester en route to **28** could therefore not be ruled out.

When the hydrogenolysis was performed in *N*,*N*-dimethylformamide, the [8,7]-lactone seemed to be much less prone to rearrange into **28**. Furthermore, an equilibrium was found to exist (~2:1) between the [8,9]- and [8,7]-lactones in *N*,*N*-dimethylformamide-d_7 solution; pure **28** rearranged slowly into the corresponding [8,7]-lactone, as monitored by ^1H NMR over 2 weeks. These data indicate that the lactone rearrangements in bis-sialic acid residues can take place both via an intermediate ester and by direct nucleophilic attack of the hydroxyl group on the lactone carbonyl; an open form intermediate is therefore not required.

Conclusions

The novel sialyl donor **6** is a powerful sialylating agent even with sterically hindered and unreactive acceptors,

Scheme 5



[a] (a) AIBN, Ph_3SnH, toluene, 110 °C, Ar; (b) H_2, Pd/C, EtOH, rt.

such as **10** and **11**. With less hindered acceptors, such as **17**, **20**, and **22**, the donor **6** gives approximately the same glycosylation yields as conventional sialic acid thioglycoside donors (e.g. **13** and **16**) but the stereoselectivity is much higher. The high selectivity is especially useful when diastereomeric products of sialylation are difficult to separate, as is often the case with oligosaccharides consisting of more than two monosaccharide units.

Experimental Section

General. NMR spectra were recorded with 500 and 300 MHz spectrometers. Assignment of ^1H NMR spectra was achieved using 2D-methods. Optical rotations were measured at 20 °C. Reactions were monitored by TLC using alumina plates coated with silica gel 60 F_{254} (Merck) and visualized either by using UV light or by charring with H_3PO_4 (aqueous 10% spray solution). Preparative chromatography was performed with Merck silica gel (35–70 μm, 60 Å). $CHCl_3$ used for preparative chromatography contained 1% of EtOH. CH_2Cl_2, toluene, and THF were distilled under N_2, over CaH_2 and sodium benzophenone ketyl, respectively. MeCN and NEt_3 were stored over 3 Å molecular sieves and filtered through a column of Al_2O_3 (activity I, Merck) immediately before use. Methanol was dried over 3 Å molecular sieves >3 days before use. Compounds obtained as white powders were precipitated with *n*-hexane from a chloroform/diethyl ether solution.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (1). To a stirred, ice-cooled solution of methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (4.97 g, 9.32 mmol) in MeCN (12.5 mL) was added trimethylsilyl trifluoromethanesulfonate (3.6 mL, 19 mmol) under Ar. The reaction mixture was kept at 2 °C for 6 h, and then pyridine (10 mL) was added slowly (ice-cooling),

(20) Ando, S.; Yu, R. K.; Scarsdale, J. N.; Kusunoki, S.; Prestegard, J. H. *J. Biol. Chem.* **1989**, *264*, 3478.

followed by toluene (25 mL). The mixture was concentrated, and the residue was chromatographed (toluene/acetone, 3:1 → 2:1, gradient) to give **1** (4.05 g, 92%) as a white foam. The ^{1}H NMR data were in agreement with those reported.[3a]

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-3-(phenylthio)-2,3,5-trideoxy-D-*erythro*-β-L-*gluco*-2-nonulopyranosonate (2) and Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-3-(phenylthio)-2,3,5-trideoxy-D-*erythro*-β-L-*manno*-2-nonulopyranosonate (3). To a solution of **1** (4.05 g, 8.55 mmol) in CH_2Cl_2 (30 mL) was added benzenesulfenyl chloride (2.6 mL, 23 mmol) under Ar, and the mixture was left with protection from light at 20 °C. After 7 days, the mixture was concentrated and the residue was chromatographed ($CHCl_3$/acetone, 40:1 → 12:1 → 8:1 → 3:1) to give **2** (2.99 g, 57%), **3** (0.979 g, 19%), and unreacted **1** (0.405 g, 10%), all as white powders. The ^{1}H NMR data were in agreement with those reported.[9]

Methyl 5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-3-(phenylthio)-D-*erythro*-α-L-*gluco*-2-nonulopyranosonate (4) and Methyl 5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-3-(phenylthio)-D-*erythro*-β-L-*gluco*-2-nonulopyranosonate (5). To a stirred solution of **2** (358 mg, 0.579 mmol) in HOAc (2.5 mL) were added Ac_2O (0.26 mL) and mercuric acetate (227 mg, 0.712 mmol), and the mixture was kept at 40 °C for 18 h. Toluene (10 mL) was added, and the solution was concentrated. The residue was dissolved in $CHCl_3$/Et_2O (1:4, 20 mL) and washed with an aqueous 10% solution of KI (8 mL) and brine (4 mL). The organic phase was concentrated, and the residue was chromatographed (toluene/acetone, 4:1 → 3:1, gradient) to give **4** (124 mg) and a mixture of **4** and **5** (3.6:1, 234 mg) as white powders. The powders were combined to give a mixture of **4** and **5** (5.2:1, 358 mg, 96%). Compound **4**: $[\alpha]_D$ +17.3 (*c* 0.99, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51−7.28 (m, 5 H), 5.47 (d, 1 H, *J* = 10.2 Hz), 5.36 (dd, 1 H, *J* = 2.3, 6.5 Hz), 5.18 (dd, 1 H, *J* = 11.0, 10.1 Hz), 5.11 (ddd, 1 H, *J* = 2.5, 5.8 Hz), 4.97 (dd, 1 H, *J* = 10.9 Hz), 4.34 (q, 1 H, *J* = 10.1 Hz), 4.34 (dd, 1 H, *J* = 12.5, 2.5 Hz), 4.02 (dd, 1 H, *J* = 5.8, 12.5 Hz), 3.84 (s, 3 H), 3.62 (d, 1 H, *J* = 11.0 Hz), 2.10, 2.07, 2.03, 2.01, 1.89, 1.86 (s, 3 H each); MS calcd for $C_{28}H_{36}NO_{14}S$ (M + H$^+$) 642.1857, found 642.1849. Compound **5**: $[\alpha]_D$ +6.7 (*c* 0.93, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39−7.23 (m, 5 H), 5.51 (d, 1 H, *J* = 10.0 Hz), 5.37 (dd, 1 H, *J* = 2.3, 4.0 Hz), 5.28 (dd, 1 H, *J* = 11.0, 10.4 Hz), 4.97 (ddd, 1 H, *J* = 2.4, 6.8, 4.0 Hz), 4.51 (dd, 1 H, *J* = 12.4, 2.4 Hz), 4.28 (q, 1 H, *J* = 10.1 Hz), 4.13 (dd, 1 H, *J* = 6.8, 12.4 Hz), 4.00 (dd, 1 H, *J* = 10.7, 2.3 Hz), 3.67 (s, 3 H), 3.46 (d, 1 H, *J* = 11.0 Hz), 2.21, 2.14, 2.04, 2.03, 1.95, 1.88 (s, 3 H each); HRMS calcd for $C_{28}H_{36}NO_{14}S$ (M + H$^+$) 642.1857, found 642.1859.

Methyl [Ethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-thio-3-(phenylthio)-2,3,5-trideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosid]onate (6). (a) To a stirred mixture of **4** and **5** (5.2:1, 337 mg, 0.525 mmol) in CH_2Cl_2 (2.5 mL) were added ethanethiol (0.078 mL, 1.05 mmol) and BF_3·Et_2O (0.34 mL, 2.7 mmol) under Ar, and the mixture was left at rt overnight. Pyridine/H_2O (4:1, 1 mL) was added under ice-cooling, stirring was continued for 10 min, and toluene (10 mL) was added. The mixture was concentrated, and the residue was chromatographed (toluene/acetone, 3:1) to give **6** (315 mg, 93%) as a white powder: $[\alpha]_D$ +61.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56−7.25 (m, 5 H), 5.36 (d, 1 H, *J* = 10.1 Hz), 5.31 (m, 1 H), 5.28 (dd, 1 H, *J* = 1.9, 7.9 Hz), 5.17 (dd, 1 H, *J* = 11.2, 10.1 Hz), 4.31 (dd, 1 H, *J* = 2.4, 12.4 Hz), 4.10 (q, 1 H, *J* = 10.1 Hz), 4.10 (dd, 1 H, *J* = 4.6 Hz), 3.89 (s, 3 H), 3.81 (dd, 1 H, *J* = 10.9 Hz), 3.42 (d, 1 H, *J* = 11.2 Hz), 2.83 (dq, 1 H, *J* = 12.1, 7.5 Hz), 2,77 (dq, 1 H, *J* = 12.1, 7.5 Hz), 2.15, 2.12, 2.04, 1.94, 1.86 (s, 3 H each), 1.24 (t, 3H); HRMS calcd for $C_{28}H_{38}NO_{12}S_2$ (M + H$^+$) 644.1835, found 644.1824.

(b) Compound **5** (60.3 mg, 0.094 mmol) was dissolved in CH_2Cl_2 (0.6 mL) and treated with ethanethiol (0.014 mL, 0.19 mmol) and BF_3·Et_2O (0.060 mL, 0.48 mmol) as described above. Workup and purification gave **6** (52 mg, 86%) as a white powder.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-8,9-*O*-(*p*-methoxybenzylidene)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (8) and Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-7,9-*O*-(*p*-methoxybenzylidene)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (9). To a stirred solution of **7**[11] (206 mg, 0.487 mmol) in MeCN (2.5 mL) were added anisaldehyde dimethyl acetal (0.2 mL, 1.2 mmol) and (±)-camphor-10-sulfonic acid (15 mg, 0.064 mmol) at rt. After 1 h, Et_3N (0.020 mL) was added. The mixture was concentrated, and the residue was chromatographed (CH_2Cl_2/MeOH, 40:1 → 25:1 → 12:1, gradient) to give **8** (154 mg, 58%) and **9** (95 mg, 36%) both as white powders. Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.16 (d, 1 H, *J* = 8.1 Hz), 5.90 (d, 1 H, *J* = 7.9 Hz), 5.89, 5.77 (s, 1 H each), 3.80, 3.80, 3.75, 3.73 (s, 3 H each), 2.72−2.62 (m, 1 H), 2.04, 2.00 (s, 3 H each), 1.84−1.73 (m, 1 H); HRMS calcd for $C_{25}H_{40}NO_{10}Si$ (M + H$^+$) 542.2373, found 542.2428. A sample of **8** was acetylated: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.45 (dd, 1 H, *J* = 1.9, 4.1 Hz), 5.19, 5.10 (d, 1 H, *J* = 9.7 Hz), 5.01−4.87 (m, 1 H), 2.18, 2.10, 2.03, 2.03, 1.89, 1.86 (s, 3 H each). Compound **9**: $[\alpha]_D$ −56.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (d, 2 H, *J* = 8.8 Hz), 6.90 (d, 2 H), 5.75 (d, 1 H, *J* = 6.3 Hz), 5.42 (s, 1 H), 3.82, 3.80 (s, 3 H each), 3.41 (m, 1 H), 2.73 (dd, 1 H, *J* = 13.3, 4.7 Hz), 1.97 (s, 3 H), 0.88 (m, 2 H), 0.00 (s, 9 H); HRMS calcd for $C_{25}H_{40}NO_{10}Si$ (M + H$^+$) 542.2373, found 542.2413. A sample of **9** was acetylated: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28 (ddd, 1 H, *J* = 5.2, 10.0 Hz), 4.91 (ddd, 1 H, *J* = 4.6, 12.3, 10.5 Hz), 2.14, 2.05, 1.96 (s, 3 H each).

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-4-*O*-benzoyl-3,5-dideoxy-9-*O*-(*p*-methoxybenzyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (10). (a) To a stirred solution of **8** (191 mg, 0.353 mmol) in CH_2Cl_2 (3 mL) was added Et_3N (0.15 mL, 1.1 mmol) under Ar. The temperature was lowered to −50 °C, and benzoyl chloride (0.049 mL, 0.42 mmol) was added. The mixture was left at −30 °C for 16 h, and H_2O (0.012 mL) was added. The cooling bath was removed, and the mixture was stirred vigorously for 10 min and then applied directly onto a short column (2 cm) of silica gel. Elution with toluene/acetone (5:1, 150 mL), followed by concentration and drying in vacuo, gave a crude product which was dissolved in tetrahydrofuran (6 mL). After the mixture was cooled to 0 °C under Ar, BH_3Me_3N (119 mg, 1.63 mmol) and $AlCl_3$ (235 mg, 1.76 mmol) were added with vigorous stirring. After 10 min at 0 °C, the mixture was allowed to attain rt (5 min). Et_2O (8 mL) and ice−water (8 mL) were added, followed by CH_2Cl_2 (30 mL) and 0.1 M aqueous NaCl (30 mL). The organic phase was separated, and the aqueous phase was washed with CH_2Cl_2 (2 × 30 mL). The organic phases were combined, concentrated, and chromatographed (toluene/acetone, 4:1) to give **10** (165 mg, 72%) as a white foam: $[\alpha]_D$ −40.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05−6.83 (m, 9 H), 6.23 (d, 1 H, *J* = 8.0 Hz), 5.18 (ddd, 1 H, *J* = 4.9, 10.5, 12.0 Hz), 4.18−4.07 (m, 2 H), 3.95 (m, 1 H), 3.83 (dd, 1 H, *J* = 2.4, 9.9 Hz), 3.82, 3.79 (s, 3 H each), 3.62 (dd, 1 H, *J* = 6.0, 9.9 Hz), 3.61−3.55 (m, 2 H), 3.41 (m, 1 H), 2.72 (dd, 1 H, *J* = 12.9, 4.9 Hz), 2.15 (dd, 1 H, *J* = 12.0, 12.9 Hz), 1.91 (s, 3 H), 0.88 (m, 2 H), 0.00 (s, 9 H); HRMS calcd for $C_{32}H_{46}NO_{11}Si$ (M + H$^+$) 648.2840, found 648.2859.

(b) Treatment of **9** (84.2 mg, 0.156 mmol) as described above gave **10** (41.1 mg, 41%) as a white powder.

2-(Trimethylsilyl)ethyl 2,3,6-Tri-*O*-benzyl-4-*O*-{2,4,6-tri-*O*-benzyl-3-*O*-[methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-3-(phenylthio)-D-*erythro*-α-L-*gluco*-2-nonulopyranosyl]onate]-β-D-galactopyranosyl]-β-D-glucopyranoside (12). (a) To a mixture of the sialyl donor **6** (94.3 mg, 0.146 mmol), the lactoside acceptor **11**[12] (213 mg, 0.217 mmol), and activated 3 Å molecular sieves (210 mg) was added MeCN (1.9 mL), and the mixture was stirred at rt for 40 min. A solution of silver trifluoromethanesulfonate (62.5 mg, 0.243 mmol) in MeCN (0.3 mL) was added under Ar, and the temperature was lowered to −40 °C. A solution of methanesulfenyl bromide[21] in $ClCH_2CH_2Cl$ (0.085 mL, 0.24 mmol, 2.8 M) was added, and the mixture was stirred for 80 min at −40 °C. iPr_2NH (0.050 mL) was added, the stirring was continued for 5 min, and the mixture was allowed to reach

(21) Dasgupta, F.; Garegg, P. J. *Carbohydr. Res.* **1988**, *177*, C13.

rt. The mixture was filtered (Celite), washed (acetone), and concentrated, and the residue was chromatographed (toluene/acetone, 4:1) to give recovered **11** (113 mg) as a syrup and **12** (153.2 mg, 67%) as a white powder: $[\alpha]_D$ +2.2 (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.40–7.10 (m, 35 H), 5.53 (ddd, 1 H, *J* = 2.5, 5.6, 8.1 Hz), 5.34 (dd, 1 H, *J* = 9.9, 11.3 Hz), 5.33 (dd, 1 H, *J* = 2.5, 8.1 Hz), 5.25 (d, 1 H, *J* = 10.1 Hz), 4.67 (d, 1 H, *J* = 7.6 Hz), 4.56 (dd, 1 H, *J* = 9.9, 2.7 Hz), 4.35 (d, 1 H, *J* = 7.8 Hz), 4.32 (dd, 1 H, *J* = 12.7, 2.5 Hz), 4.21 (q, 1 H, *J* = 10.0 Hz), 3.98 (dd, 1 H, *J* = 5.6, 12.7 Hz), 3.94 (dd, 1 H, *J* = 10.8, 2.5 Hz), 3.82 (s, 3 H), 3.72 (dd, 1 H, *J* = 7.6, 9.9 Hz), 3.52 (t, 1 H, *J* = 9.0 Hz), 3.40 (d, 1 H, *J* = 11.3 Hz), 2.08, 1.97, 1.92, 1.87, 1.84 (s, 3 H each), 1.04 (m, 2 H), 0.03 (s, 9 H); HRMS calcd for $C_{85}H_{102}NO_{23}SiS$ (M + H^+) 1564.6332, found 1564.6370.

(b) To a mixture of **6** (204.8 mg, 0.318 mmol), **11** (472 mg, 0.48 mmol), and activated AW300 molecular sieves (450 mg) was added MeCN (4.0 mL), and the mixture was stirred at rt for 1.5 h. A solution of *N*-iodosuccinimide (118.7 mg, 0.528 mmol) in acetonitrile (0.8 mL) was added under Ar, and the temperature was lowered to −40 °C. Trifluoromethanesulfonic acid (0.013 mL, 0.15 mmol) was added, and the mixture was stirred for 70 min at −40 °C. iPr_2NH (0.2 mL) was added, and the stirring was continued for 5 min. The mixture was filtered (Celite), washed (acetone), and concentrated. The residue was dissolved in $CHCl_3/Et_2O$ (1:5, 60 mL) and washed with 0.1 M aqueous NaCl (60 mL). The organic phase was concentrated, and the residue was chromatographed (toluene/acetone, 6:1 → 4:1, gradient) to give **11** (244 mg) and **12** (284.6 mg, 57%).

2-(Trimethylsilyl)ethyl 2,3,6-Tri-*O*-benzyl-4-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-β-D-galactopyranosyl]-β-D-glucopyranoside (14) and 2-(Trimethylsilyl)ethyl 2,3,6-Tri-*O*-benzyl-4-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-β-D-galactopyranosyl]-β-D-glucopyranoside (15). (a) To a mixture of the sialyl donor **13** (59.1 mg, 0.099 mmol), **11** (146 mg, 0.148 mmol), and activated 3 Å molecular sieves (160 mg) was added MeCN/CH_2Cl_2 (1:1, 1.2 mL), and the mixture was stirred at rt for 45 min. A solution of silver trifluoromethanesulfonate (31.3 mg, 0.122 mmol) in MeCN (0.3 mL) was added under Ar, and the temperature was lowered to −60 °C. Methanesulfenyl bromide[21] in $ClCH_2CH_2Cl$ (0.045 mL, 0.13 mmol, 2.8 M) was added dropwise over 5 min, and the mixture was stirred for 1 h at −60 °C. iPr_2NH (0.050 mL) was added, and the stirring was continued for 5 min. The mixture was allowed to attain rt, filtered (Celite), washed (acetone), and concentrated. The residue was chromatographed (toluene/acetone, 4:1) to give **11** (77 mg, 78 mmol) and **15** (5.0 mg, 3.5%) as syrups and **14** (52.0 mg, 36%) as a white powder. Compound **14**: $[\alpha]_D$ −11.0 (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.38–7.10 (m, 30 H), 5.47 (ddd, 1 H, *J* = 8.4, 2.7, 5.5 Hz), 5.32 (dd, 1 H, *J* = 2.0, 8.4 Hz), 5.18 (d, 1 H, *J* = 10.1 Hz), 4.93 (m, 1 H), 4.59 (d, 1 H, *J* = 7.6 Hz), 4.38 (d, 1 H, *J* = 7.8 Hz), 4.33 (dd, 1 H, *J* = 12.4, 2.7 Hz), 4.08 (q, 1 H, *J* = 10.1 Hz), 4.07 (dd, 1 H, *J* = 3.2, 9.8 Hz), 3.99 (dd, 1 H, *J* = 5.5, 12.4 Hz), 3.91 (dd, 1 H, *J* = 10.7, 2.0 Hz), 3.72 (d, 1 H, *J* = 3.2 Hz), 3.71 (s, 3 H), 3.66 (dd, 1 H, *J* = 9.8, 7.6 Hz), 3.55 (t, 1 H, *J* = 9.0 Hz), 3.40 (dd, 1 H, *J* = 9.1, 7.8 Hz), 2.45 (dd, 1 H, *J* = 13.2, 4.8 Hz), 2.12 (1 H), 2.12, 2.02, 1.99, 1.91, 1.88 (s, 3H each), 1.05 (m, 2 H), 0.03 (s, 9 H); HRMS calcd for $C_{79}H_{98}NO_{23}Si$ (M + H^+) 1456.6299, found 1456.6296.

Compound **15**: $[\alpha]_D$ +1.5 (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.40–7.08 (m, 30 H), 5.16–5.13 (m, 2 H), 5.05 (ddd, 1 H, *J* = 11.6, 4.6, 10.4 Hz), 4.99 (dd, 1 H, *J* = 2.2, 12.5 Hz), 4.54 (d, 1 H, *J* = 7.6 Hz), 4.37 (d, 1 H, *J* = 7.8 Hz), 3.97 (dd, 1 H, *J* = 10.0, 9.4 Hz), 3.86 (dd, 1 H, *J* = 11.1, 1.9 Hz), 3.78 (dd, 1 H, *J* = 10.0, 7.6 Hz), 3.69 (d, 1 H, *J* = 10.3 Hz), 3.52 (s, 3 H), 3.39 (dd, 1 H, *J* = 7.8, 9.4 Hz), 2.71 (dd, 1 H, *J* = 13.6, 4.6 Hz), 2.06, 2.02, 1.98, 1.92, 1.64 (s, 3 H each), 1.81 (dd, 1 H, *J* = 11.6, 13.6 Hz), 1.04 (m, 2 H), 0.03 (s, 9 H); HRMS calcd for $C_{79}H_{98}NO_{23}Si$ (M + H^+) 1456.6299, found 1456.6267.

(b) To a mixture of the sialyl donor **16** (109.6 mg, 0.210 mmol), **11** (312 mg, 0.317 mmol), and activated AW300 molecular sieves (360 mg) was added MeCN (2.6 mL), and the mixture was stirred at rt for 1.5 h. A solution of *N*-iodosuccinimide (69.2 mg, 0.308 mmol) in MeCN (0.65 mL) was added under Ar, and the temperature was lowered to −40 °C. Trifluoromethanesulfonic acid (0.012 mL, 0.137 mmol) was added, and the reaction mixture was stirred for 1.5 h at −40 °C. iPr_2NH (0.13 mL) was added, and the stirring was continued for 5 min, after which the reaction mixture was allowed to reach rt. The mixture was filtered (Celite), washed (acetone), and concentrated. The residue was dissolved in $CHCl_3/Et_2O$ (1:5, 42 mL) and washed with 0.1 M aqueous NaCl (40 mL). The organic phase was concentrated, and the residue was chromatographed (toluene/acetone, 6:1 → 4:1, gradient) to give **11** (225 mg), **15** (11.7 mg, 3.8%), and **14** (90.2 mg, 29%).

(c) To a stirred solution of **12** (184.1 mg, 118 mmol) and azoisobutyronitrile (15 mg, 0.092 mmol) in toluene (1.5 mL) was added a solution of triphenyltin hydride in toluene (0.70 mL, 1.2 mmol, 1.9 M) under Ar. After refluxing for 14 h, the mixture was cooled to rt and applied directly on a silica gel column. Elution (toluene/acetonitrile, 4:1 → 2:1, gradient) gave recovered **12** (21.7 mg, 12%) and **14** (141.7 mg, 83%), both as white powders.

2-(Trimethylsilyl)ethyl 2-Azido-6-*O*-benzoyl-2-deoxy-4-*O*-[methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-3-(phenylthio)-D-*erythro*-α-L-*gluco*-2-nonulopyranosyl]onate]-β-D-galactopyranoside (18). A solution of **6** (36.2 mg, 0.056 mmol) and **17** (23.5 mg, 0.057 mmol) in MeCN (0.55 mL) was stirred for 1 h at rt with 3 Å molecular sieves (0.16 g) under Ar. A solution of silver trifluoromethanesulfonate (18.2 mg, 0.071 mmol) in MeCN (0.25 mL) was added, and the temperature was lowered to −40 °C. A solution of methanesulfenyl bromide[21] in $ClCH_2CH_2Cl$ (0.025 mL, 0.070 mmol, ~2.8 M) was added in five portions during 10 min. After 3 h, Et_3N (0.2 mL) was added, the stirring was continued for 5 min at −40 °C, and toluene (5 mL) was added. The mixture was filtered (Celite), washed (toluene/acetone, 1:1), and concentrated. The residue was chromatographed (toluene/acetone, 3:1) to give **18** (42.9 mg, 77%) as a white powder: $[\alpha]_D$ +13 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.06–7.26 (m, 10 H), 5.49 (m, 1 H), 5.43 (ddd, *J* = 8.7, 2.8, 5.7 Hz), 5.38 (m, 1 H), 5.30 (m, 1 H), 4.58 (dd, 1 H, *J* = 5.2, 11.5 Hz), 4.55 (dd, 1 H, *J* = 7.5, 11.5 Hz), 4.30 (dd, 1 H, *J* = 10.0, 3.1 Hz), 4.28 (m, 2 H), 4.25 (dd, 1 H, *J* = 12.6, 2.8 Hz), 4.24 (d, 1 H, *J* = 8.2 Hz), 4.05 (bd, 1 H, *J* = 3.1 Hz), 4.02 (dd, 1 H, *J* = 5.7, 12.6 Hz), 3.98 (m, 1 H), 3.87 (s, 3 H), 3.78 (ddd, 1 H, *J* = 0.9, 5.2, 7.5 Hz), 3.61 (m, 1 H), 3.52 (d, 1 H, *J* = 11.3 Hz), 3.38 (dd, 1 H, *J* = 8.2, 10.0 Hz), 2.12, 2.05, 2.02, 2.01, 1.90 (s, 3 H each), 1.02 (m, 2 H), −0.01 (s, 9 H); $J_{C1'-H3'}$ = 5.9 Hz, cf. ref 17; HRMS calcd for $C_{44}H_{59}N_4O_{18}SSi$ (M + H^+) 991.3314, found 991.3321. A sample of **18** was acetylated: ^1H NMR (500 MHz, $CDCl_3$) δ 5.46 (bd, 1 H, *J* = 3.6 Hz), 4.86 (dd, 1 H, *J* = 10.0, 3.6 Hz), 4.41 (d, 1 H, *J* = 8.2 Hz), 3.66 (dd, 1 H, *J* = 8.2, 10.0 Hz), 3.36 (d, 1 H, *J* = 11.4 Hz), 2.10, 2.08, 2.05, 1.96, 1.93, 1.86 (s, 3H each).

2-(Trimethylsilyl)ethyl 2,3,6-Tri-*O*-benzyl-4-*O*-[2,6-di-*O*-benzyl-3-*O*-[methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-3-(phenylthio)-D-*erythro*-α-L-*gluco*-2-nonulopyranosyl]onate]-β-D-galactopyranosyl]-β-D-glucopyranoside (21). A mixture of **6** (73.2 mg, 0.114 mmol), the lactoside acceptor **20**[22] (152.1 mg, 0.170 mmol), MeCN (1.4 mL), and 3 Å molecular sieves (0.25 g) was stirred for 30 min at rt under Ar. A solution of silver trifluoromethanesulfonate (41.8 mg, 0.163 mmol) in MeCN (0.3 mL) was added, and the temperature was lowered to −40 °C. A solution of methanesulfenyl bromide[21] in $ClCH_2CH_2Cl$ (0.057 mL, 0.16 mmol, ~2.8 M) was added in portions during 10 min. After 30 min, iPr_2NH (0.050 mL) was added, and the stirring was continued for 5 min at −40 °C. The reaction mixture was filtered through a short silica gel column (toluene/acetone, 2:1). The eluate was concentrated, and the residue was chromatographed (toluene/acetone, 6:1 → 4:1, gradient) to give recovered **20** (79.6 mg) as a syrup and **21** (118.2 mg, 71%) as a white powder: $[\alpha]_D$ +17.0 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.44–7.17 (m, 30

(22) Hasegawa, A.; Nagahama, T.; Ohki, H.; Kiso, M. *J. Carbohydr. Chem.* **1992**, *11*, 699.

H), 5.40 (ddd, 1 H, J = 7.9, 2.6, 5.8 Hz), 5.32 (dd, 1 H, J = 2.2, 7.9 Hz), 5.30 (d, 1 H, J = 9.6 Hz), 5.28 (dd, 1 H, J = 10.2, 11.2 Hz), 4.57 (d, 1 H, J = 7.8 Hz), 4.36 (d, 1 H, J = 7.8 Hz), 4.35 (dd, 1 H, J = 3.3, 9.3 Hz), 4.31 (dd, 1 H, J = 12.5, 2.6 Hz), 4.28 (q, 1 H, J = 10.1 Hz), 4.12 (dd, 1 H, J = 10.8, 2.2 Hz), 4.00 (m, 1 H), 3.97 (dd, 1 H, J = 5.8, 12.5 Hz), 3.93 (d, 1 H, J = 9.1 Hz), 3.89 (bs, 1 H), 3.84 (s, 3 H), 3.59 (m, 1 H), 3.55 (t, 1 H, J = 9.1 Hz), 3.44 (dd, 1 H, J = 9.3, 7.8 Hz), 3.38 (dd, 1 H, J = 7.8, 9.1 Hz), 3.34 (d, 1 H, J = 11.2 Hz), 2.65 (bs, 1 H), 2.05, 1.97, 1.96, 1.92, 1.89 (s, 3 H each), 1.03 (m, 2 H), 0.02 (s, 9 H); $J_{C1''-H3''}$ = 6.0 Hz, cf. ref 17; HRMS calcd for $C_{78}H_{96}NO_{23}SSi$ (M + H^+) 1474.5863, found 1474.5894. A sample of **21** was acetylated: ^1H NMR (500 MHz, $CDCl_3$) δ 5.38 (d, 1 H, J = 3.3 Hz), 4.83 (dd, 1 H, J = 9.3, 3.3 Hz), 4.78 (d, 1 H, J = 7.7 Hz), 4.36 (d, 1 H, J = 7.8 Hz), 3.57 (t, 1 H, J = 9.0 Hz), 3.52 (dd, 1 H, J = 7.7, 9.3 Hz), 3.40 (dd, 1 H, J = 7.8, 9.0 Hz), 2.08, 1.96, 1.95, 1.87, 1.85, 1.80 (s, 3 H each).

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-4-*O*-benzoyl-3,5-dideoxy-9-*O*-(*p*-methoxybenzyl)-8-*O*-[methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3-(phenylthio)-3,5-dideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosid]onate]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (24). A mixture of **6** (201 mg, 0.312 mmol), the acceptor **10** (128.1 mg, 0.198 mmol), 3 Å molecular sieves (0.25 g), and MeCN (1.0 mL) was stirred for 30 min at rt under Ar. A solution of silver trifluoromethanesulfonate (111 mg, 0.432 mmol) in MeCN (0.25 mL) was added, and the temperature was lowered to −40 °C. A solution of methanesulfenyl bromide[21] in $ClCH_2CH_2Cl$ (0.16 mL, 0.44 mmol, ~2.8 M) was added in portions during 10 min. After 2 h, iPr_2NH (0.070 mL) was added, and the stirring was continued for 10 min at −40 °C. The reaction mixture was filtered (Celite, acetone) and concentrated. The residue was chromatographed (toluene/acetone, 4:1 → 2:1, gradient) to give recovered **10** (47 mg, 38%) as a syrup and **24** (69.1 mg, 28%) as a white powder: $[\alpha]_D$ +11 (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.03−6.84 (m, 14 H), 6.12 (d, 1 H, J = 9.0 Hz), 5.41 (t, 1 H, J = 10.3 Hz), 5.39 (ddd, 1 H, J = 2.7, 7.4, 8.7 Hz), 5.30 (d, 1 H, J = 10.0 Hz), 5.26 (m, 1 H), 5.23 (dd, 1 H, J = 1.7, 8.7 Hz), 4.89 (dt, 1 H, J = 2.1, 7.4 Hz), 4.40 (d, 1 H, J = 11.4 Hz), 4.39 (dd, 1 H, J = 12.3, 2.7 Hz), 4.30 (q, 2 H, J = 10.0 Hz), 4.23 (d, 1 H, J = 11.4 Hz), 4.09 (dd, 1 H, J = 10.8, 1.7 Hz), 4.07 (dd, 1 H, J = 7.4, 12.3 Hz), 4.03−4.00 (m, 2 H), 3.90−3.81 (m, 2 H), 3.81, 3.81, 3.79 (s, 3 H each), 3.75 (m, 1 H), 3.67 (d, 1 H, J = 10.3 Hz), 3.39 (m, 1 H), 2.77 (dd, 1 H, J = 12.9, 5.1 Hz), 2.18, 2.09, 2.07, 1.91, 1.90, 1.88 (s, 3 H each), 0.79 (t, 2 H, J = 7.8 Hz), −0.04 (s, 9 H); $J_{C1'-H3'}$ = 5.9 Hz, cf. ref 17; HRMS calcd for $C_{58}H_{77}N_2O_{23}SSi$ (M + H^+) 1229.4407, found 1229.4384.

2-(Trimethylsilyl)ethyl 4-*O*-[3-*O*-[Sodium (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)-onate]-β-D-galactopyranosyl]-β-D-glucopyranoside (25). Compound **14** (67.6 mg, 0.0464 mmol) was dissolved in HOAc (6 mL), and the mixture was hydrogenated (H_2, 1 atm, Pd/C, 10%, 61 mg) overnight. The mixture was filtered (Celite, HOAc) and coconcentrated with MeOH/H_2O (2:1) and MeOH, and the residue was dried in vacuo. The dried residue was dissolved in dry MeOH (1 mL), and methanolic sodium methoxide (0.007 mL, ~2 M) was added at rt under Ar. After 2 h, Duolite C26 (H^+) resin was added. The mixture was filtered and concentrated, and the residue was dissolved in H_2O (0.5 mL) and treated with aqueous sodium hydroxide (0.024 mL, 0.048 mmol, 2.013 M) for 35 min at rt. The mixture was applied to a Sephadex G10 (Pharmacia) column. The material was eluted with water, and the fractions containing the product were first freeze-dried and then dried with a good oil pump to give **25** (34.5 mg, 98%) as a white foam: $[\alpha]_D$ −6.3 (c 1.00, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.53 (d, 1 H, J = 7.9 Hz), 4.49 (d, 1 H, J = 8.0 Hz), 4.11 (dd, 1 H, J = 9.9, 3.1 Hz), 4.03 (ddd, 1 H, J = 5.2, 10.0, 12.6 Hz), 3.95 (d, 1 H, J = 3.1 Hz), 3.27 (t, 1 H, J = 8.6 Hz), 2.75 (dd, 1 H, J = 4.7, 12.5 Hz), 2.02 (s, 3 H), 1.80 (t, 1 H, J = 12.2 Hz), 1.07 (dt, 1 H, J = 5.5, 12.8 Hz), 0.97 (dt, 1 H, J = 5.3, 12.8 Hz), 0.02 (s, 9 H); HRMS calcd for $C_{28}H_{51}NO_{19}SiNa$ (M + H^+) 756.2722, found 756.2723.

2-(Trimethylsilyl)ethyl 4-*O*-[3-*O*-[Sodium (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)-onate]-β-D-galactopyranosyl]-β-D-glucopyranoside (26). Compound **15** (22.2 mg, 0.015 mmol) was treated as above to give **26** (11.0 mg, 96%) as a white foam: $[\alpha]_D$ −16 (c 0.85, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.48 (d, 1 H, J = 8.0 Hz), 4.47 (d, 1 H, J = 7.7 Hz), 4.24 (d, 1 H, J = 3.1 Hz), 4.17 (m, 1 H), 4.02 (ddd, 1 H, J = 5.3, 10.0, 12.5 Hz), 3.28 (dd, 1 H, J = 8.9, 8.0 Hz), 2.45 (dd, 1 H, J = 13.0, 4.7 Hz), 2.04 (s, 3 H), 1.68 (dd, 1 H, J = 11.8, 13.0 Hz), 1.06 (dt, 1 H, J = 5.5, 12.9 Hz), 0.97 (dt, 1 H, J = 5.2, 12.8 Hz), 0.02 (s, 9 H); HRMS calcd for $C_{28}H_{51}NO_{19}SiNa$ (M + H^+) 756.2722, found 756.2723.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-4-*O*-benzoyl-3,5-dideoxy-9-*O*-(*p*-methoxybenzyl)-8-*O*-[5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosyloyl-1′ → 7-lactone]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (27). To a stirred solution of **24** (111.8 mg, 0.090 mmol) and azoisobutyronitrile (14.7 mg, 0.895 mmol) in toluene (1.5 mL) was added a solution of triphenyltin hydride in toluene (0.5 mL, ~2 M, ~1 mmol) under Ar. After refluxing for 16 h, the mixture was allowed to reach rt and then applied directly to a silica gel column and chromatographed (toluene/acetone, 4:1 → 3:1, gradient) to give **27** as a white powder (70.2 mg, 71%): $[\alpha]_D$ −56 (c 0.98, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.01−6.92 (m, 9 H), 5.64 (ddd, 1 H, J = 5.6, 11.2, 10.3 Hz), 5.39−5.32 (m, 2 H), 5.27 (d, 1 H, J = 10.4 Hz), 5.04 (ddd, 1 H, J = 4.5, 12.2, 10.4 Hz), 4.97 (d, 1 H, J = 9.6 Hz), 4.94 (dt, 1 H, J = 4.7, 7.7 Hz), 4.59 (d, 1 H, J = 11.0 Hz), 4.49 (d, 1 H, J = 11.0 Hz), 4.49 (d, 1 H, J = 4.7 Hz), 4.40 (q, 1 H, J = 10.1 Hz), 4.23 (dd, 1 H, J = 2.4, 12.5 Hz), 4.21 (q, 1H, J = 10.1 Hz), 4.14 (d, 1 H, J = 10.6 Hz), 3.98 (dd, 1 H, J = 4.8, 12.5 Hz), 3.93 (dd, 1 H, J = 2.2, 10.7 Hz), 3.92 (d, 2 H, J = 7.9 Hz), 3.88 (s, 3 H), 3.82 (s, 3 H), 3.75 (m, 1 H), 3.39 (m, 1 H), 2.67 (dd, 1 H, J = 12.6, 4.5 Hz), 2.36 (dd, 1 H, J = 13.3, 5.6 Hz), 2.15, 2.11, 2.03, 1.99, 1.89, 1.66 (s, 3 H each), 2.06 (t, 1 H, J = 12.8 Hz), 1.79 (dd, 1 H, J = 11.2, 13.3 Hz), 0.81 (m, 2 H), −0.01 (s, 9 H); HRMS calcd for $C_{51}H_{69}N_2O_{22}Si$ (M + H^+) 1089.4111, found 1089.4100.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-4-*O*-benzoyl-3,5-dideoxy-8-*O*-[5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosyloyl-1′ → 9-lactone]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (28). A solution of **27** (14.8 mg, 0.0136 mmol) in EtOH (5 mL) was hydrogenated (H_2, 1 atm, Pd/C, 10%, 12.1 mg) for 12 h. The mixture was filtered (Celite, toluene/methanol, 1:1, 30 mL), the filtrate was concentrated, and the residue was chromatographed (toluene/acetone, 3:1 → 2:1, gradient) to give **28** (11.2 mg, 85%) as a colorless solid: $[\alpha]_D$ −47 (c 0.95, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.05−7.46 (m, 5 H), 6.22 (d, 1 H, J = 7.9 Hz), 5.51 (ddd, 1 H, J = 5.5, 11.5, 10.4 Hz), 5.35 (d, 1 H, J = 9.5 Hz), 5.35 (dd, 1 H, J = 2.3, 8.2 Hz), 5.30 (ddd, 1 H, J = 4.9, 11.8, 10.5 Hz), 5.16 (ddd, 1 H, J = 2.8, 5.5, 8.2 Hz), 4.77 (d, 1 H, J = 4.8 Hz), 4.65−4.58 (m, 2 H), 4.38 (ddd, 1 H, J = 8.2, 4.3, 9.3 Hz), 4.32 (dd, 1 H, J = 12.6, 2.8 Hz), 4.24 (q, 1 H, J = 10.1 Hz), 4.10 (dt, 1 H, J = 10.5, 7.9 Hz), 4.07 (dd, 1 H, J = 5.5, 12.6 Hz), 3.90 (m, 1 H), 3.86 (dd, 1 H, J = 10.6, 2.3 Hz), 3.84 (s, 3 H), 3.77 (dd, 1 H, J = 1.6, 10.5 Hz), 3.61 (ddd, 1 H, J = 1.6, 4.8, 8.2 Hz), 3.55 (m, 1 H), 2.78 (dd, 1 H, J = 12.7, 4.9 Hz), 2.50 (dd, 1 H, J = 13.5, 5.5 Hz), 2.15, 2.09, 2.05, 2.04, 1.98, 1.90 (s, 3 H each), 1.99 (dd, 1 H, J = 11.5, 13.5 Hz), 0.91 (m, 2 H), 0.02 (s, 9 H); HRMS calcd for $C_{43}H_{61}N_2O_{21}Si$ (M + H^+) 969.3536, found 969.3553.

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Supplementary Material Available: ^1H NMR spectra and ^1H NMR data with assigned signals for all title compounds described in the Experimental Section (22 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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III

Synthesis of a Bis(sialic acid) 8,9-Lactam

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Methyl [2-(trimethylsilyl)ethyl 5-acetamido-9-azido-4-*O*-benzoyl-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate was sialylated with methyl (ethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-thio-3-(phenylthio)-2,3,5-trideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosid)onate to give the corresponding bis(sialic acid) derivative in 23% yield. Removal of protecting groups, reduction of the azido group to an amino group, and removal of the auxiliary thiophenyl group gave the desired bis(sialic acid) 8,9-lactam. Comparison of the ^{1}H NMR spectra and energy-minimized (MM3) structures of the bis(sialic acid) lactam with those of the corresponding bis(sialic acid) lactone showed the conformations of the two compounds to be very similar (RMS = 0.027 Å).

Introduction

Glycosphingolipids that contain sialic acid moieties (gangliosides) are present on the surface of mammalian cells.[1] Tumor cells often carry abnormal amounts of some gangliosides, which has led to the concept of gangliosides as tumor-associated antigens.[2] It is well known that gangliosides form δ-lactones under acidic conditions,[3] and furthermore, the high density of gangliosidic sialic *acid* on many tumor cells may induce the formation of lactones *in vivo*. The δ-lactones were suggested to be the true immunogens in the preparation of antiganglioside antibodies, but the hydrolytic lability of the lactones would make them poor immunogens due to the difficulty of maintaining a high *in vivo* concentration during immunization.[2b,4]

We have prepared ganglioside lactams[5] (G_{M1-4}) and used them as immunogens in order to raise antibodies that cross-react with the corresponding lactones on murine melanoma cells. This work demonstrated that G_{M3}-lactone is indeed present on the cell surface.[6] The lactams have proved to be hydrolytically stable and structurally similar to their lactone counterparts.[5]

Ganglioside G_{D3}, which carries two sialic acid moieties, is abnormally frequent on human malignant melanoma cells as compared to healthy cells.[7] G_{D3} is capable of forming several lactones, of which the 8,9-lactone is formed most readily (Figure 1).[8] We now describe the synthesis of a bis(sialic acid) lactam (**1**), suitable for further elaboration into G_{D3}-lactam and other ganglioside lactams containing a bis(sialic acid) moiety. The synthesis is based on our novel sialyl donor[9] **12** and acceptor **6**.

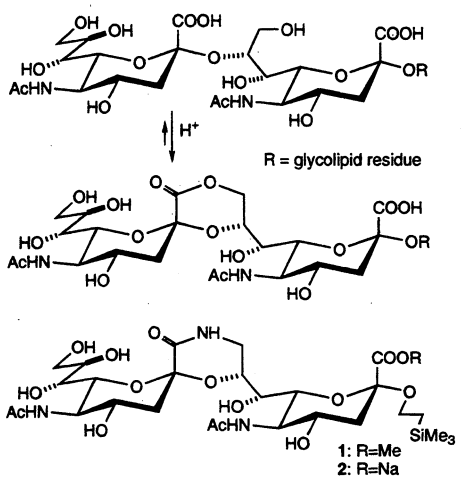

Figure 1. Bis(sialic acid) lactone and the synthetic lactam analogs **1** and **2**.

Results and Discussion

Our synthetic strategy was based on stereoselective 8-*O*-sialylation of the acceptor **6**, rather than introduction of a nitrogen functionality in the 9-position of commercially available bis(sialic acid) (for numbering, see Figure 2). The latter forms a quite stable 8,9-lactone,[10]

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(1) (a) Hakomori, S. *Ann. Rev. Biochem.* **1981**, *50*, 733. (b) Karlsson, K.-A. In *Biological Membranes;* Chapman, D., Ed.; Academic Press: London, 1982; Vol. 4, pp 1–74. (c) Gigg, J.; Gigg, R. *Top. Curr. Chem.* **1990**, *154*, 77. (d) Sweeley, C. C. In *Biochemistry of Lipids, Lipoproteins and Membranes*; Vance, D. E., Vance, J., Eds.; Elsevier Science Publishers B. V.: Oxford, 1991; pp 327–361.

(2) (a) Yogeeswaran, G. *Adv. Cancer Res.* **1983**, *38*, 289. (b) Hirabayashi, Y.; Hanaoka, A.; Matsumoto, M.; Matsubara, T.; Tagawa, M.; Wakabayashi, S.; Taniguchi, M. *J. Biol. Chem.* **1985**, *260*, 13328. (c) Hakomori, S. *Chem. Phys. Lipids* **1986**, *42*, 209.

(3) (a) Wiegandt, H. *Physiol. Biol. Chem. Exp. Pharmacol.* **1966**, *57*, 190. (b) Gross, S. K.; Williams, M. A.; McCluer, R. H. *J. Neurochem.* **1980**, *34*, 1351. (c) Riboni, L.; Sonnino, S.; Acquotti, D.; Malesci, A.; Ghidoni, R.; Egge, H.; Mingrino, S.; Tettamanti, G. *J. Biol. Chem.* **1986**, *261*, 8514. (d) Bassi, R.; Riboni, L.; Sonnino, S.; Tettamanti, G. *Carbohydr. Res.* **1989**, *193*, 141. (e) Maggio, B.; Ariga, T.; Yu, R. K. *Biochemistry* **1990**, *29*, 8729.

(4) Nores, G. A.; Dohi, T.; Taniguchi, M.; Hakomori, S. *J. Immunol.* **1987**, *139*, 3171.

(5) (a) Ray, A. K.; Nilsson, U.; Magnusson, G. *J. Am. Chem. Soc.* **1992**, *114*, 2256. (b) Wilstermann, M.; Kononov, L. O.; Nilsson, U.; Ray, A. K.; Magnusson, G. *J. Am. Chem. Soc.* **1995**, *117*, 4742.

(6) (a) Ding, K.; Rosén, A.; Ray, A. K.; Magnusson, G. *Glycoconjugate. J.* **1992**, *9*, 303. (b) Magnusson, G.; Ding, K.; Nilsson, U.; Ray, A. K.; Rosén, A.; Sjögren, H.-A. In *Complex Carbohydrates in Drug Research;* Bock, K., Clausen, H., Eds.; Munksgaard: Copenhagen, **1994**; pp 89–100.

(7) (a) Livingston, P. J.; Ritter, G.; Calves, M. J. *Cancer Immunol. Immunother.* **1989**, *29*, 179. (b) Pukel, C. S.; Lloyd, K. O.; Travassos, L. R.; Dippold, W. G.; Oettgen, H. F.; Old, L. J. *J. Exp. Med.* **1982**, *155*, 1133. (c) Portoukalian, J.; Zwingelstein, G.; Abdul-Malak, N.; Doré, J. F. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 916.

(8) Ando, S.; Yu, R. K.; Scarsdale, J. N.; Kusunoki, S.; Prestegard, J. H. *J. Biol. Chem.* **1989**, *264*, 3478.

(9) (a) Ercégovic, T.; Magnusson, G. *J. Chem. Soc., Chem. Commun.* **1994**, 831. (b) Ercégovic, T.; Magnusson, G. *J. Org. Chem.* **1995**, *60*, 3378.

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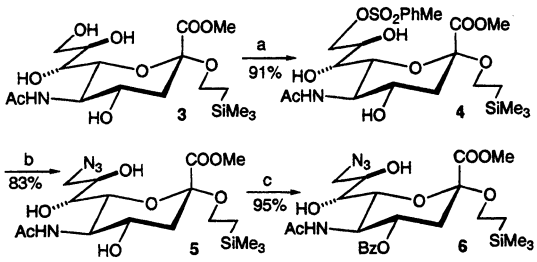
Scheme 1[a]



[a] Key: (a) *p*-MePhSO$_2$Cl, CH$_2$Cl$_2$/pyridine, −80 °C, 48 h; (b) NaN$_3$, 18-crown-6, DMF, 60 °C, 20 h; (c) BzCl, Et$_3$N, −30 °C, 18 h.

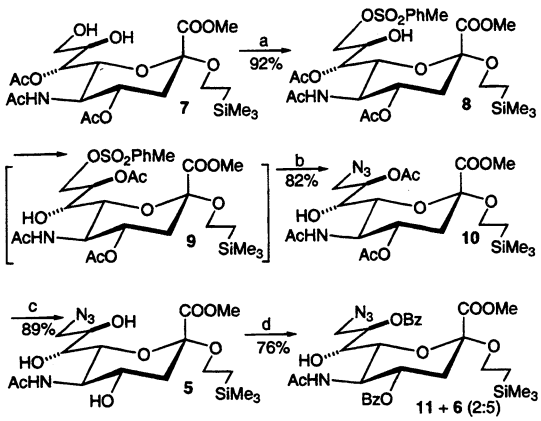
and it is by no means trivial to exploit this for regioselective introduction of nitrogen at C-9.

The sialic ester glycoside **3**[11] was treated with 2 equiv of *p*-toluenesulfonyl chloride at −80 °C, which gave the 9-*O*-tosylate **4** (91%) after chromatography (Scheme 1). Regioselective 9-*O*-tosylations of sialic ester glycosides have been conducted earlier.[12] Treatment of the tosylate **4** with sodium azide in *N,N*-dimethylformamide in the presence of 18-crown-6 ether as catalyst gave the 9-azido compound **5** (83%).

At this point, the reactivity order was not settled for the three secondary hydroxyl groups in **5**. Treatment of **5** with benzoyl chloride in dichloromethane/pyridine at −80 °C (Scheme 2) gave the desired monobenzoate **6** (55%) and the dibenzoate **11** (21%). In order to improve the yield of **6**, alternative benzoylation procedures were investigated. Regioselective *O*-benzoylation has been performed using benzoyl cyanide/triethylamine or 1-(benzoyloxy)benzotriazole/triethylamine.[13] Since benzoyl cyanide is reported to be unreactive in the absense of triethylamine in aprotic solvents,[13a] we thought that benzoyltriethylammonium cyanide was the reactive species needed for regioselective benzoylation to occur. Hence, we expected that the new combination benzoyl chloride/triethylamine (Scheme 1) would constitute a viable alternative. Treatment of **5** with the latter reagent combination in dichloromethane at −30 °C gave the desired sialyl acceptor **6** (95%) as the only product formed. The order of reactivity of the four hydroxyl groups in **3** was thus established to be HO-9 >> HO-4 > HO-8 >> HO-7.

Prior to the successful preparation of **6** (Scheme 1), we investigated the regioselective introduction of a 9-azido group in the known diol **7**[14] (Scheme 2). Treatment of **7** with a large excess of *p*-toluenesulfonyl chloride gave the monotosylate **8** (92%). However, treatment of **8** with lithium azide in tetrahydrofuran, using 15-crown-5 ether as catalyst, caused the 7-*O*-acetyl group to migrate to the 8-position, and **10** (82%) was the only compound isolated. Monitoring the reaction by TLC revealed the intermediate formation of a strongly UV-absorbing compound,

Scheme 2[a]



[a] Key: (a) *p*-MePhSO$_2$Cl, pyridine, 0 → 20 °C, 22 h; (b) LiN$_3$, 15-crown-5, THF, 45 °C, 24 h; (c) MeONa, MeOH, 20 °C, 2 h; (d) BzCl, CH$_2$Cl$_2$/pyridine, −80 °C, 96 h.

which had disappeared when the reaction was complete. It is thus expected that the transformation of **8** into **10** proceeds *via* the tosylate **9** (Scheme 2). ^{1}H-NMR analysis of the reaction mixture supported this assumption. Tetrabutylammonium azide in acetonitrile gave rapidly the desired product according to TLC analysis. However, rearrangement into **10** occurred during workup of the reaction mixture. De-*O*-acetylation of **10** gave **5** (89%), identical with the product obtained by azide treatment of **4** (Scheme 1). As a consequence of these studies, acyl protection of HO-7 in sialic acids should be avoided and sialylations in the 8-position be performed with acceptors such as **6** that have both HO-7 and HO-8 unprotected, as shown in Scheme 3.

The joining of two sialic acid units *via* an α2→8 glycosidic bond in high yield and αβ selectivity is difficult, and consequently, many synthetic approaches have been published.[15] In a recent paper, we reported the synthesis of the novel sialyl donor **12**, which was found to be superior to conventional donors. Sialylation yields were higher, even with sterically hindered and unreactive sialyl acceptors, and the αβ-selectivity was virtually complete.[9] Compound **12**, which carries an auxiliary phenylthio substituent[16] in the 3-position, was synthesized from *N*-acetylneuraminic acid in six steps and 47% overall yield.[9]

The 9-azido diol **6** was sialylated by the donor **12** at −40 °C in acetonitrile, using methyl sulfenyl bromide[17] /silver trifluoromethane sulfonate as promotor, to give the α2→8 bis(sialoside) **13** in 28% yield (Scheme 3). When the concentration of the reactants was halved, the yield of **13** was reduced to 23%. However, the main part (63%) of acceptor **6** was thus recovered, and the yield, based on consumed **6**, was raised from 47% to 64% when the more diluted reaction mixture was employed. According to TLC and NMR analysis, additional bis(sialosides) were formed, but these were extremely labile and could not be isolated; their identity remains un-

(10) (a) Abbas, S. Z.; Sugiyama, S.; Diakur, J.; Pon, R. A.; Roy, R. *J. Carbohydr. Chem.* **1990**, *9*, 891. (b) Hasegawa, A.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1993**, *12*, 371.

(11) Hasegawa, A.; Ito, Y.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1989**, *8*, 125.

(12) (a) Warner, T. G. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 1323. (b) Isecke, R.; Brossmer, R. *Tetrahedron* **1994**, *50*, 7445.

(13) (a) Holy, A.; Sovcek, M.; *Tetrahedron Lett.* **1971**, 185. (b) Abbas, S. A.; Haines, A. H. *Carbohydr. Res.* **1975**, *39*, 358. (c) Kim, S.; Chang, H.; Kim, W. J. *J. Org. Chem.* **1985**, *50*, 1751.

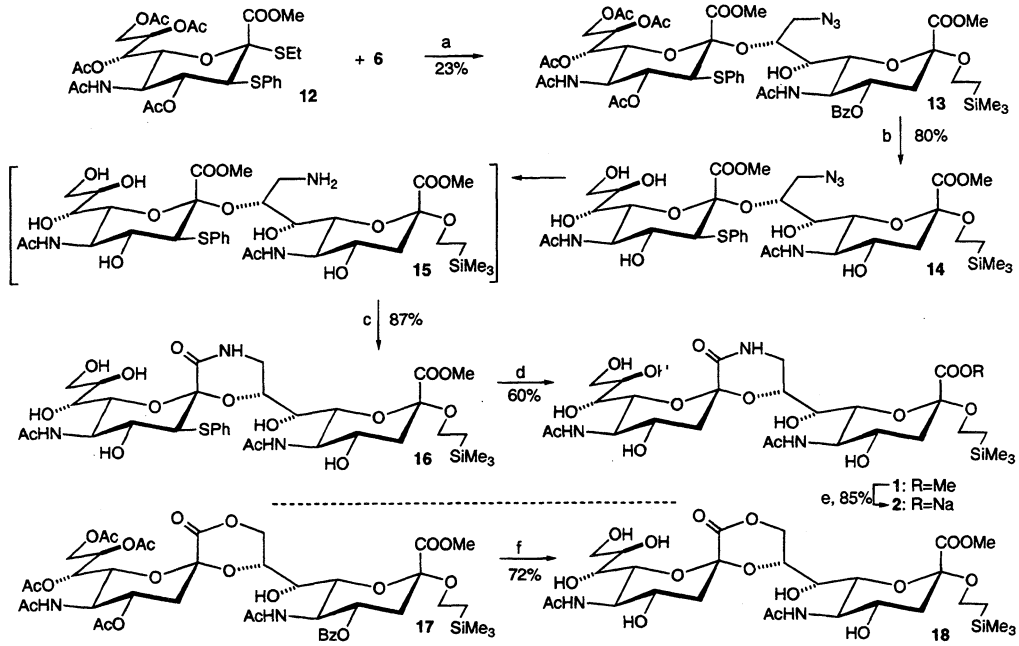
(14) Hasegawa, A.; Ito, Y.; Morita, M.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1989**, *8*, 135.

(15) (a) Okamoto, K.; Kondo, T.; Goto, T. *Tetrahedron Lett.* **1986**, *43*, 5229. (b) Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1988**, *7*, 501. (c) Tsvektov, Y. E.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, *35*, 8583.

(16) Ito, Y.; Ogawa, T. *Tetrahedron* **1990**, *46*, 89.

(17) Dasgupta, F.; Garegg, P. J. *Carbohydr. Res.* **1988**, *177*, C13.

Scheme 3[a]



[a] Key: (a) MeSBr, CF_3SO_3Ag, 3 Å MS, MeCN, −40 °C, 2 h; (b) MeONa, MeOH/toluene, 20 °C, 16 h; (c) Ph_3P, THF/H_2O, 40 °C, 20 h; (d) Ra-Ni, EtOH, 20 °C, 3 h, then Ph_3P, 1 h; (e) NaOH, MeOH/H_2O, 20 °C, 24 h, Sephadex G10; (f) MeONa, MeOH, 20 °C, 24 h, then HOAc, 20 °C, 22 h, freeze-drying.

known. All of the donor **12** was destroyed in the reaction. Since **12** gave high yields (71−77%) with galactoside and lactoside diol acceptors,[9] the low yields obtained with sialic acid diol acceptors indicate that the latter are highly hindered. More stable, yet highly reactive, sialyl donors are thus required in order to obtain high yields in sialylations of sialic acid-derived acceptors in the 8-position. Such donors remain to be developed.

In our previous syntheses of ganglioside lactams, we found that the conditions used for reduction of the azide functionality caused the intermediate amino ester to lactamize.[5] However, reduction of the 9-azido group of **13**, using hydrogen sulfide as reducing agent, gave almost no lactamization. De-*O*-acylation of **13** (→ **14**, 80%), followed by reduction of **14** with triphenylphosphine, gave the desired 8,9-lactam **16** (presumably via the amine **15**) in 87% yield.

Reductive removal of the auxiliary phenylthio substituent of **16** was performed with Raney nickel in ethanol. Initial attempts gave the desired lactam **1** in <20% yield, due to absorption of **1** to the surface of the Raney nickel particles. However, addition of triphenylphosphine to the reaction mixture prior to filtration caused desorption of **1** and an acceptable yield of 60% was obtained. The yield might well be improved by optimizing the type and amount of the desorption agent. It deserves to be mentioned that the phenylthio group could not be removed by traditional tin hydride reagents;[16,18] either several products were formed or no reaction occurred.

Hydrolysis of the methyl ester function of **1** gave the sodium salt **2** in 85% yield. The successful synthesis of **1** has provided useful information for our planned synthesis of the tetrasaccharidic G_{D3}-ganglioside lactam. Furthermore, compound **1** is well suited as starting material for the preparation of neoglycoprotein antigens, similar in scope to G_{M3}-lactam-BSA used for raising monoclonal antibodies that crossreacted with G_{M3}-lactone on cell surfaces.[6]

The bis(sialic acid) lactone **17**[9b] was de-*O*-acylated, and the crude product was freeze-dried from an acetic acid solution to give lactone **18** (72%) for comparison with **1** concerning the solution conformations of the two compounds. The lactone ring of **18** was partially opened by methanol to give an equilibrium mixture (~9:1) of **18** and the corresponding bis(methyl ester), as indicated in Figure 2C. FAB mass spectroscopy of the mixture gave peaks at *m/z* 697.3 (**18**) and *m/z* 729.3 (bis(methyl ester)), thus confirming the presence of the bis(methyl ester).

Comparison of the ^{1}H-NMR spectra (Figure 2) of **1** and **18** showed that coupling constants diagnostic of the sialic acid- and lactam/lactone-ring conformations are very similar (Figure 2 and Table 1). The conformational similarity was corroborated by a molecular mechanics calculation [MM3(92)][19] of **1** and **18** (Figure 2). Superimposition and RMS-fitting of the low energy conformations of **1** and **18** (using all ring atoms) showed them to have very similar overall shapes (rms = 0.027 Å), as depicted in Figure 2.

(18) Kondo, T.; Abe, H.; Goto, T. *Chem. Lett.* **1988**, 1657.

(19) (a) Burkert, U.; Allinger, N. L. *Molecular Mechanics*; American Chemical Society: Washington, D.C., 1982. (b) The MM3 force-field: Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551. (c) Molecular construction and energy minimization were made with the MacMimic/MM3 package: InStar Software, Ideon Research Park, S-22370 Lund, Sweden.

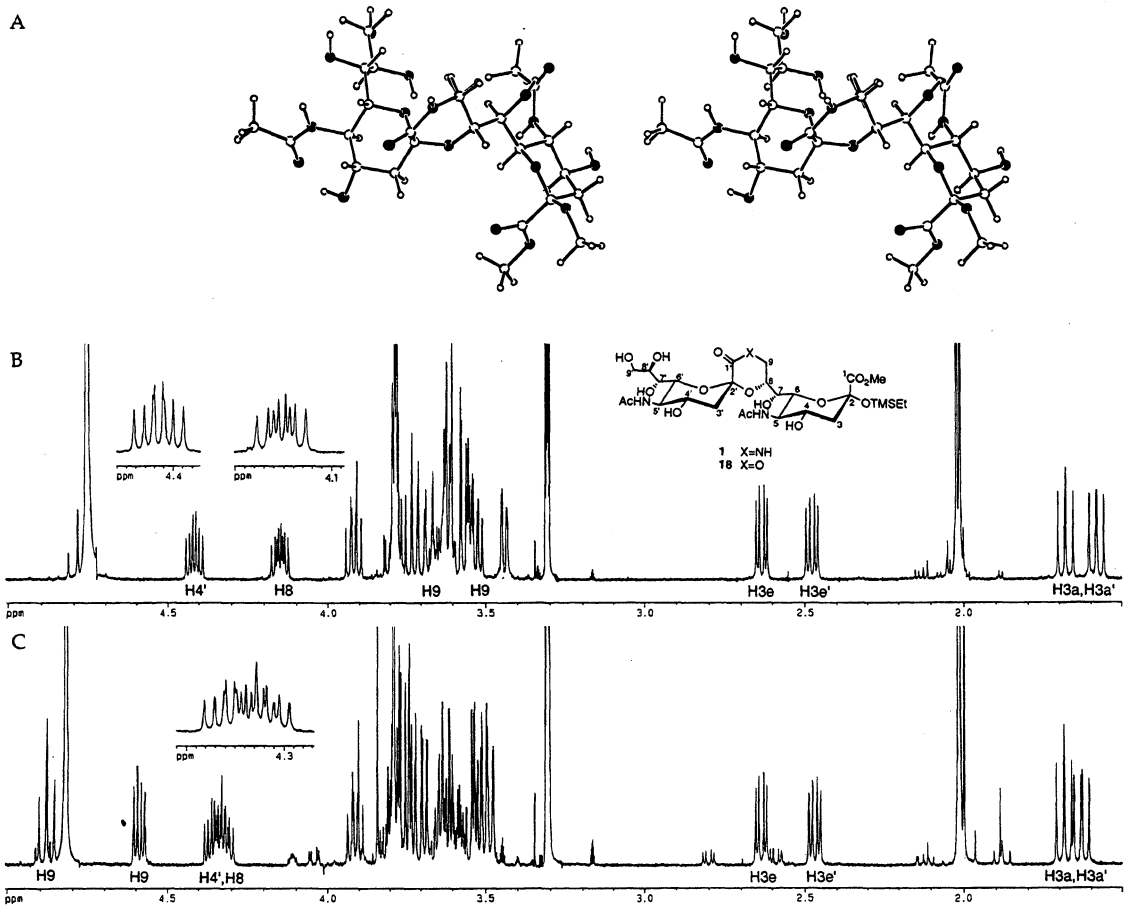


Figure 2. (A) Stereoview of the superimposed low energy [MM3(92)][19] conformers of the methyl glycosides that correspond to **1** and **18**. (B) ^{1}H-NMR (500 MHz, CD_3OD) spectrum of **1**. (C) ^{1}H-NMR (500 MHz, CD_3OD) spectrum of **18** (contaminated by ~10% of the corresponding bis(methyl ester), obtained by reaction of **18** with the solvent).

Table 1. Selected ^{1}H NMR coupling constants for 1 and 18

	coupling constant (Hz)	
coupling[a]	1	18
H_{3a}–H_{3e}	12.6	12.7
H_{3a}–H_4	11.9	12.3
H_{3e}–H_4	4.6	4.6
H_7–H_8	8.6	7.7
H_{9A}–H_{9B}	13.1	11.8
H_8–H_{9A}	11.0	11.6
H_8–H_{9B}	5.9	5.2
$H_{3'a}$–$H_{3'e}$	12.7	13.1
$H_{3'a}$–$H_{4'}$	10.9	11.2
$H_{3'e}$–$H_{4'}$	5.4	5.4

[a] Assignments were made by COSY and HETCOR procedures.

The ^{1}H NMR spectra of **1** and **18** require some additional comments. In both compounds, the ring hydrogen H-4′ is observed at an unusually large chemical shift (~4.4 ppm). This is probably due to close proximity between H-4′ and the carbonyl oxygen of the lactam and lactone rings. The H-4′–O-distance is ~2.6 Å in the energy-minimized structures obtained by the MM3-calculations. Such oxygen-induced chemical shifts have been observed in other saccharides.[20]

As with the mono(sialic acid)-containing lactams reported earlier,[5] bis(sialic acid)-containing lactams also seem to be good substitutes for the natural sialic acid lactones, thus making the lactams potentially useful as hydrolytically stable immunogens for the preparation of, e.g., anti-lactone antibodies.

Experimental Section

General. NMR spectra were recorded at 500 and 300 MHz. Assignment of ^{1}H NMR spectra was achieved using 2D-methods (COSY, HETCOR). Optical rotations were measured at +20 °C. Chemical shifts are expressed in ppm using residual $CHCl_3$, CHD_2OD as reference. Reactions were monitored by TLC using alumina plates coated with silica gel 60 F_{254} (Merck) and visualized using either UV light or by charring with H_3PO_4 (aqueous 10% spray solution). Preparative chromatography was performed with Merck silica gel (35–70 μm, 60 Å). CH_2Cl_2, toluene, and THF were distilled under N_2 over CaH_2 and sodium benzophenone ketyl, respectively. MeCN and NEt_3 were stored over 3 Å molecular sieves and filtered through a column of Al_2O_3 (activity I, Merck) im-

(20) (a) Thögersen, H.; Lemieux, R. U.; Bock, K.; Meyer, B. *Can. J. Chem.* **1982**, *60*, 44. (b) Bock, K.; Frejd, T.; Kihlberg, J.; Magnusson, G. *Carbohydr. Res.* **1988**, *176*, 253. (c) Grönberg, G.; Nilsson, U.; Bock, K.; Magnusson, G. *Carbohydr. Res.* **1994**, *257*, 35.

mediately before use. Methanol was dried over 3 Å molecular sieves >3 days before use. Compounds obtained as white powders were precipitated with *n*-hexane from a chloroform/diethyl ether solution.

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-9-amino-3,5,9-trideoxy-8-*O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate 1′→9-Lactam (1). Compound **16** (8.7 mg, 0.0108 mmol) was stirred vigorously with Raney-Ni (1 mL, 50% slurry in water) in ethanol (4 mL) at room temperature for 3 h. Triphenylphosphine (0.35 g) was added, and the stirring was continued for 1 h. The reaction mixture was filtered through glass fiber paper (Whatman) on a Celite pad (MeOH). The eluate was concentrated, and the residue was chromatographed (CH_2Cl_2/MeOH/H_2O 40:10:1) to give **1** (4.5 mg, 60%) as a white solid: $[\alpha]_D$ −36° (*c* 0.34, CH_3OH); IR (neat) 1738, 1680, 1635 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 4.42 (ddd, 1 H, *J* = 10.9, 5.4, 10.0 Hz), 4.15 (ddd, 1 H, *J* = 5.9, 8.6, 11.0 Hz), 3.92 (m, 1 H), 3.79 (s, 3 H), 3.73 (t, 1 H, *J* = 10.3 Hz), 3.69 (dd, 1 H, *J* = 13.1 Hz), 3.62 (m, 1 H), 3.56 (dd, 1 H, *J* = 1.5, 10.5 Hz), 3.53 (dd, 1 H), 3.44 (m, 1 H), 2.64 (dd, 1 H, *J* = 4.6, 12.6 Hz), 2.48 (dd, 1 H, *J* = 12.7 Hz), 2.02, 2.01, (2 s, 6 H), 1.68 (dd, 1 H, *J* = 11.9 Hz), 1.58 (dd, 1 H), 0.88 (m, 2 H), 0.01 (s, 9 H); ^{13}C NMR (CD_3OD) δ 175.8, 175.4, 171.1, 170.7, 100.7, 99.0, 74.5, 74.1, 73.5, 72.3, 72.0, 69.1, 69.0, 65.4, 62.9, 54.4, 53.8, 42.8, 42.0, 41.9, 23.0, 22.7, 19.2. HR FAB-MS calcd for $C_{28}H_{50}N_3O_{15}Si$ (M + H) 696.3011, found 696.3019.

Sodium {2-(Trimethylsilyl)ethyl 5-acetamido-9-amino-3,5,9-trideoxy-8-*O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate 1′→9-Lactam (2). To a stirred solution of **1** (4.3 mg, 0.0062 mmol) in methanol (0.3 mL) were added water (0.2 mL) and aqueous sodium hydroxide (0.050 mL, 0.2654 M, 0.0133 mmol). After 24 h at room temperature, the mixture was chromatographed (Sephadex G10, H_2O). The eluate was freeze-dried to give **2** (3.7 mg, 85%) as a white foam: $[\alpha]_D$ +6.2° (*c* 0.32, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.33 (ddd, 1 H, *J* = 5.4, 10.0, 11.0 Hz), 4.23 (ddd, 1 H, *J* = 4.7, 6.4, 11.1 Hz), 4.07 (dd, 1 H, *J* = 1.0, 10.6 Hz), 3.91 (t, 1 H, *J* = 10.1 Hz), 3.86 (t, 1 H, *J* = 10.2 Hz), 3.83 (m, 1 H), 3.83 (dd, 1 H, *J* = 2.4, 11.8 Hz), 3.76 (dd, 1 H, *J* = 1.2, 10.5 Hz), 3.70 (dd, 1 H, *J* = 1.2, 6.6 Hz), 3.63 (m, 1 H), 3.54 (m, 1 H), 3.54 (dd, 1 H, *J* = 1.1, 9.3 Hz), 2.66 (dd, 1 H, *J* = 4.4, 12.2 Hz), 2.58 (dd, 1 H, *J* = 13.2 Hz), 2.06, 2.05 (2 s, 6 H), 1.78 (dd, 1 H, *J* = 11.4 Hz), 1.59 (t, 1 H, *J* = 12.2 Hz), 0.94 (m, 2 H), 0.02 (s, 9 H); HR FAB-MS calcd for $C_{27}H_{47}N_3O_{15}SiNa$ (M + H): 704.2674, found 704.2675.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-9-*O*-(*p*-toluenesulfonyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (4). A stirred solution of **3**[11] (244.5 mg, 0.577 mmol) in dry dichloromethane/pyridine 2:1 (4.5 mL) was cooled to −70 °C under an argon atmosphere, and *p*-TsCl (226 mg, 1.18 mmol) was added. The reaction was left at −80 °C without stirring for 2 d, after which water (0.1 mL) was added. Stirring for 10 min at −70 °C and 30 min at rt followed by concentration to a brown syrup which upon chromatography (dichloromethane/methanol 25:1) gave **4** (303 mg, 91%) as a white foam: $[\alpha]_D$ −8.6° (*c* 0.93, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3/CD_3OD$ ~97:3) δ 7.76 (d, 2 H, *J* = 8.3 Hz), 7.32 (d, 2 H), 4.30 (dd, 1 H, *J* = 2.2, 10.0 Hz), 4.12 (d, 1 H, *J* = 5.7 Hz), 4.00 (ddd, 1 H, *J* = 9.2 Hz), 3.79 (m, 1 H), 3.78 (s, 3 H), 3.66 (t, 1 H, *J* = 10.1 Hz), 3.47 (m, 1 H), 3.42 (dd, 1 H, *J* = 1.7 Hz), 3.36−3.26 (m, 2 H), 2.69 (dd, 1 H, *J* = 13.0, 4.7 Hz), 2.41 (s, 3 H), 2.00 (s, 3 H), 1.79 (t, 1 H, *J* = 11.4 Hz), 0.82 (m, 2 H), 0.00 (s, 9 H); HR FAB-MS calcd for $C_{24}H_{40}NO_{11}SSi$ (M + H^+) 578.2091, found 578.2096.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (5). (a) A solution of **4** (256.0 mg, 0.443 mmol), 18-crown-6 ether (44.4 mg, 0.168 mmol), and sodium azide (148 mg, 2.27 mmol) in dry *N*,*N*-dimethylformamide (1.5 mL) was stirred vigorously at 60 °C for 20 h after which the reaction mixture was filtered, coconcentrated with toluene, and chromatographed (CH_2Cl_2 → CH_2Cl_2/EtOH 12:1, gradient) to give **5** (165 mg, 83%) as a white powder: $[\alpha]_D$ +1.1° (*c* 0.95, $CHCl_3$); IR (neat) 2090 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3/CD_3OD$ ~97:3) δ 3.97 (ddd, 1 H, *J* = 9.0, 2.6, 6.3 Hz), 3.82 (m, 1 H), 3.78 (s, 3 H), 3.66 (t, 1 H, *J* = 10.1 Hz), 3.57 (dd, 1 H, *J* = 12.7 Hz), 3.45 (m, 1 H), 3.40 (dd, 1 H, *J* = 1.9 Hz), 3.37−3.27 (m, 3 H), 2.69 (dd, 1 H, *J* = 13.1, 4.7 Hz), 1.99 (s, 3 H), 1.79 (t, 1 H, *J* = 12.5 Hz), 0.82 (m, 2 H), −0.05 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 173.6, 169.9, 98.6, 73.7, 70.6, 69.5, 68.3, 62.0, 53.6, 53.2, 53.0, 40.8, 29.7, 23.2, 18.0. HR FAB-MS calcd for $C_{17}H_{33}N_4O_8Si$ (M + H) 449.2067, found 449.2069.

(b) To a stirred solution of **10** (233 mg, 0.437 mmol) in dry methanol (3 mL) was added a solution of NaOMe in methanol (0.050 mL, ~2 M) under N_2. After 2 h at rt, Amberlite IR-120 was added and the reaction mixture was filtered, washed with methanol, concentrated, and chromatographed (toluene/EtOH 15:1 → 10:1, gradient) to give **5** (175 mg, 89%) as a white powder.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-9-azido-4-*O*-benzoyl-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (6) and Methyl [2-(Trimethylsilyl)-ethyl 5-acetamido-9-azido-4,8-di-*O*-benzoyl-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (11). (a) A stirred solution of **5** (132 mg, 0.295 mmol) in dry dichloromethane/pyridine 2:1 (1.5 mL) was cooled to −70 °C under Ar, and benzoyl chloride (0.034 mL, 0.29 mmol) was added. The mixture was stirred vigorously for 20 min and then kept at −80 °C for 3 d, after which additional benzoyl chloride (0.007 mL, 60 μmol) was added. After 24 h at −80 °C, water (0.050 mL) was added, and the mixture was concentrated and chromatographed (toluene/EtOH 80:1 → 50:1 → 10:1, gradient) to give **11** (41 mg, 21%) and **6** (90 mg, 55%) as white powders and recovered **7** (20 mg, 15%) as a syrup. Compound **6**: $[\alpha]_D$ −31° (*c* 1.00, $CHCl_3$); IR (neat) 2090, 1720 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3/CD_3OD$ ~97:3) δ 7.99, 7.60, 7.45 (5 H), 5.12 (ddd, 1 H, *J* = 12.2, 4.9, 10.5 Hz), 4.14−4.04 (m, 2 H), 3.90 (m, 1 H), 3.86 (s, 3 H), 3.63 (dd, 1 H, *J* = 2.5, 12.8 Hz), 3.52−3.45 (m, 2 H), 3.41 (dd, 1 H, *J*′ = 6.3 Hz), 3.39 (m, 1 H), 2.82 (dd, 1 H, *J* = 12.9 Hz), 2.10 (t, 1 H), 1.91 (s, 3 H), 0.86 (m, 2 H), −0.01 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 173.0, 169.5, 167.5, 133.9, 129.8, 128.7, 128.6, 98.4, 73.9, 70.5, 69.6, 69.4, 62.1, 53.6, 53.3, 51.7, 37.5, 23.0, 17.9; HR FAB-MS calcd for $C_{24}H_{37}N_4O_9Si$ (M + H) 553.2329, found 533.2330.

Compound **11**: $[\alpha]_D$ +8.2° (*c* 1.00, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.06−7.40 (m, 10 H), 6.21 (d, 1 H, *J* = 10.1 Hz), 5.58 (ddd, 1 H, *J* = 8.4, 2.9, 4.2 Hz), 5.18 (ddd, 1 H, *J* = 12.1, 4.7, 10.5 Hz), 4.13 (q, 1 H), 3.95 (m, 1 H), 3.87−3.80 (m, 3 H), 3.75 (dd, 1 H, *J* = 10.5, 1.8 Hz), 3.37 (m, 1 H), 3.31 (s, 3 H), 2.70 (dd, 1 H, *J* = 12.7 Hz), 2.10 (t, 1 H), 1.95 (s, 3 H), 0.91 (m, 2 H), 0.02 (s, 9 H); HR FAB-MS calcd for $C_{31}H_{41}N_4O_{10}Si$ (M + H) 657.2592, found 657.2583.

(b) To a stirred solution of **5** (200.4 mg, 0.447 mmol) in dichloromethane (3 mL) was added dry triethylamine (0.090 mL, 0.65 mmol) at rt under Ar. After cooling to −50 °C and addition of benzoyl chloride (0.055 mL, 0.48 mmol), the mixture was left at −30 °C for 18 h. Methanol (0.010 mL) was added, the mixture was concentrated, and the residue was chromatographed (toluene/EtOH 80:1 → 40:1, gradient) to give **6** (234 mg, 95%) as a white powder.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-4,7-di-*O*-acetyl-3,5-dideoxy-9-*O*-(*p*-toluenesulfonyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (8). To an ice-cooled, stirred solution of **7**[14] (235.8 mg, 0.465 mmol) in dry pyridine (5 mL) was added *p*-toluenesulfonyl chloride (0.30 g, 1.6 mmol) under N_2. After 5 h at 0 °C, the mixture was left at room temperature for 17 h. Methanol was added, the mixture was concentrated, and the residue was chromatographed (toluene/EtOH 30:1) to give **8** (284 mg, 92%) as a white powder: $[\alpha]_D$ −3.3° (*c* 1.01, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.80, 7.33 (2 d, 4 H), 5.24 (d, 1 H, *J* = 10.1 Hz), 4.98 (dd, 1 H, *J* = 2.2, 8.7 Hz), 4.81 (ddd, 1 H, *J* = 12.1, 4.8, 10.3 Hz), 4.17 (m, 1 H), 4.11 (q, 1 H, *J* = 10.3 Hz), 4.08 (dd, 1 H, *J* = 2.8, 10.8 Hz), 3.95 (dd, 1 H, *J*′ = 6.7 Hz), 3.85 (s, 3 H), 3.89−3.78 (m, 2 H), 3.40 (m, 1 H), 2.67 (dd, 1 H, *J* = 13.0 Hz), 2.44 (s, 3 H), 2.08, 2.03 (2 s, 6 H), 1.99 (t, 1 H), 1.85 (s, 3 H), 0.89 (m, 2 H), 0.02 (s, 9 H); HR FAB-MS calcd for $C_{28}H_{44}NO_{13}SSi$ (M + H) 662.2302, found 662.2299.

A sample of **8** was treated with acetic anhydride/pyridine to give the fully acetylated derivative: ^1H NMR (300 MHz,

$CDCl_3$) δ 5.35–5.27 (m, 2 H), 4.35 (dd, 1 H, J = 2.4, 11.5 Hz), 4.01 (dd, 1 H, J = 7.1 Hz), 2.07, 2.06, 2.02 (3 s, 9 H), 1.88 (s, 3 H).

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-4,8-di-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (10). To a stirred solution of **8** (20.0 mg, 0.0302 mmol) in dry tetrahydrofuran (0.6 mL) was added lithium azide (22 mg, 0.45 mmol) and 15-crown-5 ether (0.010 mL). After 24 h at 45 °C, the mixture was filtered and concentrated and the residue was chromatographed (toluene/EtOH 50:1) to give **10** (13.2 mg, 82%) as a colorless syrup: $[\alpha]_D$ −26° (*c* 0.37, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.91 (d, 1 H, J = 7.5 Hz), 5.28 (ddd, 1 H, J = 9.1, 2.9, 3.8 Hz), 4.90 (ddd, 1 H, J = 12.2, 4.6, 10.2 Hz), 4.86 (d, 1 H, J = 4.7 Hz), 3.95 (m, 1 H), 3.89 (m, 1 H), 3.78 (s, 3 H), 3.77–3.59 (m, 4 H), 3.24 (dt, 1 H, J = 7.3, 9.2 Hz), 2.59 (dd, 1 H, J = 12.8 Hz), 2.16, 2.10, 2.01 (3 s, 9 H), 1.95 (t, 1 H), 0.86 (m, 2 H), 0.01 (s, 9 H); HR FAB-MS calcd for $C_{21}H_{37}N_4O_{10}Si$ (M + H) 533.2279, found 533.2264.

A sample of **10** was treated with acetic anhydride/pyridine to give the fully acetylated derivative: ^1H NMR (300 MHz, $CDCl_3$) δ 5.34–5.28 (m, 2 H), 3.66–3.62 (m, 2 H), 2.17, 2.16, 2.03 (3 s, 9 H), 1.88 (s, 3 H).

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-9-azido-4-*O*-benzoyl-3,5,9-trideoxy-8-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3-(phenylthio)-3,5-dideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosid)onate]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate (13). A solution of **6** (84.7 mg, 0.153 mmol) and **12** (160.1 mg, 0.249 mmol) in acetonitrile (1.6 mL) was stirred for 40 min at rt with 3 Å molecular sieves (0.2 g). Silver trifluoromethanesulfonate (72.8 mg, 0.283 mmol) in acetonitrile (0.3 mL) was added under Ar, and the temperature was lowered to −40 °C, after which a solution of methyl sulfenyl bromide[17] in 1,2-dichloromethane (0.103 mL, 2.8 M, 0.28 mmol) was added dropwise over a period of 10 min. After 2 h, diisopropylamine (0.1 mL) was added, the stirring was continued for 5 min at −40 °C, and the mixture was allowed to attain room temperature. The mixture was filtered through a short column of silica gel (toluene/acetone 1:1), the eluate was concentrated, and the residue was chromatographed (toluene/acetone 8:1 → 6:1 → 4:1 → 3:1 → 2:1, gradient) to give recovered **6** (53.6 mg, 63%) as a syrup and **13** (40.5 mg, 23%) as a white powder: $[\alpha]_D$ +14.5° (*c* 1.00, $CHCl_3$); IR (neat) 2095, 1735, 1660 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.03–7.23 (m, 10 H), 6.05 (d, 1 H, J = 8.8 Hz), 5.47 (dd, 1 H, J = 10.6, 9.9 Hz), 5.38 (ddd, 1 H, J = 2.8, 6.4, 8.9 Hz), 5.33 (d, 1 H, J = 9.5 Hz), 5.27 (m, 1 H), 5.26 (dd, 1 H, J = 1.6 Hz), 4.75 (dt, 1 H, J = 2.4, 7.3 Hz), 4.37 (dd, 1 H, J = 12.3 Hz), 4.20 (q, 2 H), 4.15 (dd, 1 H, J = 10.9 Hz), 4.12 (dd, 1 H), 3.99 (broad d, 1 H), 3.94 (dd, 1 H, J = 13.3 Hz), 3.88 (m, 1 H), 3.84, 3.82 (2 s, 6 H), 3.78 (dd, 1 H, J = 10.5, 1.4 Hz), 3.50 (d, 1 H), 3.48 (m, 1 H), 3.40 (dd, 1 H), 2.77 (dd, 1 H, J = 12.8, 4.9 Hz), 2.19, 2.10, 2.07, 2.03 (4 s, 12 H), 2.09 (1 H), 1.92, 1.89 (2 s, 6 H), 0.89 (m, 2 H), 0.02 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 172.2, 171.1, 171.0, 170.6, 170.1, 169.7, 168.3, 168.2, 166.8, 135.4, 133.5, 132.0, 129.8, 129.3, 128.9, 128.5, 127.5, 100.7, 98.6, 75.4, 75.3, 73.0, 72.1, 69.5, 69.4, 68.5, 67.2, 62.7, 62.2, 58.1, 52.5, 52.2, 51.1, 50.0, 37.8, 23.2, 23.0, 20.9, 20.8, 20.7, 20.6, 18.1; HR FAB-MS calcd for $C_{50}H_{68}N_5O_{21}SSi$ (M + H) 1134.3897, found 1134.3956.

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-9-azido-3,5,9-trideoxy-8-*O*-[methyl (5-acetamido-3,5-dideoxy-3-(phenylthio)-D-*erythro*-α-L-*gluco*-2-nonulopyranosid)onate]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate (14). To a stirred solution of **13** (62.7 mg, 0.0553 mmol) in toluene (0.8 mL) was added methanol (1.6 mL) and sodium methoxide in methanol (0.020 mL, 0.04 mmol, ~2 M) under Ar at room temperature. After 16 h, the mixture was neutralized with acetic acid and concentrated, and the residue was chromatographed (CH_2Cl_2/MeOH 25:1 → CH_2Cl_2/MeOH/H_2O 60:10:1, gradient) to give **14** (38 mg, 80%) as a white solid: $[\alpha]_D$ −36° (*c* 1.0, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 7.67–7.19 (m, 5 H), 4.63 (dt, 1 H, J = 6.7 Hz), 4.12 (t, 1 H, J = 10.1 Hz), 4.04 (dd, 1 H, J = 10.6 Hz), 4.00 (dd, 1 H, J = 1.4, 1.9 Hz), 3.86, 3.79 (2 s, 6 H), 3.77 (dd, 1 H, J = 1.5, 7.3 Hz), 3.76 (dd, 1 H), 3.58 (dd, 1 H, J = 2.8, 13.1 Hz), 3.47 (dd, 1 H, J = 9.2 Hz), 3.41 (m, 1 H), 3.33 (d, 1 H), 2.65 (dd, 1 H, J = 4.6, 12.8 Hz), 2.02, 2.02 (2 s, 6 H), 1.66 (dd, 1 H, J = 11.6 Hz), 0.84 (m, 2 H), 0.01 (s, 9 H); HR FAB-MS calcd for $C_{35}H_{56}N_5O_{16}SSi$ (M + H) 862.3212, found 862.3224.

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-9-amino-3,5,9-trideoxy-8-*O*-[5-acetamido-3,5-dideoxy-3-(phenylthio)-D-*erythro*-α-L-*gluco*-2-nonulopyranosyl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate 1′→9-Lactam (16). To a stirred solution of **14** (17.9 mg, 0.0208 mmol) in tetrahydrofuran/water 8:1 (0.23 mL) was added triphenylphosphine (22.1 mg, 0.0843 mmol), and the mixture was kept at 40 °C for 20 h. Chloroform/methanol (~1:1) was added, the mixture was concentrated, and the residue was chromatographed (CH_2Cl_2/MeOH/H_2O 50:10:1) to give **16** (14.5 mg, 87%) as a white solid: $[\alpha]_D$ −44° (*c* 1.0, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 7.63–7.16 (m, 5 H,), 4.39 (t, 1 H, J = 10.1 Hz), 4.22 (dt, 1 H, J = 5.4, 9.9 Hz), 3.90 (t, 1 H, J = 10.3 Hz), 3.90 (m, 1 H), 3.68 (s, 3 H), 3.58 (ddd, 1 H, J = 11.8, 4.6, 9.9 Hz), 3.44 (m, 1 H), 3.00 (d, 1 H, J = 10.4 Hz), 2.58 (dd, 1 H, J = 12.7 Hz), 2.01, 2.00 (2 s, 6 H), 1.69 (dd, 1 H), 0.86 (m, 2 H), 0.01 (s, 9 H); HR FAB-MS calcd for $C_{34}H_{54}N_3O_{15}SSi$ (M + H) 804.3045, found 804.3024.

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-8-*O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate 1′→9-Lactone (18). To a solution of **17**[9b] (14.0 mg, 0.0145 mmol) in methanol (0.7 mL) was added sodium methoxide in methanol (0.003 mL, ~2 M, ~0.006 mmol), and the mixture was left at room temperature for 24 h and then neutralized with acetic acid, concentrated, and dried *in vacuo* for 3 h. The crude product was dissolved in acetic acid (0.5 mL), and the mixture was left at room temperature for 22 h. The mixture was freeze-dried and the residue was chromatographed (SiO_2, CH_2Cl_2/MeOH/H_2O 50:10:1 → 40:10:1, gradient) to give **18** as a syrup (7.2 mg, 72%): $[\alpha]_D$ −64° (*c* 0.14, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 4.88 (t, 1 H, J = 11.7 Hz), 4.59 (dd, 1 H, J = 5.2, 11.8 Hz), 4.36 (ddd, 1 H, J = 5.4, 11.2, 10.3 Hz), 4.32 (ddd, 1 H, J = 7.7, 11.6 Hz), 3.92 (m, 1 H), 3.79 (s, 3 H), 3.75 (t, 1 H, J = 10.3 Hz), 3.73 (dd, 1 H, J = 10.4, 1.2 Hz), 3.69 (dd, 1 H, J = 8.0 Hz), 3.60 (m, 1 H), 3.53 (m, 1 H), 3.52 (dd, 1 H, J = 1.7, 10.6 Hz), 3.49 (dd, 1 H, J = 9.3 Hz), 2.64 (dd, 1 H, J = 4.6, 12.7 Hz), 2.47 (dd, 1 H, J = 5.4, 13.1 Hz), 2.02, 2.01 (2 s, 6 H), 1.69 (t, 1 H, J = 12.3 Hz), 1.63 (dd, 1 H, J = 11.2 Hz), 0.88 (m, 2 H), 0.02 (s, 9 H); HR FAB-MS calcd for $C_{28}H_{49}N_2O_{16}Si$ (M + H) 697.2851, found 697.2865.

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Supporting Information Available: ^1H NMR spectra and ^1H NMR data with assigned signals for all title compounds described in the Experimental Section (30 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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IV

A study of the donor properties of sialyl phosphites having an auxiliary 3-(*S*)-phenylseleno group.

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Dedicated to the memory of professor Göran Magnusson

Abstract

Two phosphite sialyl donors, each having an auxiliary 3-(*S*)-phenylseleno group, were prepared and evaluated. The phenylseleno group was introduced via a new mode of generating phenylselenenic acid ("PhSeOH"). Although the sialyl donors provided fair yields (32-76%) of the desired sialosides in glycosylations of the reactive acceptor 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose, no sialylated products could be obtained with less reactive acceptors. The presence of a 5-*N*-acetylacetamido group on the phosphite sialyl donor did not appear to improve its sialylating capability. The weak C-Se bond, possibly in combination with a steric hindrance which disfavors α-nitrilium ion formation, seem to explain the unsuccessful sialylations of the less reactive acceptors.

Keywords: Sialic acid phosphites, auxiliary phenylseleno group, phenylselenenic acid, long-range coupling

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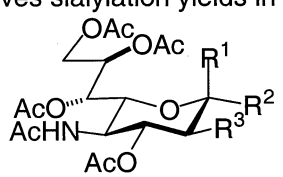
Introduction

Gangliosides are glycosphingolipids characterised by the presence of sialic acid (*N*-acetylneuraminic acid, Neu5Ac), which terminates the saccharide chain via an α-glycosidic bond *e.g.* to a galactose or sialic acid residue [1]. No β-glycosides of sialic acid have been found in nature. Due to the observed biological importance of gangliosides *inter alia* as tumor-associated antigens and receptors for various bacterial and viral pathogens, there is an ongoing demand for powerful methods of performing α-sialylations [2]. Since it is rather difficult to accomplish an α-sialylation in high yield, many researchers have addressed this problem over the years. In short, donors for α-sialylation seem to have evolved through four main stages. The first was the optimisation of the anomeric leaving group, where β-phosphites and α/β-thioglycosides (**1** and **2**, respectively; Fig. 1) gave the most useful results [3]. However, such sialyl donors are prone to eliminate and form a glycal (**6**) when unreactive acceptors are to be sialylated. They also yield α/β product mixtures [4].

The second stage was the introduction of an auxiliary 3-(*S*)-substituent to suppress glycal formation and improve the α/β selectivity [5]. In this respect, the phenylseleno, phenylthio and thiobenzoyloxy groups gave the best results [6]. Although giving fair to good α-sialylation yields, only halosugars such as **3** were at first equipped with such auxiliary substituents, thereby providing some room for continued development. The logical third stage was then to combine the best anomeric leaving group with the best auxiliary substituent, thereby providing *e.g.* the further improved sialyl donors **4** and **5** [7]. The fourth stage was the recent discovery that transformation of the 5-acetamido group of sialyl donors to a 5-*N*-acetylacetamido group improves sialylation yields in general [8].



1 $R^1 = OP(OEt)_2$, $R^2 = CO_2Me$, $R^3 = H$

2 $R^1 = CO_2Me$, $R^2 = SMe$ (α/β 1:1), $R^3 = H$

3 $R^1 = Cl$, $R^2 = CO_2Me$, $R^3 = SPh$

4 $R^1 = CO_2Me$, $R^2 = SEt$, $R^3 = SPh$

5 $R^1 = OP(OEt)_2$, $R^2 = CO_2Me$, $R^3 = OC(S)Ph$

Figure 1. Some typical available sialyl donors.

We now report a study of the phosphite sialyl donors **9** and **17** (Scheme 1 and 3, respectively) each having an auxiliary 3-(*S*)-phenylseleno group. These sialyl donors were anticipated to have the following properties: *i)* convenient activation by use of a catalytic amount of an acidic promotor [3a-b]; *ii)* stereospecific and high-yielding sialylation also of unreactive acceptors; *iii)* easy removal of the phenylseleno group due to the weak C-Se bond (243 kJ/mol) as compared to the C-S and C-O bonds (272 and 336 kJ/mol, respectively). Aspect *iii)* was deemed to be of considerable importance, particularly because the removal of an auxiliary phenylthio group can be rather difficult [9].

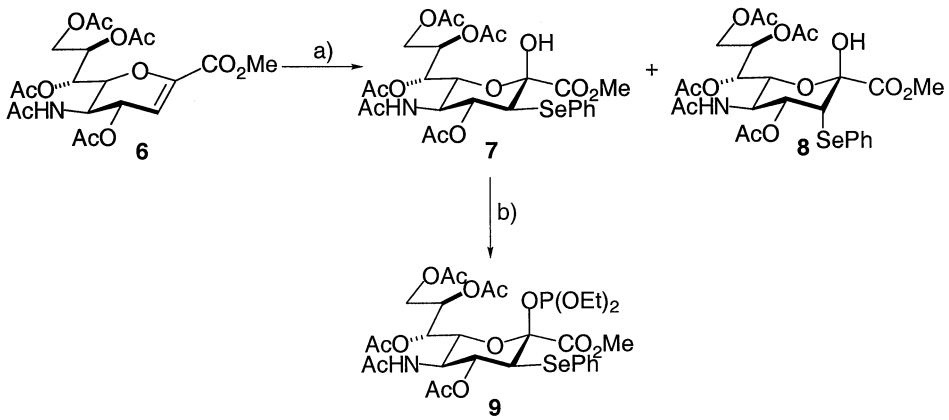
Results and Discussion

*Preparation of the sialyl donor **9** (Scheme 1).* —As starting material the readily available glycal **6** [10] was used, to which phenylselenenic acid ("PhSeOH") was added via *in situ* generation by mixing phenylselenenyl chloride, water and silver trifluoromethanesulfonate in tetrahydrofuran and keeping the reaction mixture at 0˚C for seven days. The desired *exo* adduct **7** was provided in 51% yield and easily separated from its diastereomeric *endo* adduct **8** obtained in 35% yield. The original Sharpless method of generating phenylselenenic acid in a neutral equilibrium system by reacting diphenyl diselenide with hydrogen peroxide [11] gave a similar result, although here the addition reaction was very slow (weeks required at room temperature). Our method of generating phenylselenenic acid thus provides a higher reaction rate than the original method, and it may serve as an attractive alternative in other systems. The method generates trifluoromethanesulfonic acid, so an obvious proviso is that a potential substrate should not be too acid sensitive (but *vide infra*).

Direct treatment of the *endo* adduct **8** with trifluoromethanesulfonic acid in tetrahydrofuran led to partial epimerisation into the *exo* adduct **7** (~25% yield) and formation of numerous by-products, including the glycal **6**. Studies by others have shown that highly reactive electrophilic species, such as bromine and $(PhS)_3SbCl_6$, prefer *endo* addition to sialic acid glycals [5,7b] . In summary, these observations support our assumption that the obtained **7**/**8** ratio is the result of a direct addition of phenylselenenic acid and not of any significant epimerisation. A weak buffering effect may thus be present in our system. In contrast to the results reported for the tetra-*O*-benzylated analogues **21** and **22** [6b], no epimerisation was observed under basic conditions (DBU).

Treatment of the hemiketal **7** with diethyl phosphorochloridite in the

presence of *N*-ethyldiisopropylamine in acetonitrile afforded the α-phosphite sialyl donor **9** in 88% yield. Unexpectedly, compound **9** was found to be stable over several months if stored as an amorphous powder under argon at -30°C.



Scheme 1. Synthesis of the sialyl donor **9**. Reagents: (a) PhSeCl, H_2O, AgOTf, THF, 0°C (51% **7** and 35% **8**, respectively). (b) $ClP(OEt)_2$, $EtN(i\text{-}Pr)_2$, CH_3CN, 0°C (88%).

Acceptors used in the present study. —The study of the sialyl donors **9** and **17** was performed with the acceptors **10** [12], **11** [13] and **12** [7d], each chosen for a particular reason (Fig. 2). The diisopropylidenyl galactoside **10** was chosen due to its high reactivity, which should easily provide a sialylated product. The hexabenzyl lactoside **11** and the 9-*O*-benzylated sialic acid glycal **12** were chosen due to their low reactivity and consequent importance in establishing the full potential of sialyl donors. Indeed, the usefulness of any sialyl donor is mainly governed by its ability to α-sialylate unreactive acceptors. As a general rule, sialyl donors having an auxiliary group should be reserved only for cases where low yields of an α-sialylated product are expected. Sialyl donors such as **1** and **2** are acceptable in the α-sialylation of reactive acceptors, such as **10**, whereas their α-sialylation yields are expected to be impractically low (if any) for the acceptors **11** and **12**.

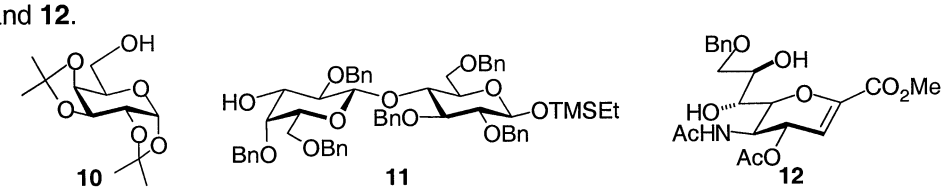
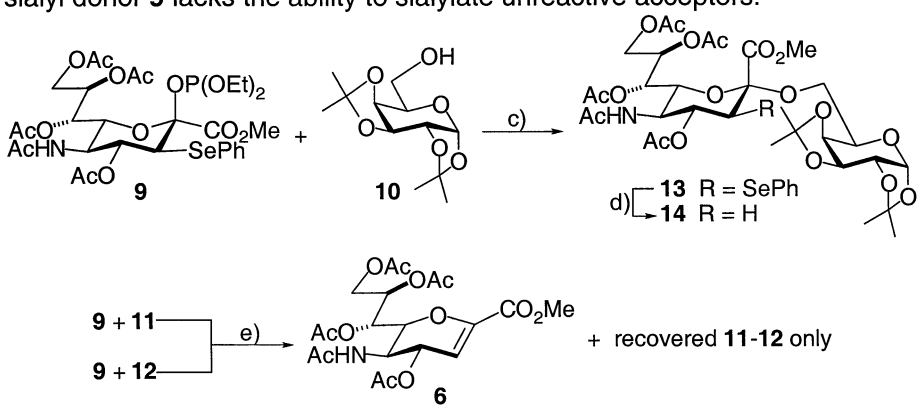


Figure 2. Acceptors used in the evaluation of the sialyl donors **9** and **17**.

*Evaluation of the sialyl donor **9**.* —The optimum conditions for activating sialyl phosphites are well established, and they normally involve treatment with a catalytic amount of either trimethylsilyl trifluormethanesulfonate or trifluoromethanesulfonic acid in acetonitrile or dichloromethane at a temperature between -40 and -78˚C [3b]. Hence, our first trial was to treat the sialyl donor **9** with the galactoside **10** in acetonitrile at -40˚C in the presence of a catalytic amount of trimethylsilyl trifluormethanesulfonate, which afforded the desired β-sialoside **13** in 76% yield (Scheme 2). The only by-product observed was the glycal **6**. This result was promising, particularly in view of that a tetra-*O*-benzylated 2-fluoro analogue of **9** afforded β-sialylated product in 46% yield with the galactoside **10** [6b]. However, when the sialyl donor **9** was treated with the more demanding acceptors **11** and **12** under *exactly* the same conditions, not even a trace of β-sialylated product could be obtained. The only products obtained were the glycal **6**, recovered pure acceptor and trace amounts of trimethylsilyl-*O*-protected acceptor. In contrast to the expected result and despite several modifications in line with the optimum conditions referred to above, the sialyl donor **9** was consistently unable to provide any glycoside with the acceptors **11** and **12**. As a comparison, the available sialyl donor **4** having an auxiliary 3-(*S*)-phenylthio group provided the β-sialylated product in 67% yield with the acceptor **11** [7a]. In summary, we therefore had to conclude that the sialyl donor **9** lacks the ability to sialylate unreactive acceptors.

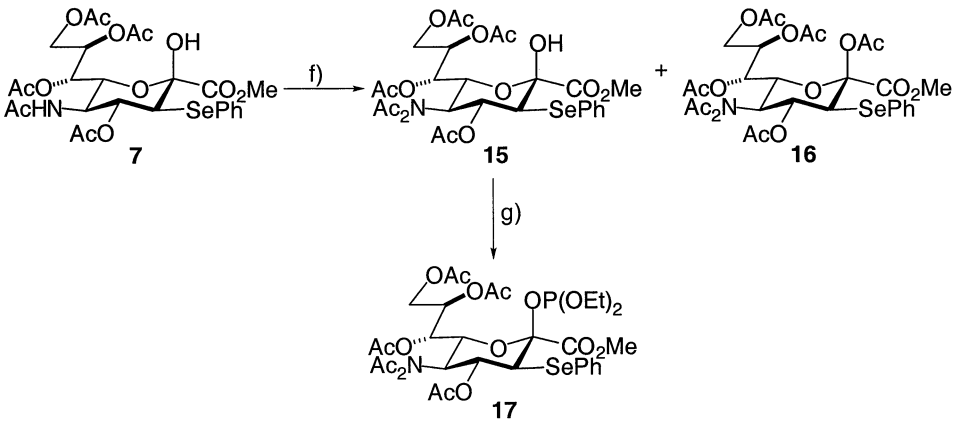


Scheme 2. Evaluation of sialyl donor **9** with the acceptors **10-12**. Reagents: (c) TMSOTf (cat.), MeCN, AW 300, -40˚C (76%). (d) Ph_3SnH, AIBN, $C_6H_5CH_3$, reflux (92%). (e) TMSOTf (cat.) or TfOH (cat.), MeCN or CH_2Cl_2 or MeCN/CH_2Cl_2, AW 300 (if any), -78˚C → -40˚C.

The auxiliary phenylseleno group of **13** was conveniently removed by treatment with triphenyltin hydride/azoisobutyronitrile in refluxing toluene for 1 h, thereby providing the known α-sialoside **14** [14] in 92% yield (Scheme 2). Removal of phenylthio groups under these conditions normally requires refluxing at least over night, and a significant amount of starting material is nevertheless often recovered [9a-b]. At least the problem of easy removal could thereby be well met by the sialyl donor **9**.

*Preparation of the sialyl donor **17** (Scheme 3).* —In our attempts to synthesize an improved analogue of **9**, we observed that the 5-acetamido group of the hemiketal **7** could be selectively acetylated before its anomeric hydroxyl group. Treating the hemiketal **7** with isopropenyl acetate in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate at 65°C over night thus gave the desired 5-*N*-acetylacetamido hemiketal **15** in 66% yield, together with the peracetylated analogue **16** in 19% yield. ^{1}H NMR analysis of an aliquot of the reaction mixture confirmed that the **15**/**16** ratio formed was ~3:1. We also noticed that it was necessary to keep the concentrations of both the hemiketal **7** and the catalyst at a fairly low level (below 0.08 M and 0.016 M, respectively) in order to avoid uncontrollable formation of **16**. Careful attempts to selectively remove the anomeric *O*-acetyl group of **16** under acidic conditions (heating in toluene/acetic acid/water 500:200:1) were unsuccessful and primarily led to *N*-deacetylation. We therefore concluded that the anomeric (tertiary) hydroxyl group of **7** is indeed very sterically hindered and quite unreactive under the acetylating conditions set forth above. This type of selective acetylation may perhaps be observed in other 3-(*S*)-substituted Neu5Ac derivatives, but this remains to be tested.

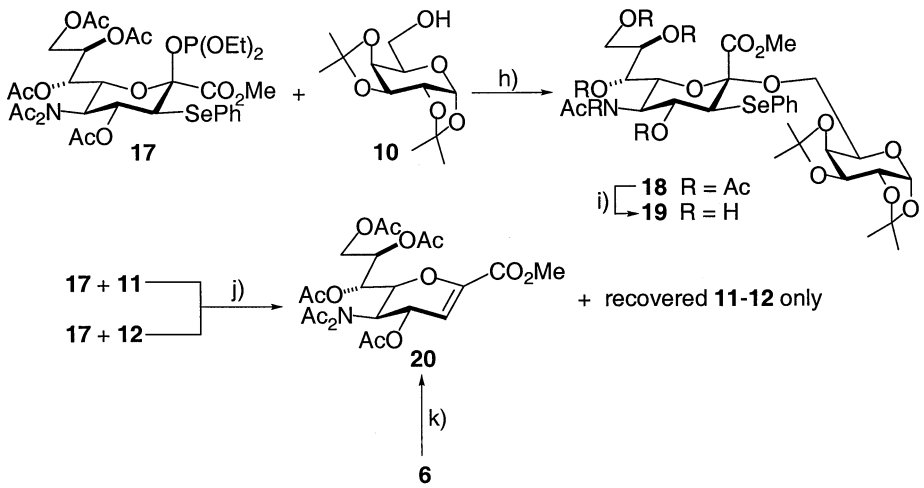
Treatment of the 5-*N*-acetylacetamido hemiketal **15** with diethyl phosphorochloridite in the presence of *N*-ethyldiisopropylamine in acetonitrile then gave the corresponding α-phosphite sialyl donor **17** in 85% yield. This compound was very acid-sensitive and required the presence of a base, such as triethylamine, during work-up in order not to hydrolyze into the hemiketal **15**.



Scheme 3. Synthesis of the 5-*N*-acetylacetamido sialyl donor **17**. Reagents: (f) $CH_3(CH_2{=})COAc$, $TsOH{\cdot}H_2O$, 65°C (66% **15** and 19% **16**, respectively). (g) $ClP(OEt)_2$, $EtN(i\text{-}Pr)_2$, CH_3CN, 22°C then work-up in the presence of Et_3N (85%).

Evaluation of the 5-N-*acetylacetamido sialyl donor **17**.* —When the sialyl donor **17** was treated with the galactoside **10** under the same conditions as those used for the sialyl donor **9**, the desired β-sialoside **18** was obtained in about 50% yield. However, ^1H NMR analysis revealed that the product **18** was no more than 80% pure and contaminated *inter alia* by the corresponding 5-*N*-acetylacetamido glycal **20** (Scheme 4). Since we failed to obtain even a trace of pure **18** by conventional chromatography, the crude product **18** was subjected to deacetylation by treatment with sodium methoxide in dichloromethane/methanol, whereby the deacetylated β-sialoside **19** could be obtained pure in a total yield of 32% (calculated from **17**). In view of this result, it was not surprising that compound **17** was incapable of sialylating the acceptors **11** and **12** in the presence of trimethylsilyl trifluormethanesulfonate in acetonitrile at -40°C, but instead provided only the glycal **20** and recovered acceptor as main products. Clearly, the 5-*N*-acetylacetamido group of **17** did not confer any improved sialylating power as compared to the donor **9**.

We also prepared the 5-*N*-acetylacetamido glycal **20** in 90% yield directly from the glycal **6** by treating the latter with isopropenyl acetate under standard conditions (Scheme 4).



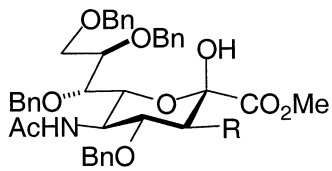
Scheme 4. Evaluation of the 5-*N*-acetylacetamido sialyl donor **17** with the acceptors **10**-**12**. Reagents: (h) TMSOTf (cat.), MeCN, -40˚C (~50% crude **18**). i) NaOMe, MeOH/CH_2Cl_2 1:1, 22˚C (32% from **17**). (j) TMSOTf (cat.), MeCN, AW 300 (if any), -40˚C. (k) $CH_3(CH_2{=})COAc$, $TsOH{\cdot}H_2O$, 65˚C (90%).

General observations regarding the 3-(S)-phenylseleno group. —The main reason for the results above is probably the weak C-Se bond, but we also believe that steric hindrance was of some importance. The latter consideration is supported by the unexpected long-range *J* couplings of 1.0-1.9 Hz observed in ^{1}H NMR analysis of the 3-(*S*)-phenylseleno compounds **7**, **9**, **15** and **17** (Table 1). Such long-range *J* couplings have been observed in other carbohydrates [15], where they are considered indicative of a so-called "W-conformation", in this case for the atom sequence H(3)-C(3)-C(2)-O-R (R=H or $P(OEt)_2$). We have found no reports in the literature of such long-range *J* couplings in other Neu5Ac hemiketals or phosphites carrying a 3-(*S*)-bromine, iodine, phenylthio (compound **22**) or thiobenzoyloxy (compound **5**) substituent [5,6b,7d]. However, a long-range *J* coupling of 1.5 Hz was reported for the tetra-*O*-benzylated hemiketal **21**, which carries a 3-(*S*)-phenylseleno substituent [6b].

Table 1
NMR analysis of long-range *J* couplings in 3-(*S*)-SePh substituted Neu5Ac derivatives

Compound	*J*C(1),H(3)/Hz[a]	*J*H(3),OH/Hz	*J*H(3),P/Hz	Configuration[b]
7	2.3[c]	1.6[d]	-	α
9	<1.0[e]	-	1.1[e]	α
15	1.5[c]	1.9[d]	-	α
17	1.5[e]	-	1.0[e]	α
21	-	1.5[d]	-	α

[a] Measured according to ref. 16. [b] Anomeric (non-carboxyl) substituent.
[c] In CD_3OD. [d] In $CDCl_3$. [e] In C_6D_6. - = inaccessible or not measured.



21 R = SePh
22 R = SPh

These NMR results show that a 3-(*S*)-phenylseleno group confers a fairly rigid "W-conformation" in Neu5Ac derivatives. The rotation around the C(2)-OR bond thus appears to be restricted, probably due to steric interference from the phenylseleno group. For compound **15**, this steric effect may explain the selective *N*-acetylation of the 5-acetamido group over the anomeric hydroxyl group. It appears likely that the phenylseleno group also sterically disfavors the formation of an α-nitrilium ion in a nitrile solvent [17], and this would in turn reduce the β–sialylating capability beyond what is expected purely on the basis of the weak C-Se bond.

Concluding remark. —In general, reactive acceptors tend to give lower α/β ratios than less reactive acceptors when sialylated with a donor lacking an auxiliary 3-(*S*)-group [18]. Hence, in the not too uncommon situation where a reactive acceptor yields an undesired α/β mixture, our donor **9** might provide an

alternative due to its convenient preparation, high β–stereoselectivity and easy phenylseleno group removal.

Experimental

General methods. —NMR spectra were recorded at 400 or 500 MHz. Assignment of ^{1}H NMR spectra was achieved using 2D-methods (COSY, HETCOR). Optical rotations were measured at 22°C. Chemical shifts are expressed in ppm using residual $CHCl_3$, C_6HD_5 or CHD_2OD as reference. Reactions were monitored by TLC using alumina plates coated with silica gel 60 F254 (Merck) and visualized using either UV light or by charring with H_3PO_4 (aqueous 5% dip solution). Preparative chromatography was performed with Amicon silica gel (35-70 μm, 60 Å). THF was sonicated and filtered through a column of Al_2O_3 (activity I, Merck) immediately before use. CH_2Cl_2, toluene and EtN(*i*-Pr)$_2$ were stored over 4 Å molecular sieves (3 Å for MeCN) and filtered through a column of Al_2O_3 as above. Compounds obtained as white powders were precipitated with *n*-hexane from a chloroform/diethyl ether (~1:2) solution. All reactions were carried out under an argon atmosphere. Anomeric configurations of Neu5Ac residues were determined in accordance with ref 16.

*Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-3-phenylseleno-*D-erythro-α-L-gluco*-2-nonulopyranosonate* (**7**) *and methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-3-phenylseleno-*D-erythro-α-L-manno*-2-nonulopyranosonate* (**8**). —To a stirred, ice-cooled solution of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (**6**, 264 mg, 0.558 mmol) in tetrahydrofuran (1.40 mL) was added PhSeCl (192 mg, 1.11 mmol) immediately followed by H_2O (22 mg, 1.22 mmol) and AgOTf (257 mg, 1.12 mmol). The reaction mixture was stirred vigorously for an additional 5 min, and then kept dark at 2°C. After 7 days, Et_3N (0.30 mL) was added under ice-cooling, after which the reaction mixture was filtered (Celite), washed with toluene/acetone 1:1 (4 x 10 mL) and concentrated. The residue was chromatographed (toluene/acetone, 4:1 → 3:1, gradient) to give **7** (185 mg, 51%, pure α) and **8** (127 mg, 35%, α/β ~12/1), both as white powders. Data for compound **7**: $[\alpha]_D$ +28° (*c* 0.99, $CHCl_3$). ^{1}H NMR (CD_3OD): δ 7.56-7.27 (m, 5 H, SePh), 5.41 (dd, 1 H, $J_{4,5}$ 10.2 Hz, $J_{3,4}$ 11.1 Hz, H-4), 5.37 (dd, 1 H, $J_{6,7}$ 2.4 Hz, $J_{7,8}$ 6.0 Hz, H-7), 5.12 (ddd, 1 H, $J_{8,9A}$ 2.6 Hz, $J_{8,9B}$ 6.6 Hz, H-8), 4.41 (dd, 1 H, $J_{9A,9B}$ 12.4 Hz, H-9A), 4.38 (dd, 1 H, $J_{5,6}$ 10.5 Hz, H-6), 4.03 (dd, 1 H, H-9B), 4.01 (bt, 1 H, H-5), 3.79 (s, 3 H, CO_2Me), 3.58 (d, 1 H, H-3), 2.07, 2.05,

1.99, 1.80, 1.75 (s, 3 H each, Ac); ^{13}C NMR (CD_3OD): δ 172.2, 171.4, 170.8, 170.7, 170.6, 168.6 ($J_{C1,H3}$ 2.3 Hz, C-1), 133.5, 130.5, 129.2, 127.7, 97.8, 74.7, 70.9, 70.3, 68.3, 62.4, 52.6, 50.8, 50.3, 21.6, 19.8, 19.7, 19.6, 19.5. HR FAB-MS for $C_{26}H_{33}NO_{13}SeNa$ (M + Na): Calcd 670.1017. Found 670.1031.

Data for compound **8**: $[\alpha]_D$ -3.6° (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.87-7.22 (m, 5 H, SePh), 6.19 (d, 1 H, $J_{5,NH}$ 9.3 Hz, NH), 5.57 (dd, 1 H, $J_{3,4}$ 4.1 Hz, $J_{4,5}$ 10.3 Hz, H-4), 5.35 (dd, 1 H, $J_{6,7}$ 1.8 Hz, $J_{7,8}$ 3.0 Hz, H-7), 5.28 (ddd, 1 H, $J_{8,9A}$ 2.4 Hz, $J_{8,9B}$ 8.6 Hz, H-8), 4.95 (dd, 1 H, $J_{9A,9B}$ 12.4 Hz, H-9A), 4.44 (q, 1 H, H-5), 4.35 (dd, 1 H, $J_{5,6}$ 10.7 Hz, H-6), 4.17 (dd, 1 H, H-9B), 3.94 (d, 1 H, H-3), 3.82 (s, 3 H, CO_2Me), 2.24, 2.09, 2.03, 1.93, 1.69 (s, 3 H each, Ac); ^{13}C NMR ($CDCl_3$): δ 172.8, 171.8, 171.0, 170.9, 170.8, 168.8, 134.4, 130.3, 129.5, 128.3, 98.1, 73.8, 72.3, 70.5, 69.5, 63.4, 53.1, 52.1, 47.4, 23.6, 21.6, 21.3, 21.2, 20.7. HR FAB-MS for $C_{26}H_{33}NO_{13}SeNa$ (M + Na): Calcd 670.1017. Found 670.1034.

Methyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-2*-O-*(diethyl phosphite)-3-phenylseleno*-D-erythro-α-L-gluco-*2-nonulopyranosonate* (**9**). —To a stirred, ice-cooled solution of compound **7** (145 mg, 0.224 mmol) in MeCN (0.50 mL) was added EtN(*i*-Pr)$_2$ (0.12 mL, 0.70 mmol) followed by dropwise addition of ClP(OEt)$_2$ (0.070 mL, 0.49 mmol). After 4 h, *t*-BuOH (0.035 mL) was added dropwise under ice-cooling, after which the reaction mixture was concentrated. The residue was chromatographed (toluene/acetone, 6:1) on a short column (<15 cm) to give **9** (152 mg, 88%) as a white powder. $[\alpha]_D$ +22° (*c* 0.98, C_6H_6). ^{1}H NMR (C_6D_6): δ 7.44-6.83 (m, 5 H, SePh), 5.81 (dd, 1 H, $J_{6,7}$ 2.4 Hz, $J_{7,8}$ 3.7 Hz, H-7), 5.75 (t, 1 H, *J* 10.5 Hz, H-4), 5.59 (m, 1 H, H-8), 5.04 (dd, 1 H, $J_{8,9A}$ 2.2 Hz, $J_{9A,9B}$ 12.4 Hz, H-9A), 4.84 (dd, 1 H, $J_{5,6}$ 10.7 Hz, H-6), 4.75 (q, 1 H, *J* 10.3 Hz, H-5), 4.60 (d, 1 H, $J_{5,NH}$ 10.2 Hz, NH), 4.38 (dd, 1 H, $J_{8,9B}$ 6.9 Hz, H-9B), 4.31-4.07 (m, 4 H, 2OCH_2CH$_3$), 4.00 (dd, 1 H, $J_{3,4}$ 10.9 Hz, $J_{3,P}$ 1.1 Hz, H-3), 3.32 (s, 3 H, CO_2Me), 1.92, 1.88, 1.72, 1.55, 1.44 (s, 3 H each, Ac), 1.33 (t, 3 H, *J* 7.1 Hz, OCH$_2$CH_3), 1.26 (t, 3 H, *J* 7.1 Hz, OCH$_2$CH_3); ^{13}C NMR (C_6D_6): δ 170.8, 170.4, 170.3, 170.2, 169.6, 167.2 ($J_{C1,H3}$ <1.0 Hz, C-1), 133.8, 129.5, 128.7, 127.8, 101.6, 74.2, 73.7, 73.5, 68.6, 63.0, 59.9, 59.8, 58.8, 58.7, 53.1, 51.6, 51.0, 22.8, 21.2, 20.8, 20.6, 20.4, 17.4, 17.4, 17.2, 17.2. HR FAB-MS for $C_{30}H_{42}NO_{15}PSeNa$ (M + Na): Calcd 790.1354. Found 790.1362.

1,2;3,4-Di-O-*isopropylidene-6*-O-*[methyl [5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-3-phenylseleno*-D-erythro-β-L-gluco-*2-nonulopyranosyl]onate]*-α-D-*galactopyranose* (**13**). —To a mixture of compound **9** (51.8 mg, 0.068 mmol),

1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose (74.3 mg, 0.285 mmol) and activated AW300 molecular sieves (0.30 g) was added MeCN (0.50 mL), and the mixture was vigorously stirred at rt for 10 min. The temperature was then lowered to -40°C, and TMSOTf (5.0 μL, 0.027 mmol) was added. After 80 min Et_3N (0.060 mL) was added, after which the reaction mixture was filtered (Celite), washed with $CHCl_3$/acetone 1:1 (2 x 10 mL) and concentrated. The residue was chromatographed (toluene/acetone, 4:1) to give **13** (46 mg, 76%) as a white powder. $[\alpha]_D$ -16° (*c* 1.00, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.68-7.24 (m, 5 H, SePh), 5.54 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1), 5.38 (d, 1 H, $J_{5',NH}$ 9.5 Hz, NH), 5.37-5.31 (m, 2 H, H-4', H-8), 5.28 (dd, 1 H, $J_{6,7}$ 1.8 Hz, $J_{7,8}$ 7.6 Hz, H-7), 4.60 (dd, 1 H, $J_{2,3}$ 2.4 Hz, $J_{3,4}$ 7.9 Hz, H-3), 4.31 (dd, 1 H, H-2), 4.27 (dd, 1 H, $J_{8,9A}$ 2.7 Hz, $J_{9A,9B}$ 12.5 Hz, H-9A), 4.23 (dd, 1 H, $J_{4,5}$ 1.7 Hz, H-4), 4.20-4.09 (m, 3 H, H-5', H-6', H-9B), 4.02 (dd, 1 H, $J_{5,6A}$ 6.2 Hz, $J_{6A,6B}$ 9.0 Hz, H-6A), 3.96 (dt, 1 H, H-5), 3.88 (dd, 1 H, $J_{5,6B}$ 5.6 Hz, H-6B), 3.85 (s, 3 H, CO_2Me), 3.25 (d, 1 H, $J_{3',4'}$ 11.4 Hz, H-3'), 2.09, 2.09, 2.04, 2.02, 1.88, 1.51, 1.44, 1.36, 1.33 (s, 3 H each, Ac, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 171.2, 170.7, 170.4, 170.2, 169.8, 168.3 ($J_{C1',H3'}$ 6.1 Hz, C-1), 134.9, 130.7, 129.2, 128.1, 109.4, 108.7, 101.3, 96.5, 73.5, 72.5, 71.1, 70.9, 70.8, 69.4, 67.5, 67.2, 63.8, 62.3, 52.7, 52.6, 50.3, 26.4, 26.2, 25.2, 24.9, 23.4, 21.1, 21.0, 20.9. HR FAB-MS for $C_{38}H_{51}NO_{18}SeNa$ (M + Na): Calcd 912.2169. Found 912.2157.

*1,2;3,4-Di-O-isopropylidene-6-O-[methyl [5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D-glycero-α-D-galacto-*2-nonulopyranosyl]onate]-α-*D-*galactopyranose* (**14**). —To a stirred solution of compound **13** (45.1 mg, 0.051 mmol) in toluene (1.0 mL) was added AIBN (12.5 mg, 0.076 mmol) and Ph_3SnH (0.13 mL, 0.51 mmol). After refluxing for 1 h, the reaction mixture was allowed to reach rt and then applied directly to a silica gel column and chromatographed ($CHCl_3$/acetone, 30:1 → 10:1, gradient) to give **14** (34.0 mg, 92%) as a white powder. The ^1H NMR data were in agreement with those reported [14].

*Methyl 4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamido)-3,5-dideoxy-3-phenylseleno-*D-erythro-α-L-gluco-*2-nonulopyranosonate* (**15**) *and methyl 2,4,7,8,9-penta-O-acetyl-5-(N-acetylacetamido)-3,5-dideoxy-3-phenylseleno-*D-erythro-α-L-gluco-*2-nonulopyranosonate* (**16**). —To a stirred solution of compound **7** (50.5 mg, 0.078 mmol) in isopropenyl acetate (1.0 mL) was added *p*-TsOH·H_2O (3.0 mg, 0.016 mmol), and the reaction mixture was then stirred at 65°C. After 14 h Et_3N (0.060 mL) was added and the reaction mixture was

concentrated in the presence of toluene. The residue was chromatographed repeatedly (toluene/acetone, 15:1) to give **15** (35.5 mg, 66%) and **16** (10.9 mg, 19%), both as white powders. Data for compound **15**: $[\alpha]_D$ +64° (*c* 1.00, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.54-7.24 (m, 5 H, SePh), 5.92 (dd, 1 H, $J_{3,4}$ 10.7 Hz, $J_{4,5}$ 10.2 Hz, H-4), 5.31-5.26 (m, 2 H, H-6, H-8), 5.11 (dd, 1 H, $J_{6,7}$ 1.7 Hz, $J_{7,8}$ 8.1 Hz, H-7), 4.44 (d, 1 H, $J_{OH,3}$ 1.9 Hz, OH-2), 4.33 (t, 1 H, $J_{5,6}$ 10.2 Hz, H-5), 4.18 (dd, 1 H, $J_{8,9A}$ 2.5 Hz, $J_{9A,9B}$ 12.5 Hz, H-9A), 4.06 (dd, 1 H, $J_{8,9B}$ 6.0 Hz, H-9B), 3.83 (s, 3 H, CO_2Me), 3.69 (dd, 1 H, H-3), 2.38, 2.28, 2.12, 2.09, 2.02, 1.76 (s, 3 H each, Ac); ^{13}C NMR ($CDCl_3$): δ 174.4, 173.7, 170.9, 170.4, 170.3, 169.9 ($J_{C1,H3}$ 1.5 Hz, C-1), 168.6, 133.8, 130.1, 129.3, 127.9, 97.2, 72.0, 69.0, 68.1, 67.4, 62.4, 57.7, 53.9, 50.2, 28.3, 26.6, 21.1, 21.1, 21.0, 20.6. HR FAB-MS for $C_{28}H_{35}NO_{14}SeNa$ (M + Na): Calcd 712.1120. Found 712.1136.

Data for compound **16**: $[\alpha]_D$ +19° (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.56-7.27 (m, 5 H, SePh), 5.94 (dd, 1 H, $J_{4,5}$ 9.8 Hz, $J_{3,4}$ 10.7 Hz, H-4), 5.18 (dd, 1 H, $J_{6,7}$ 1.9 Hz, $J_{7,8}$ 5.5 Hz, H-7), 5.00 (m, 2 H, H-6, H-8), 4.43 (dd, 1 H, $J_{8,9A}$ 2.6 Hz, $J_{9A,9B}$ 12.4 Hz, H-9A), 4.40 (t, 1 H, $J_{5,6}$ 10.0 Hz, H-5), 4.19 (dd, 1 H, $J_{8,9B}$ 6.1 Hz, H-9B), 3.71 (s, 3 H, CO_2Me), 3.35 (d, 1 H, H-3), 2.38, 2.32, 2.21, 2.13, 2.02, 2.01, 1.89 (s, 3 H each, Ac); ^{13}C NMR ($CDCl_3$): δ 174.5, 174.1, 171.0, 170.8, 170.4, 170.3, 168.1, 165.2 ($J_{C1,H3}$ 1.5 Hz, C-1), 134.6, 129.6, 129.4, 128.5, 100.6, 71.7, 70.7, 70.1, 68.0, 61.9, 57.5, 53.5, 50.8, 28.5, 26.8, 21.4, 21.3, 21.3, 21.2, 20.9. HR FAB-MS for $C_{30}H_{37}NO_{15}SeNa$ (M + Na): Calcd 754.1225. Found 754.1230.

Methyl 4,7,8,9-tetra-O-*acetyl-5-(*N-*acetylacetamido)-3,5-dideoxy-2*-O-*(diethyl phosphite)-3-phenylseleno*-D-erythro-α-L-gluco-*2-nonulopyranosonate* (**17**). —To a stirred, ice-cooled solution of compound **15** (38.7 mg, 0.056 mmol) in MeCN (0.80 mL) was added $EtN(i\text{-}Pr)_2$ (0.040 mL, 0.23 mmol) and then $ClP(OEt)_2$ (0.023 mL, 0.16 mmol) dropwise. After 30 min the reaction mixture was allowed to reach rt. After 2.5 h, *t*-BuOH (0.040 mL) was added under ice-cooling, and the reaction mixture was concentrated. The residue was chromatographed (toluene/acetone/Et_3N, 15:1:0.075) on a silica gel column, which had been preconditioned with toluene/Et_3N 200:1, to give **17** (38.6 mg, 85%) as a white powder. $[\alpha]_D$ +46° (*c* 1.00, C_6H_6). ^1H NMR (C_6D_6): δ 7.47-6.82 (m, 5 H, SePh), 6.49 (t, 1 H, $J_{4,5}$ 10.5 Hz, H-4), 5.97 (dd, 1 H, $J_{6,7}$ 1.7 Hz, $J_{5,6}$ 10.2 Hz, H-6), 5.73 (dd, 1 H, $J_{7,8}$ 5.3 Hz, H-7), 5.65 (ddd, 1 H, $J_{8,9A}$ 2.5 Hz, $J_{8,9B}$ 6.0 Hz, H-8), 4.92 (t, 1 H, H-5), 4.84 (dd, 1 H, $J_{9A,9B}$ 12.5 Hz, H-9A), 4.57 (m, 1 H, OCH_2CH_3), 4.43 (dd, 1 H, H-9B), 4.43 (m, 1 H, OCH_2CH_3), 4.22 (m, 2 H,

OCH_2CH_3), 4.06 (dd, 1 H, $J_{3,P}$ 1.0 Hz, $J_{3,4}$ 10.6 Hz, H-3), 3.29 (s, 3 H, CO_2Me), 2.07, 1.91, 1.89, 1.77, 1.68, 1.48 (s, 3 H each, Ac), 1.47 (t, 3 H, *J* 7.0 Hz, OCH_2CH_3), 1.26 (t, 3 H, *J* 7.0 Hz, OCH_2CH_3); ^{13}C NMR (C_6D_6): δ 174.2, 173.7, 170.8, 170.4, 170.4, 169.6, 167.7 ($J_{C1,H3}$ 1.5 Hz, C-1), 134.1, 130.9, 129.6, 128.9, 101.7, 72.6, 72.3, 71.1, 68.7, 62.5, 61.1, 60.9, 58.5, 58.3, 53.2, 53.0, 28.3, 26.7, 21.4, 20.9, 20.8, 20.4, 17.5, 17.5, 17.4, 17.4. HR FAB-MS for $C_{32}H_{44}NO_{16}PSeNa$ (M + Na): Calcd 832.1461. Found 832.1441.

*1,2;3,4-Di-O-isopropylidene-6-O-[methyl (4,7,8,9-tetra-O-acetyl-5-N-acetylacetamido-3,5-dideoxy-3-phenylseleno-*D-erythro-*β*-L-gluco-*2-nonulopyranosyl)onate]-α-*D-*galactopyranose* (**18**) *and 1,2;3,4-di-O-isopropylidene-6-O-[methyl (5-acetamido-3,5-dideoxy-3-phenylseleno-*D-erythro-*β*-L-gluco-*2-nonulopyranosyl)onate]-α-*D-*galactopyranose* (**19**). —To a stirred solution of compound **17** (34.9 mg, 0.043 mmol) and 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose (21.5 mg, 0.083 mmol) in MeCN (0.40 mL) at -40°C was added TMSOTf (3.0 μL, 0.017 mmol). After 40 min *t*-BuOH (0.040 mL) and Et_3N (0.060 mL) were added, and the reaction mixture was concentrated. The residue was chromatographed (toluene/acetone, 15:1 → 8:1, gradient) to give crude **18** (22 mg, ~50%) as a syrup. ^1H NMR ($CDCl_3$): δ 7.73-7.24 (m, 5 H, SePh), 5.92 (t, 1 H, $J_{3',4'}$=$J_{4',5'}$ 9.6 Hz, H-4'), 5.55 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1), 5.31 (m, 1 H, H-8), 5.10 (dd, 1 H, $J_{6',7}$ 1.7 Hz, $J_{7,8}$ 7.4 Hz, H-7), 5.02 (dd, 1 H, $J_{5',6'}$ 10.2 Hz, H-6'), 4.60 (dd, 1 H, $J_{2,3}$ 2.5 Hz, $J_{3,4}$ 7.8 Hz, H-3), 4.32 (dd, 1 H, H-2), 4.15 (dd, 1 H, $J_{8,9B}$ 5.3 Hz, $J_{9A,9B}$ 12.5 Hz, H-9B), 3.88 (s, 3 H, CO_2Me), 3.25 (d, 1 H, H-3'), 2.38, 2.27 (s, 3 H each, Ac_2N), 2.11, 2.08, 2.03, 1.96 (s, 3 H each, 4OAc), 1.49, 1.45, 1.35, 1.33 (s, 3 H each, $2C(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 174.6, 173.8, 170.7, 170.2, 170.1, 170.0, 167.4 ($J_{C1',H3'}$ 5.9 Hz, C-1), 135.0, 131.3, 129.3, 128.1, 109.5, 108.8, 101.2, 96.6, 71.9, 71.4, 70.9, 70.8, 70.0, 69.3, 67.6, 67.4, 64.3, 61.9, 57.5, 53.8, 52.7, 28.2, 26.4, 26.3, 26.2, 25.3, 24.7, 21.2, 21.1, 20.9, 20.8. To crude compound **18** (22 mg) dissolved in CH_2Cl_2/MeOH 1:1 (1.0 mL) was then added 1 M NaOMe in MeOH (0.025 mL, 0.025 mmol) at rt. After 1.5 h acetic acid (0.040 mL) was added, and the reaction mixture was concentrated. The residue was chromatographed ($CHCl_3$/EtOH, 10:1 → 5:1, gradient) to give **19** (9.9 mg, 32% from **17**) as a white powder. $[\alpha]_D$ -75° (*c* 0.50, CH_3OH). ^1H NMR (CD_3OD): δ 7.77-7.23 (m, 5 H, SePh), 5.46 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1), 4.58 (dd, 1 H, $J_{2,3}$ 2.5 Hz, $J_{3,4}$ 7.9 Hz, H-3), 4.32 (dd, 1 H, H-2), 4.22 (dd, 1 H, $J_{4,5}$ 1.6 Hz, H-4), 4.05 (dd, 1 H, $J_{3',4'}$ 10.5 Hz, $J_{4',5'}$ 9.8 Hz, H-4'), 3.99-3.94 (m, 2 H, H-5, H-6A), 3.94 (dd, 1 H, $J_{5',6'}$

10.7 Hz, H-5'), 3.81 (s, 3 H, CO_2Me), 3.79 (dd, 1 H, $J_{6',7}$ 1.4 Hz, H-6'), 3.79-3.71 (m, 3 H, H-6B, H-8, H-9A), 3.62 (dd, 1 H, $J_{8,9B}$ 5.1 Hz, $J_{9A,9B}$ 11.0 Hz, H-9B), 3.45 (dd, 1 H, $J_{7,8}$ 9.1 Hz, H-7), 3.08 (d, 1 H, H-3'), 1.99 (s, 3 H, NAc), 1.46, 1.37, 1.32 (3 s, 12 H, $2C(CH_3)_2$); ^{13}C NMR (CD_3OD): δ 174.9, 169.9, 135.9, 133.1, 130.1, 128.7, 110.5, 110.1, 102.7, 97.9, 74.1, 73.5, 72.6, 72.2, 72.1, 71.8, 70.4, 68.9, 65.2, 64.4, 57.9, 54.2, 52.9, 29.7, 26.7, 26.5, 25.4, 24.9, 22.8. HR FAB-MS for $C_{30}H_{43}NO_{14}SeNa$ (M + Na): Calcd 744.1746. Found 744.1734.

*Methyl 4,7,8,9-tetra-O-acetyl-5-(*N*-acetylacetamido)-2,3,5-trideoxy-*D-glycero-D-galacto-*non-2-enopyranosonate* (**20**). —To a stirred solution of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (**6**, 89.7 mg, 0.189 mmol) in isopropenyl acetate (2.0 mL) was added *p*-$TsOH{\cdot}H_2O$ (1.8 mg, 0.010 mmol), and the reaction mixture was then stirred at 65°C. After 16 h, Et_3N (0.10 mL) was added and the reaction mixture was concentrated in the presence of toluene. The residue was chromatographed (toluene/acetone, 15:1) to give **20** (88.1 mg, 90%) as a white powder. $[\alpha]_D$ +51° (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.08 (dd, 1 H, $J_{3,4}$ 2.8 Hz, $J_{4,5}$ 9.3 Hz, H-4), 5.94 (d, 1 H, H-3), 5.35 (ddd, 1 H, $J_{7,8}$ 6.1 Hz, $J_{8,9A}$ 2.9 Hz, $J_{8,9B}$ 6.3 Hz, H-8), 5.23 (dd, 1 H, $J_{6,7}$ 1.7 Hz, H-7), 5.16 (dd, 1 H, $J_{6,7}$ 1.7 Hz, $J_{5,6}$ 10.1 Hz, H-6), 4.56 (t, 1 H, H-5), 4.53 (dd, 1 H, $J_{9A,9B}$ 12.5 Hz, H-9A), 4.18 (dd, 1 H, H-9B), 3.81 (s, 3 H, CO_2Me), 2.39 (bs, 6 H, Ac_2N), 2.11, 2.07, 2.05, 2.03 (s, 3 H each, 4OAc); ^{13}C NMR ($CDCl_3$): δ 170.8, 170.6, 170.2, 170.0, 161.8, 146.6, 109.2, 76.5, 70.4, 68.0, 67.5, 62.1, 55.3, 52.8, 21.0, 21.0, 21.0, 20.9. HR FAB-MS for $C_{22}H_{29}NO_{13}Na$ (M + Na): Calcd 538.1537. Found 538.1528.

Acknowledgements

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References

[1] L. Svennerholm, *J. Lipid Res.*, 5 (1964) 533-551.

[2] (a) K. Okamoto, T. Goto, *Tetrahedron*, 46 (1990) 5835-5856. (b) M.P. DeNinno, *Synthesis*, (1991) 583-593. (c) B.G. Davis, *J. Chem. Soc., Perkin Trans. 1*, 14 (2000) 2137-2160.

[3] (a) T.J. Martin, R. Brescello, A. Toepfer, R.R. Schmidt, *Glycoconj. J.*, 10 (1993) 16-25. (b) H. Kondo, S. Aoki, Y. Ichikawa, R.L. Halcomb, H. Ritzen, C-H. Wong, *J. Org. Chem.*, 59 (1994) 864-877. (c) A. Marra, P. Sinaÿ,

Carbohydr. Res., 195 (1990) 303-308. (d) A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida, M. Kiso, *Carbohydr. Res.*, 212 (1991) 277-280.

[4] (a) W. Birberg, H. Lönn, *Tetrahedron Lett.*, 32 (1991) 7453-7456. (b) T. Ercegovic, G. Magnusson, *J. Chem. Soc., Chem. Commun.*, (1994) 831-832.

[5] K. Okamoto, T. Kondo, T. Goto, *Bull. Chem. Soc. Jpn.*, 60 (1987) 631-637.

[6] (a) T. Kondo, H. Abe, T. Goto, *Chem. Lett.*, (1988) 1657-1660. (b) Y. Ito, T. Ogawa, *Tetrahedron*, 46 (1990) 89-102. (c) J.C. Castro-Palomino, Y.E. Tsvetkov, R. Schneider, R.R. Schmidt, *Tetrahedron Lett.*, 38 (1997) 6837-6840.

[7] (a) T. Ercegovic, G. Magnusson, *J. Org. Chem.*, 60 (1995) 3378-3384. (b) Z.-G. Wang, X.-F. Zhang, Y. Ito, Y. Nakahara, T. Ogawa, *Bioorg. Med. Chem.*, 4 (1996) 1901-1908. (c) V. Martichonok, G.M. Whitesides, *J. Am. Chem. Soc.*, 118 (1996) 8187-8191. (d) J.C. Castro-Palomino, Y.E. Tsvetkov, R.R. Schmidt, *J. Am. Chem. Soc.*, 120 (1998) 5434-5440.

[8] (a) A.V. Demchenko, G.-J. Boons, *Tetrahedron Lett.*, 39 (1998) 3065-3068. (b) A.V. Demchenko, G.-J. Boons, *Chem. Eur. J.*, 5 (1999) 1278-1283. (c) N. Hossain, G. Magnusson, *Tetrahedron Lett.*, 40 (1999) 2217-2220.

[9] (a) K.C. Nicolaou, C.W. Hummel, Y. Iwabuchi, *J. Am. Chem. Soc.*, 114 (1992) 3126-3128. (b) T. Ercegovic, G. Magnusson, *J. Org. Chem.*, 61 (1996) 179-184. (c) U. Ellervik, H. Grundberg, G. Magnusson, *J. Org. Chem.*, 63 (1998) 9323-9338. (d) K.M. Halkes, P.M. St. Hilaire, A.M. Jansson, C.H. Gotfredsen, M. Meldal, *J. Chem. Soc., Perkin Trans. 1*, (2000) 2127-2133.

[10] (a) P. Meindl, H. Tuppy, *Monatsch. Chem.*, 100 (1969), 1295-1306. (b) A. Claesson, K. Luthman, *Acta Chem. Scand., Ser. B*, 36 (1982) 719-720. (c) W. Schmidt, R. Christian, E. Zbiral, *Tetrahedron Lett.*, 29 (1988) 3643-3646.

[11] K.B. Sharpless, R.F. Lauer, *J. Am. Chem. Soc.*, 8 (1973) 2697-2699.

[12] O.T. Schmidt, *Methods Carbohydr. Chem.*, 2 (1963) 318-325.

[13] A. Kameyama, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.*, 193 (1989) c1-c5.

[14] H. Paulsen, H. Tietz, *Carbohydr. Res.*, 125 (1984) 47-64.

[15] (a) J.C. Jochims, G. Taigel, *Tetrahedron Lett.*, No. 52 (1968) 5483-5488. (b) Y.-H. Lee, F. Picardt, S.J. Pilkis, *Arch. Biochem. Biophys.*, 322 (1995) 357-360.

[16] H. Hori, T. Nakajima, Y. Nishida, H. Ohrui, H. Meguro, *Tetrahedron Lett.*, 29 (1988) 6317-6320.

[17] V. Martichonok, G.M. Whitesides, *Carbohydr. Res.*, 302 (1997) 123-129.

[18] V. Martichonok, G.M. Whitesides, *J. Org. Chem.*, 61 (1996) 1702-1706.

V

Iodine Monochloride/Silver Trifluoromethanesulfonate (ICl/AgOTf) as a Convenient Promoter System for *O*-Glycoside Synthesis

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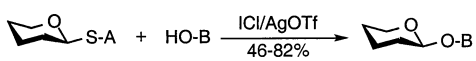
ABSTRACT



A = Me, Et, Ph, C(S)OEt
B = saccharide moiety

The novel promoter system iodine monochloride/silver trifluoromethanesulfonate (ICl/AgOTf) was evaluated with various thioglycoside donors and saccharide acceptors, and *O*-glycosides were obtained in 46-82% yield. Several practical advantages of the ICl/AgOTf system over known promoter systems were observed, such as convenient handling of the reagents and absence of by-products related to *N*-succinimide.

O-Glycosides, such as glycosphingolipids, are biologically significant *inter alia* as tumor-associated antigens and receptors for various bacterial and viral pathogens.[1] Consequently, numerous methods of *O*-glycosylation have been developed, of which thioglycoside methodology is one of the most versatile and widespread.[2] In short, such methodology employs a thioglycoside donor (*e.g.* having an alkylthio aglycon) which is *O*-glycosidically condensed with a carbohydrate acceptor in the presence of a thiophilic agent, where the latter is generated by a promoter system and transforms the aglycon into a good leaving group. There are numerous promoter systems for thioglycoside activation[2,3], of which sulfenylhalides/silver trifluoromethanesulfonate, such as PhSCl/AgOTf[4] and MeSBr/AgOTf,[5] or *N*-iodosuccinimide

† Deceased in June 2000.

(1) (a) Davis, B.G. *J. Chem. Soc., Perkin Trans. 1* **2000**, *14*, 2137-2160. (b) DeNinno, M.P. *Synthesis* **1991**, 583-593.

(2) *Carbohydrate Chemistry*, 1st ed.; Boons, G-J., Ed.; Blackie Academic & Professional: London, 1998; and references cited therein.

(3) (a) *Modern Methods in Carbohydrate Synthesis*; Khan, S. H.; O'Neill, R. A., Eds.; Harwood Academic Publishers: Amsterdam, 1996. (b) *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997.

(4) Martichonok, V.; Whitesides, G.M. *J. Org. Chem.* **1996**, *61*, 1702-1706.

(NIS) combined with either trifluoromethanesulfonic acid (TfOH)[6] or silver trifluoromethanesulfonate[7] appear to be the most commonly used. These systems are substantially comparable as regards yield of the desired *O*-glycosylated product. Hence, practical aspects such as preparation time, reagent handling and ease of reagent/product purification play an important role in the choice of promoter system. This is especially true for non-specialists entering the present field. Since sulfenyl halides are toxic, malodorous and unstable, thus also requiring preparation by the practitioner immediately before the reaction at hand, they are seldom a first choice. As a consequence, the *N*-iodosuccinimide systems are often preferred. However, those systems generate *N*-succinimide, which can be troublesome to separate from the desired *O*-glycoside product by conventional silica gel chromatography. Furthermore, it has recently been reported that *N*-succinimidyl glycosides are potential by-products, especially with reactive donors and unreactive acceptors.[8]

Iodine and its interhalogen compounds have been used for thioglycoside activation, *e.g.* in the preparation of glycosyl halides.[9] We now report the novel promoter system iodine monochloride/silver trifluoromethanesulfonate (ICl/AgOTf). This combination was found to have none of the aforementioned drawbacks and provides both efficient and practical[10] means of performing *O*-glycoside synthesis with thioglycoside donors.

In order to evaluate the full potential of the present promoter system, we decided to perform the glycosylations with some representative substrates. A considerable variety of protective groups was thereby also subjected to the reaction conditions (Scheme 1 and Table 1).[11]

The results set forth demonstrate the general applicability of the present method, and the yields obtained are all comparable to those obtained by other methods. Moreover, since it has been reported that iodine monochloride/silver tetrafluoroborate in methanol can iodinate even deactivated aromatic rings,[12] we find it noteworthy that no aromatic addition of iodine was observed in the glycosylations (see **7** and **13** in particular). All the reactions appeared to be very rapid, also for the least reactive donor **4**, albeit they were allowed to progress at least one hour for practical reasons. Also note that the iodonium species generated by this method selectively reacts with an anomeric methylthio group in the presence of a 2-methoxyphenylthio ring substituent (donor **11**).[13]

In summary, we believe that the efficiency and convenient handling of the ICl/AgOTf promoter system can provide an attractive alternative in *O*-glycoside synthesis with thioglycoside donors.

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Supporting Information Available: Complete experimental details and ^{1}H NMR spectra for compounds **11-13** as well as HR FAB-MS spectra for compound **13**.

(5) Dasgupta, F.; Garegg, P.J. *Carbohydr. Res.* **1988**, *177*, C13-C17.

(6) (a) Veeneman, G.H.; Van Leeuwen, S.H.; Van Boom, J.H. *Tetrahedron Lett.* **1990**, *31*, 1331-1334. (b) Konradsson, P.; Mootoo, D.R.; McDevitt, R.E.; Fraser-Reid, B. *J. Chem. Soc., Chem. Comm.* **1990**, 270-272.

(7) Konradsson, P.; Udodong, U.E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313-4316.

(8) (a) Turek, D. Ph.D. Thesis, Stockholm University, Stockholm, Sweden, 2000. (b) Andersson, M.; Konradsson, P.; Oscarsson, S.; Öhberg, L. *J. Carbohydr. Chem.*, in press.

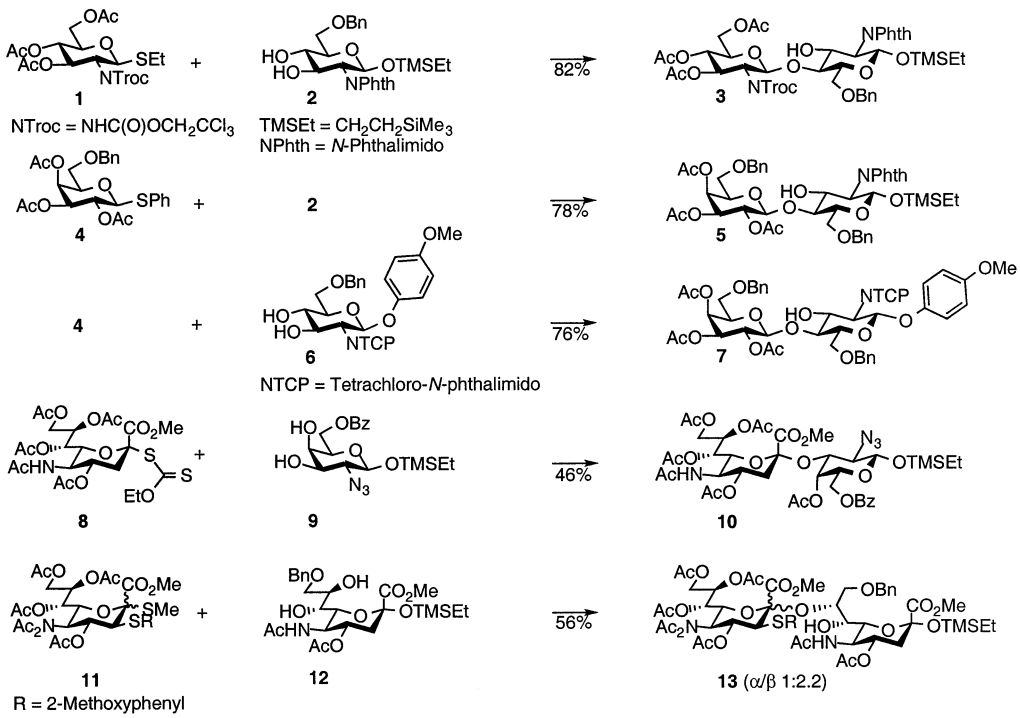
(9) (a) Ravindranathan Kartha, K.P.; Cura, P.; Aloui, M.; Readman, S.K.; Rutherford, T.J.; Field, R.A. *Tetrahedron: Asymmetry.* **2000**, *11*, 581-593. (b) Ravindranathan Kartha, K.P.; Field, R.A. *Tetrahedron Lett.* **1997**, *38*, 8233-8236.

(10) Iodine monochloride is commercially available as a dry 1.0 M solution in dichloromethane. Silver trifluoromethanesulfonate is soluble in acetonitrile and easily dried *in vacuo*. The entire procedure is substantially free from offensive odors.

(11) **Representative experimental procedure:** A stirred solution of ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (**1**; 76 mg, 0.15 mmol) and 2-(trimethylsilyl)ethyl 6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2**; 50 mg, 0.10 mmol) in dichloromethane (0.75 mL) was cooled to -45°C under an argon atmosphere. A solution of silver trifluoromethanesulfonate (77 mg, 0.30 mmol) in acetonitrile (0.50 mL) was added followed by dropwise addition of a 1.0 M solution of iodine monochloride in dichloromethane (0.25 mL, 0.25 mmol) during 10 min. After 2 h, diisopropylamine (0.20 mL, 1.4 mmol) was added and stirring was continued for 20 min. The reaction mixture was filtered and concentrated under reduced pressure. The residue was chromatographed (heptane/ethyl acetate, 3:1 → 1:1) on silica gel to give 2-(trimethylsilyl)ethyl 6-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-β-D-glucopyranoside (**3**; 78.2 mg, 82%) as a syrup.

(12) Jacob, L.A.; Chen, B.L.; Stec, D. *Synthesis* **1993**, *6*, 611-614.

(13) Using MeSBr/AgOTf as alternative promoter system gave the same diastereomeric ratio, and the mixture was inseparable by conventional silica gel chromatography.



Scheme 1. Evaluation of ICl/AgOTf as Promoter System for Various *O*-Glycosylations with Thioglycoside Donors

Table 1. Reaction Data for Scheme 1

donor	acceptor	mole ratio[a]	reaction conditions[b]	product[c]	yield[d,e] (%)
1[14]	**2**[14]	1.5:1.0:2.5:3.0	MeCN/CH_2Cl_2 1:2, -45°C, 2 h	**3**[14]	82
4[15]	**2**	1.3:1.0:2.5:3.0	MeCN/CH_2Cl_2 5:12, -78°C, 1 h	**5**[15]	78
4	**6**[15]	1.4:1.0:2.5:3.0	MeCN/CH_2Cl_2 1:2, -78°C, 3 h	**7**[15]	76
8[16]	**9**[17]	1.5:1.0:1.5:1.5	MeCN/CH_2Cl_2 4:3, MS 3Å, -78°C, 2.5 h	**10**[17]	46
11[18]	**12**[18]	1.0:1.6:1.4:1.4	MeCN, MS 3Å, -40°C, 2 h	**13**[18]	56

[a] Donor/acceptor/ICl/AgOTf. [b] Solvents and molecular sieves were dried and activated, respectively, using conventional methods. [c] NMR data were in agreement with those reported in the literature. [d] Based on the substrate (donor or acceptor) present in the smallest amount. [e] Unoptimized yields.

(14) Ellervik, U.; Magnusson, G. *Carbohydr. Res.* **1996**, *280*, 251-260.
(15) Ellervik, U.; Magnusson, G. *J. Org. Chem.* **1998**, *63*, 9314-9322.
(16) Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1990**, *195*, 303-308.
(17) Wilstermann, M.; Kononov, L.O.; Nilsson, U.; Ray, A.K.; Magnusson, G. *J. Am. Chem. Soc.* **1995**, *117*, 4742-4754. The crude product was acetylated to facilitate purification.
(18) Ercegovic, T. Ph.D. Thesis, The Lund Institute of Technology, University of Lund, Lund, Sweden, 2001, in press. Available at http://www.lub.lu.se/dissdb/ in .pdf format as of February 27, 2001.

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