Recent Advances in *O*-Sialylation

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I. Introduction

Sialic acids are a diverse family of naturally occurring 2-keto-3-deoxy-nononic acids that are involved in a wide range of biological processes.[1,2] The *C*-5-amino derivative represents the long-known neuraminic acid, and its amino function can either be acetylated (Neu5Ac) or glycolylated (Neu5Gc). The hydroxyls of these derivatives can be further acetylated, most commonly at *C*-9 but di- and tri-*O*-acetylated derivatives are also known. Lactoylation or phosphorylation may also occur at *C*-9, while *C*-8 hydroxyl can be methylated or sulfated. The most abundant sialic acid is, however, *N*-acetylneuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid). 3-Deoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (KDN) is an important form of sialic acid that does not have an amino functionality.[2]

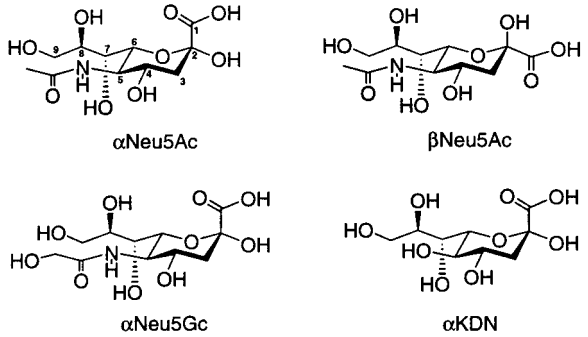

Figure 1.

The sugar ring of Neu5Ac has a $^2C_5$ conformation in which the bulky side chain and *C*-5 acetamido moiety adopt equatorial orientations (Figure 1). Most sialic acids occur as glycosides of oligosaccharides, glycoproteins, and glycolipids. The natural equatorial glycosides are classified as the α-anomers, whereas the unnatural axial ones are the *β*-glycosides. In *N*-linked glycoproteins, sialic acids appear essentially as terminal sugars α(2 → 3) or α(2 → 6)-linked to galactosides or α(2 → 6)-linked to *N*-acetyl-galactosaminides {i.e., Neu5Acα(2 → 3)Gal, Neu5Acα(2 → 6)Gal, and Neu5Acα(2 → 6)Gal*N*Ac}, whereas in *O*-linked glycoproteins, often terminal Neu5Acα(2 → 6)Gal*N*Ac moieties can be found. The disialosyl structures Neu5Acα(2 → 8)Neu5Ac and Neu5Acα(2 → 9)Neu5Ac have also been found as constituents of glycoproteins and lipids.

Neu5Ac or Neu5Gc also occur in linear homopolymers where they are usually linked internally by α-(2 → 8), α(2 → 9) or alternating α(2 → 8)/α(2 → 9) glycosidic linkages.[1,3−6] These polysialic acids are found in glycoproteins of embryonic neural membranes, where they play a role as neural cell adhesion molecules. They are also found in fish eggs and in the capsule of certain bacteria such as *Neisseria meningitidis* group B. An α(2 → 4)-linked homopolymer of 5,7-diacetamido-8-*O*-acetyl-3,5,7,9-tetradeoxy-D-*glycero*-D-*galacto*-nonulosonic acid,[7] α(2 → 5)-linked derivatives,[8,9] as well as galactose-substituted subterminal Neu5Ac[10] have also been described. Another unusual polysialic acid is composed of Neu5Gc moieties, which are glycosidically linked to the hydroxyl of the glycolyl moiety.[11]

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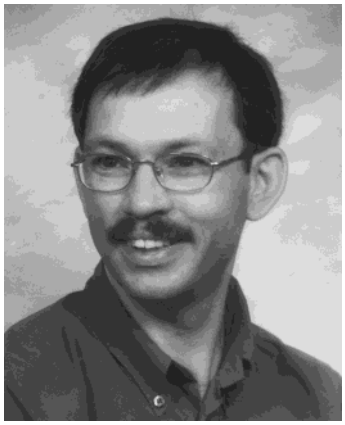
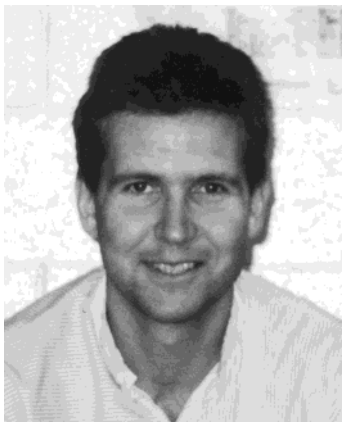


Professor Geert-Jan Boons received his B.Sc. degree from the University of Leiden, where he also obtained his Ph.D. degree under the direction of Jacque van Boom. He was a Ramsey Fellow working with Steven Ley FRS at Imperial College London and the University of Cambridge (U.K.). In 1994, he joined the faculty of the School of Chemistry of the University of Birmingham (U.K.), where he was promoted to Professor of Bioorganic Chemistry in 1998. In the same year, he moved to the Complex Carbohydrate Research Center (University of Georgia, Athens), where he is a Professor of Chemistry. His current interests are in the areas of bioorganic and synthetic chemistry with an emphasis on the development of efficient methods for glycoconjugate synthesis, preparation of oligosaccharides and glycoconjugates of medical or biological importance, and studying carbohydrate–protein interactions.



Alexei Demchenko received his Ph.D. degree from the Russian Academy of Sciences in 1993 under the direction of Professor N. K. Kochetkov. His thesis was entitled "Stereospecific 1,2-*cis*-glycosylation with 1,2-*trans*-thiocyanates". Then he continued for two more years working with Professor Kochetkov at the Institute of Organic Chemistry as a Research Associate. In 1995 he joined Professor G. J. Boons' group at the University of Birmingham, U.K., as a BBSRC postdoctoral research fellow. In 1998 he moved with Professor Boons to the University of Georgia as a Research Associate, where his research interests focus on the synthesis of complex oligosaccharides for immunological studies, investigation of stereo- and regioselectivity of glycosylation, as well as exploration of chemical *O*-sialylation of sterically hindered hydroxy groups and application for the synthesis of Neu5Ac(2 → 8)Neu5Ac-linked oligomers.

Cell-type expression of sialyltransferases leads to specific sialylation patterns of glycoconjugates that can determine the makeup of cells.[12,13] Striking differences have been found in the sialylation pattern of cells during development, activation, aging, and oncogenesis. As terminal substituents of cell surface glycoproteins and glycolipids, sialic acids are ideally positioned to participate in carbohydrate−protein interactions that mediate recognition phenomena. Indeed, they serve as ligands for microbial toxins, for microbial adhesion which mediate attachment to a host cell, and for animal lectins that are important for cell−cell adhesion.

Sialic acids also play important roles as masks to prevent biological recognition.[5] For example, acetylation of the *C*-9 hydroxyl prevents this monosaccharide to be a receptor for the attachment of influenza A and B viruses.[14] Modifications of sialic acids can interfere with the mode of cell interaction. For example, *O*-acetylation or *N*-acetyl hydroxylation hinders the action of sialidases, leading to longer lifetimes of rat erythrocytes.[15]

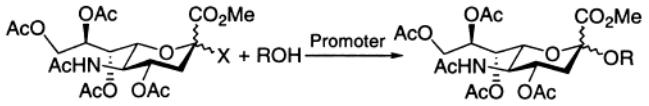
Advances in both chemical and enzymatic syntheses have provided reliable routes to the production of many complex sialosides. These compounds are of key importance to determine the biological roles of these glycoconjugates. This review surveys recent progress in chemical and enzymatic sialylation.

Interglycosidic bond formation is generally achieved by condensing a fully protected glycosyl donor, which bears a potential leaving group at its anomeric center, with a suitably protected glycosyl acceptor that often contains only one free hydroxyl group.[16−22] The glycosylation can result in a α- and/or β-anomer, and the stereocontrol of this condensation reaction is one of the most challenging aspects in oligosaccharide chemistry. The nature of a protecting group at *C*-2 of the glycosyl donor is a major determinant of the anomeric selectivity. A protecting group at *C*-2, which can perform neighboring group participation during glycosylation, will favor the formation of a 1,2-*trans*-glycosidic linkage. On the other hand, the reaction conditions (e.g., solvent, temperature, and promoter) will determine the anomeric selectivity when a nonassisting functionality is present at *C*-2. Also, the constitution of the glycosyl donor and acceptor (e.g., type of saccharide, leaving group at the anomeric center, protection, and substitution pattern) have a major effect on the α/β selectivity.

Glycosides of *N*-acetyl neuraminic acid can be introduced by similar glycosylation approaches, and these coupling procedures are classified as direct methods.[23−26] The use of glycosyl donors of Neu5Ac is, however, complicated by the fact that no neighboring *C*-3 functionality is present to direct the stereochemical outcome of glycosylations. In addition, the deoxy moiety in combination with the electron-withdrawing carboxylic acid at the anomeric center makes these derivatives prone to 2,3-elimination (formation of glycal). Also, glycosylations of Neu5Ac take place at a sterically hindered tertiary oxocarbenium ion intermediate. The most successful glycosyl donors of Neu5Ac use rather unusual anomeric leaving groups. For example, anomeric fluorides and trichloroacetimidates are most widely applied for glycosidation of common glycosyl donors, whereas sialyl donors rarely or never possess these leaving groups. On the other hand, the rather unusual phosphites and xanthates are very attractive leaving groups for sialyl donors (Scheme 1).

The difficulties of glycosidations of Neu5Ac have been addressed by indirect methods. These approaches require several synthetic steps for the formation of *O*-sialosides and will be discussed in section III.

Scheme 1



X = Br, Cl, F, SMe, SEt, SPh, SC(=S)OEt, OP(OR")$_2$, OCOR'

II. Direct Chemical Glycosylations

Direct *O*-sialylations include those methods which lead to the formation of *O*-sialosides in one synthetic step involving the coupling of a glycosyl acceptor having a free hydroxyl with a glycosyl donor with an appropriate leaving group at *C*-2.

A. 2-Halogeno Derivatives

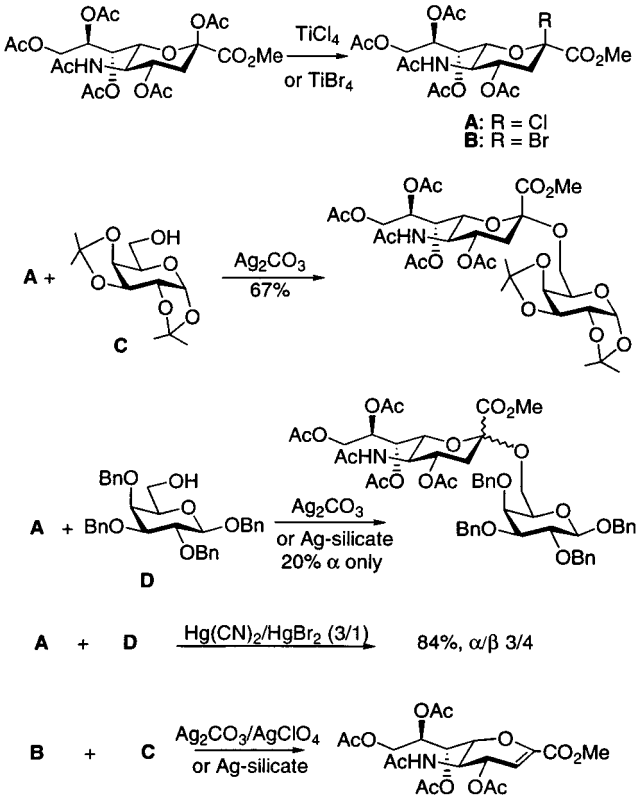
2-Chloro derivatives of Neu5Ac were the first compounds to be used for the glycosidation of sialic acid. This approach was the main tool for the synthesis of compounds containing *N*-acetylneuraminic acid from the 1960s through 1980s. Nowadays, the use of these derivatives is limited to the glycosylation of simple alcohols. 2-Bromide and 2-fluoride derivatives of Neu5Ac have also been used as glycosyl donors but results were disappointing. In this section, the most important aspects of the use of 2-halogeno derivatives will be discussed. More comprehensive discussions can be found in previous review articles.[23,24]

1. 2-Chloro Derivatives

2-Chloro derivatives of Neu5Ac have a reasonable shelf life when handled with care and stored in a freezer. Several methods have been reported for their synthesis. For example, they can be prepared from the corresponding *C*-2 acetate by the treatment with HCl/AcCl,[27] $TiCl_4$,[28] or AcCl/MeOH.[29] The first glycosides of *N*-acetylneuraminic acid were obtained by coupling simple alcohols (e.g., methanol, benzyl alcohol, substituted benzyl alcohols, etc.) with the free acid of an acetochloro derivative of Neu5Ac using Ag_2CO_3 (classical Koenigs−Knorr) or $Hg(CN)_2/HgBr_2$ (Helferich modification) as promoter.[30] The corresponding α-ketosides were obtained in modest yields (30−50%). The methyl ester of acetochloroneuraminate is a much more stable derivative (compound **A**, Scheme 2) and in the presence of the promoter Ag_2CO_3[27] proved to be a far superior glycosyl donor. This glycosyl donor found wide application, and its properties have been discussed in detail in several review articles.[23,24,31−35]

In 1971, the first synthesis of a disaccharide containing Neu5Ac was accomplished by Khorlin and co-workers.[36] Several Neu5Ac(2 → 6), (2 → 3)Glc, and Neu5Ac → Gal derivatives were synthesized by a Ag_2CO_3-promoted glycosylation of glycosyl donor **A** in $CHCl_3$ (Scheme 2). Further studies by Paulsen and co-workers[37] led to the conclusion that silver-promoted glycosylations give only satisfactory results when applied to highly reactive hydroxyls (e.g., acceptor **C**, Scheme 2). Mainly glycal (2,3-dehydro derivative) formation by competing elimination was

Scheme 2



observed when applied to less reactive secondary or sterically hindered primary hydroxyl groups (e.g., **D**), and in these cases only traces of desired disaccharides were isolated. They found, however, that the formation of glycal can be suppressed by employing $Hg(CN)_2/HgBr_2$ as the promoter.[37] This activation method gave disaccharides in reasonable yields although as mixtures of anomers. For example, a Neu5Ac(2 → 6)-Gal derivative was obtained in an excellent yield of 84% as a 3/4 mixture of α/β-anomers. A similar glycosylation approach was employed for the first synthesis of a Neu5Ac(2 → 9)Neu5Ac-linked dimer (41%, α/β 2/1),[38] whereas Neu5Ac(2 → 3)Gal derivatives were obtained with rather low efficiency (15%, α/β 2/3),[39] presumably due to lower reactivity of secondary hydroxyls leading mainly to elimination of the Neu5Ac donor.

Another approach for the synthesis of *O*-glycosides of Neu5Ac is based on activation of acetochloroneuraminic acid or its methyl ester with insoluble polymer-based silver salts, such as silver polymaleate and polymethacrylate.[40] Insoluble silver salts were introduced for glycosylations with inversion of configuration, and these promoters proved to be of particular value for the preparation of β-mannosides. When these silver salts were applied to the coupling of acetochloroneuraminic acid with simple alcohols, the corresponding α-glycosides were isolated in reasonable yields (51−64%). These conditions gave slightly higher yields for the free acid compared to the methyl ester. Silver salicilate, which is another polymeric catalyst, was also applied,[41] and when simple alcohols were glycosylated, only α-anomers were obtained in good yields (84−89%). In most cases, these glycosylations were complete within a few

minutes and, therefore, found application for the synthesis of several simple glycosides of Neu5Ac.[42−46]

Other promoters have also been applied for the glycosylation of the 2-chloro derivatives of Neu5Ac. It was shown that the mild Lewis acid promoter $ZnBr_2$ gives better results (62−76%, α/β 2−4/1) than $SnCl_2$, $Sn(OTf)_2$, $ZnCl_2$, ZnI_2, or $Zn(OTf)_2$.[47] Phase-transfer catalyses proved to be efficient for the synthesis of aryl glycosides.[48] Important advantages of this approach include cheap and nonhazardous catalysts {$BnNEt_3Cl$ vs Ag_2CO_3 or Hg(II) salts}, short reaction times (0.5 h vs many hours or days), high stereoselectivities, and generally good yields (40−70%). Unfortunately, this method is only applicable for the glycosylation of water-soluble aglycons. Some other promoters such as silver trifluoromethanesulfonate (AgOTf),[49,50] silver zeolite,[51] Bu_4NHSO_4,[52] I_2/2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ),[53] and diisopropylethylamine (*i*-Pr_2NEt, DIPEA)[54] were introduced. It has also been shown that glycosylation of aliphatic alcohols can be effectively performed in the absence of added promoter for both free acid and corresponding methyl ester.[55]

O-Sialylation with high levels of regioselectivity were achieved by tin-mediated glycosylations.[56] Thus, treatment of the unprotected *O*-(2-trimethysilyl)ethyl (*O*-TMS-ethyl, *O*TE) galactoside with Bu_2SnO, followed by reaction with 2-chloride in the presence of Bu_4NBr, gave regioselectively a Neu5Ac(2 → 6)Gal-linked disaccharide (59%) as an α/β-mixture (3/2).

2-Chloro derivatives of Neu5Ac were used for the formation of a dimeric (2 → 8)-linked structure,[57,58] synthesis of 3-deoxy-D-glycero-D-galacto-2-nonulopyranosonic acid (KDN) glycosides,[59−62] synthesis of *C*-[63−65] and *S*-glycosides.[34,35,66−72] Several other examples of the use of 2-chloro derivatives of Neu5Ac in the oligosaccharide synthesis have been reported.[28,29,70,73−98]

It can be concluded that the application of 2-chloro derivatives of Neu5Ac gives, in general, good yields when coupled with simple or primary sugar alcohols.[99] As a matter of fact, these methods offer the most reliable approach for the synthesis of simple glycosides of Neu5Ac (see, for example, the synthesis of the strategically important *O*TE glycosides[100]). More complex glycosides require modern glycosylation methods which will be discussed in the subsequent sections.

2. 2-Bromo and 2-Fluoro Derivatives

2-Bromo derivatives have found limited application in chemical *O*-sialylation, most likely due to their high reactivity and, therefore, low chemical stability. 2-Bromides of Neu5Ac (**B**, Scheme 2) can be obtained by treatment of 2-*O*-acetyl derivatives with $TiBr_4$[28] or by a simplified procedure which involves treatment with AcBr/MeOH in $CHCl_3$.[29] In general, glycosylations with these bromides give low yields of glycosylation products mainly due to competing elimination to give a 2,3-dehydro product (**B** + **C**, Scheme 2). Only coupling of the bromide with highly reactive monosaccharide glycosyl acceptors having a 6-hydroxy group in the presence of a silver catalyst gave reasonable yields of α-linked disaccharides. Glycosylation of less reactive alcohols gave lower yields compared to the use of sialyl chloride.[28,101] Methyl glycosides, however, can be synthesized in the presence of γ-collidine or its salts in high yield (89%) and good anomeric selectivity (α/β 9/1).[29] The stereoselectivity was compromised (α/β 2/1) when applied to the synthesis of benzyl glycosides; no glycal formation was observed in these reactions.

The 2-bromo derivative of KDN is more stable than the analogous Neu5Ac derivative and found wider application for the synthesis of KDN-glycosides.[62,83,102,103]

2-Fluoro derivatives of Neu5Ac have found limited application for glycoside formation. The fluorides can be synthesized from the corresponding 2-chloro derivatives by reaction with AgF in MeCN to give a mixture of anomers (65%, α/β 3/1)[104,105] or by a direct method involving treatment of 2-*O*-acetyl precursors with HF/pyridine (80−90%, only β-anomer).[104,106,107] They were used to overcome the relatively low stability of 2-chloro and 2-bromo derivatives, but unfortunately, $BF_3{\cdot}OEt_2$-promoted glycosylations gave consistently unnatural β-anomers as the predominant or exclusive product in moderate yields.[106,107] These findings limit the application of 2-fluoro derivatives as glycosyl donors for direct glycosylation. Fluorides were utilized for the synthesis of *S*-glycosides of Neu5Ac.[105]

B. 2-Thio Derivatives

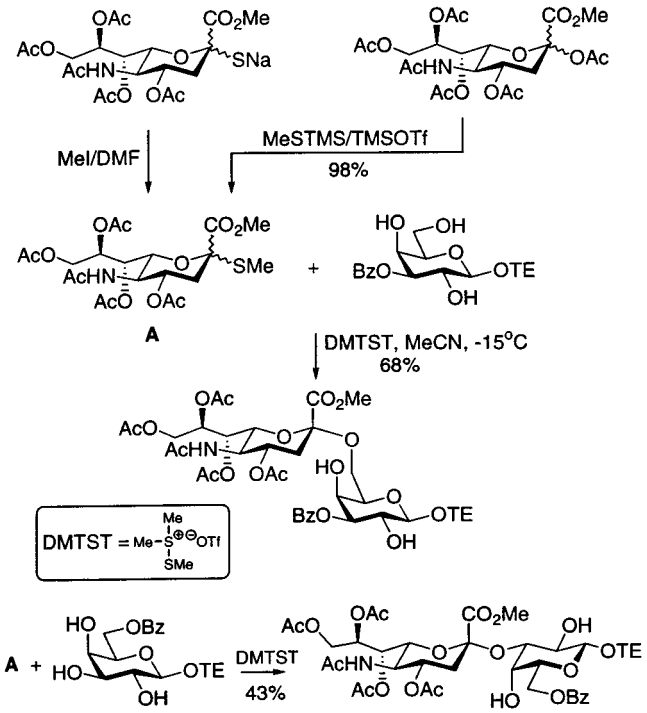
The most important 2-thio derivatives for glycosidation of Neu5Ac include *S*-alkyl (methyl, ethyl) and *S*-aryl (phenyl and substituted phenyl) glycosides and glycosyl xanthates (ethoxydithiocarbonate). These derivatives have been widely applied for the synthesis of sialic acid-containing oligosaccharides and glycoconjugates. Several reviews discuss these glycosyl donors.[17,18,21,35,108−113] Early results of the application of *S*-xanthates has also been surveyed.[17] Thioglycosides can be activated under mild conditions in the presence of at least an equimolar amount of a thiophilic promoter.[112] Alkyl(aryl) thioglycosides have emerged as versatile building blocks for oligosaccharide synthesis. Due to their excellent chemical stability, anomeric alkyl(aryl) thio groups offer efficient protection of anomeric centers and are compatible with many reaction conditions often employed in carbohydrate chemistry. However, in the presence of soft electrophiles, thioglycosides can be activated and used in direct glycosylations. The most commonly used activating reagents include methyl trifluoromethanesulfonate (MeOTf), dimethyl(methylthio) sulfonium trifluoromethanesulfonate (DMTST), *N*-iodosuccinimide (NIS)−trifluoromethanesulfonic acid (triflic acid, TfOH), iodonium dicollidine perchlorate (IDCP), and phenyl selenyl trifluoromethanesulfonate (PhSeOTf). Another attractive feature of thioglycosides is that they can be transformed into a range of other glycosyl donors.

1. 2-Thioalkyl Derivatives

a. Thiomethyl Glycosides. The 2-thiomethyl sialyl derivatives are attractive glycosyl donors. Originally these compounds were synthesized by methyl-

ation of a sodium salt of 2-thiol derivatives of Neu5Ac with MeI in DMF (Scheme 3).[114] This method was

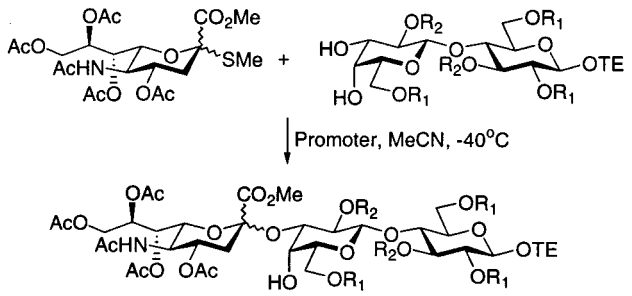
Scheme 3



substituted by a very efficient procedure which involves the direct conversion of a *C*-2 anomeric acetate into 2-thiomethyl TMS-derivatives by the treatment with methylthiotrimethylsilane (TMSSMe) in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-triflate, TMSOTf) in 1,2-dichloroethane (DCE).[115] This approach gives a fully acetylated thioglycoside (**A**, Scheme 3) in almost quantitative yield (98%) as a 1/1 mixture of α/*β*-anomers. In glycosylations, the α- and *β*-anomers have very comparable glycosyl donor properties and therefore do not need to be separated.[116] The synthesis of 2-thiomethyl glycosides of Neu5Ac and the application as sialyl donors was thoroughly developed by Hasegawa and co-workers.[114,117−119]

Glycosylation of 6-hydroxy derivatives of galactosides in the presence of DMTST gave the corresponding (2 → 6)-linked disaccharides (68%, Scheme 3).[120−122] The best α-anomeric selectivities were obtained when the participating solvent acetonitrile (MeCN) was employed at low temperature.[114] These reaction conditions were also used for the glycosylation of secondary sugar hydroxyls such as *C*-3 of galactosides and *C*-3′ of lactosides. Good yields (∼50%) and high α-anomeric selectivities were achieved when glycosyl acceptors were partially protected (glycosylations of diol and triols, Schemes 3 and 4). The best results were obtained by *C*-3′ regioselective glycosylations of a 2′,3′,4′-trihydroxy derivative of lactosides rather than the use of similar 3′,4′-diols (Scheme 4).[120−122] The regioselectivity of this glycosylation is due to the greater reactivity of the equatorial alcohol compared to the axial *C*-4 hydroxyl. Furthermore, the *C*-2 hydroxyl has a lower nucleophilicity due to the electron-withdrawing effect of the adjacent anomeric

Scheme 4



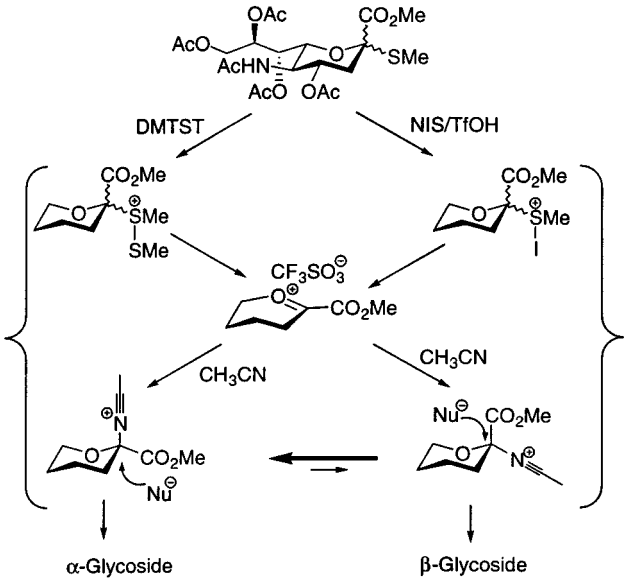
center. When a similar glycosylation was performed with a galactosyl acceptor having only a free *C*-3 hydroxyl group, the yield and anomeric stereoselectivity were significantly reduced. It should also be realized that procedures for the preparation of glycosyl acceptors having several free hydroxyls are often easier to conduct and, hence, may offer shorter routes to oligosaccharides.

Further improvement came with the application of the highly reactive promoters NIS/catalytic TfOH which proved to be especially valuable when applied to glycosylations of sterically more hindered hydroxyls.[123] For example, NIS/TfOH-mediated glycosylation of 3′,4′-diol of a lactoside gave a much higher yield of the (2 → 3)-linked product (69%) and improved anomeric stereoselectivity (α/*β* 6/1) compared to a similar glycosylation promoted by DMTST (Scheme 4). The resulting glycosylation products can be easily converted into glycosyl donors by conversion of the temporary anomeric *O*TE functionality into a thiomethyl leaving group (acetylation of the free hydroxyls with Ac_2O/pyridine followed by acetolysis of the anomeric *O*-(2-trimethylsilyl)ethyl group by treatment with $BF_3{\cdot}Et_2O/Ac_2O$ and conversion of the resulting anomeric acetyl group into a thiomethyl moiety by treatment with TMSSMe/TMSOTf). The promoter system NBS/Bu_4NOTf was proposed as an alternative for NIS/TfOH and was found to be equally effective.[124]

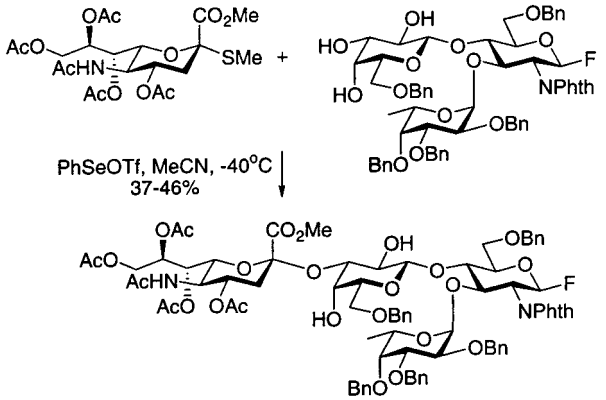
Reaction mechanisms of activation of thioglycosides of Neu5Ac by either DMTST or NIS/TfOH in MeCN have been proposed[123] (Scheme 5) and involve the generation of an electrophilic species ($^+$SMe or I^+), which reacts with the lone pair of sulfur resulting in the formation of a sulfonium intermediate. The sulfonium moiety is an excellent leaving group and can be displaced by a hydroxyl of a glycosyl acceptor or alternatively by nitrogen of acetonitrile to give a nitrilium ion. This nitrilium ion adopts a preferred axial (*β*) configuration. Nucleophilic substitution of the nitrilium ion with an alcohol gives predominantly equatorial α-glycosides. It has been observed that less reactive (secondary) alcohols give much higher α-selectivity than primary alcohols.

PhSeOTf-promoted glycosylations of *S*-methyl sialosides were developed by Ogawa and co-workers.[125] Good yields and anomeric stereoselectivities were achieved when performed in MeCN at low temperature.[116] This promoter was applied for an orthogonal

Scheme 5



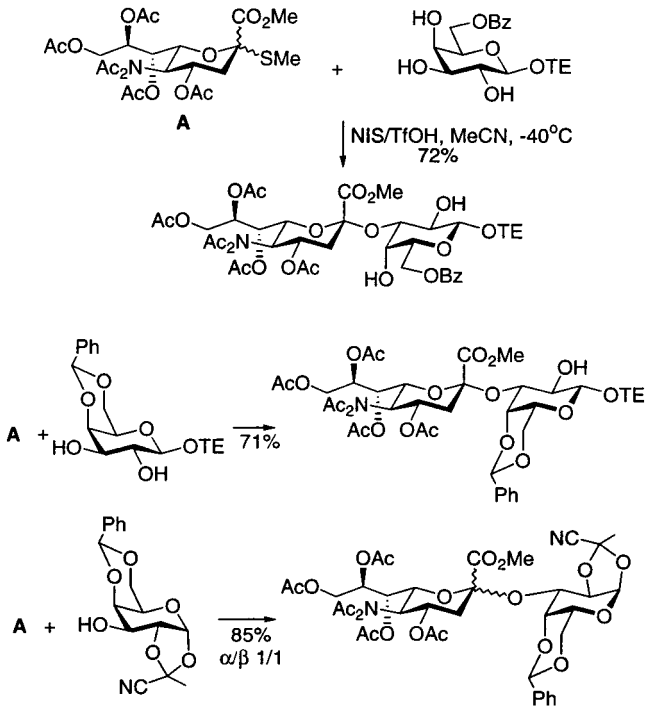
Scheme 6



glycosylation strategy whereby a tetrasaccharide (Scheme 6) was obtained in good yield by coupling a 2-thiomethyl sialosyl donor with a glycosyl fluoride trisaccharide acceptor. The orthogonal glycosylation strategy exploits the finding that a thioglycoside can be activated without affecting an anomeric fluoride and vice versa.[93,98] This feature is attractive since the methodology allows complex oligosaccharides to be assembled without extensive manipulations at the anomeric center. This also reduces the number of synthetic steps and therefore increases overall efficiency. For example, acetylation of the free hydroxyls of a tetrasaccharide (Scheme 6) gives a glycosyl fluoride which can be used as a glycosyl donor in a subsequent glycosylation with a thioglycosyl acceptor.

Recently, a significantly more reactive 2-thioglycosyl donor of Neu5Ac was introduced which bears a di-*N*-acetyl (*N*-acetylacetamido) functionality at *C*-5.[126] It was observed that the additional *N*-acetyl moiety of the glycosyl donor dramatically increases its reactivity, resulting in improved yields of glycosylation products (Scheme 7). For example, an NIS/TfOH-promoted coupling of 2-(trimethylsilyl)ethyl 6-*O*-benzoyl-*β*-D-galactopyranoside with mono-*N*-acetylated glycosyl donor proceeded with high regioselectivity to give, after a reaction time of 2 h, an α(2 → 3)-linked

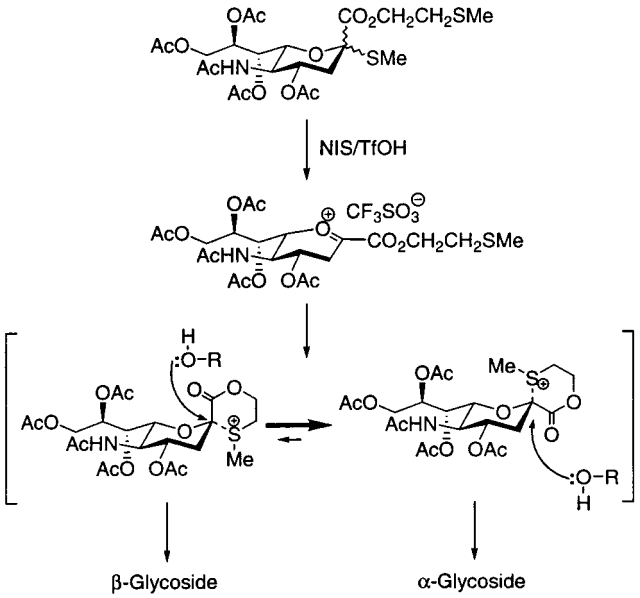
Scheme 7



disaccharide in a yield of 61%.[123] The application of the novel di-*N*-acetylated glycosyl donor, under similar reaction conditions, afforded a similar disaccharide within 5 min in an improved yield of 72%. Moreover, only a small excess of donor was required to achieve the high yield (1.1 mol equiv of the donor instead of the conventional 1.7−2.0 mol equiv). Excellent yields were also obtained when a 4,6-*O*-benzylidene-protected galactoside was used as glycosyl acceptor. Furthermore, when a 1,2-cyanoethylidene derivative was applied as glycosyl acceptor, the corresponding disaccharide was obtained in high yield (85%); however, the anomeric stereoselectivity was compromised (α/*β* 1/1). The additional *N*-acetyl function can be easily cleaved under Zémplen deacetylation conditions with concomitant *O*-acetyl group removal. The highly reactive glycosyl donor was also applied for the direct synthesis of (2 → 8)- and (2 → 9)-linked dimers of *N*-acetylneuraminic acid (see sections IV.A and IV.B).[127]

An interesting attempt to control the stereoselectivity of glycosylation of Neu5Ac was reported employing long-range participation.[128] In this approach, the carboxyl at *C*-1 of 2-thiomethyl Neu5Ac was protected as a 2-thioethyl ester. It was envisaged that activation of the anomeric center of the Neu5Ac donor would give an oxonium-ion intermediate, which would be stabilized by long-range participation of the thiomethyl moiety resulting in the formation of a sulfonium intermediate (Scheme 8). Glycosylation of the thermodynamically more stable *β*-sulfonium intermediate should give mainly formation of α-glycosides. Reasonable yields and α-anomeric selectivities were achieved when applied to primary as well as secondary glycosyl acceptors. The proposed mechanism was supported by the finding that anomeric selectivities were not affected by reaction solvents, and even when the glycosylation was performed in

Scheme 8



ethylene glycol dimethyl ether (DME), mainly α-linked disaccharides were formed although in moderate yields (20−50%). Application of PM3 calculations to the α-and *β*-sulfonium ion revealed an energy difference of 1.2 kcal/mol. This energy difference is probably not large enough to achieve high anomeric selectivities. The method was applied to the preparation of a taxol−sialyl conjugate.[129]

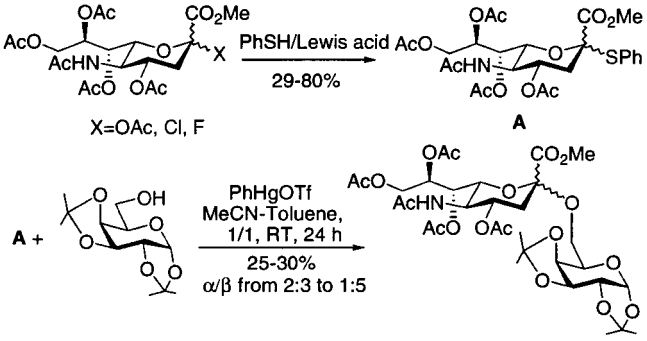
The 2-thiomethyl sialyl donor has been applied for the synthesis of many oligosaccharides and glycoconjugates, and in particular, Hasagawa and coworkers have synthesized a large number of complex molecules.[96,130−169] This sialyl donor was also used for the solid support synthesis of sialyl Lewis^x.[170] In this case, the glycosylation was performed in MeCN at −15 °C and promoted with DMTST. The glycosylation was repeated twice to give the desired tetrasaccharide in a yield of 68%.

b. 2-Thioethyl Glycosides. Thioethyl glycosides of Neu5Ac are readily available by treatment of the corresponding 2-*O*-acetyl derivative with ethanethiol in the presence of $BF_3{\cdot}Et_2O$ (75% yield, α/*β* 1/24).[171] The anomerically pure α-glycoside was obtained from the 2-chloro derivative using phase-transfer catalyst conditions ($EtSH/Bu_4NHSO_3/CH_2Cl_2$/1 M aq Na_2CO_3). Under these conditions, the displacement proceeds via a clean S_N2 mechanism.[172] DMTST was the first promoter to be used for the activation of *S*-ethyl glycosides,[173] and Neu5Ac(2 → 6)Gal derivatives were prepared in yields of 15−32% as mixtures of anomers (α/*β* 3/1). Other promoters that were applied include NIS/TfOH,[174−177] NBS/Bu_4NOTf,[176] and NIS/TMSOTf.[178−180] These studies suggest that *S*-methyl and *S*-ethyl derivatives give very similar yields and anomeric selectivities, but no proper comparative study has been reported.

2. 2-Thioaryl Derivatives

2-Thiophenyl glycosides of Neu5Ac are easily available by reaction of thiophenol with 2-*O*-acetyl, 2-chloro, or 2-fluoro derivatives of Neu5Ac.[171,181] In general, 2-thioaryl glycosyl donors are activated by using similar promoter systems as employed for *S*-alkyl derivatives. The first glycosylation of a 6-hydroxyl of a galactoside employed PhHgOTf as the promoter and afforded disaccharides in modest yields (25−30%) and poor anomeric selectivity (see Scheme 9).[181] The

Scheme 9

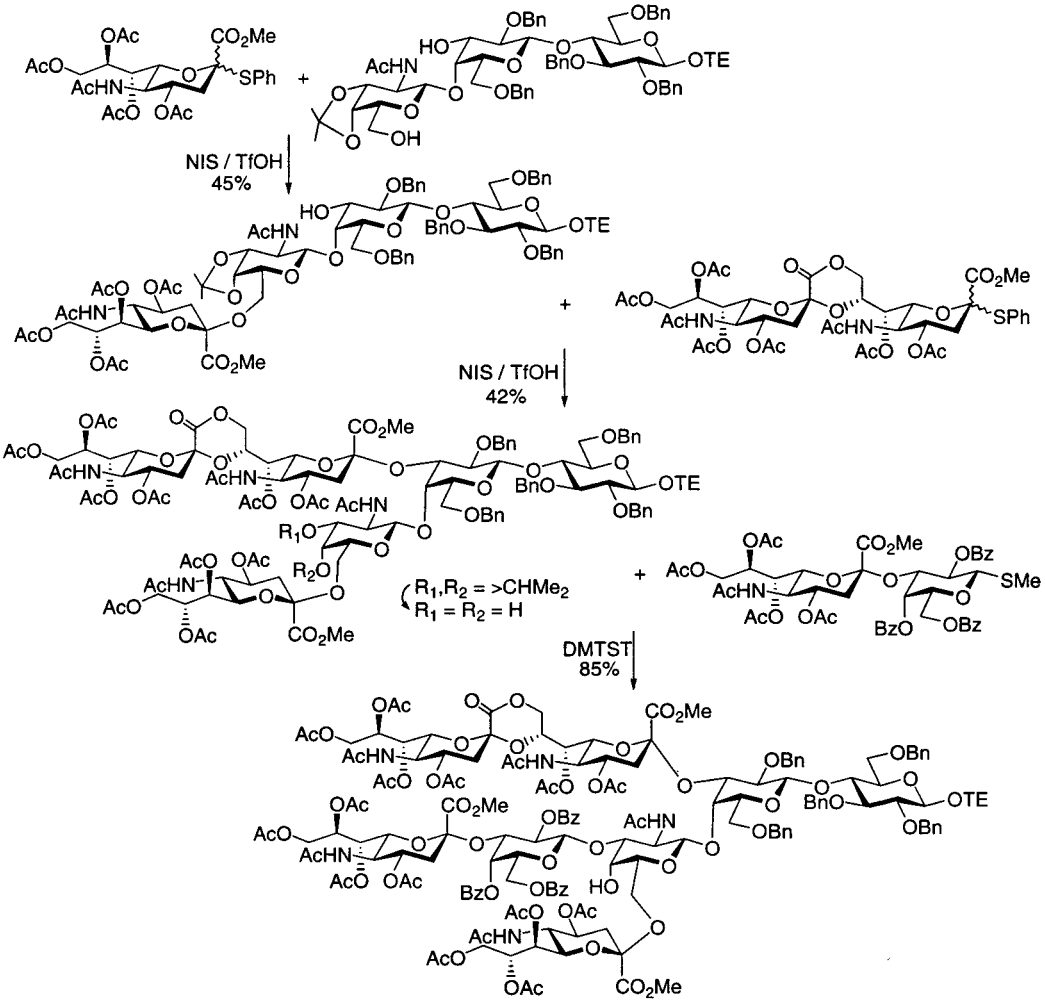


application of NIS/TfOH as the promoter system at low temperature gave much improved results.[182−184] Several other activators including NBS, I_2/Bu_4NOTf, and DMTST have been used.[185] In some cases, 2-thiophenyl glycosides proved to be more efficient than the corresponding 2-thiomethyl derivatives.[165] Higher yields were obtained with a benzoylated glycosyl donor.[186]

2-Thiophenyl glycosides were also employed for the introduction of KDN glycosides, and in this case, NIS/TMSOTf was used as the promoter.[149,187−189] The most valuable use of thiophenyl glycosides for *O*-sialylation was for the formation of (2 → 8)-linked oligomeric sialyl units.[165,190−198] This important glycosylation will be discussed below (section IV.A).

An example of a highly convergent synthesis of an oligosaccharide that contains four sialyl moieties is shown in Scheme 10.[199] Thus, NIS/TfOH-promoted glycosylation of a trisaccharide having a 3′,6″-diol with a 2-thiophenyl glycosyl donor of Neu5Ac gave the expected α(2 → 6)-linked tetrasaccharide in a 45% yield. The glycosylation was highly regioselective and took place only at the more reactive primary hydroxyl. Subsequent glycosylation of the resulting tetrasaccharide with a dimeric sialyl donor under similar reaction conditions afforded a hexasaccharide (42%). Removal of the 3,4-*O*-isopropylidene acetal followed by DMTST-promoted glycosylation with a Neu5Acα(2 → 3)Gal*S*Me donor furnished the desired octasaccharide in exceptional 85% yield. This compound was subsequently converted into a glycosyl donor required for the introduction of a ceramide moiety. This example illustrates three different ways for introducing sialic acid fragments into complex oligosaccharides. The first glycosylation entails an NIS/TfOH-mediated regioselective glycosylation of a monomeric 2-thiophenyl sialyl donor. In the second glycosylation, a 2-thiophenyl donor is employed also but in this case the donor is a more complex dimeric Neu5Acα(2 → 8)Neu5Ac derivative (section IV.A). The last glycosylation step cannot be classified as an *O*-sialylation because it uses a galactosyl donor that has a Neu5Ac moiety at C-3. Nevertheless, the latter block synthetic approach is important for the synthesis of complex sialylated oligosaccharides because

Scheme 10



the sialic acid unit is introduced into simpler structure prior to complex oligosaccharide assembly. In general, such a strategy is more easily achieved.

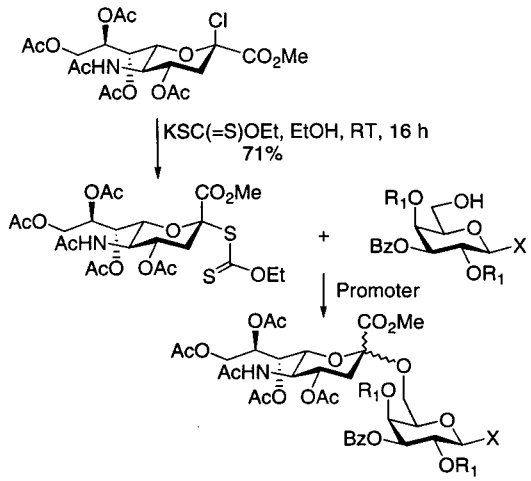
Synthesis and use of *p*-substituted 2-thioaryl glycosides has been described by Roy and co-workers.[141,172] It was found that a *p*-methoxyphenyl 2-thio derivative has very similar glycosyl donor properties compared to an unsubstituted *S*-phenyl derivative. On the other hand, an anomeric *p*-nitrophenyl substituent was inert (latent) toward activation with NIS/TfOH. This moiety, however, could be easily converted into the active *p*-acetamidophenyl by the reaction with $SnCl_2$/EtOH followed by *N*-acetylation.[141] Such a derivative could be activated with NIS/TfOH as the promoter system. Tuning the anomeric reactivity of glycosides offers an attractive way to synthesize complex oligosaccharides involving a minimal number of protecting group manipulations.

2-Thiophenyl glycosyl donors have been applied for the synthesis of many sialyl-containing oligosaccharides.[156,197,200−221]

3. 2-Xanthates

2-(Ethoxy)dithiocarbonate or 2-xantho derivative of Neu5Ac have been synthesized from the 2-chloride by the reaction with potassium ethoxydithiocarbonate in EtOH (Scheme 11).[171] Sialyl xanthate is a stable crystalline material which has a good shelf

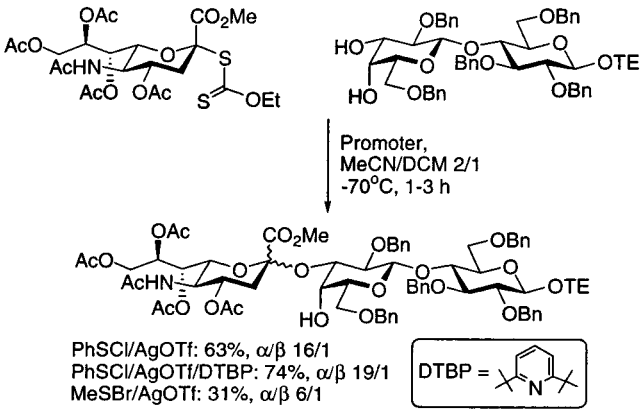
Scheme 11



live. A DMTST-promoted glycosylation of the 6-hydroxyl of a galactosyl acceptor (Scheme 10) afforded a (2 → 6)-linked disaccharide in a 64% yield as a 3/1 mixture of α/β-anomers.[173] A 2-thioalkyl glycosyl donor gave a lower yield (32%) of the disaccharide when reacted under similar conditions illustrating the advantageous properties of 2-xanthates.

Other promoter systems such as NIS/TfOH were also successfully used;[110,176,222] however, a major breakthrough came with the introduction of methylsulfenyl trifluoromethanesulfonate (MeSOTf) as the promoter. This highly reactive thiophilic reagent can be generated in situ by reaction of methylsulfenyl bromide (MeSBr) with AgOTf. It activates 2-xanthates at low temperature (−70 °C), and the best results were obtained when a mixture of MeCN/CH_2Cl_2 (3/2 v/v) was used as the reaction solvent.[223] An attractive feature of this glycosylation protocol is that sialyl xanthates can be selectively activated in the presence of thioglycosides. A successful coupling of a 2-xanthate donor with a thioglycosyl acceptor allowed an oligosaccharide to be synthesized by a highly efficient orthogonal assembly approach (see, for example, Scheme 11). Phenyl sulfenyl trifluoromethanesulfonate (PhSOTf) obtained in a similar fashion from PhSCl and AgOTf proved to be another efficient promoter, especially when applied in combination with the hindered base 2,6-di(*tert*-butyl)-pyridine (DTBP) at low temperatures (−70 °C).[224] For example, a trisaccharide was obtained in an excellent yield of 74% mainly as the α-anomer (α/*β* 19/1, Scheme 12). It has been shown that this promoter

Scheme 12



performs better in terms of yields and stereoselectivities compared to MeSOTf.

The reaction mechanism of xanthate activation is similar to that of thioglycosides.[224] Several interesting examples of the application of sialyl xanthates for oligosaccharide synthesis have been reported.[151,152,176,225−240]

4. Other 2-Thio Derivatives

Several other 2-thio derivatives of Neu5Ac have been reported.[171,172] For example, phenyl sulfones were used for the synthesis of *C*-sialosides[241−244] using SmI_2 as the activator. A sodium salt of a 2-thiol derivative was employed for the synthesis of *S*-linked oligosaccharides.[148,245−247]

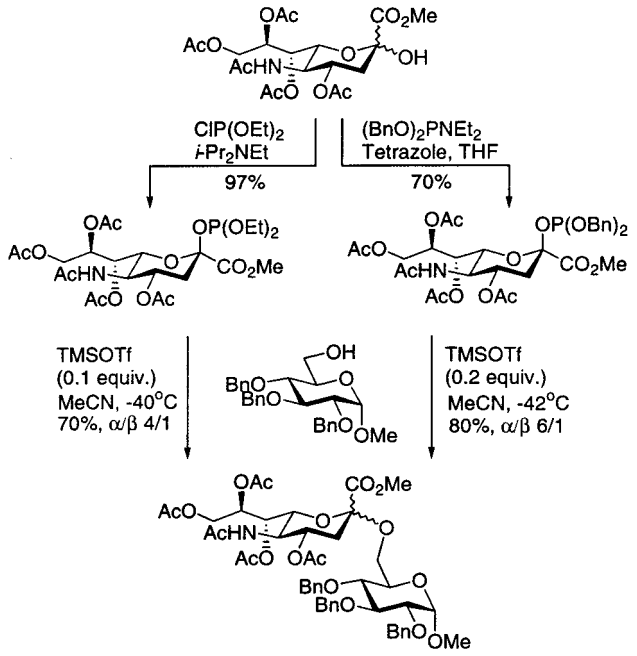
(1-Phenyl-1*H*-tetrazol-5-yl)thioglycosides were developed and used.[61,248−250] These derivatives were synthesized from the corresponding 2-hydroxy derivative of Neu5Ac and *S*,*S*′-bis(1-phenyl-1*H*-tetrazol-5-yl)dithiocarbonate in the presence of 4-(dimethylamino)pyridine (DMAP) in MeCN as a 1/2 mixture of α/*β*-anomers (76%). Glycosylation of 6′-hydroxyl of a modified lactoside derivative in the presence of AgOTf afforded a trisaccharide in a fair yield and stereoselectivity. It was observed that *β*-2-thio-derivative gives better yields (21%) and α-stereoselectivity (α/*β* 2/1) than when the analogous α-anomer was used as starting material. Even higher yields (up to 54%) were obtained when the condensation was performed in CH_2Cl_2; however, in this case the main product was the unwanted *β*-anomer (α/*β* 1/5). Similar derivatives were applied for the synthesis of KDN glycosides.[251]

C. 2-Phosphites

Sialyl phosphites, which were independently introduced by Schmidt[252] and Wong,[253] have found wide application in chemical *O*-sialylation.[22,108,110,254] Sialyl phosphites are very reactive glycosyl donors and only require a catalytic amount of TMSOTf (usually 10−20 mol %) for their activation.

Diethyl *β*-sialyl phosphites can be prepared in high yields (97%) by reaction of a properly protected 2-hydroxyl derivative of Neu5Ac with $ClP(OEt)_2$ in the presence of the hindered base *i*-Pr_2NEt (Scheme 13).[252,255] Glycosylation of a 6-hydroxyl derivative of

Scheme 13

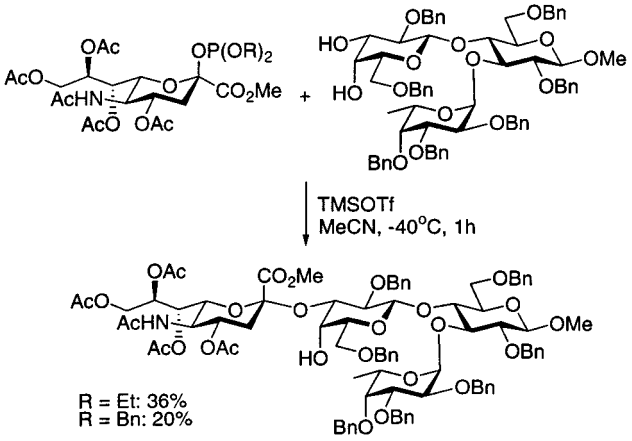


a glucoside in the presence of TMSOTf (0.1 equiv) in MeCN at −40 °C afforded a (2 → 6)-linked disaccharide as a 4/1 mixture of α/*β*-anomers (70%). The application of a dibenzyl phosphite derivative to the same model coupling gave a somewhat higher yield and improved anomeric selectivity (80%, α/*β* 5/1,[253,256] α/*β* 6/1,[257] Scheme 13).

The dibenzyl phosphite methodology has not found wide application, probably due to the fact that the reagent dibenzyl *N*,*N*-diethylphosphoro amidite (DDP) required for its introduction is not commercially available. Moreover, the reaction of a 2-hydroxysialyl derivative with DDP in the presence of tetrazole provided the desired phosphite in a yield of 70% yield, whereas a yield of 97% was obtained for the diethyl

analogue. The glycosyl donor properties of these two anomeric phosphites were directly compared in a synthesis of the tetrasaccharide sialyl Lewis^x, and in this case it was found that the diethyl phosphite was more efficient (36%) than the dibenzyl analogue (20%, Scheme 14).[258,259]

Scheme 14



Several other examples of the use of 2-(di-ethyl)-phosphite[93,99,158,163,167,176,222,260−270] and dibenzyl phosphite[99,271,272] derivatives of Neu5Ac have been reported. It is noteworthy that the *C*-1 benzyl ester of Neu5Ac gave a higher α-selectivity than those protected as a methyl ester.[257] Other sialyl phosphites −O(*n*-Bu), −OCH_2CH_2Cl, −OCH_2CCl_3, −$O(CH_2)_3O$-, O−$CH_2CH_2Me_2CH_2O$-,[255] and 1,2-*O*-cyclopentyl[273] were found to be less efficient than their OEt or OBn counterparts. However, dimethyl phosphite proved to be rather reactive,[274] especially when promoted with $ZnCl_2/AgClO_4$ in CH_2Cl_2 at room temperature, providing sialosides in high yields (83−85%) and good β-stereoselectivity (α/β 1/5−6) when performed with simple acceptors.

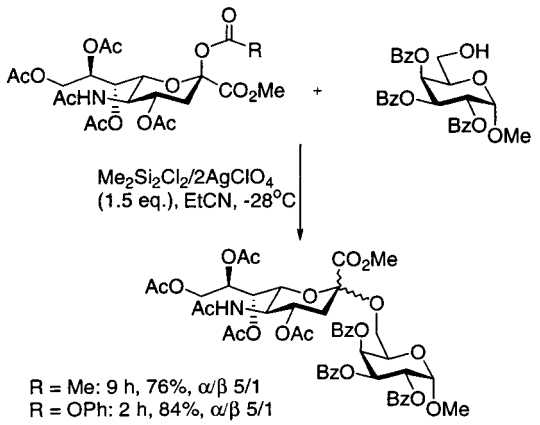
2-Phosphate derivatives of Neu5Ac {-OP(=O)$(OR)_2$, R = Et, Bn} were investigated as glycosyl donors. Only low yields of glycosylation products (20−35%) were obtained when a stoichiometric amount of TMSOTf was used.[255,256]

D. Miscellaneous Direct Chemical Methods

Sialylation by Fischer glycosylation is only applicable for the synthesis of simple glycosides and gives mainly β-glycosides. This type of reaction was first reported in 1965,[275] when β-(*n*-amyl)- (24%) and *n*-hexyl-glycosides (50%) were obtained from free *N*-acetylneuraminic acid by reaction with an alcohol in the presence of dry HCl at 70−80 °C. This simple glycosylation approach found limited application since it only provides access to unnatural β-sialosides. For example, (methylthio)methyl sialosides could be synthesized by this approach.[273] The method was also applied for the synthesis of methyl glycosides of KDN using either cation-exchange resin (Dowex-50, H^+)[250,276] or HCl, formed in situ from AcCl and MeOH.[277] Both anomers were formed under these reaction conditions. The formation of furanose derivatives was also detected when the glycosylations were performed at higher temperatures (70 °C).

Application of 2-*O*-acyl derivatives of Neu5Ac as glycosyl donors have been reported, but their use is rather limited due to either low yields or poor anomeric selectivity. For example, anomeric acetates give mainly β-sialosides when activated with a Lewis acid in either MeCN or CH_2Cl_2.[47,278] Modest α-selectivities (α/β 2/1) were achieved when TMSCl/$Zn(OTf)_2$ was used as the promoter and MeCN as the reaction solvent[47] or $SiCl_4/AgClO_4$ in EtCN.[279] A 2-*O*-phenoxycarbonyl derivative was found to be more reactive than the corresponding 2-*O*-acetyl derivative (Scheme 15).[279] In this case, glycosylation of a 6-hydroxyl of a

Scheme 15



galactoside in the presence of $Me_2SiCl_2/2AgClO_4$ gave a (2 → 6)-linked disaccharide in a yield of 84% (76% for acetate) as a mixture of anomers (5/1 α/β). Other 2-*O*-acyl derivatives such as (2-methoxy)ethoxy carbonyl, *o*-methoxybenzoyl, or *p*-nitrobenzoyl have been used but are of minor interest as glycosyl donors due to their reported lower reactivity.[279]

E. Evaluation of the Different Direct Chemical Methods

The anomeric chloride of Neu5Ac offers the most reliable glycosyl donor for the preparation of glycosides of simple alcohols. Sometimes they give lower yields compared to 2-thiomethyl donors but are still the glycosyl donor of choice because of higher anomeric selectivities.[96] For glycosylation of primary sugar alcohols, the chloride gives higher yields and anomeric selectivity compared to 2-*O*-acetyl derivatives.[47]

2-Thioalkyl, 2-thiophenyl, 2-xanthate, and 2-(dibenzyl)- or 2-(diethyl)phosphites are the leaving groups of choice when more complex hindered sugar alcohols need to be sialylated. The best results are obtained when saccharide acceptors have a free diol or triol, and in many of these cases the sialylations proceed with excellent regioselectivities in combination with high yields and anomeric selectivities.

In some studies, a 2-thiomethyl derivative of Neu5Ac gave better α-stereoselectivity[141] or higher yields[156] compared to the use of a similar 2-thiophenyl donor. In other cases, however, the 2-thiophenyl glycosyl donor gave higher yields and/or stereoselectivities.[109,163,165] Despite these anomalies, it can be concluded that 2-thioalkyl/aryl derivatives of Neu5Ac give the highest yields and α-selectivities when

glycosylated with a 2,3,4-triol of a galactoside. Often the anomeric selectivity was somewhat compromised when 2,3- or 3,4-diols were glycosylated. This problem was addressed by employing a more reactive 2-thiomethyl donor that has a *N*-acetylacetamido moiety at *C*-5.[126] Several reports indicate that 2-xanthate of Neu5Ac give higher yields and α-selectivities than 2-thioalkyl derivatives.[151,173]

When applied to secondary alcohols, the 2-(diethyl)-phosphite donor gives better results than the benzylated analogue.[258,259] In some cases, a 2-(diethyl)-phosphite gives higher yields than the use of similar 2-xanthate[110,222] or 2-thiomethyl derivatives.[110] In another case, the 2-thiomethyl glycosyl donor proved to be more efficient.[163] In general, for glycosylations of secondary hydroxyls, diethyl or dibenzyl phosphites are preferred over the 2-chloro derivative;[99] however, controversial observations have also been reported.[93]

The data presented in this survey clearly show that there is no method that gives satisfactory results for a wide range of glycosyl acceptors. Certainly, more active phosphite donors produce better results when applied for the glycosylation of sterically hindered alcohols, whereas thio derivatives can be successfully applied for the regio/stereoselective glycosylation of polyols, such as a 2,3,4-trihydroxy derivative of galactosides.

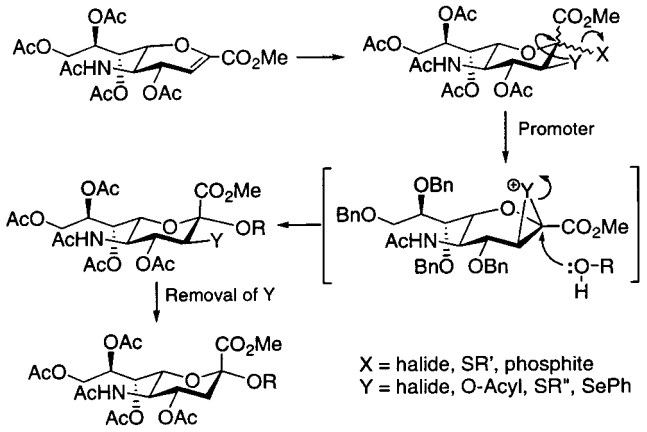
III. Indirect Chemical Methods

Indirect methods afford *O*-sialosides in two or more synthetic steps, one of which may be a glycosylation. Most of these approaches involve modified sialyl donors that have a participating functionality at *C*-3. *O*-Sialosides have also been obtained by total synthesis.

A. Participating Auxiliaries at *C*-3

Several glycosyl donors of Neu5Ac have been prepared that have an auxiliary at *C*-3. These auxiliaries control the anomeric selectivity of a glycosylation by neighboring group participation, leading to the formation of 2,3-*trans*-glycosides (Scheme 16). Thus, α-glycosides will be obtained in the case

Scheme 16



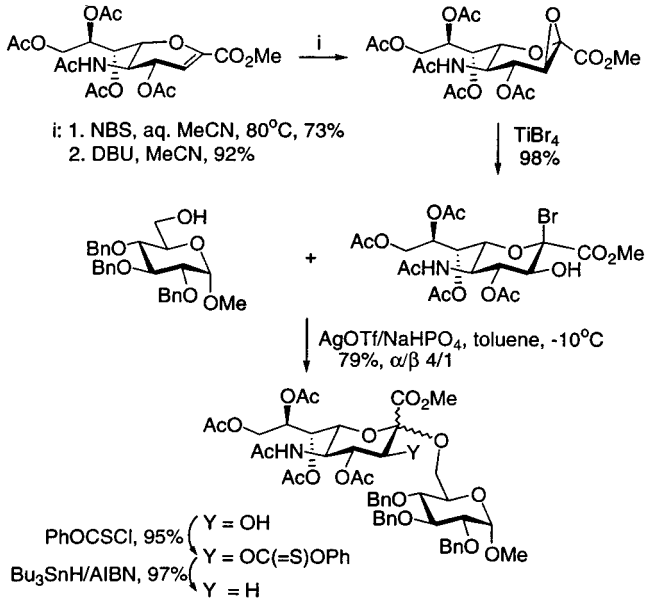
of equatorial auxiliaries, whereas *β*-glycosides will be formed when the auxiliary is in an axial position. Auxiliaries should also prevent 2,3-eliminations, which are often a major side reaction of direct *O*-sialylations. Apart from these properties, an auxiliary should be easily installed prior to and removed after a glycosylation.

Usually, auxiliaries are introduced by a chemical modification of a 2,3-dehydro derivative of Neu5Ac either through a 2,3-oxirane derivative or by an addition reaction to the double bond. The 2,3-dehydro derivative is easily accessible in high yield (81%) by elimination of the methyl ester of acetochloroneuraminic acid by the treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[280]

1. 3-O-Auxiliaries

Several approaches have been reported for the synthesis of 3-*O*-derivatives of Neu5Ac. For example, synthesis of a 3-hydroxy-substituted compound in combination with a halide leaving group at *C*-2 (Cl, Br, F) was accomplished by a three-step reaction sequence starting from the glycal.[280,281] Bromohydrination of the 2,3-dehydro derivative with *N*-bromosuccinimide (NBS) in aqueous MeCN yielded predominantly a diaxially substituted 3-bromo-2-hydroxy derivative (73%), which was treated with DBU to give a 2,3-anhydro derivative (92%, Scheme 17). The

Scheme 17



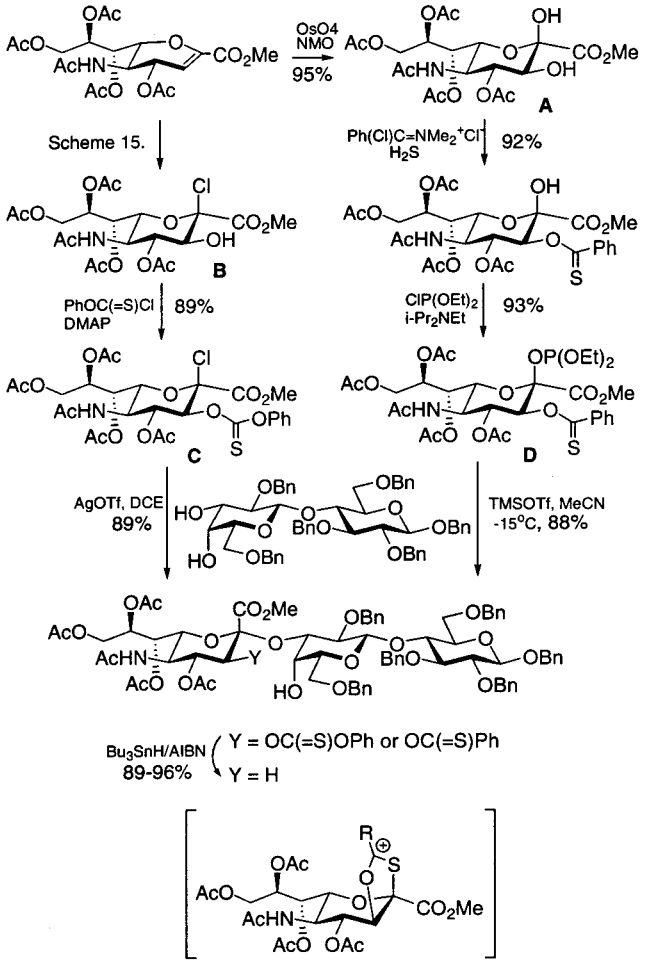
epoxy ring could be opened with $TiBr_4$, $TiCl_4$, or $BF_3 \cdot Et_2O$ to afford 2-bromo, 2-chloro, or 2-fluoro-3-hydroxy derivatives, respectively, in high yields (95−98%). Coupling of the bromo derivative with a 6-hydroxyl of a glucoside in the presence of AgOTf/$NaHPO_4$ in aprotic solvent at −10 °C gave a (2 → 6)-linked disaccharide as a mixture of anomers (79%, α/β 4/1).[281,282] No glycosylation of the *C*-3′ hydroxyl was observed. The auxiliary was removed by phenoxythiocarbonylation in the presence of DMAP (95%) followed by reduction with Bu_3SnH in the presence of 2,2′-azobisisobutyronitrile (AIBN, 97%) to give a (2 → 6)-linked derivative of Neu5Ac. The efficiency of the approach was compromised when applied to the synthesis of Neu5Ac(2 → 3)Gal deriva-

tives, and in this case a complex mixture of α/β-anomers was obtained.

The 2-chloro derivative proved to be less efficient than the 2-bromo analogue, whereas the 2-fluoro derivative did not react in the presence of either $BF_3 \cdot Et_2O$ or $AgOTf/NaHPO_4$. The 2-hydroxy auxiliary approach was efficiently applied for the first synthesis of (2 → 8)-linked dimeric structures as well as (2 → 9)-linked derivatives[283,284] and will be discussed below (sections VI. A.1, 2). Other applications of the 2-bromo derivatives include the synthesis of *O*-aryl-[285] and *C*-glycosides.[64]

As evident from the examples discussed above, a *C*-3 hydroxy auxiliary does not provide efficient neighboring group participation, especially when applied to the glycosylation of secondary hydroxyls. To ensure a more efficient anomeric control, a 3-*O*-thiocarbamate functionality was introduced prior to glycosylation into 3-hydroxy-2-chloro derivative of Neu5Ac (**B**, Scheme 18) to provide a 2-chloro glycosyl

Scheme 18

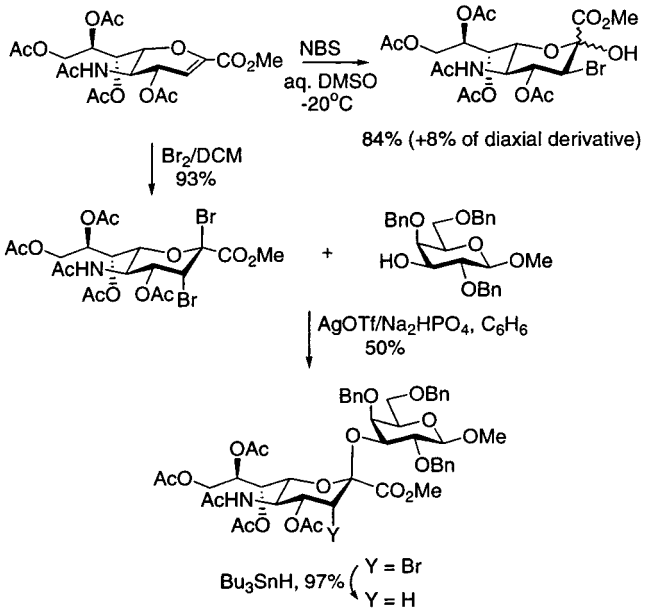


donor (**C**). Alternatively, 2,3-diol (**A**), which was obtained by stereoselctive dihydroxylation, was reacted with $Ph(Cl)C{=}NMe_2^+Cl^-$ in the presence of H_2S to give a 3-*O*-thiocarbonyl derivative. The anomeric hydroxyl was subsequently converted into a phosphite leaving group by treatment with $(EtO)_2PCl$ to afford a sialyl donor (**D**). It was anticipated that these auxiliaries would provide the neighboring group participation through a five-membered cyclic intermediate (Scheme 18).[266,286] Indeed, excellent α-stereoselectivities and high yields were achieved when the donors **C** and **D** were used. After glycosylation, the thiocarbonyl moiety could be readily reduced by a standard procedure. For the synthesis of a Neu5Ac-(2 → 3)Gal disaccharide, a 2-phosphite was equally efficient as a 2-chloro leaving group; however, the donor **D** proved to be the donor of choice for the synthesis of (2 → 8)-linked dimers (section IV. A).[286]

2. 3-Bromo Auxiliaries

Addition of bromine to the double bond of glycal of Neu5Ac gave a diaxially substituted 2,3-dibromo derivative, which was immediately used for glycosylations in the presence of $AgOTf/NaHPO_4$ (Scheme 19).[280,287,288] Due to the axial orientation of the

Scheme 19

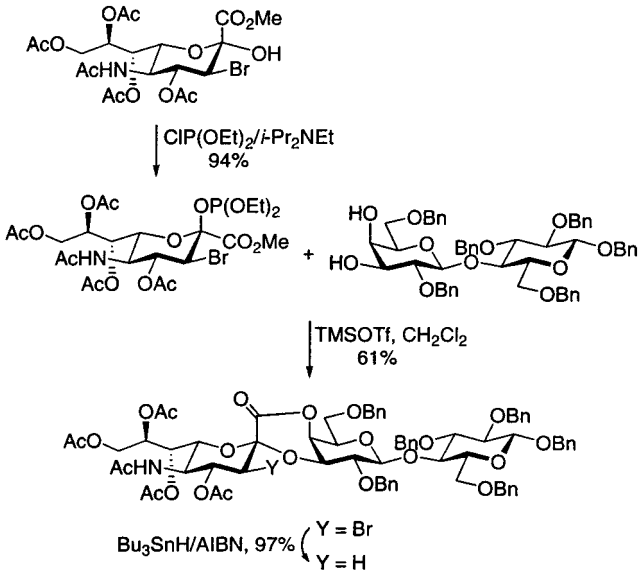


bromide substituent at *C*-3, only unnatural β-linked disaccharides were formed (50−70%). The bromo auxiliary could be removed by reduction with Bu_3SnH to furnish β-*O*-sialosides.

Alternatively, the addition of NBS to glycal at low temperature (−20 °C) in aqueous dimethyl sulfoxide (DMSO) predominantly afforded a diequatorial substitution product (84%) with bromo substituent at *C*-3 and hydroxyl at the anomeric center.[280] However, at that time this substrate did not find application due to the absence of suitable methods for conversion of 2-hydroxy into an appropriate leaving group. However, a decade later, this compound was converted into a *C*-2 diethyl phosphite derivative (Scheme 20),[286] which was glycosylated with a lactosyl acceptor to give a glycosylation product in a reasonable yield (61%) with excellent α-stereoselectivity. When the donor was applied to the glycosylation of unreactive alcohols, i.e., for the synthesis of a (2 → 8)-linked sialyl dimer, only unwanted β-anomer was isolated. Thus, in the latter case, the bromide did not perform neighboring group participation and the glycosylation proceeds through an anomeric oxocarbenium ion.

A chemoenzymatic approach was applied for the synthesis of 3(ax)-bromo donor with 2-dibenzyl phos-

Scheme 20



phite leaving group, which also led to the formation of β-glycosides.[257]

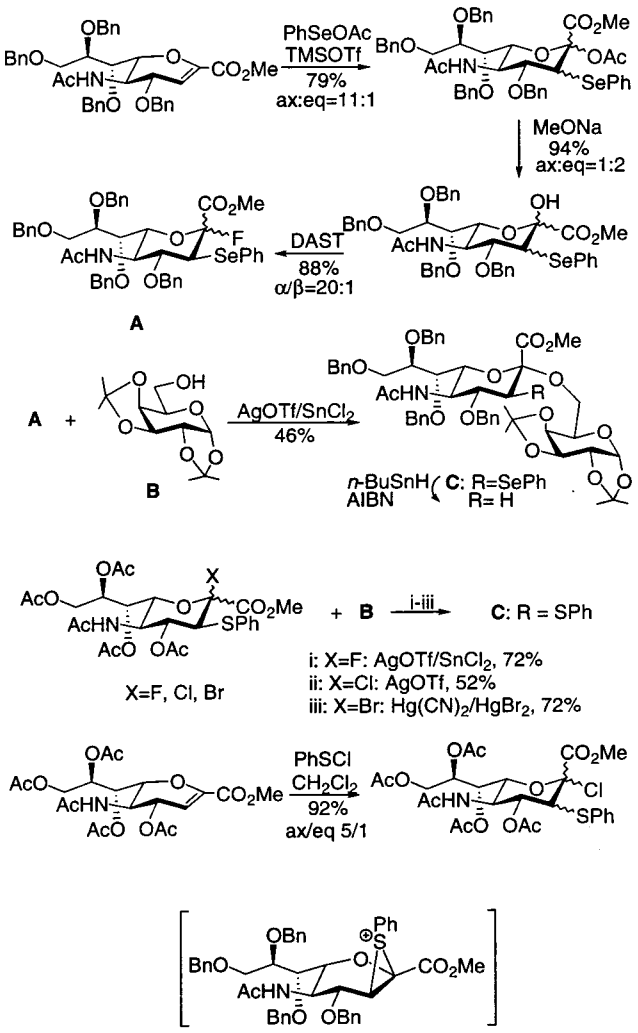
3. 3-Thio and 3-Seleno Auxiliaries

A 3-thio moiety provides one of the most reliable auxiliaries and has been studied most extensively among the indirect methods. A detailed discussion or comparison of these methods has not been reported; however, some aspects of this approach have been surveyed.[31,113,289,290]

The first glycosylations with 3-*S*-phenyl- and 3-*Se*-phenyl-substituted derivatives of Neu5Ac were performed by Ito and Ogawa.[291−293] Electrophilic addition of PhSeOAc (generated in situ from PhSeCl and AgOAc) to the double bond of the 2,3-dehydro derivative of Neu5Ac afforded mainly a 2-*O*-acetyl-3-*Se*-phenyl derivative (3ax/3eq = 11/1, Scheme 21).[291,293] To obtain naturally occurring α-sialosides, the glycosyl donor requires an equatorially substituted auxiliary at *C*-3. Therefore, the *C*-3-seleno derivative was epimerized with concomitant 2-*O*-deacetylation by treatment with sodium methoxide to afford 2/1 mixture of eq/ax isomers at *C*-3. The two epimers were separated by column chromatography, and treatment of the desired equatorial isomer with (dimethylamino)sulfur trifluoride (DAST) gave a 2-fluoro derivative. The axial intermediate was recycled by isomerization. The glycosyl donor properties of the 2-fluoride were investigated. For example, glycosylation of 1,2:3,4-di-*O*-isopropylidene galactose in the presence of $AgOTf/SnCl_2$ in CCl_4 afforded a α-(2 → 6)-linked product in a 46% yield (Scheme 21). The only detectable byproduct was the 2,3-dehydro derivative. When AgOTf/*n*-Bu_3SnOTf was used as promoter, the glycosylation process was complicated by a sequential elimination−addition process of cationic selenium species such as PhSeOTf that were formed in situ. These reactions caused epimerization of *C*-3 resulting in the formation of a substantial amount of β-linked product.

The 2-fluoro-3-thiophenyl derivative proved to be a more efficient glycosyl donor than the corresponding 3-seleno compound.[292,293] Thus, glycosylation of

Scheme 21



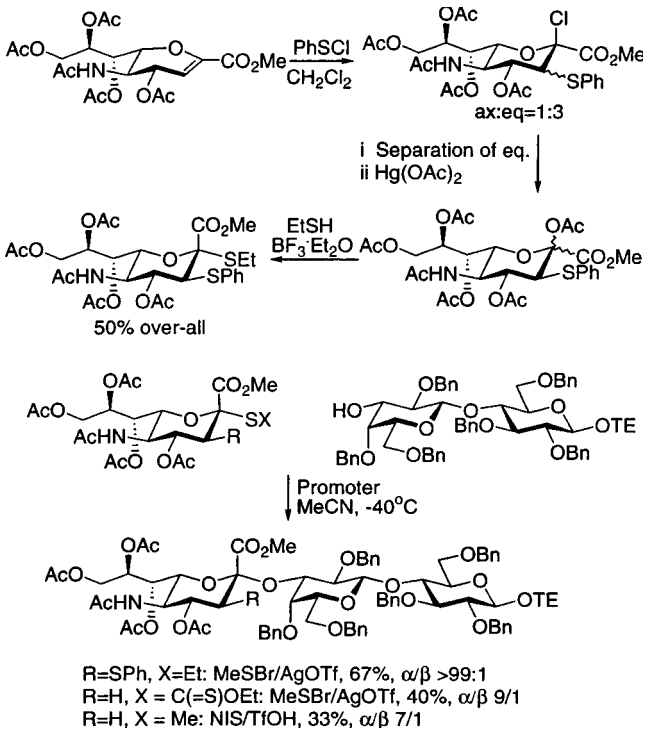
the 2-fluoro-3-thiophenyl derivative with the same acceptor under similar reaction conditions gave the α(2 → 6)-linked disaccharide in a 72% yield (46% for 3-seleno analog). $Hg(CN)_2/HgBr_2$-promoted glycosylation of the 2-bromo-3-thiophenyl derivative was equally effective (72%), whereas an AgOTf-promoted glycosylation of a 2-chloro analogue gave a modest yield of 52%. It was proposed that these glycosylations proceed through an episulfonium (or episelenium) intermediate (Scheme 21), which provides excellent α-stereoselectivity.[293] The 2-fluoro-3-thiophenyl neuraminyl donor was synthesized as follows: 2,3-dehydro derivative was treated with NBS to afford a 2-hydroxy-3-bromo derivative (eq/ax = 1/4). The epimers were separated and individually treated with thiophenol to substitute the 3-bromo group. The resulting axial 3-*S*-phenyl isomer was epimerized with DBU and combined with the equatorially 3-*S*-phenyl-substituted product. The resulting compound was treated with DAST to afford an anomeric fluoride in an overall yield of 70%. In a similar fashion, 2-chloro and 2-bromo derivatives were obtained.[292,293]

This multistep procedure for the synthesis of the sialyl donor having 3-thiophenyl auxiliary was substantially improved.[294] It was shown that direct addition of PhSCl to the double bond of 2,3-dehydro derivative afforded predominantly the 3-equatorial

isomer (77%), along with 15% of the 3-axial derivative, when the addition was performed in CH_2Cl_2 at 30 °C. As noted above, the glycosylation properties of the 2-chloro derivative are not as favorable as that of 2-bromo and 2-fluoro-analogues, making this synthetic route less attractive. Both selenophenyl and thiophenyl functionalities can be readily reduced with *n*-Bu_3SnH in the presence of AIBN.

Sialyl donors having an *S*-alkyl leaving group proved to be highly efficient for the formation of glycosidic linkages (See section II. B.1) Not surprisingly, extensive efforts have been directed toward the design and synthesis of 3-*S*-phenyl-substituted donors bearing *S*-alkyl leaving group at *C*-2. It was found that these derivatives could be synthesized from glycal via the known 2-chloro-3-thiophenyl derivative (Scheme 22). The 2-chloro function was

Scheme 22

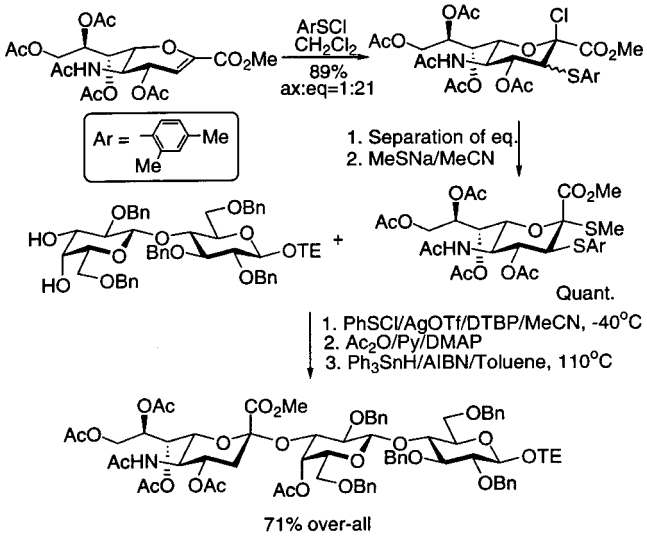


replaced by a thioethyl moiety by treatment with Hg(OAc)$_2$ to give an anomeric acetyl moiety which was substituted by ethane thiol in the presence of $BF_3 \cdot Et_2O$. The resulting compound was synthesized in a 50% overall yield starting from glycal and proved to be a highly efficient glycosyl donor.[230,295] For example, MeSBr/AgOTf-mediated glycosylation of a sterically hindered 3′-hydroxyl of a lactoside at −40 °C afforded the desired α-linked trisaccharide in 67% yield (Scheme 22). This method compares very favorably with other popular glycosyl donors, especially when applied to the glycosylation of sterically hindered alcohols. Indeed, direct glycosylations of either 2-xanthate or *S*-methyl derivatives of *N*-acetylneuraminic with the lactosyl acceptor gave in both cases a trisaccharide as a mixture of anomers in yields of 40% (α/*β* 9/1) and 33% (α/*β* 7/1), respectively.[230,295]

Another synthetic approach for the introduction of the 2-thioalkyl leaving group into a 3-thio-substituted Neu5Ac derivative involves electrophilic addition of $(PhS)_3SbCl_6$ to the double bond of the glycal in MeCN followed by epimerization of the axial *C*-3 substituent with DBU to give a 2-hydroxy-3(eq)-*S*-phenyl derivative. This compound was *O*-acetylated at *C*-2 and reacted with TMSSMe to furnish the desired 2-thiomethyl-3-thiophenyl glycosyl donor in an overall yield of 50−60%.[296] It has been shown that a 2-*S*-methyl derivative is a far more efficient glycosyl donor than a 2-bromo analogue.[296]

Often additions of other electrophiles of the type "PhSX" lead to mixtures of epimers which require separation or epimerization prior to glycosylation. This makes the synthesis and application of these compounds inconvenient. It has been reported that reaction of sialyl glycal with 2,4-dimethylbenzenesulfenyl chloride in CH_2Cl_2 affords the corresponding 2-chloro-3-thioaryl derivative with good stereoselectivity and high yield (ax/eq 1/21, 89%, Scheme 23).[297]

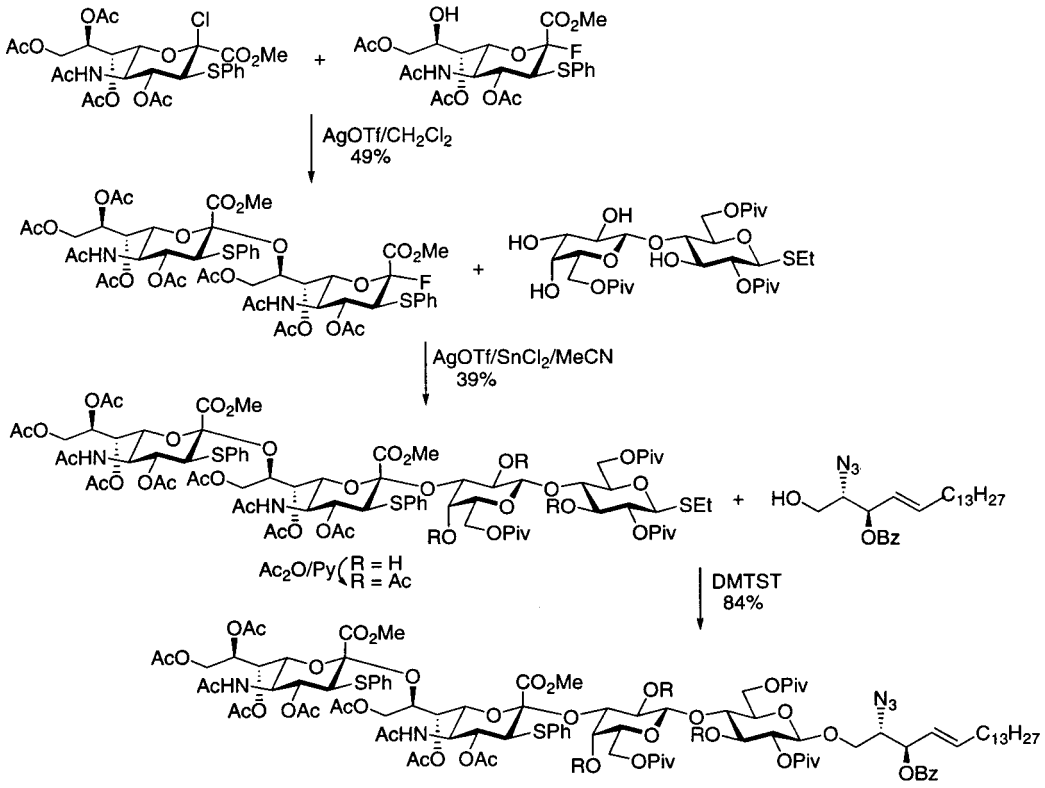
Scheme 23



Moreover, the formed chloride is a crystalline material and, hence, can be simply purified by crystallization. The glycosyl donor properties of this compound were investigated by glycosylation with the 3,4-dihydroxy derivative of a lactoside, and the desired *O*-sialoside was isolated in an excellent yield of 71% after *O*-acetylation and reductive removal of the *S*-aryl auxiliary.

2-Chloro and 2-fluoro derivatives both having a 3-*S*-phenyl moiety were employed for the preparation of a tetrasaccharide using an elegant orthogonal glycosylation strategy (Scheme 24).[298,299] It was established that the glycosyl fluoride is much more stable under basic as well as weakly acidic conditions required for protecting group manipulations but also to conditions required for activation of anomeric chlorides and bromides. Therefore, coupling of a sialyl glycosyl fluoride acceptor having an 8-hydroxyl with a fully protected 2-chloro sialyl donor in the presence of AgOTf gave a (2 → 8)-linked dimer in a 49% yield. As in the previous cases, the α-selectivity was achieved by neighboring group participation of the 3-thiophenyl moiety. The obtained dimer could be used in a subsequent glycosylation without a need for any chemical manipulations, and glycosylation

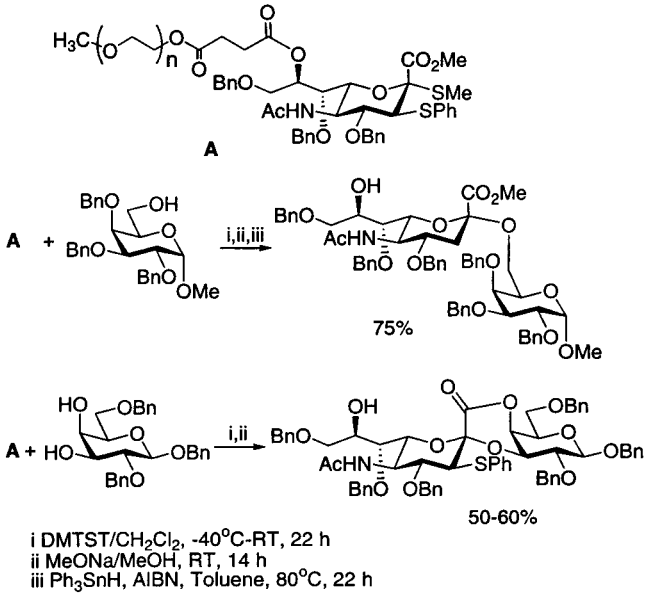
Scheme 24



with a thiolactosyl acceptor in the presence of AgOTf/$SnCl_2$ gave a tetrasaccharide in 39% yield. This reaction exploits the finding that glycosyl fluorides can be activated in the presence of thioglycosyl acceptors. However, the thioglycoside can be activated using a thiophilic reagent, and in this case, the promoter DMTST was used in a coupling with an azidosphingosine acceptor to give a glycosyl azidosphingosine.

A 2,3-dithiosubstituted derivative was also used for the solid-supported synthesis of sialyl glycosides (Scheme 25).[300] The glycosyl donor was attached to the soluble polymer poly(ethylene glycol) (PEG) via a succinyl linker. Glycosylation of a 6-hydroxy derivative of a galactoside in the presence of DMTST, followed by cleavage from the polymer support by base treatment, gave the target disaccharide in 70% yield. The 3-thiophenyl moiety was conveniently removed to furnish a (2 → 6)-linked *O*-sialoside. A trace amount of the β-anomer was also isolated. This approach was also used for the synthesis of (2 → 3)-linked derivatives. (Scheme 25). To date, this is the only example whereby a glycosyl donor of a modified Neu5Ac was attached to a polymeric support and successfully glycosylated illustrating the synthetic potential of these pseudo sialosyl donors.

Scheme 25

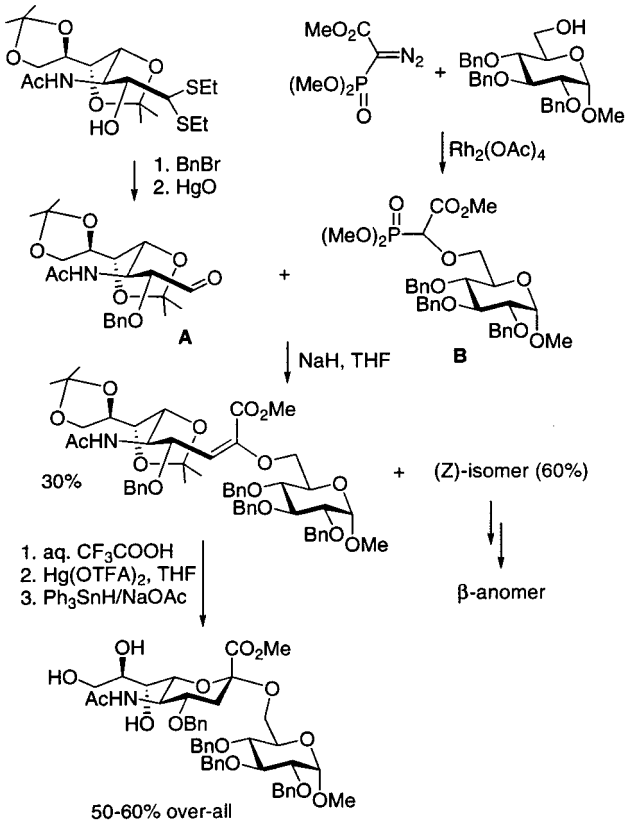


3-Thio auxiliaries have been applied for the synthesis of several sialylated oligosaccharides.[237,289,290,301−317]

B. Miscellaneous Indirect Methods

One report deals with the synthesis of *O*-sialosides that does not use a traditional glycosylation.[318] In this case, an intramolecular oxymercuration−demercuration reaction was used for the stereocontrolled synthesis of α- and β-*O*-sialosides (Scheme 26). A known dithioacetal was converted into an aldehyde (**A**, Scheme 26) by sequential benzylation and dethioacetalation with $HgO/BF_3{\cdot}Et_2O$ in an overall yield of 80%. A $Rh_2(OAc)_4$-catalyzed reaction of diazotrimethylphosphonoacetate with a *C*-6 hydroxyl of a glucoside afforded the corresponding phosphonate (**B**). Horner−Wittig condensation of **A** with **B** gave a separable mixture of *E*- (30%) and *Z*-enones (60%). Both isomers were treated with aqueous trifluoroacetic acid to cleave the isopropylidene acetals, and treatment of the resulting compound with Hg(II) induced cyclization. Demercuration of *C*-3′ with Ph_3-

Scheme 26

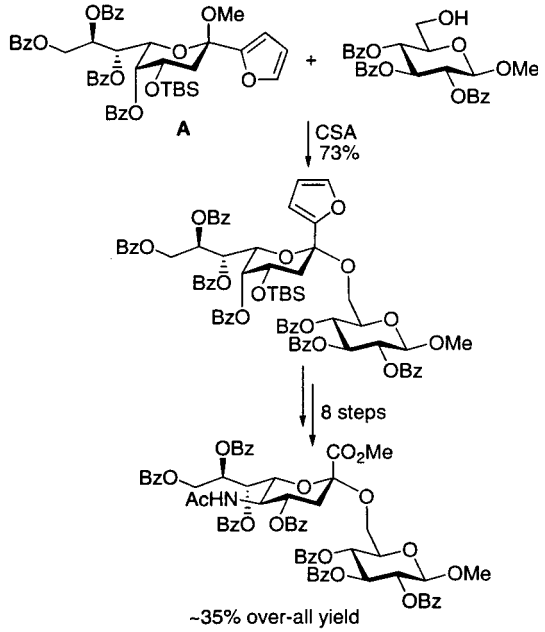


SnH in the presence of NaOAc gave α- and *β*-sialosides, respectively.

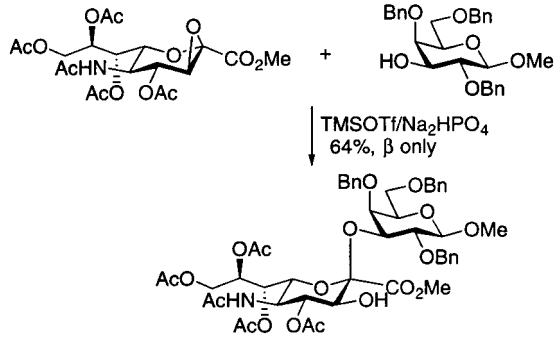
Another nonorthodox approach was reported by Danishefsky and co-workers.[319−321] They employed a furan as a masking functionality of the *C*-1 carboxylic acid. It was postulated that during glycosylation the furanyl moiety stabilizes the carbocation intermediate as well as provides good stereoselectivity. In each case, the equatorial glycoside was obtained stereoselectively as the result of a thermodynamic equilibration. Furthermore, the *C*-5 acetamido functionality was introduced after the glycosylation step. Thus, *trans*-glycosylation of **A** (Scheme 27)[319] in the presence of camphorsulfonic acid (CSA) gave an equatorially substituted disaccharide in yields ranging between 53% and 73% depending on the glycosyl acceptor. The furan ring was converted into the corresponding methyl ester by oxidation with RuO_4, followed by esterification with diazomethane. Introduction of the acetamido functionality was achieved by desilylation to give a 4′-hydroxyl, which was treated with potassium carbonate to induce 5′ → 4′ *O*-benzoate migration. The resulting *C*-5′ hydroxyl was converted into a triflate, which was displaced with Bu_4NN_3. Reduction of the *C*-5′azido group followed by *N*-acetylation gave the desired α-sialoside in a 35% yield over eight synthetic steps.

2,3-Anhydro derivatives have also been applied for *O*-sialylation.[322] As shown above, these compounds are easily accessible from a 2,3-dehydro derivative in two synthetic steps (Scheme 17). The best promoter for glycosylation proved to be TMSOTf, especially when buffered with Na_2HPO_4 in DCE at −20 °C. This condensation provided a *C*-3′-hydroxyl-

Scheme 27



Scheme 28



substituted *β*(2 → 3)-linked disaccharide (Scheme 28) in 64% yield. A similar stereoselectivity was observed when a 6-hydroxy derivative of galactose was used as a glycosyl acceptor. However, when highly reactive acceptors such as methanol were used, predominantly α-glycosides were formed. 2,3-Dehydro derivatives were also used as glycosyl donors for the synthesis of methyl glycosides.[323] Thus, treatment of the glycal with NBS in methanol gave a 1/1 mixture of diaxial/diequatorial 2-*O*-methyl-3-bromo derivatives (97%). The 3-bromo moiety can be efficiently removed with Bu_3SnH.

The methods described in this section did not find wide application for the synthesis of sialosides and are only of interest from a methodological point of view.

C. Comparison of Indirect Chemical Methods

Among the indirect methods, glycosyl donors which possess a *S*-phenyl participating auxiliary at *C*-3 and thioalkyl leaving group at *C*-2 generally give the best yields and anomeric selectivities, especially when applied for the glycosylation of sterically hindered alcohols.[296,315] A 3-*O*-phenylthiocarbonyl auxiliary also gives excellent yields and stereoselectivities.[286]

The major drawbacks of indirect methods are the additional chemical steps required for introduction

and removal of the auxiliary at *C*-3. Furthermore, stereoselective introduction of a *C*-3 auxiliary is problematic in many cases, and often mixtures of diastereoisomers need separation and/or epimerization.

IV. Synthesis and Introduction of Dimers

Neu5Ac or Neu5Gc also occur in linear homopolymers where they are usually linked internally by α-(2 → 8), α(2 → 9) or alternating α(2 → 8)/α(2 → 9) glycosidic linkages. These polysialic acids as well as some unusual structures are found in glycoproteins of embryonic neural membranes where they play a role of neural cell adhesion molecules.[1,3−9,11]

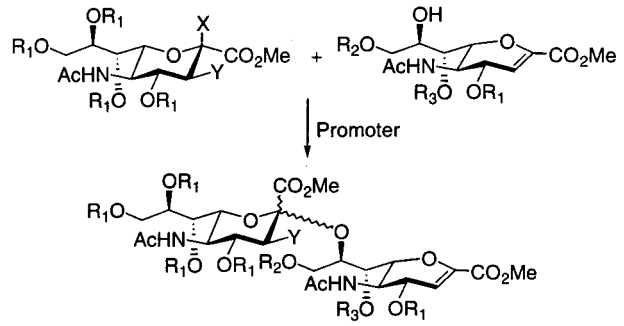
The dimer Neu5Acα(2 → 8)Neu5Ac is a principal terminal constituent of a number of glycoconjugates. It plays important roles in numerous biological phenomena being a tumor-associated antigen and a receptor for bacterial toxins and viruses. The dimeric structure is important for these biological properties since removal of one of the Neu5Ac residues, to give a monomeric Neu5Ac substitution, often leads to a dramatic or total loss of activity.

A. (2 → 8)-Linked Derivatives

The synthesis of oligosaccharides that contain (2 → 8)-linked fragments is complicated by the low reactivity of the C-8 hydroxyl of Neu5Ac. To date, several successful syntheses of this dimer have been reported. Most of these approaches are based on the use of indirect glycosylation methods (i.e., neuraminyl donors that bear a participating auxiliary at *C*-3).

The first synthesis of a (2 → 8)-linked dimer was accomplished by employing a 2-bromo-3-hydroxy-substituted glycosyl donor (Scheme 29).[283,284] Glycosylation of this donor with a suitably protected acceptor in the presence of AgOTf and Na_2HPO_4 afforded the dimer in a 34% yield as 3/1 mixture of α/β-anomers. The 3-hydroxy auxiliary was converted into a phenoxythiocarbonyl moiety, which was removed by reduction. It is noteworthy that this approach allows an easy access to complex oligosaccharides, since the 2,3-double bond of the coupling product could be converted into a glycosyl donor for a subsequent glycosylation.

Scheme 29



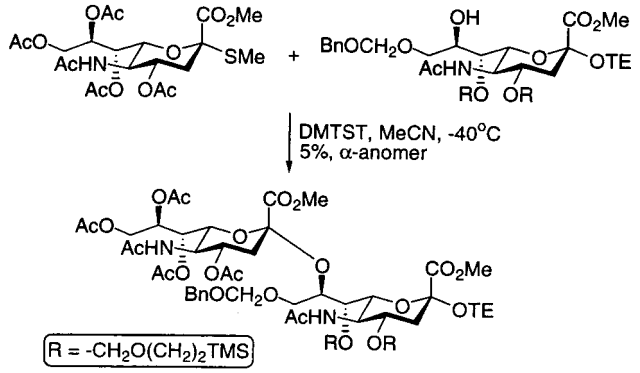
The use of the diaxially substituted 2,3-dibromo derivative afforded the anomerically pure but unnatural β(2 → 8)-linked derivative in a 58% yield.[287] These results were significantly improved by applying the 2-bromo-3eq-phenylthio-substituted donor.[301,306] In this case, the dimer was obtained as only the α-anomer in a good yield of 64% (Scheme 29). Further improvements came by the application of glycosyl donors that have a 2-phosphite leaving group and a 3-*O*-phenylthiocarbonyl participating functionality.[286] By employing this donor, the α-anomer was also formed in a stereoselective manner but in an improved yield of 83%. Surprisingly, under similar conditions 3eq-bromo-2-phosphite donor gave only β-linked product in 58% yield.[286] These results suggest that the 3-*O*-phenylthiocarbonyl group is a more efficient participating functionality than the 3-bromo moiety.

A 2-thioethyl leaving group in combination with a 3-thiophenyl participating auxiliary afforded α(2 → 8)-linked product in a disappointing yield of 28%.[230,313] This result, however, was significantly improved by using a glycosyl donor that has an additional activating *N*-acetyl function at the acetamido group of *C*-5.[126,127] In this case, the α-linked dimer was obtained in an improved yield of 44%.[316]

As discussed above, an orthogonal glycosylation approach proved to be very efficient for the preparation of this class of compounds (see Scheme 24, section III.A.3).[298,299]

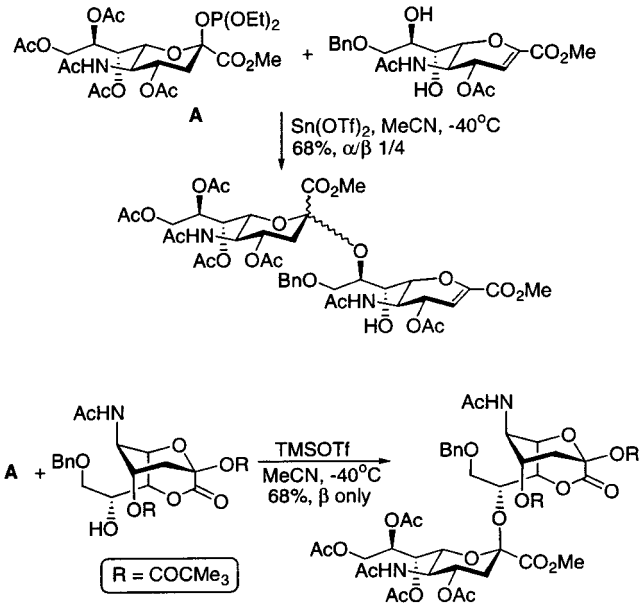
Early attempts to prepare (2 → 8)-linked dimers by direct methods gave very disappointing results. For example, direct glycosylation of a 8-hydroxyl sialyl acceptor with a 2-thiomethyl neuraminyl donor in the presence of DMTST in MeCN at −40 °C afforded only trace amounts (5%) of the desired α-linked dimer (Scheme 30).[114] Glycosyl phosphites proved to be more effective for a direct sialylation (section II.C). In this case, (2 → 8)-linked derivatives were obtained in good yields (22−68%) (Scheme 31).[260,266] Unfortunately, predominantly the unnatural β-anomers were formed. It is noteworthy that the low reactivity of the *C*-8 hydroxyl was addressed by employing an acceptor modified as a 1,7-lactone.

Scheme 30



The 8-hydroxyl of Neu5Ac is of low nucleophilicity due to a combination of obvious steric effects, interactions with the acetamido group at *C*-5, and/or the presence of an internal hydrogen bond between the *C*-8 hydroxyl and *C*-1 carboxyl or 2-*O*R (R = Me, Ac,

Scheme 31



H etc.) moieties (Figure 2). The introduction of the internal 1,7-lactone results in a change of ring conformation favoring $^5C_2$. In this conformation, the unfavorable intramolecular interactions are removed. Some of these interactions may also be removed by the introduction of the 2,3-dehydro moiety in the acceptor. In this case, the *C*-1 carbonyl is remote from *C*-8 hydroxyl and no hydrogen bonding can take place. The improvement of glycosyl-accepting properties resulted in good yields of coupling products, but unfortunately mainly β-anomeric selectivity were obtained.

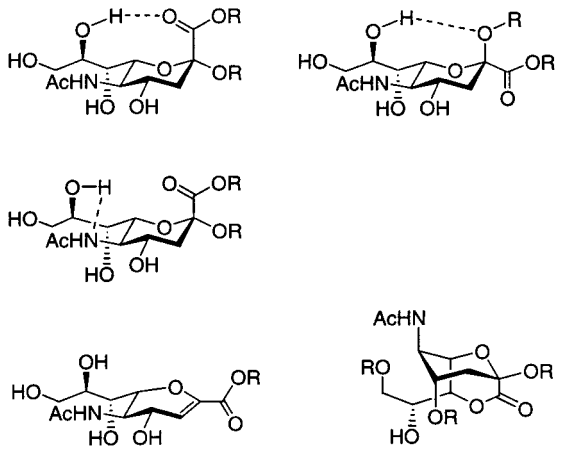
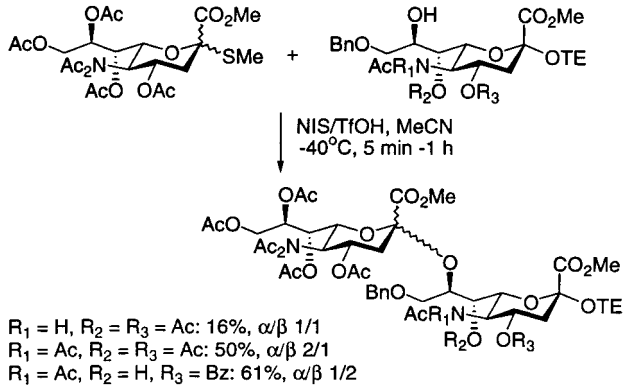


Figure 2.

Recently, another way to address the difficulties of direct synthesis of Neu5Acα(2 → 8)Neu5Ac dimers was reported.[127] As discussed above, an additional *N*-acetyl moiety at *C*-5 of a glycosyl donor dramatically increases its reactivity and gives higher yields in glycosylations.[126] It was shown that this highly reactive donor can successfully be applied to the synthesis of Neu5Acα(2 → 8)Neu5Ac dimers. For example, coupling of a 2-thiomethyl-5-*N*-acetylacetamido glycosyl donor with the *C*-8 hydroxyl of a mono-*N*-acetylated acceptor in the presence of NIS/TfOH in MeCN at −40 °C gave a dimer as a mixture of

Scheme 32

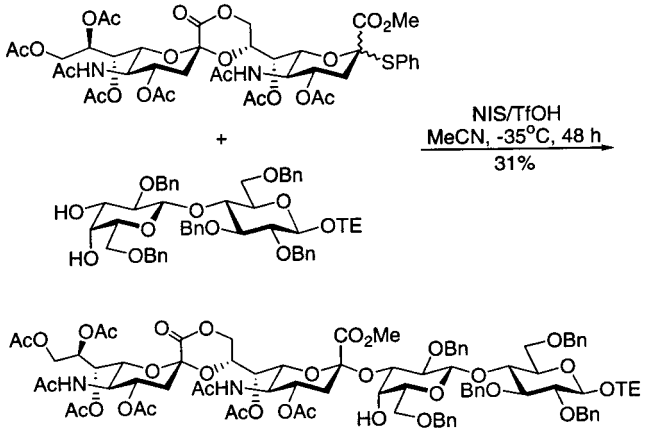


anomers in modest yield (16%, α/β 1/1, Scheme 32). This result, however, was an improvement compared to the previously reported attempts using a similar acceptor.[114] The application of a di-*N*-acetylated glycosyl acceptor under identical reaction conditions gave the dimer in a much improved yield and stereoselectivity (50%, α/β 2/1). These results illustrate that high yields of coupling products can be obtained when the 5-acetamido moieties of both the neuraminyl glycosyl donor and acceptor are derivatized into *N*-acetylacetamido functionalities. An attempt to reduce steric hindrance around *O*-8 by applying a 7,8-diol as an acceptor resulted in an excellent yield (61%) of coupling product, but in this case mainly β-anomer was formed (α/β 1/2). Schmidt and co-workers made a similar observation when they minimized steric hindrance around the *C*-8 hydroxyl.[266] These findings are remarkable since reduction of steric hindrance around *C*-3 of a galactosyl acceptor results in higher α-stereoselectivities.

A completely different strategy for obtaining oligosaccharides that have a Neu5Acα(2 → 8)Neu5Ac moiety was developed and has as an attraction that it avoids the difficulties of the chemical formation of the α(2 → 8) linkage. In this case, mild acid treatment of colominic acid,[324] which is a homopolymer of Neu5Acα(2 → 8)Neu5Ac moieties, results in glycosidic bond cleavage and gives a low yield of dimer. This dimer can be separated from higher oligomers and monomers and then be converted into a glycosyl donor by chemical manipulations. Unfortunately, such a donor gives lower yields of coupling products compared to a corresponding monomeric glycosyl donor, especially when applied for the glycosylation of secondary sugar alcohols. For example, a NIS/TfOH-mediated coupling of a 2-thiophenyl dimeric derivative with the *C*-3 hydroxyl of a lactoside afforded a (2 → 3)-linked product in a yield of 31% (Scheme 33).[190,191] A higher yield of 50% was achieved in a similar coupling.[192] Trimeric as well as tetrameric glycosyl donors have also been coupled with a lactosyl acceptor to give oligosaccharides in a reasonable yields of 49%[193] and 35%,[198] respectively.

2-Thiomethyl[143] and 2-chloro dimeric derivatives[57,58] have also been employed. It was, however, observed that these compounds (especially halides) give lower yields compared to similar 2-thiophenyl donors.[165,194,195,197,199]

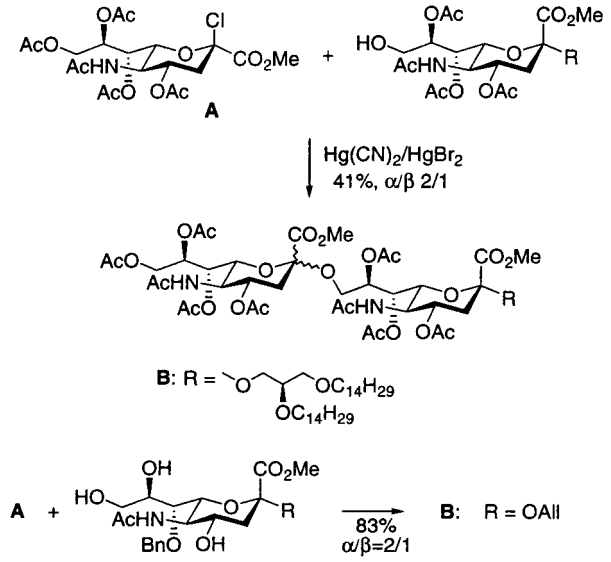
Scheme 33



B. (2 → 9)-Linked Derivatives

Synthesis of (2 → 9)-linked oligomers is relatively straightforward due to the high reactivity of the primary *C*-9 hydroxyl group. It is not surprising that the first synthesis of a dimeric structure was accomplished by the coupling a 9-hydroxyl and a sialyl chloride to give a (2 → 9)-linked dimer in reasonable yield as a mixture of anomers (α/*β*-anomers 2/1, 41%, Scheme 34).[38] The yield was significantly improved

Scheme 34



(83%, α/*β* 2/1) by employing a partially protected acceptor that has less steric hindrance around *C*-9.[74] In a similar fashion, a 2-thiomethyl sialyl donor was successfully applied for the glycosylation of a 7,8,9-triol glycosyl acceptor.[130] The (2 → 9)-linked dimer was obtained with high regioselectively in a yield of 67% as a 2/1 mixture of α/*β* anomers. The *N*-acetylacetamido approach[127] gave (2 → 9)-linked derivatives in yields of 67−98% (α/*β* 2.0−2.5/1).

In the above-discussed cases, the anomeric stereoselectivities are far from being satisfactory. Therefore, indirect methods, using a participating auxiliary at *C*-3, were employed. Thus, the 2-bromo-3-hydroxy glycosyl donor was coupled with an acceptor in the presence of $AgOTf/Na_2HPO_4$.[283,284] Unfortunately, the (2 → 9)-linked dimer was isolated in 63% yield as a 2/1 mixture of α/*β*-anomers. It is to be expected that other indirect methods give more efficient anomeric control in the glycosylation of a *C*-9 hydroxyl.

The synthesis of an α(2 → 9)-*S*-linked dimer was accomplished by reaction of the sodium salt of a 2-thio sialyl derivative with 9-brominated acceptor.[246]

V. Enzymatic Methods

The need for increasingly efficient methods for oligosaccharide synthesis has stimulated the development of enzymatic approaches.[6,25,26,254,271,325−338] The enzymatic methods bypass the need for protecting groups since they control both the regio- and stereoselectivities of glycosylations. Two fundamentally different approaches for enzymatic oligosaccharide synthesis have been developed: (i) the use of glycosyltransferases and (ii) the application of glycosyl hydrolases.

Glycosyltransferases are essential enzymes for oligosaccharide biosynthesis. These enzymes can be classified as enzymes of the Leloir and those of the non-Leloir pathway. The glycosyltransferases of the Leloir pathway are involved in the biosynthesis of most *N*- and *O*-linked glycoproteins in mammalians and utilize sugar nucleotide mono- or diphosphates as glycosyl donors. In contrast, glycosyltransferases from the non-Leloir pathway use sugar phosphates as substrates.

Glycosyltransferases are highly regio- and stereoselective enzymes and have been successfully applied for enzymatic synthesis of oligosaccharides, which can be isolated from milk, serum, and organ tissues and are most commonly purified by affinity column chromatography using immobilized sugar nucleotide diphosphates. Several glycosyltransferases have been cloned and overexpressed and are now readily available in reasonable quantities. However, the number of easily available glycosyltransferases is still very limited. Furthermore, these enzymes are highly substrate specific, and therefore, the possibilities of preparing analogues are limited.

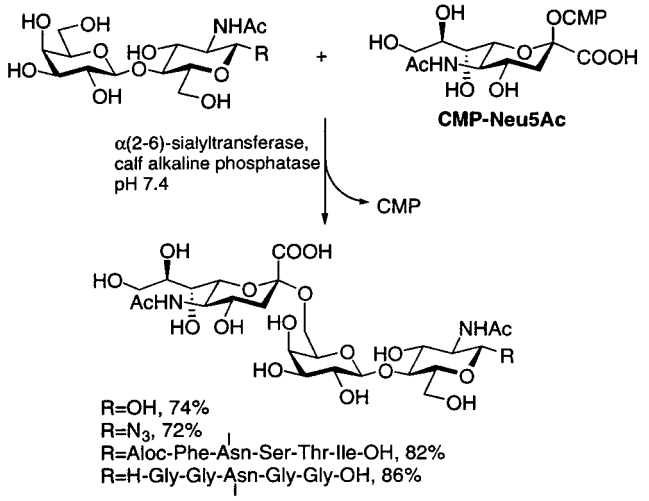
Nature employs glycosyl hydrolases for the degradation of oligosaccharides. However, the reverse hydrolytic activity of these enzymes can also be exploited in glycosidic bond formation. This method allows the preparation of several di- and trisaccharides. Glycosyl hydrolases are much more readily available than glycosyltransferases but are generally less regiooselective and the transformations are lower yielding.

A. Sialyltransferases

Several α(2 → 6) and α(2 → 3)-sialyltransferases have been used for oligosaccharide synthesis.[339−347] These enzymes transfer the neuraminic acid moiety of activated CMP−Neu5Ac to the *C*-6 or *C*-3 hydroxyl of terminal galactosides and *N*-acetyl galactosides (Scheme 35). The α(2 → 8)-sialyltransferase is involved in the synthesis of α(2→8)-linked polysialic acids.

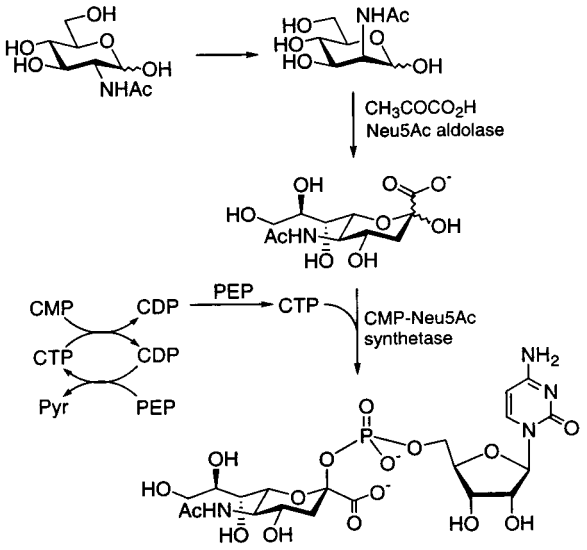
CMP−Neu5Ac has been obtained by chemical and enzymatic approaches. The most attractive procedure involves the CMP−Neu5Ac synthase-catalyzed con-

Scheme 35



densation of Neu5Ac with CTP.[348,349] An enzymatic conversion of cheap CMP gave CTP and an aldolase-mediated condensation of *N*-acetyl mannosamine with pyruvate gave Neu5Ac. The CTP and Neu5Ac were used as crude preparations, and the only purification step in the synthesis is the final separation of CMP−Neu5Ac from the reaction mixture by ion-exchange chromatography (Scheme 36).

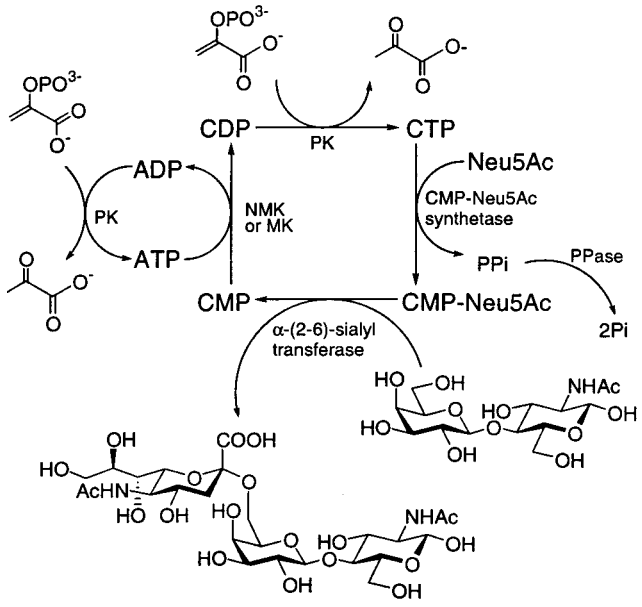
Scheme 36



Another attractive way to enzymatically synthesize sialylated oligosaccharides involves the in situ regeneration of CMP−Neu5Ac.[350,351] This procedure only requires a catalytic amount of CMP−Neu5Ac, and the CMP that is formed after the sialyl transferase is in situ regenerated by a series of enzymatic transformations (Scheme 37). An even more sophisticated procedure involves the in situ enzymatic synthesis of Neu5Ac by an aldolase-mediated reaction of Man*N*Ac with pyruvate coupled with the regeneration of CMP−Neu5Ac. This procedure was employed for the synthesis of Neu5Acα(2 → 3)Galβ(1 → 4)-Glc*N*Acβ1-R and Neu5Acα(2 → 6)Galβ(1 → 4)-GlcNAcβ1-R derivatives.

α(2 → 6)-Sialyltransferases have been isolated from porcine liver, bovine colostrum, and *Photobacterium*

Scheme 37



damsela.[352,353] α(2 → 3)-Sialyltransferases have been obtained from porcine liver and porcine submaxillary glands. The latter enzyme has also been cloned and overexpressed.[354]

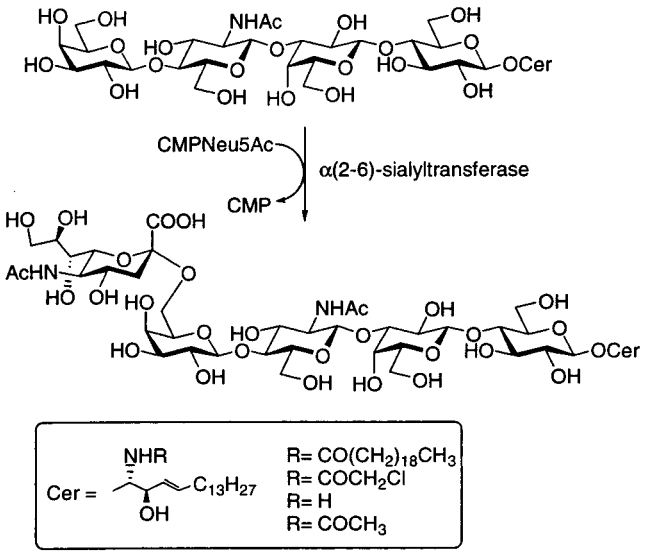
Sialyltransferases accept particular modifications of substrates,[352,355−363] and the use of these acceptors has led to the synthesis of modified sialylated oligosaccharides. Several sialyl transferases with different substrate specificities have been characterized and purified.[364−367] The most striking example is that of the α(2 → 6)-sialyltransferases of *Photobacterium damsela*.[352,353,368] Furthermore, this bacterial enzyme also transfers Neu5Ac to 2′-fucosyllactose and 3′-sialyllactose and can use both *O*- and *N*-linked glycoproteins as a substrate.[353]

The combined use of glycosidases and sialyltransferases have also been reported.[369,370] In this approach, the synthesis of Galβ(1 → 3)GalNAc was catalyzed by a β-galactosidase and used *p*-nitrophenyl β-galactoside and *N*-acetylglucosamine as the substrates. The lactosamine was sialylated by a α(2 → 3)-sialyltransferase coupled with regeneration in situ of CMP−Neu5Ac. A similar approach was used for the preparation of Neu5Acα(2 → 3)Galβ(1 → 4)-GlcNAc.

Sialyltransferases have also been used for the synthesis of neolacto series gangliosides.[371] It was observed that a purified α(2 → 6)sialyltransferase would not accept a neutral glycosphingolipid paragloboside as an acceptor (Scheme 38, R = $CO(CH_2)_{18}CH_3$). This result was rather surprising since the oligosaccharide of the glycolipid is known to be an excellent substrate for the enzyme. Thus, the ceramide has a very profound effect on the enzymatic transformation. Modified ceramides that have no substitution or an acetyl ($COCH_3$) or chloroacetyl ($COCH_2Cl$) moiety at the amino function proved to be good substrates for the enzyme.

Clustered acceptors proved to be appropriate substrates for sialyltransferases.[372−374] A trimeric β-lactosyl cluster based on 2-nitro-2-(hydroxymethyl)-propane-1,3-diol is an effective acceptor for rat-liver

Scheme 38



α(2 → 3)-sialyltransferase. Its K_m was comparable to those for monomeric lactosyl and *N*-acetyllactosamine acceptors, whereas its V_{max} was only 1% of that measured for the Lac*N*Ac acceptor. While the V_{max} was relatively low, a preparative-scale sialylation afforded a trimeric cluster of the GM3 oligosaccharide in good yield. Also di-, tetra-, and octavalent sialyl Lewis^x (SLe^x) ligands were enzymatically prepared starting from hypervalent dendritic L-lysine cores that have a 2-acetamido-2-deoxy-D-glucose attachment.

Sialyltransferases have been employed for the chemoenzymatic synthesis of several oligosaccharides. Most of the procedures involve the chemical synthesis of a complex oligosaccharide. After removal of the protecting groups, a sialyl moiety is introduced by employing a sialyl transferase. This approach has been used for the synthesis of the core sialyl-containing hexasaccharide found on *O*-linked glycoproteins,[375] a complex type biantennary *N*-glycan,[376] GM3,[377,378] ^{13}C-enriched GM3, Lewis^x and sialyl Lewis^x,[379] a spacer-modified sialyl Lewis^x,[374,380] and a *Streptococcus* group B capsular oligosaccharide.[381−383] A dimethyloctylsilylethyl lactoside proved to be an appropriate substrate for α(2 → 3)-sialyltransferase, but the turnover rate was only 2% of that reported for β-D-Gal-(1 → 3)-D-Glc*N*Ac.[384] The aliphatic aglycon allowed easy purification by reverse-phase column chromatography but could easily be removed by treatment with TFA or BF_3OEt_2. Sialyltransferases have also been used for the polymer-supported synthesis of sialooligosaccharides.[385−388]

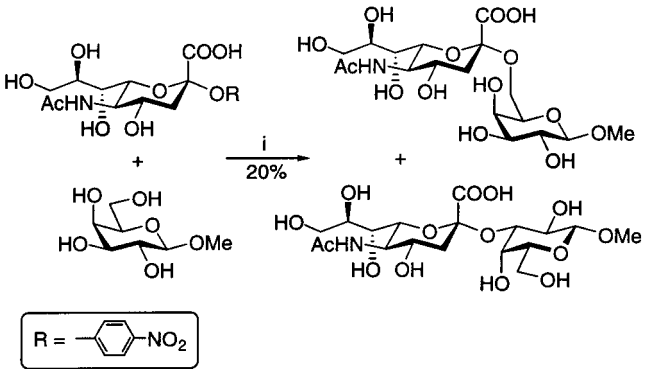
Sialyltransferases accept some modifications of CMP−Neu5Ac and therefore can be used for the incorporation of modified sialic acids. It was shown that a α(2 → 3)-sialyltransferase accepted CMP-sialic acid analogues that have a *N*-glycolyl, benzyloxycarbamate, or hydroxyl at *C*-5.[389] A 5-acetamido-3,5,9-tri-deoxy-β-D-*glycero*-D-*galacto*-nonulopyranosidonic acid analogue could also be incorporated.[390] Incorporation of the latter unit into glycoproteins should aid structure determination by X-ray crystallography. The modified donors for these enzymatic glycosylations were prepared by chemical methods. CMP 3-fluoroneuraminic acid is an inhibitor of α(2 → 6) sialyltransferase. The enzyme recognizes this compound, but the 3-fluoro moiety destabilizes the oxycarbenium ion that is formed in the transition state.[391] A CMP-9-fluoro-[3-^{13}C]-Neu5Ac derivative has been synthesized by a combined chemical and enzymatic approach, and this substrate could be used for the sialylation of a glycoprotein using an α(2 → 6)sialyl transferase.[392] Only 60% of the galactosides of the glycoprotein were sialylated, and those that had not reacted were removed by a galactoside. The ^{13}C and fluoride label enabled NMR experiments to be performed on the neoglycoprotein.

B. Sialidases

Sialidases are glycohydrolases that catalyze the release of terminal sialic acids α-glycosidically linked to glycoproteins, glycolipids, and polysaccharides. These enzymes have also been used for the synthesis of sialosides by *trans*-glycosylation.

Thiem and co-workers reported the first example of such a transformation.[393] They employed immobilized sialidase from *Vibrio cholerae* as the catalyst, *p*-nitrophenyl Neu5Ac as the glycosyl donor, and several galactosides as acceptors. Both α(2 → 3) and α(2 → 6) glycosides were formed in yields ranging from 14% to 24% (Scheme 39). The ratio of the two

Scheme 39[a]



[a] Conditions: (i) immobilized enzyme, buffer pH 5.5 (NaOAc/$CaCl_3$/NaN_3), in the presence or absence of DMSO.

regioisomers depended upon the acceptor substrate used but in each case a preference for the formation of (2 → 6)-linked derivatives was observed.

The origin of the sialidase influences the regioselectivity of the transformations. Sialidases from *Clostridium perfringens*, *Arthrobacter ureafaciens*, and *Vibrio cholerae* gave mainly α(2 → 6)-linked sialyl *N*-acetyl lactosamine derivatives, whereas the sialidase from Newcastle disease virus gave preferential formation of the α(2 → 3)-linked regioisomer.[394] In these transformations, *p*-nitrophenyl Neu5Ac or α(2−8)-linked sialic acid dimer were used as glycosyl donors. It has been suggested that immobilization of these enzymes results in lower regioselectivities.[394]

Sialidase- catalyzed *trans*-glycosylations have also been applied for regioselective α(2 → 3) sialylations of Lewis^x and Lewis^a.[395] For these transformations, sialidase from *Salmonella typhimurium* LT2 was used and *p*-nitrophenyl Neu5Ac as the acceptor. Sialyl Lewis^x and sialyl Lewis^a were isolated in yields

of 9.3% and 12.0%, respectively. These results are significant because mammalian sialyltransferases do recognize Le^a or Le^b as acceptors.

The trimer Neu5Acα(2 → 3)Galβ(1 → 4)Glc*N*Ac was synthesized by two regioselective *trans*-glycosylations using β-galactosidase from *Bacillus circulans* and *trans*-sialidase from *Trypanosoma cruzi*.[396] A yield of 60% was achieved when 4-methylumbelliferyl Neu5Ac was used as a donor. Colominic acid (a polymer composed of α(2 → 8)-linked Neu5Ac) has been used as a donor substrate for *trans*-sialylations.[397]

A combined use of *trans*-sialidase and sialyltransferase for enzymatic synthesis of Neu5Acα(2 → 3)βGal*O*R has also been reported.[398] This method takes advantage of the *trans*-sialidase enzyme of *Trypanosoma cruzi* which has the unique property of catalyzing the reversible transfer of Neu5Ac of a donor substrate of the sequence Neu5Acα(2 → 3)-Galβ*O*R^1 to virtually any galactoside acceptor β-D-Gal-*O*R^2 to yield the new product Neu5Acα(2 → 3)Galβ*O*R^2. The substrate of this enzyme was prepared in situ using α(2 → 3)-sialyltransferase to transfer free Neu5Ac to its precursor galactoside acceptor via catalytic in situ regeneration of CMPNeu5Ac. This method circumvents the narrow substrate specificity of sialyltransferases.

VI. Other Topics

A. Modifications of N-5

N-Glycolylneuraminic acid (Neu5Gc) is another important naturally occurring neuraminic acid derivative. Several attempts to synthesize oligosaccharides that contain this derivative have been reported, and for this purpose, glycosyl donors were prepared that have an *O*-acetyl-[83,164,179,201] or *O*-benzyl-[160,168] protected *N*-glycolyl moiety {-C(=O)CH_2OAc or −C(=O)CH_2OBn}. Other functional groups at *C*-5 were introduced for biological studies.[399]

Protection of the amino group at *C*-5 with *tert*-butyloxycarbonyl (Boc),[132] benzyloxycarbonyl (CBz, Z),[45] azido,[45,162] or phthalimido (Phth) group[145] allowed access to a free *C*-5 amine upon deprotection. This amine can then be subsequently derivatized with glycolyl or other moieties. No report indicates that the use of these protecting groups results in a change of reactivity or stereoselectivity, except that the application of 5-azido-derivatized 2-thiomethyl substrate gave lower yields of coupling product (26%) compared to the use of a similar donor that has an *N*-acetamido moiety (51%).[162] As discussed above, an *N*-acetylacetamido function significantly improves the reactivity of both sialyl donor[126,127] and acceptor[127] as well as donors with a participating auxiliary at *C*-3.[316]

B. NMR Rules for the Anomeric Assignments

NMR spectroscopy offers the most efficient method to determine the anomeric configuration of sialosides, although other physical methods have been used.[1] For example, reliable anomeric assignments based on $[\alpha]_D$ determination have been reported, but this method requires both α- and β- anomers. Circular dichroism can only be used for unprotected derivatives.

For normal pyranosides, assignment of the anomeric configuration is based on measuring the following coupling constants: $^3J_{H-1,H-2}$ and $^1J_{C-1,H-1}$. This approach is not applicable for sialic acid due to the absence of an anomeric hydrogen (*C*-2). Therefore, empirical rules have been developed based on chemical shift data and coupling constants. Five different parameters can be used which include δ *H*-3eq,[400] δ *H*-4,[37,401] $J_{H-7,H-8}$,[37,322] $\Delta\delta$ {*H*-9a−*H*-9b},[322] and $J_{C-1,H-3ax}$.[402] Thus, it was observed that the *H*-3eq signals of α-linked compounds are shifted downfield (δ = 2.67−2.72 ppm) compared to those of β-anomers (δ = 2.25−2.40 ppm).[400] This rule is especially useful for unprotected sialosides and does not always apply to *O*-acetylated derivatives. Another rule, which states that the signal of *H*-4 of an α-anomer appears at higher field (δ = 4.89−4.93 ppm) than that of the corresponding β-anomer (δ = 5.68−5.81 ppm),[37] can also be applied for unprotected sialosides (δ = 3.6−3.8 ppm is characteristic for an α-glycosidic linkage, whereas δ = 3.9−4.2 ppm for β-anomers).[41,401] The $J_{H-7,H-8}$ coupling constants of α-glycosides are larger (6.2−8.5 Hz) than those of β-anomers (1.5−2.6 Hz), which suggests different conformations of the side chain.[37,322,403] The anomeric configuration of the sialosides can also be determined from the chemical shift difference of two vicinal protons at *C*-9, i.e., the $\Delta\delta$ {*H*-9a−*H*-9b} value, is smaller than 0.5 ppm for α-glycosides, whereas it is around 1.0 ppm for the corresponding β-anomers.[322] A ^{13}C NMR technique and 2D-methods can be employed for the determination of the anomeric configuration by measuring the long-range $J_{C,H}$ coupling constants. It was demonstrated that sialic acid residues having an axial carboxylic function (α-anomers) show a larger $J_{C-1,H-3ax}$ coupling constant (5.8−7.5 Hz) than the corresponding equatorial carboxyl derivative (β-anomers, 1.0−1.7 Hz).[402,404,405] Moreover, the $J_{C-2,H-3ax}$ values were found to be ∼8.0 Hz for the α-anomers and ∼4.0 Hz for the β-anomers.[406]

VII. Concluding Remarks

Despite recent progress in the chemical and enzymatic sialylation of saccharides, no approach has been reported that allows glycosylation of a wide range of acceptors in high yields and stereoselectivities. Anomeric chlorides of Neu5Ac offer the most reliable glycosyl donor for the preparation of glycosides of simple alcohols. 2-Thioalkyl, 2-thiophenyl, 2-xanthate, and 2-(dibenzyl) or 2-(diethyl)phosphites are the leaving groups of choice when more complex hindered sugar alcohols need to be sialylated. The best results are obtained when saccharide acceptors have a free diol or triol, and in many of these cases the sialylations proceed with excellent regioselectivities in combination with high yields and anomeric selectivities.

Several glycosyl donors of Neu5Ac have been prepared that have an auxiliary at *C*-3. These auxiliaries control the anomeric selectivity of a glycosylation by neighboring group participation. Gly-

cosyl donors that possess an equatorial *S*-phenyl-participating auxiliary at *C*-3 and thioalkyl leaving group at *C*-2 generally give the best yields and α-anomeric selectivities especially when applied for the glycosylation of sterically hindered alcohols. A 3-*O*-phenylthiocarbonyl auxiliary also gives excellent yields and stereoselectivities. The major drawbacks of indirect methods are the additional chemical steps required for introduction and removal of the auxiliary at *C*-3. Furthermore, the introduction of a *C*-3 auxiliary in many cases proceeds with poor stereoselectivity rate. Despite these drawbacks, the indirect methods offer the most reliable approach for the synthesis of the dimer Neu5Acα(2 → 8)Neu5Ac.

Several α(2 → 6)- and α(2 → 3)-sialyltransferases have been used for oligosaccharide synthesis, and the most attractive approach involves in situ regeneration of CMP−Neu5Ac. The substrate specificity of these enzymes precludes the synthesis of a wide range of sialooligosaccharides. Sialidases have also been used for the preparation of sialosides. These enzymes have less restricted substrate specificities, but yields and regioselectivities are in most cases low.

Recent progress allows chemical, enzymatic, or chemoenzymatic synthesis of complex sialooligosaccharides. Each synthetic target, however, should be regarded as a research project, and several reaction conditions, methods, or strategies may need to be examined to obtain an efficient synthetic approach. Most synthetic efforts have been directed to the glycosylation of Neu5Ac. Less effort has been spent on the preparation of sialosides that have an *N*-glycolyl moiety (Neu5Gc) or have an acetyl, lactoyl, methyl, or phosphate at one of the hydroxyls.

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