Sialic Acid Donors: Chemical Synthesis and Glycosylation

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Abstract: Sialic acids (or ulosonic acids) are a family of acidic ketoses (including neuraminic acid, KDN and KDO) that are found at the non-reducing terminus of many glycoconjugates. These saccharide residues are recognized ligands of protein lectins and are removed in the first step in glycoconjugate catabolism. Moreover, sialic acid containing carbohydrates, such as glycolipid gangliosides (*i.e.* GM3), glycopeptides (*i.e.* Tn) and polysaccharides (*i.e.* polysialic acid or colominic acid) play important biological roles. Thus, they represent important targets in natural product synthesis. This review examines the application of sialic acids as donors in glycosylation reactions. The synthesis of protected sialic acid donors and challenges associated with their use in the synthesis of heteronuclear and carbon glycosides are discussed.

STRUCTURE OF SIALIC ACIDS

Since the discovery of sialic acids in vertebrates by Blix and Klenk in 1941, extensive effort has focused on identifying the structure and the functional roles that these ubiquitous carbohydrates play in mammalian biology [1]. Sialic acids are a structurally unique family of 8 and 9-carbon monosaccharides and characteristically contain an anomeric carboxylate, a deoxygenated methylene C-3 ring carbon, an oligohydroxylated side chain at C-6 and are differentially functionalized at C-5 (Figure 1). Neu5Ac is the most common sialic acid and so the major portion of this review will discuss the chemical synthesis of Neu5Ac donors and their use in glycosylation.

FUNCTION OF SIALIC ACIDS

Sialic acids are present in mammalian and avian tissues predominantly in the form of lipooligosaccharides, lipopolysaccharides and as the glycan component of glycoproteins. They exist in a variety of glycosidic linkages, most typically α(2-3) and α(2-6) to galactose (or lactose), as well as α(2-8) and α(2-9) linkages in homopolymers of Neu5Ac (polysialic acid) [1, 2]. Representative sialic acid containing glycans found throughout nature are illustrated in Figure 2. The functions of these acidic glycans vary widely in animals based on the structural heterogeneity of their monosaccharide residues, their aglycone moiety and their molecular size. They are typically found capping the non-reducing termini of many glycan chains, where they are responsible for a host of carbohydrate mediated intercellular and intracellular events. These may include cell-cell interactions [2], aggregation [3, 4], and development [5].

In addition to playing a major role in host physiology and development, many pathological microbes employ sialic acids to promote infection. These opportunists have evolved to take advantage of the multi-functional role performed by sialosides. Some viruses utilize hemagglutinin, a sialic acid binding lectin, others utilize neuraminidase, an enzyme that cleaves sialic acid, to gain entry into a cell [6-9]. A prominent example of a pathogenic bacteria exploiting the presence of mammalian sialosides is *Neisseria meningitides.* This virile pathogen biosynthesizes an extracellular homopolymeric Neu5Ac capsule as camouflage to escape host immune response [7, 8]. Infectious pathogens are not the only malevolent entities that express and utilize sialoglycoconjugates. Gangliosides, an important class of sialic acid containing antigenic glycotopes, are currently being evaluated as anti-cancer agents [10]. Specific tumor types express gangliosides (GM **4** and GD series,) as well as specific glycopeptides (sTn) selectively on their cell surface [1, 11]. Thus, the potential for use of sialic acid based therapeutic agents is promising. The specific expression of these surface carbohydrate antigens by certain pathogens and tumor cell lines has resulted in a concerted effort to synthesize sialoconjugates as biological probes, small molecule inhibitors and as part of higher ordered, multivalent vaccines [12].

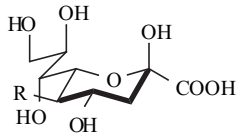

Fig. (1). Neuraminic acid, Neu5Ac, R = NHAc **1**; ketodeoxy-nonulosonic acid, KDN **2**, R = OH; and *N*-glycolyl neuraminic acid, Neu5Gc R = $HOCH_2CO$ **3**.

CHEMICAL SYNTHESIS AND GLYCOSYLATION

Glycosylation Strategies

There are numerous factors that should be considered when undertaking the synthesis of sialic acid donors. These include protection schemes, functional substitutions, promoter choice and acceptor architecture. A hastily devised scheme incorporating poor protecting group strategies, or one that utilizes incompatible chemistries can result in an

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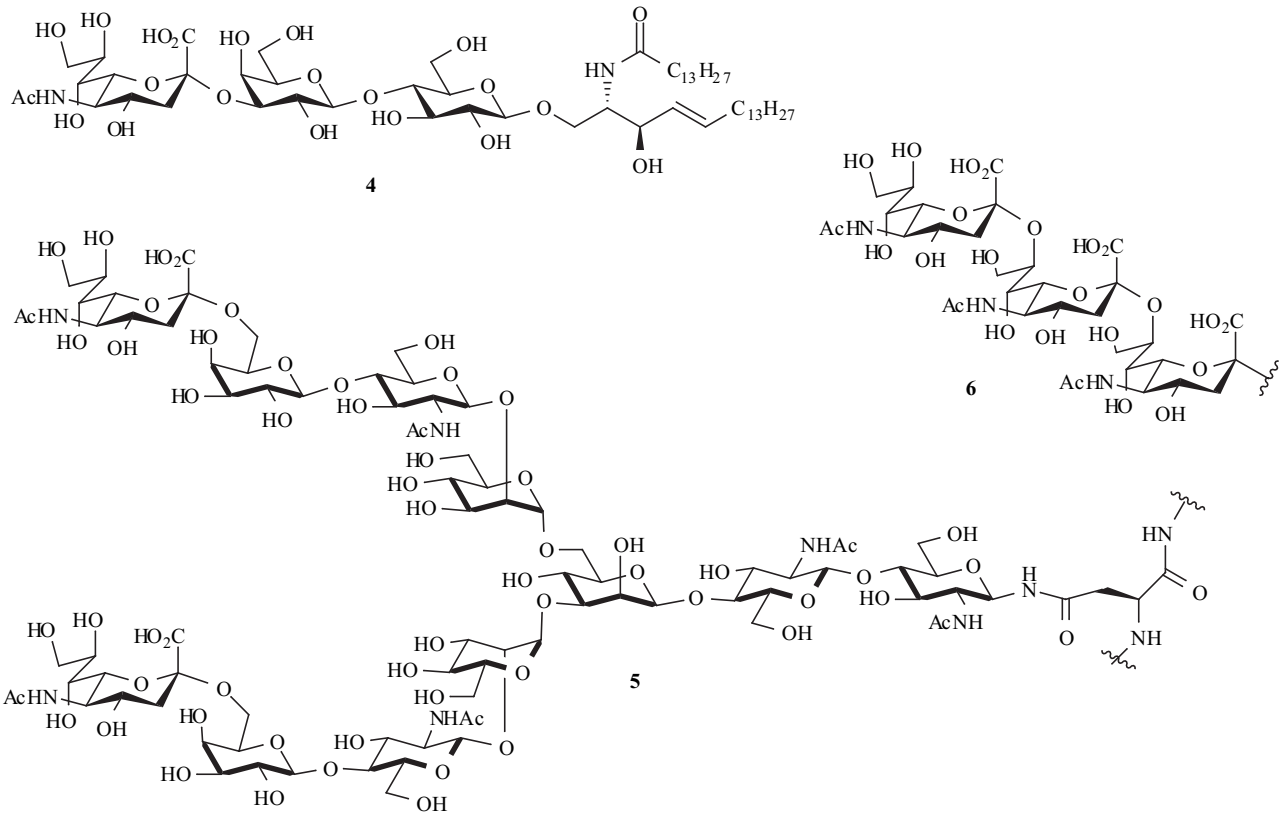
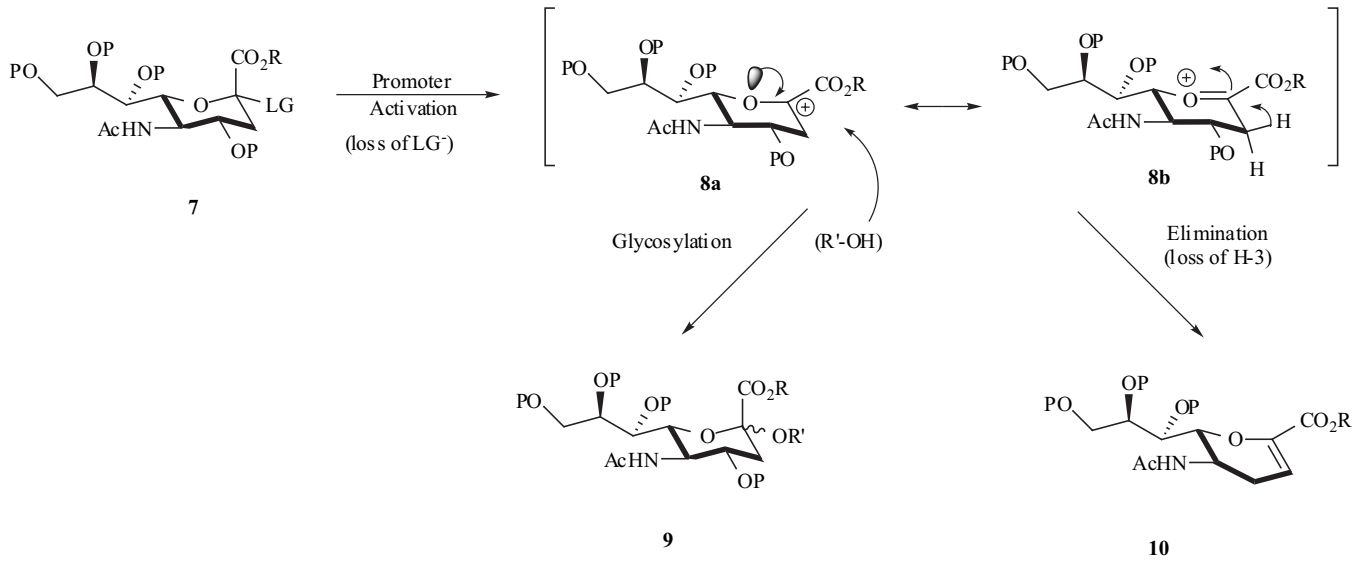


Fig. (2). Glycolipid, ganglioside GM3 **4**; glycopeptide, biantennary glycan **5**, and polysaccharide α(2→8) Polysialic Acid (Colominic Acid) **6.**

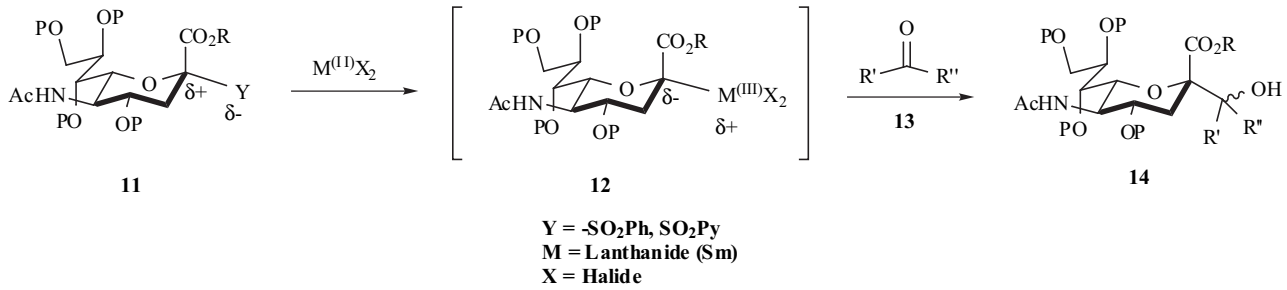
unfulfilling experience replete with frustration and multiple revisions of synthetic schemes.

Mechanistic Aspects of Glycosylation

The complicated molecular architecture of sialic acids imparts a substantial degree of difficulty in their synthesis, protection and activation. The polyfunctional nature (3-4 secondary hydroxyl groups) of these molecules, as well as their tertiary anomeric centers, complicates the job of the synthetic chemist to efficiently construct differentially glycosylated synthetic targets. Even after appropriate protection, the presence of the anomeric carboxylic acid destabilizes the formation of the oxocarbenium ion intermediate (**8a-8b**) in heteroatomic (O, N, S) glycosylation through an inductive electron withdrawing effect (Scheme 1). The steric constraints at the C-2 reactive site and the lack of an anchimeric assistance from a neighboring C-3 substituent often leads to low stereoselectivity and the formation of



Scheme 1. Heteroatom glycosylation of sialic acid donor with competing elimination.



Scheme 2. *C*-glycosylation of sialic acid donor using umpolung strategy.

side-products through a competing elimination reaction (**10**) [13].

A prominent method for preparing *C*-glycosides of sialic acid involves an umpolung (charge inversion) strategy (Scheme 2). During *O*-glycosylation, the role of the intermediary anomeric center is classically electrophilic in nature, in this *C*-glycosylation strategy the charge character of the anomeric carbon at C-2 is reversed. The newly generated anomeric anion (**12**) reacts in a remarkably different fashion. In this type of chemistry, the synthesis of both donor (**11**) and acceptor (**13**) is a departure from classical methods and involves re-thinking protection group design and manipulation as well as donor and acceptor chemistries. A more comprehensive discussion of *C*-glycosylation will be presented later in this review.

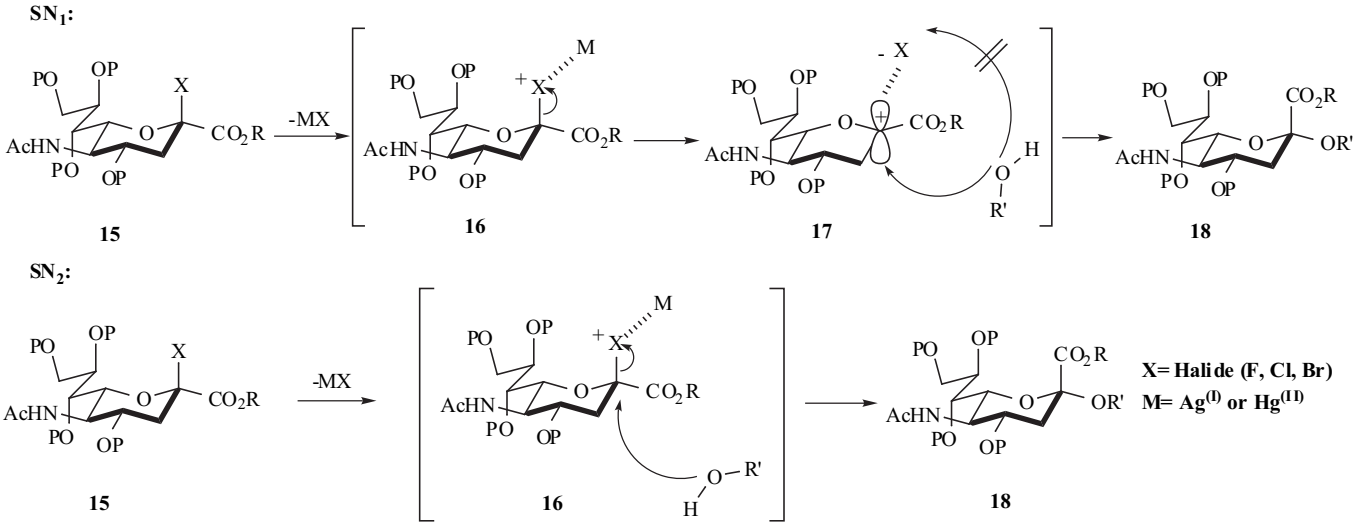
Promoter Design and Use

Heteroatomic Inter-Glycosidic Bond Formation

The promoter plays a significant role in glycosylation by facilitating the buildup of positive charge at the anomeric C-2 position. α-Glycosides of sialic acid have been prepared by the Koenigs-Knoor reaction (1901) [14] or using the Helferich modification (1962) [15]. These procedures are benchmark glycosylation protocols developed prior to the advent of modern, non-metal Lewis acid based methods.

A typical Koenigs-Knoor/Helferich sequence is outlined in Scheme 3. The substrates of choice in these reactions are β-glycosyl halides. Starting with an arbitrarily protected β-haloglycoside of Neu5Ac (**15**), metal induced activation of the anomeric position ($Ag^{(I)}$-Koenigs-Knoor, $Hg^{(II)}$-Helferich) leads to **16**. Intermediate **16** can then react through an SN_1 type mechanism where dissociation leads to an intimate ion pair (**17**). Compound **18** is arrived at by the subsequent displacement of the halide anion by an alcohol. *O*-glycoside **18** can also be formed from **16** through direct displacement via an SN_2 mechanism. Solvent polarity appears to play an important role in determining the reaction pathway. Polar solvents favor an SN_1 reaction because of their ability to solvate charged species, whereas non-polar solvents support the SN_2 mechanism. In both cases, predominantly the α-*O*-glycosidic product is formed. This is somewhat surprising as the planar nature of the cationic intermediate in the SN_1 reaction should afford an α,β anomeric mixture. A plausible and widely circulated explanation is that the leaving group in the intimate ion pair blocks the top face of the molecule hindering the approach of an incoming nucleophile, predominantly affording the α-anomeric product. The enhanced organic solvent solubility of $Hg^{(II)}$ salts, utilized in the Helferich methodology, has allowed carbohydrate chemists to investigate glycosylation reactions in increasingly non-polar organic media.

There are a number of variations on these classical methods designed to improve their scope and yield. The main differences between these variations are related to the choice of metal/counter-ion pairing promoters. The more common promoters used are AgOTf, Ag_2CO_3, HgX_2 (X =



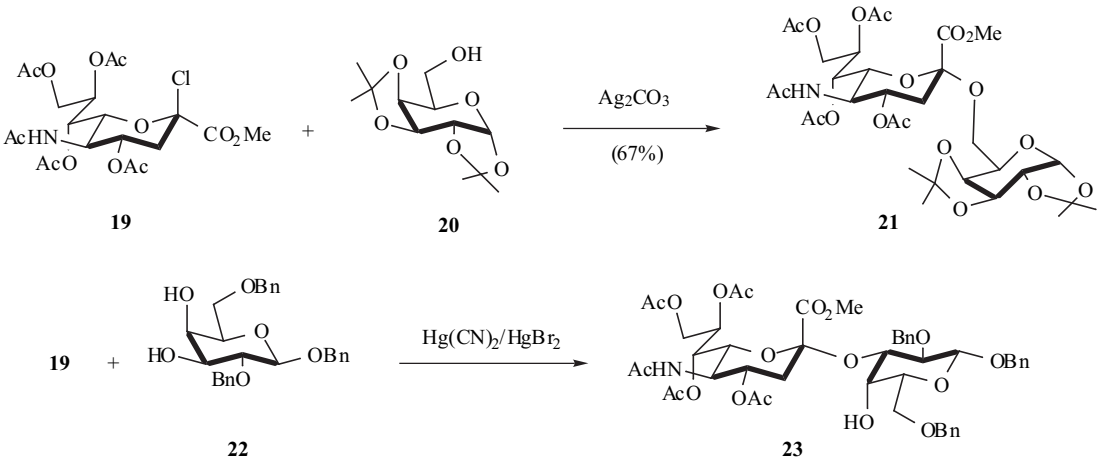
Scheme 3. Koenigs-Knoor/Helferich glycosylation of sialic acids involving SN_1 and SN_2 reactions.

halide), and $Hg(CN)_2$. In general, $Ag^{(I)}$ promoters are more active and stereoselective, while $Hg^{(II)}$ promoters afford higher yields.

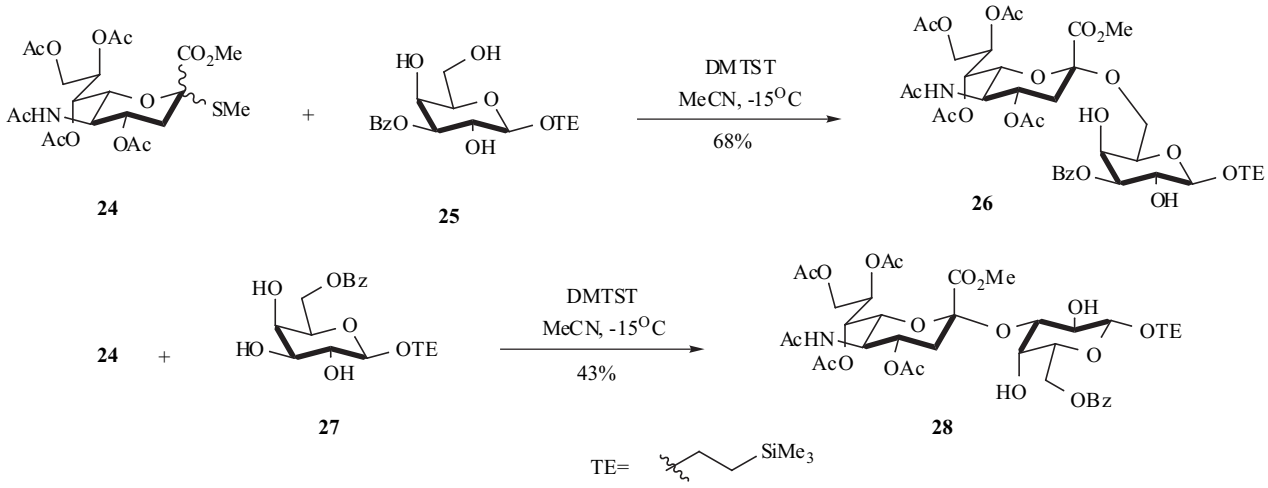
The introduction of thioalkyl and thioaryl glycosides of sialic acids by Hasegawa and Roy has ushered in a renaissance with respect to sialic acid glycosylation [16-18]. The stability advantages gained by using thioglycosides are considerable. Halide-based sialosides, especially bromides, require careful manipulation and are difficult to purify due to their extreme lability. In contrast, alkyl and aryl thiosialosides are remarkably stable, do not require such careful manipulation and can be prepared in advance and carried through multi-step syntheses until introduced to soft glycosyl initiators.

Promoter design has followed suit and a number of "thiophilic" reagents have emerged to expand the scope of the *O*-sialylation reactions. These new promoters have expanded the limited scope of the Koenigs-Knoor/Helferich processes, which offer reasonable yields and stereoselectivities when activated donors are coupled to acceptors through primary hydroxyl groups, such as in the formation of α(2→6) linkage to galactose. Chloro donor **19** reacts with acceptor **20** under classical Koenig's-Knoor conditions giving α-sialoside **21** in 67% yield (Scheme 4) [19]. In contrast, when donor **19** is brought into contact with acceptor **22** containing a less reactive secondary hydroxyl group**,** sialoside **23** is formed in only 15% yield as a mixture of anomers (α/β 2/3) [20]. The synthesis of the more common α(2→8)-neuraminyl and α(2→3)-lactosyl and galactosyl linkages requires even stronger promoters.

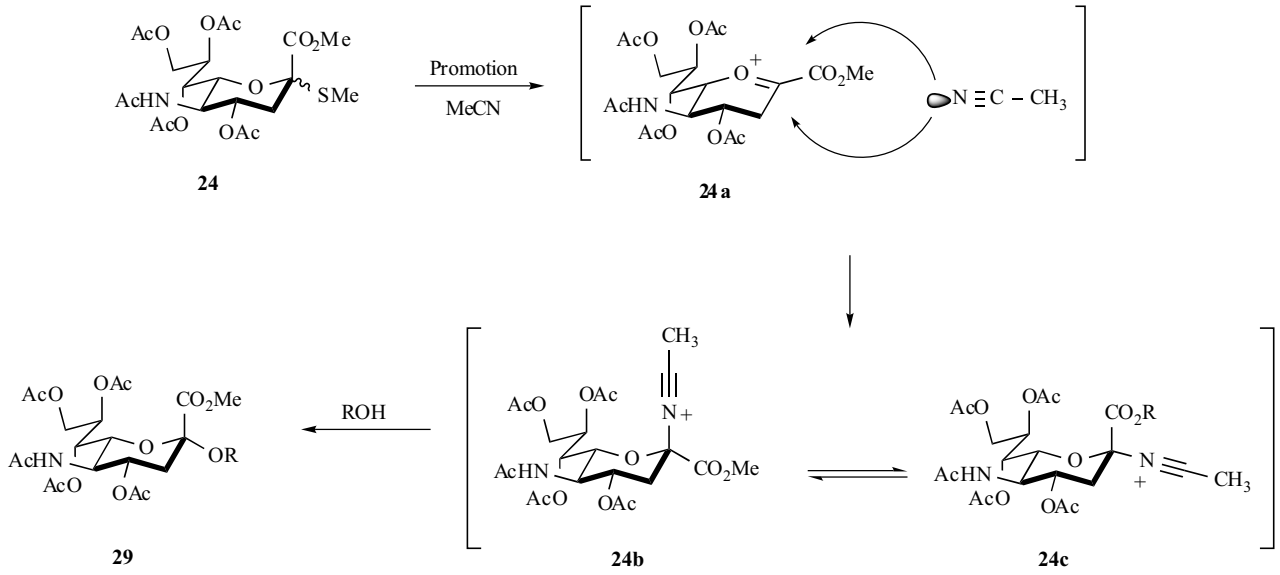
The development of electrophilic, non-metal, specific thiophilic promoters, such as dimethyl(methylthio) sulfonium trifluoromethanesulfonate (DMTST), N-iodosuccinimide (NIS), and phenyl selenyl trifluoromethane-sulfonate (PhSeOTf) has overcome many of the deficiencies observed with traditional sialylation methods. Pioneering work by Hasegawa [16] (Scheme 5) describes the use of DMTST, in combination with α/β (1:1) thiomethyl glycoside of Neu5Ac **24**, to synthesize Neu5Ac α(2→6) galactosyl disaccharide **26**, and Neu5Ac α(2→3) galactosyl disaccharide **28**. The high level of regioselectivity in the formation of the α(2→3) product was rationalized based on the increased nucleophilicity of the equatorial 3-OH group. The 2-OH group was considerably less reactive due to an inductive electron withdrawal effect of the anomeric substituent. Similar investigations were carried out using globally protected galactose residues, albeit with dramatically lower yields and stereoselectivity.



Scheme 4. Koenigs-Knoor/Helferich glycosylation with acceptor containing primary and secondary hydroxyl groups.



Scheme 5. Electrophilic, non-metal, specific thiophilic promoter dimethyl(methylthio) sulfonium trifluoromethanesulfonate (DMTST) in the synthesis of sialic acid containing disaccharides.



Scheme 6. Solvent participation in glycosylation through the formation of intermediate nitrillium ions and control of stereochemical outcome.

The high anomeric selectivity of these reactions is interesting due to the racemic nature of the donor. The absence of a stereo-controlling element in both the promoter and donor suggest participation by the solvent during the glycosylation process. The influence of solvents on anomeric selectivity has been reported extensively [21-24]. Glycosylations conducted in CH_3CN afford predominantly α-anomeric products. A mechanism has been proposed that accounts for the observed stereochemistry through a kinetically controlled β-nitrilium ion intermediate (Scheme 6). Under SN_1 conditions, activation of racemic Neu5Ac donor **24**, leads to a solvated ion pair, which is nucleophilically captured by a molecule of solvent to yield **24b** and **24c**. Equilibration of the intermediate species leads predominantly to the α-nitrilium anomer, however, under kinetic control the conversion is slow. Therefore the β-nitrilium ion, which is more reactive due to more favorable (less hindered) approach by the collapsing nucleophile, reacts with an acceptor (ROH) to form the α-sialoside **29** as the major glycosylation product.

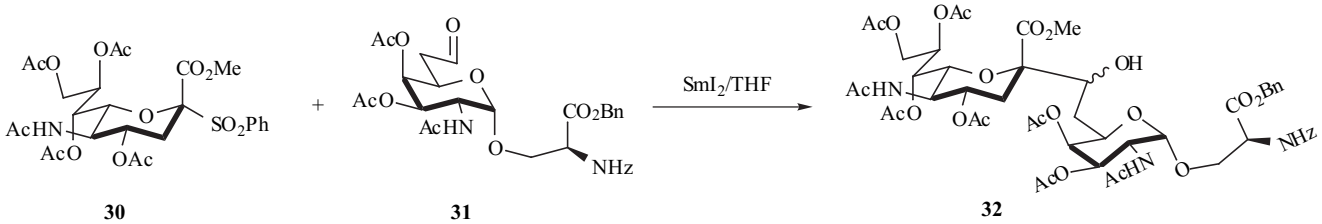
C-Glycosidic Bond Formation

Thioglycosides of sialic acid are also useful precursors in the synthesis of *C*-linked sialosides. As a result of regioselective and stereoselective demands and the charge inversion nature of *C*-glycosylation chemistry, it was necessary to develop alternative initiators to synthesize sialic acid *C*-glycosides. Samarium iodide (SmI_2) is a powerful one-electron reductant that is capable of stereoselectively forming new C-C bonds. Prototypical donors are oxidized thioaryl (phenyl or pyridyl sulfone) compounds and typical acceptors are electrophilic carbonyl-based aldehydes and ketones. Our laboratory has recently completed the synthesis of a hydroxymethylene linked isostere of the tumor carbohydrate antigen sTn (Scheme 7) [25]. *C*-glycosyl donor **30** was synthesized from Hasegawa's donor in one step using a ruthenium catalyzed periodate oxidation [26]. Glycosylation of **31** under samarium mediated reductive coupling gave exclusively the α-*C*-glycoside **32** in excellent yield. The diastereomeric mixture at the bridge hydroxymethylene carbon was chemoselectively resolved through a combination oxidation/reduction sequence.

Promoter variation and function has been extensively reviewed elsewhere. For a more expanded discussion on the availability of new glycosylation agents, please consult recent reviews done by Chappell [27], Boons [28], and Schmidt [24].

Donor Types

The choice of promoter is not the sole determinant affecting the outcome of glycosylation reactions. Structural features of both donor and acceptor have been studied and extensively manipulated in an attempt to identify important intramolecular features affecting stereoselectivity to both maximize yields and improve stereoselectivity. Due to the



Scheme 7. Synthesis of a *C*-sTn analog using samarium mediated *C*-glycosylation chemistry.

large number and variety of functional groups present in sialic acids, their derivatization or chemical conversion would be expected to drastically affect the outcome of a glycosylation, providing a serviceable chemophore map. In theory, the appropriate modification of these residues could be used to tweak a particular reaction sequence and enhance the likelihood of the desired reaction outcome.

Protecting Group Modifications

A well-planned, selective blocking group strategy remains the core of any successful venture in carbohydrate synthesis. The glycosylation reaction, often requiring a wide array of potential promoters and reaction conditions, necessitates a library of versatile protecting groups. Their stability, longevity and compatibility with the synthetic plan for both donor and acceptor is of paramount concern.

Carbohydrates are almost exclusively protected with one of three main functionalities; esters, ethers, and acetals. Each one having its own identifiable stereoelectronic effects on both the latently armed/disarmed glycosyl donor and the glycosyl acceptor. Esters typically act to disarm donors, thereby decreasing the reactivity at the anomeric position. If not for the instability and tertiary nature of the oxocarbenium ion intermediate in sialic acids, esters might be ideally suited as protecting group partners. These stereoelectronic and architectural restraints, as well as the sluggish reactivity of the activated sialic acid intermediate, often require ether protection chemistry to facilitate the formation of an armed donor. The problems here arise from the multifunctional nature of the molecule and the fairly aggressive reaction conditions usually associated with the installation of the ethereal protecting groups. The wealth and variety of reactive groups present in the sialic acids makes cross reactivity a paramount concern.

The benzyl protection described by Vasella *et al.* [29] is representative of the hazards associated with masking reactive groups in sialic acid synthesis (Scheme 8). The benzyl protected methyl ester of sialic acid target **35** was painstakingly isolated from the reaction mixture in modest yield only after a complex chromatographic separation and chemically re-working the undesired products formed.

Anomeric Variations

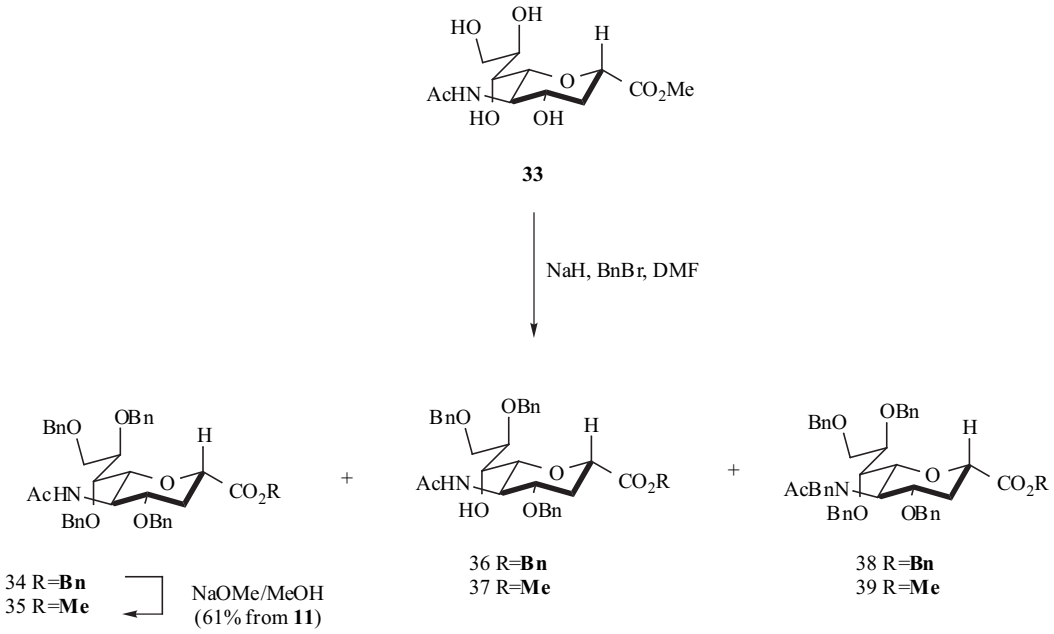
2-Halo Sialic Acids

Once considered to be the prototypical sialic acid donor, the use of halo-glycosides has diminished considerably since their first application. Chlorides were considered ideal donors because they are easily synthesized and are relatively stable. Bromides have not seen widespread use due to stability concerns, and because of their predisposition to yield anomeric mixtures and elimination products during glycosylation. Fluorides, synthesized from corresponding chlorides by reaction with AgF in MeCN [30, 31], or by conversion of 2-OAc derivatives with HF/pyridine [12, 32], have also been applied in sialoside synthesis. Initially synthesized to overcome the stability concerns associated with other halide donors, sialyl fluorides have seen limited application due to their propensity to form unnatural β-linkage [14].

2-Thio (alkyl and aryl) Sialic Acids

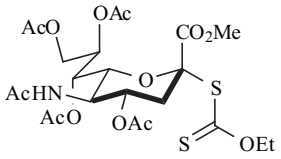
The importance of thioglycoside donors in sialic acid glycosylation has been discussed. These donors are popular with synthetic carbohydrate chemists because they are easily prepared and tolerate a variety of reaction conditions. This permits the early installation of the thioalkyl or thioaryl moiety prior to extensive chemical modification of the sialic acid molecular scaffold.

Anomeric sialyl xanthates (Figure 3) are relatively new donor designs and are finding widespread use in synthetic carbohydrate chemistry. Sinaÿ and coworkers synthesized the 2-xantho donor of sialic acid from the corresponding anomeric chloride by reaction with sodium or potassium ethoxydithiocarbonate in ethanol [33]. 2-Xanthates are



Scheme 8. Complications in the benzyl protection of Neu5Ac methyl ester.

crystalline and are considered to be more useful over their 2-thioalkyl counterparts because they often afford higher yields [34].



40

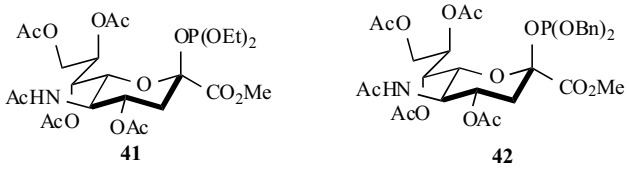
Fig. (3). Xanthate ester of Neu5Ac methyl ester **40**.

When thioaryl sialic acids are oxidized to aryl sulfones, they become useful donors for the synthesis of carbon-linked sialosides. Our group has utilized both phenyl and pyridyl sulfones in the synthesis of *C*-glycosides of Neu5Ac, KDN and KDO [26, 35-37].

2-Phosphites of Sialic Acids

Schmidt [38] and Wong [39] first introduced sialyl phosphites as *O*-glycosylation donors. Phosphite donors offer the advantage of only requiring a catalytic amount of promoter, usually TMSOTf, for glycosylation initiation, and the stereochemistry is favorably set prior to adding the glycosyl promoter. Under conditions favoring solvated ion pair formation (SN_1), or using direct nucleophilic displacement (SN_2) conditions, the preferred β-axial orientation of the phosphite group insures the predominant formation of the α-product. Yields and anomeric selectivity with phosphites are comparable to those obtained using thioalkyl (aryl) and xanthate donors.

β-Sialophosphites (Figure 4) are commonly synthesized as either the diethyl (**41**) or the dibenzyl (**42**) analogs from the reaction of a suitably protected sialic acid containing a free 2-OH group and the corresponding chloro-diethoxyphosphite and a bulky base such as i-Pr_2NEt (DIEA). Dibenzyl phosphite analogs are used much less frequently due to the lower availability of the *N,N*-diethylphosphoroamidite used in their preparation.



41 **42**

Fig. (4). β-Sialophosphite donors **41** and **42**.

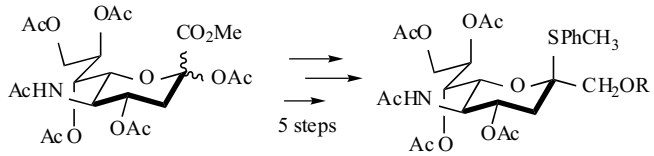
This brief overview of the 2-position substituents, common in sialyl donors, is not meant to be all-inclusive but rather an introduction. Most of the compounds and chemistry presented in upcoming sections of this review rely on the donor chemistries just covered. There is still great potential for the development of new, unique chemistry targeted at sialic acid-based glycosyl donors and their application that merits further attention.

Positional Modifications

C-1

The anomeric carboxylic acid is important because it inductively affects the formation of the oxocarbenium ion intermediate involved in heteroatom glycosylation reactions. This can have a major influence on yields, stereochemical outcomes, and reaction rates. There are several reports of efforts to affect the outcome of glycosylation by modifying the carboxylic acid carbon either by chemical conversion, or by incorporating a stereocontrolling element into the protecting component. Takahashi *et al.* were one of the first groups to explore the use of auxiliary participation [40].

Wong and co-workers have converted the carboxylic acid into a hydroxymethylene isostere to investigate the potential use of this analog as an effective sialyl donor [41]. While innovative by design, the results of this transformation are disappointing from a utilitarian standpoint. Compounds **44a-c** (Scheme 9) were synthesized from peracetylated Neu5Ac precursors **43** in 5 steps with an overall yield of 36 %. The relative reactivity values (RRV) were calculated for these new analogs against the known RRV's of other donors. Generally, an RRV improvement over unmodified anomeric carboxyl donors was observed. Sialylation (Scheme 10) of donor **44a** with galactoside acceptor **45** proceeded smoothly to give **46** in a phenomenal yield of 95%. These results, however, were unexpected and disappointing as the reaction afforded the undesired β-anomer (α:β 16:1) as the major compound [19].



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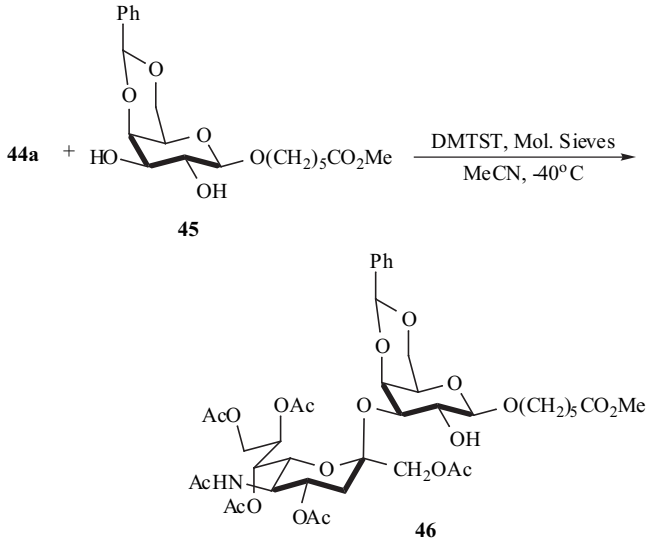
44a R=Ac
44b R= BOM
44c R=TBDPS

Scheme 9. Attempts to control stereochemical outcome of glycosylation using sterically demanding groups at the C-1position of sialic acid.

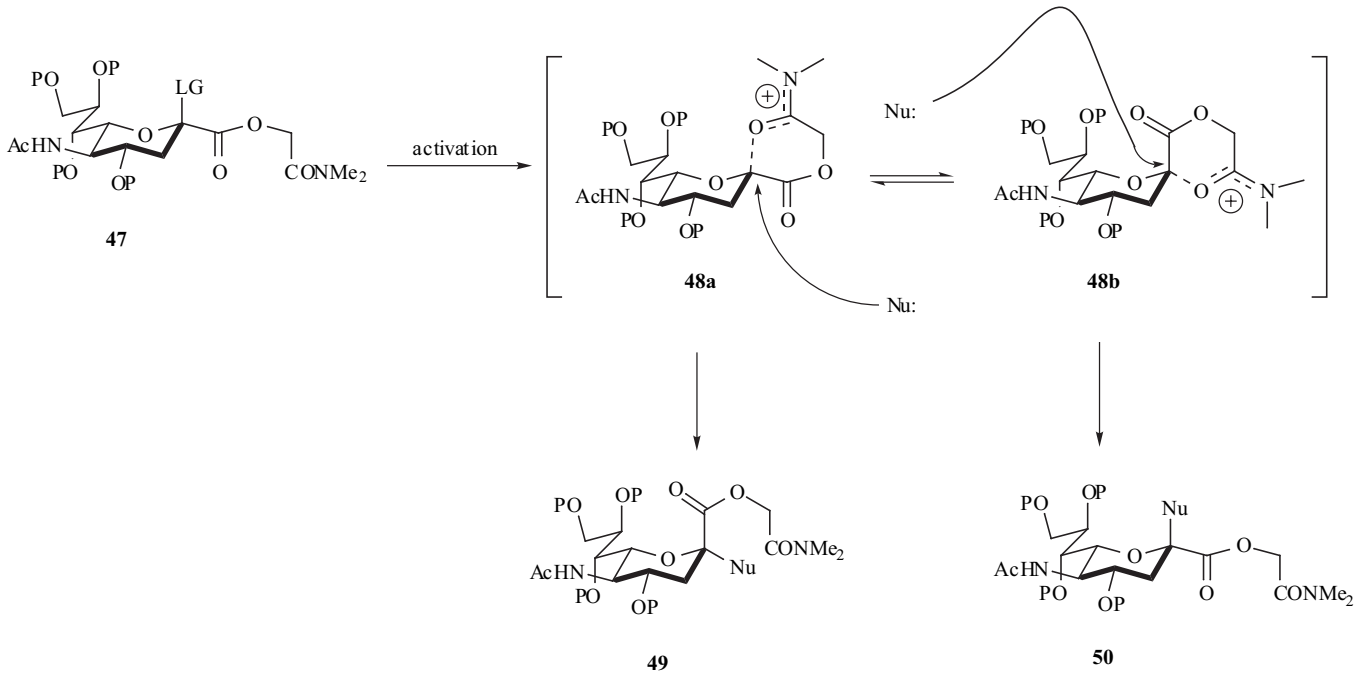


45 **46**

Scheme 10. Synthesis of the unnatural β-sialoside of galactopyranoside.

Koketsu and co-workers [37] used the *t*-butyl ester to reverse the *C*-glycosylation stereochemistry, which gave an undesired β-*C*-glycoside of the acetyl protected KDO methyl ester, in the synthesis of the first *C*-glycoside of KDO exclusively in the natural α-configuration.

Using an alternative approach, Gin and co-workers incorporated a stereocontrolling adjutant into a sialyl donor



Scheme 11. Application of a tethered *N,N*-dimethylglycolamide to control the stereochemical outcome of glycosylation reaction using a sialic acid donor. LG is leaving group and P is protecting group.

[42]. The novel *N,N*-dimethylglycolamide ester auxiliary participated favorably during the coupling process to amplify α-selectivity through the activation of β-leaving group of Neu5Ac donor **47**. Oxonium ion formation can be stabilized by the participatory *N,N*-dimethylglycolamide carbonyl group through the formation of either α or β oriented intermediates (Scheme 11). Of these two possible isomeric intermediates, **48a** predominates because of the anomeric effect. Subsequent capture of **48a** by a nucleophile leads to the formation of α-sialoside **49** as the major product. Comparative sialylation studies, using a variety of peracetylated anomeric *N,N*-dimethylglycolamide Neu5Ac donors against isosteric methyl ester protected Neu5Ac donors, confirmed the value of this participatory group in the control of stereochemical outcome. In every case, modest to excellent improvements in yield and stereoselectivity were seen in the use of the auxiliary containing donors [42]. Gin has recently published a dehydrative sialylation procedure utilizing an auxiliary ester component in conjunction with a freely deprotected hemi ketal donor **51** (Figure 5) [43].

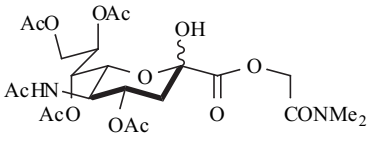


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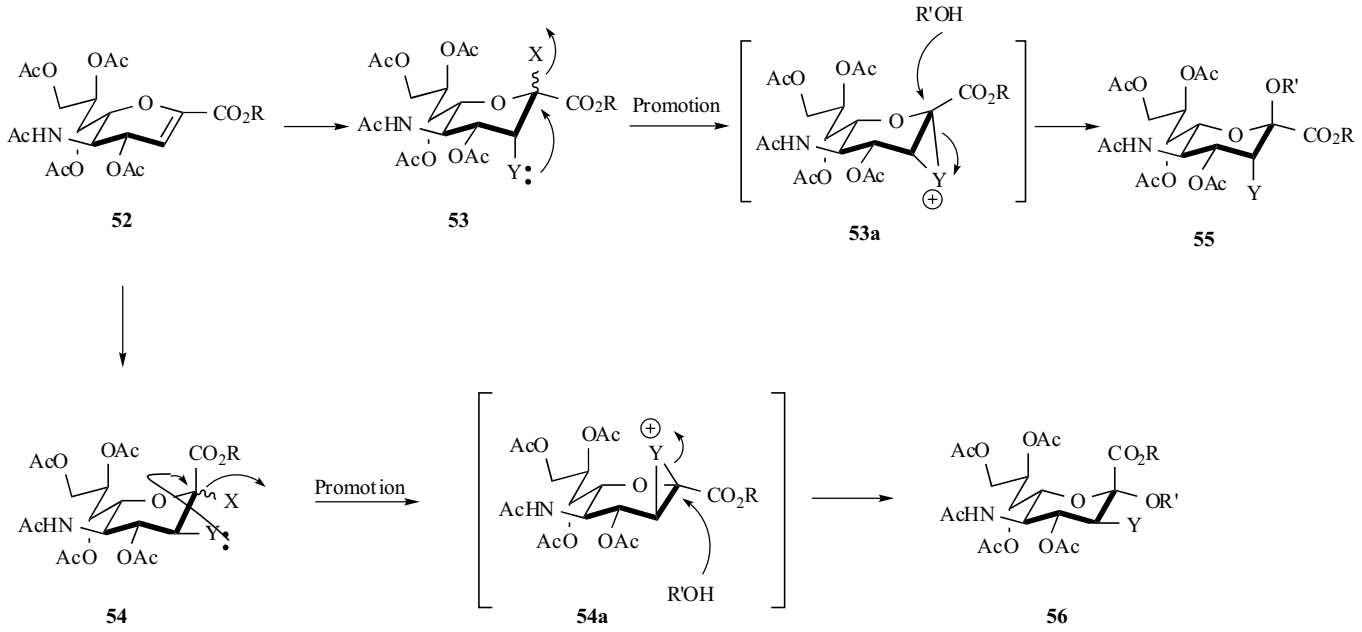
Fig. (5). Dehydrative sialylation procedure utilizes an auxiliary ester component in conjunction with a freely deprotected hemi ketal donor **51.**

C-3

The absence of a stabilizing group at C-3 compromises the stereochemical integrity of sialylation reactions. Many laboratories have sought to overcome this problem through the inclusion of an anchimeric assisting functional group in the deoxy position at C-3. Ideally, assisting groups should prevent competing 2,3-elimination reactions, which commonly occur in *O*-sialylation, and should be installed and removed with ease. C-3 auxiliaries act to enhance sialylation and control anomeric selectivity through neighboring group participation. Auxiliaries are typically introduced to the donor through the chemical transformation of 2,3-dehydro Neu5Ac analogs either through an oxirane introduction, or by addition across the glycal double bond. Scheme 12 describes the mechanistic transformation from sialyl glycal **52** through bicyclic intermediates **53a** and **54a** to glycosylation products **55** and **56**. Thus, it is imperative to control the regiochemical and stereochemical elements during the installation of the C-3 auxiliary. If the trans directed addition across the glycal leads to equatorial auxiliary installation, as in the case of **54**, the α-sialoside will be formed. If the mechanism of addition leads to axial confirmation of the auxiliary as in **53**, then the β-sialoside will be formed. Goto and coworkers were among the first to investigate this strategy with hydroxyl groups [44-46]. Using sialyl bromides, they attempted to probe the efficiency of this methodology in synthesizing α(2→8) and α(2→9) dimeric Neu5Ac linkages. Although, these initial studies produced unexceptional yields (~25-40%) some stereocontrol (α:β 3/1) was observed.

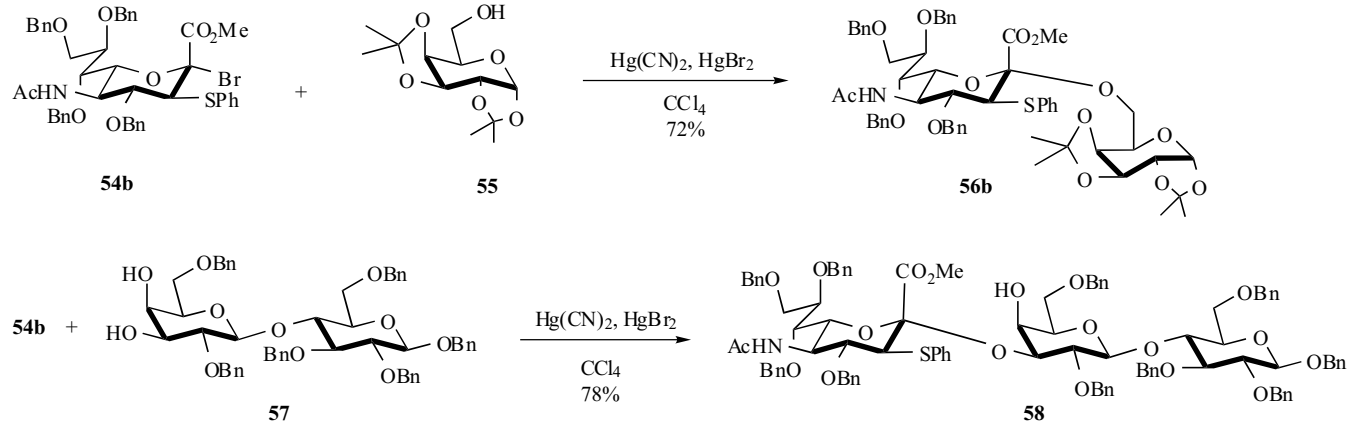
Encouraged by these results, Ogawa and co-workers expanded the scope of this methodology by employing phenylthio and phenylseleno C-3 auxiliary substituents [47, 48].

They correctly anticipated that the use of phenylthio and phenylseleno substituents could better situate and stabilize charge due to their increased size and polarizability compared to oxygen based C-3 auxiliaries. Initial attempts with phenylselenium substituents were mixed. A substantial increase in stereoselectivity was observed at the consequence



52 **53** **53a** **55** **54** **54a** **56**

Scheme 12. Stereoselective glycosyation with sailic acid based donors through the intramolecular formation of a 3-membered ring containing intermediate. X is the anomeric activating group at C-2 in the donor and Y is a participating nucleophilic group at C-3.



54b **55** **56b** **57** **58**

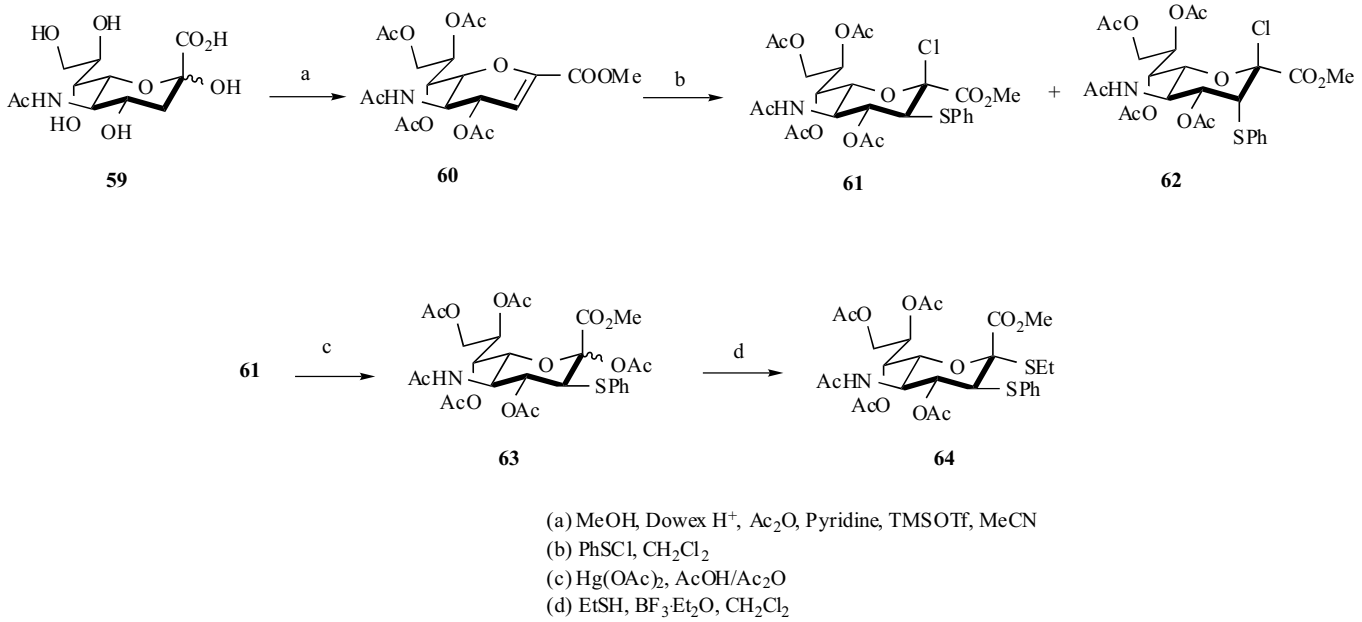
Scheme 13. Synthesis of GM-series precursors using a C-3 thiophenyl auxiliary group.

of diminished yields. This was presumably due to the elimination of a cationic selenium species yielding the 2,3-dehydro Neu5Ac derivative as the major product. A switch to phenylthio auxiliaries resolved the yield issue while maintaining the stereoselectivity element. Ogawa performed a very thorough study pairing together a variety of donors and acceptors. This study described the reactions of donor **54b** with acceptors **55** and **57** to produce the GM-series precursors **56b** and **58** (Scheme 13). These reactions produced good yields (72%-78%) and exclusively afforded α-products. The major significance of this study is illustrated by the reaction of lactosyl acceptor **57** with the auxiliary containing donor. The sterically restricted C-3' hydroxyl group would be expected to afford a dramatically lower yield compared to a relatively unhindered hydroxyl group, C-6' for acceptor **55.** Surprisingly, the yield for this reaction was slightly higher than that for the glycosylation of the less sterically hindered acceptor **55**. Problems associated with the required removal of the auxiliary were ameliorated by the use of a simple one step radical dethiophenylation using tributyltin hydride.

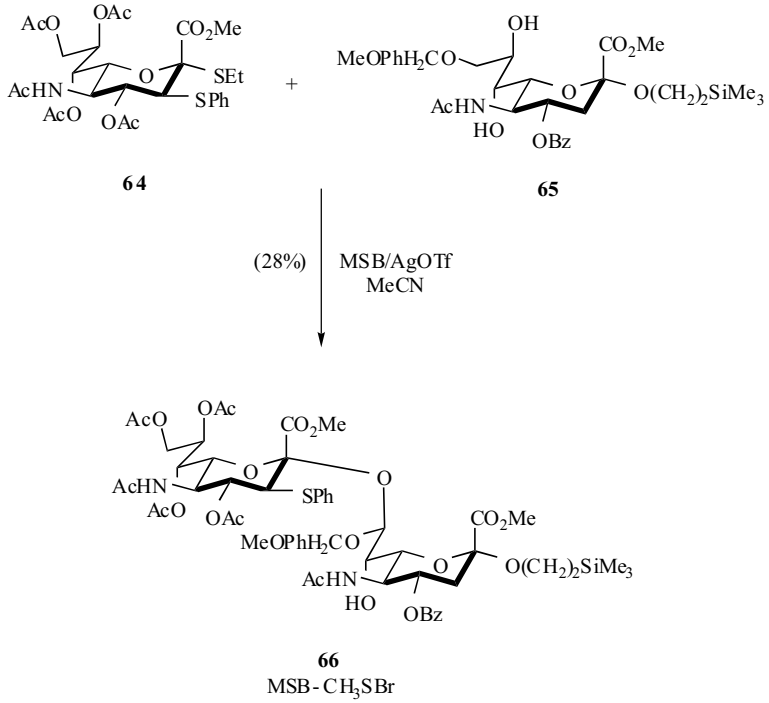
Until the work of Magnusson and co-workers, most donors using C-3 auxiliaries were halogen glycosides. This group took the next logical step in exploring the utility of anchimeric assistance by synthesizing auxiliary containing thiosialoside donors (Scheme 14) [49]. Starting from Neu5Ac (**59**), a 3 step chemical transformation lead to sialic acid glycal **60**. Introduction of the C-3 thiophenyl moiety was accomplished by addition of benzensulfenyl chloride across the glycal double bond. This resulted in a diastereomeric mixture (**61** and **62** ~3:1), which was easily resolved through chromatographic separation. Acetolysis of **61** yielded anomeric acetate **63** (α/β 1:5) in nearly quantitative yield, which was then stereoselectively converted to thiodonor **64** using $BF_3{\cdot}Et_2O$/EtSH. Chemical transformation of the sialic acid glycal **60** to **64** was accomplished in nearly 50% yield. Magnusson's effort to utilize donor **64** in the synthesis of the biologically important Neu5Ac α (2→8) Neu5Ac linkage using prefabricated acceptor **65** is illustrated in Scheme 15. Glycosylation of acceptor **65** with **64** using methanesulfenyl-bromide and silver triflate afforded **66** in 28% yield.

Although the yield compared somewhat unfavorably with standard thioglycoside donors, the real gain here is the excellent stereoselectivity (α/β ratio of >99:1). In 1999 the group revisited the synthesis of the Neu5Ac α(2→8) Neu5Ac bis sialic acid linkage. They introduced a modified thiophenyl auxiliary donor that was differentially functionalized at C-5. Glycosylation yield was improved upon by incorporating a di-*N*-acetylated moiety into the donor landscape. This synthesis will be discussed in detail later on in this review [50].

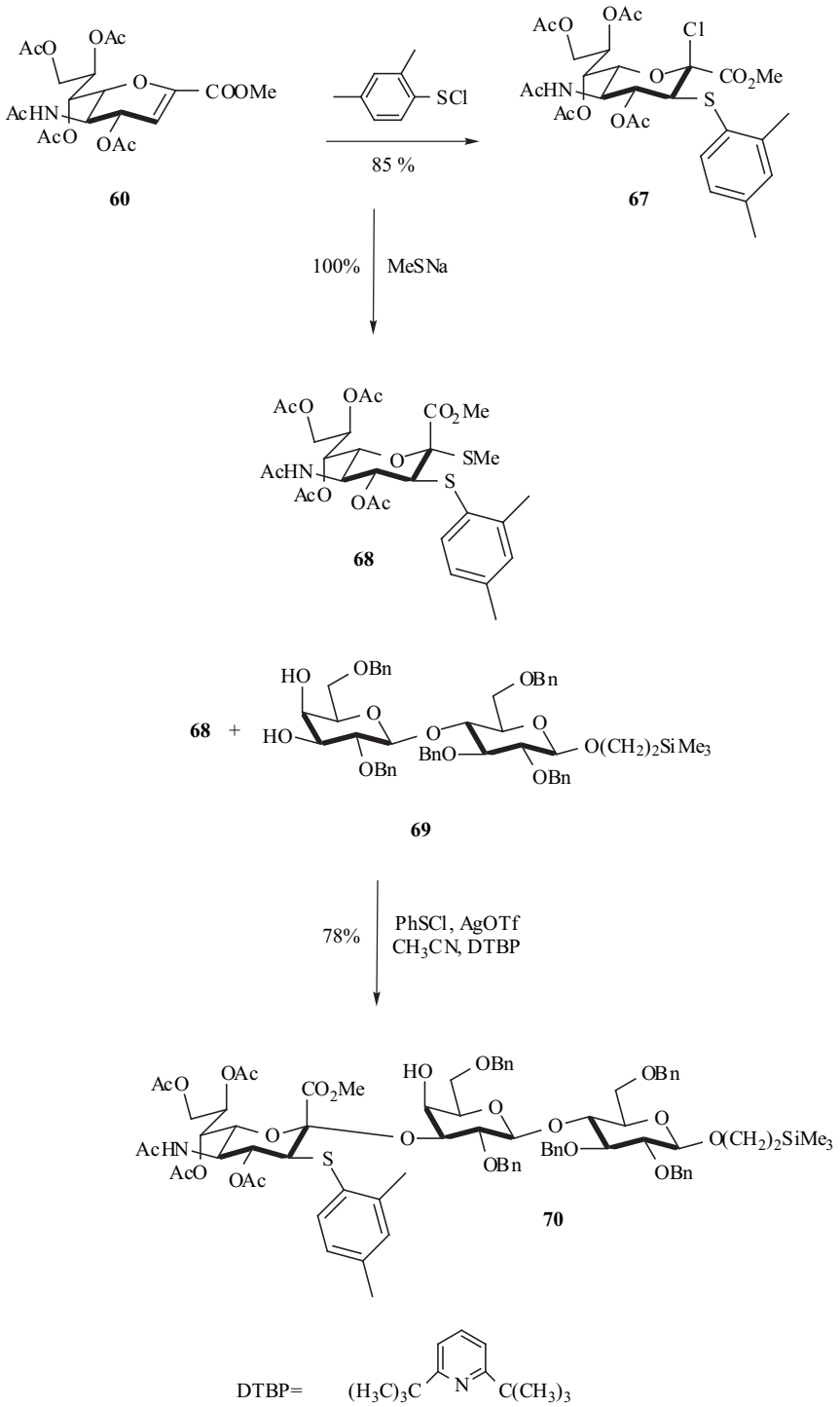
A negative aspect of the Magnusson synthesis was the lengthy conversion of glycal to functionalized thiodonor. This issue was addressed later by Whitesides and co-workers through their facile synthesis of a C-3 thioaryl isostere of Neu5Ac [51, 52]. Precursor **67** was synthesized through an electrophilic addition of 2,4-dimethylbenzenesulfenyl chloride to the glycal olefin (Scheme 16). In contrast to previous additive attempts to install the auxiliary, this sequence did not afford a mixture of C-3 conformers, presumably a result of the steric constraints placed upon the system from the methyl substituents on the aryl ring. Treatment of **67** with sodium methanethiolate gave donor **68** in 85% overall yield. Glycosylation of lactosyl acceptor **69** with donor **68** afforded trisaccharide **70** in 78% yield with no trace of the β-anomer detected.



Scheme 14. Synthesis of thioglycosyl donor containing a 3-thiophenyl auxiliary group.



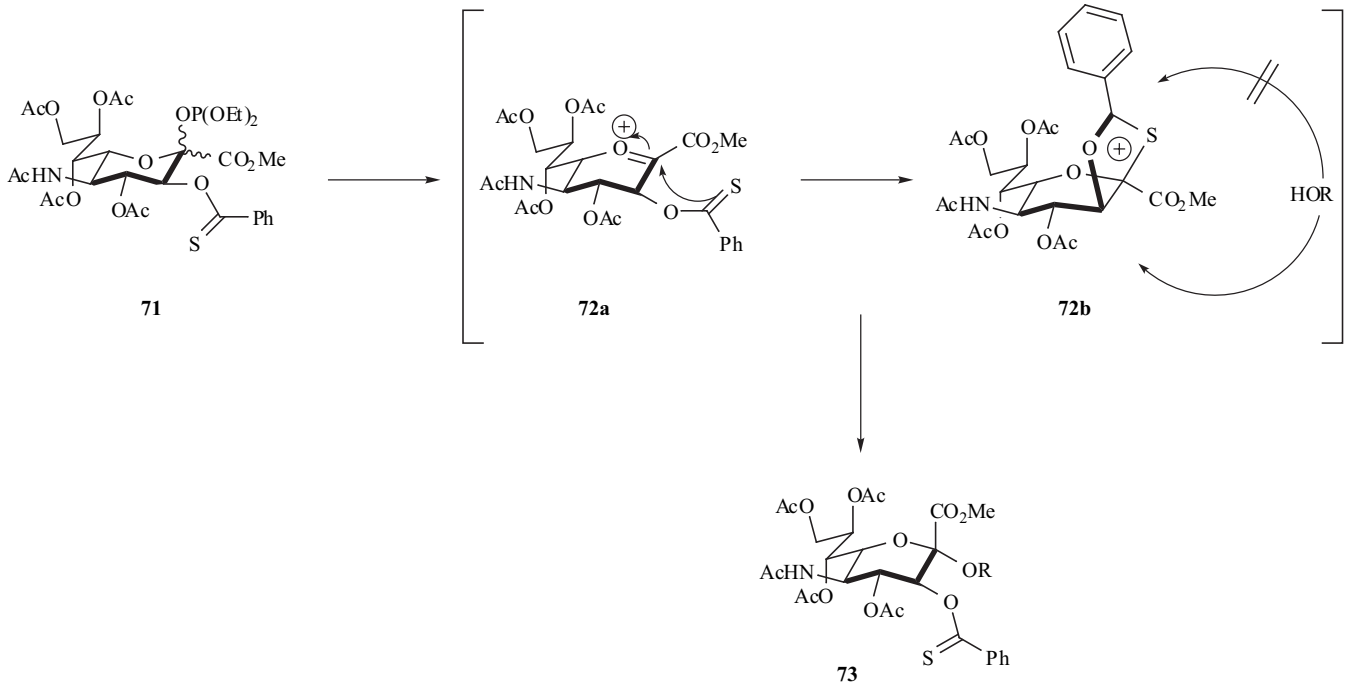
Scheme 15. Synthesis of a Neu5Ac (2→8)Neu5Ac disaccharide.



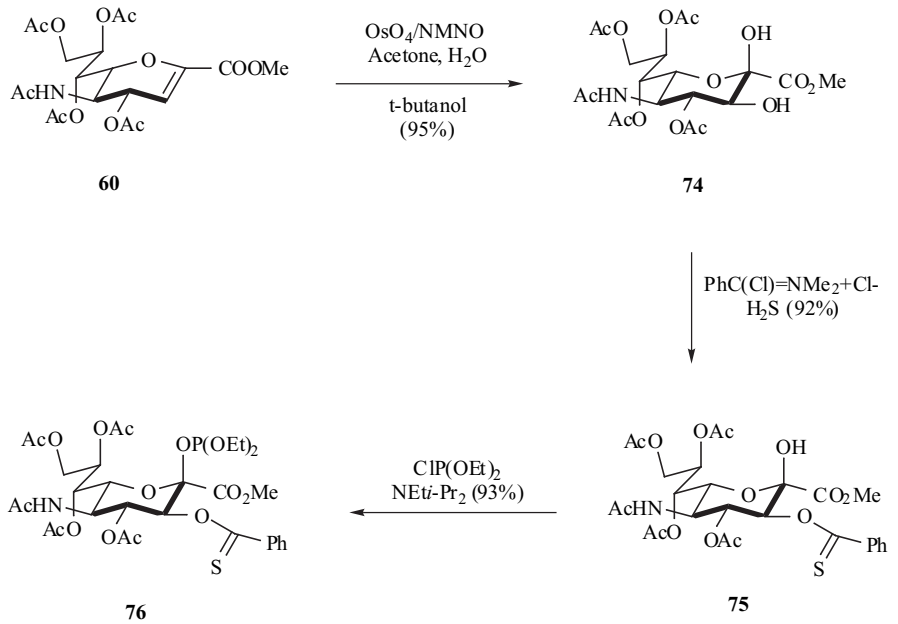
Scheme 16. Improved stereocontrol using a novel C-3 thioaryl isostere.

Advances in the development of C-3 auxiliary donors have thus far culminated in the 1998 effort by the Schmidt research group. Their goal was the synthesis of the α(2→8) bis sialic acid linkage through a C-3 thionosialyl phosphite donor [53]. The aim of this work was to improve upon previous methods through the achievement of five basic goals. First, electrophilic addition to the glycal should yield a readily usable donor. Second, activation of the donor should be accomplished through the catalytic use of promoters. Third, the ideal auxiliary should assist and direct glycosylation towards the formation of the α-anomer even with less reactive acceptors. Fourth, facile removal of the auxiliary is possible on product formation. Fifth, the donor should be designed to accommodate repeated glycosylation attempts often required to synthesize higher ordered Neu5Ac homopolymers. The optimal design of the auxiliary is at the heart of these demands. Schmidt reasoned that the inclusion of an anomeric phosphite would satisfy the demand for catalytic initiation, while a C-3 auxiliary resulting in 5,6-bicyclo intermediate would direct glycosylation. The aryloxythiocarbonyl moiety was selected as the C-3 auxiliary to satisfy the requirements. The mechanistic rationale of

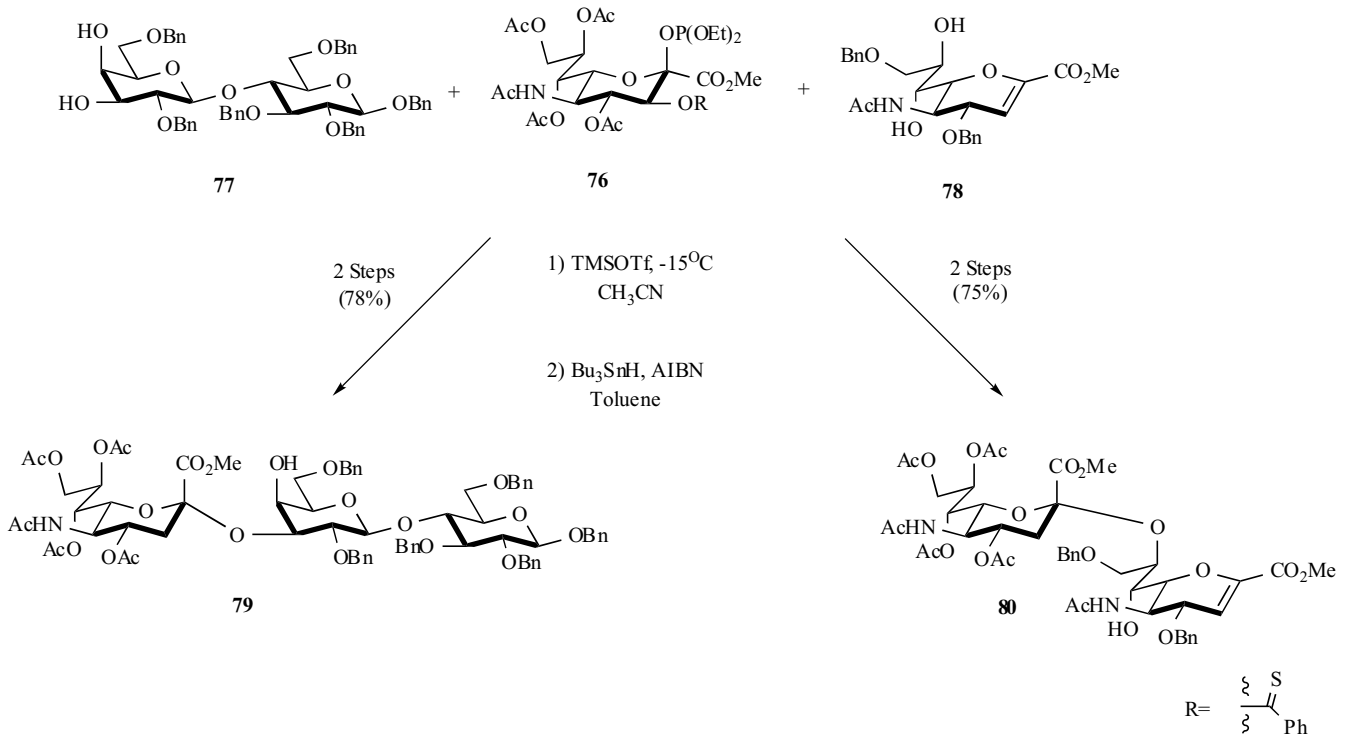
Schmidt's approach is shown in Scheme 17**.** Neu5Ac phosphite **71** is catalytically activated leading to oxonium precursor **72a**. The equatorially situated phenyloxythiocarbonyl group then collapses on the β-face of the oxonium ion resulting in a 5-membered thionocarbenium intermediate **72b**. The attacking nucleophile is directed toward the α-face leading to the desired α-sialoside **73**. Donor **71** was synthesized from Neu5Ac glycal **60** (Scheme 18). Osmium mediated syn-dihydroxylation on the β-face of glycal **60** gave **74** in nearly quantitative yield (95%). Regioselective introduction of C-3 thiocarbonate into **74** was accomplished with *N,N*-dimethyl-α-chlorobenzimidium chloride ($PhC(Cl)=NMe_2^+Cl^-$) and hydrogen sulfide to give **75** (92%). Treatment of **75** with $ClP(OEt)_2$ and ethyldiisopropyl amine gave donor **76** in an overall yield of 81% from glycal **60**. The 2 step glycosylation-dethiocarbonation of lactosyl acceptor **77** and homomonomeric acceptor **78** (Scheme 19) led to **79** and **80.** The yields and stereoselectivities compared favorably with other C-3 auxiliary donors used under similar reaction conditions with the same acceptors. Thus, Schmidt established the most complete and practical auxiliary-based strategy to date. Magnusson subsequently countered with his own comprehensive study on utility of 3-(S)-phenylseleno sialyl phosphite donors [54].



Scheme 17. A bicyclic thionocarbenium ion intermediate to control stereoselectivity of glycosylation.



Scheme 18. Synthesis of a phosphite donor containing a C-3 auxiliary group from Neu5Ac glycal.



Scheme 19. Glycosylation with a phosphite donor containing a C-3 auxiliary.

C-5

The C-5 acetamide moiety of Neu5Ac has been the target of extensive chemical modification. Boons and co-workers demonstrated the utility of difunctionalizing the C-5 position to increase yields during glycosylations [55, 56]. They demonstrated that reaction of Neu5Ac donor **81** with monomeric galactoside acceptor **82 (**1.7:1 eq. donor/acceptor) with *N*-iodosuccinimide and triflic acid produced disaccharide **83** in 2-6 hours in 61 % yield and excellent α-selectivity. Treatment of the *N,N*-diacetamido Neu5Ac donor **84** with **82 (**1.1:1 eq. donor/acceptor, optimal conditions) for 5 minutes gave **85** in 71% overall yield (Scheme 20). The resulting di-*N*-acetamido disaccharide product **85** can be *N*-deacetylated and deprotected under Zemplen conditions (NaOMe/MeOH) to obtain the desired deprotected mono-acetylated Neu5Ac containing disaccharide. Boons followed up these initial findings with a study using trifluoroacetamido protection at C-5 in the synthesis of the human melanoma associated antigen, GD3 [57]. Magnusson incorporated this C-5 di-*N*-acetamido methodology into the preparation of C-3 thiophenyl auxiliary donors (Figure 6) [29].

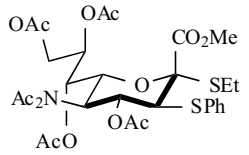
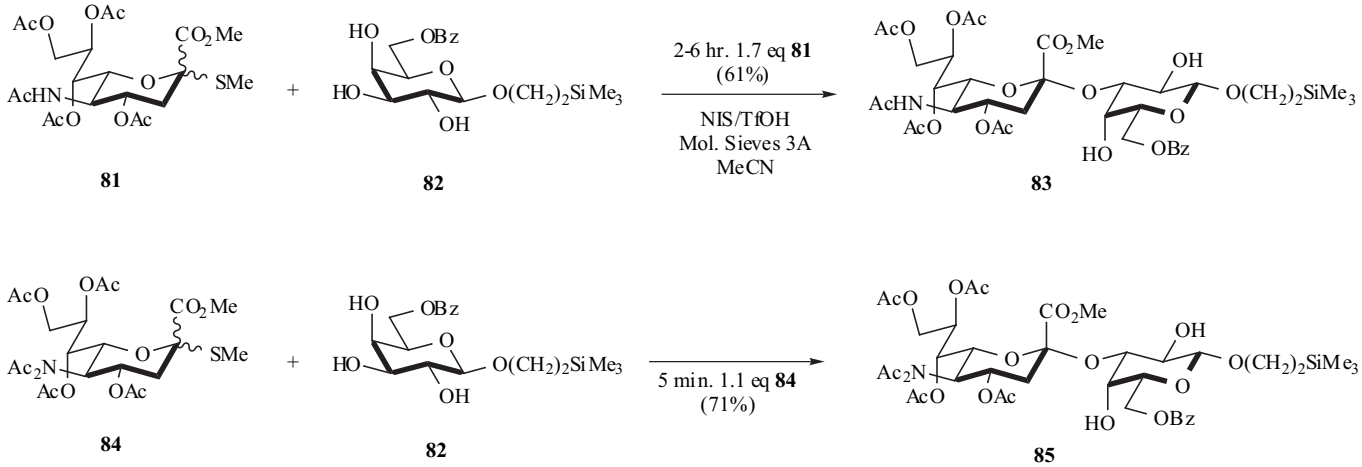
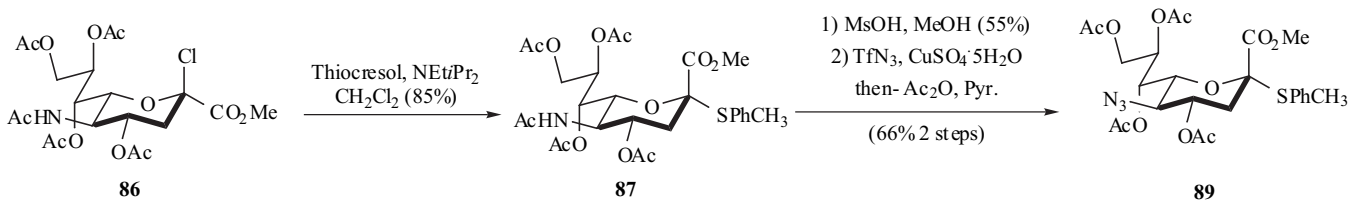


Fig. (6). Sialic acid donor containing a 3-thiophenyl auxiliary group **88.**

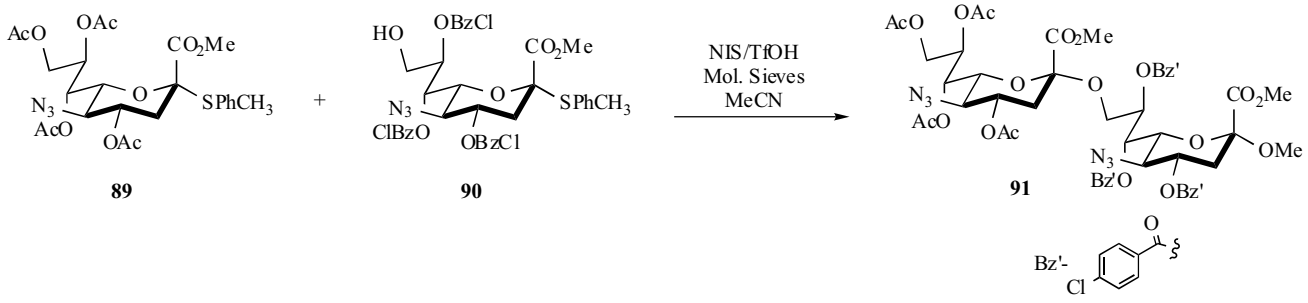


Scheme 20. Application of di-*N*-acetamido neuraminyl donor in glycosylation.



86 **87** **89**

Scheme 21. Synthesis of 5-azido neuraminyl donor.



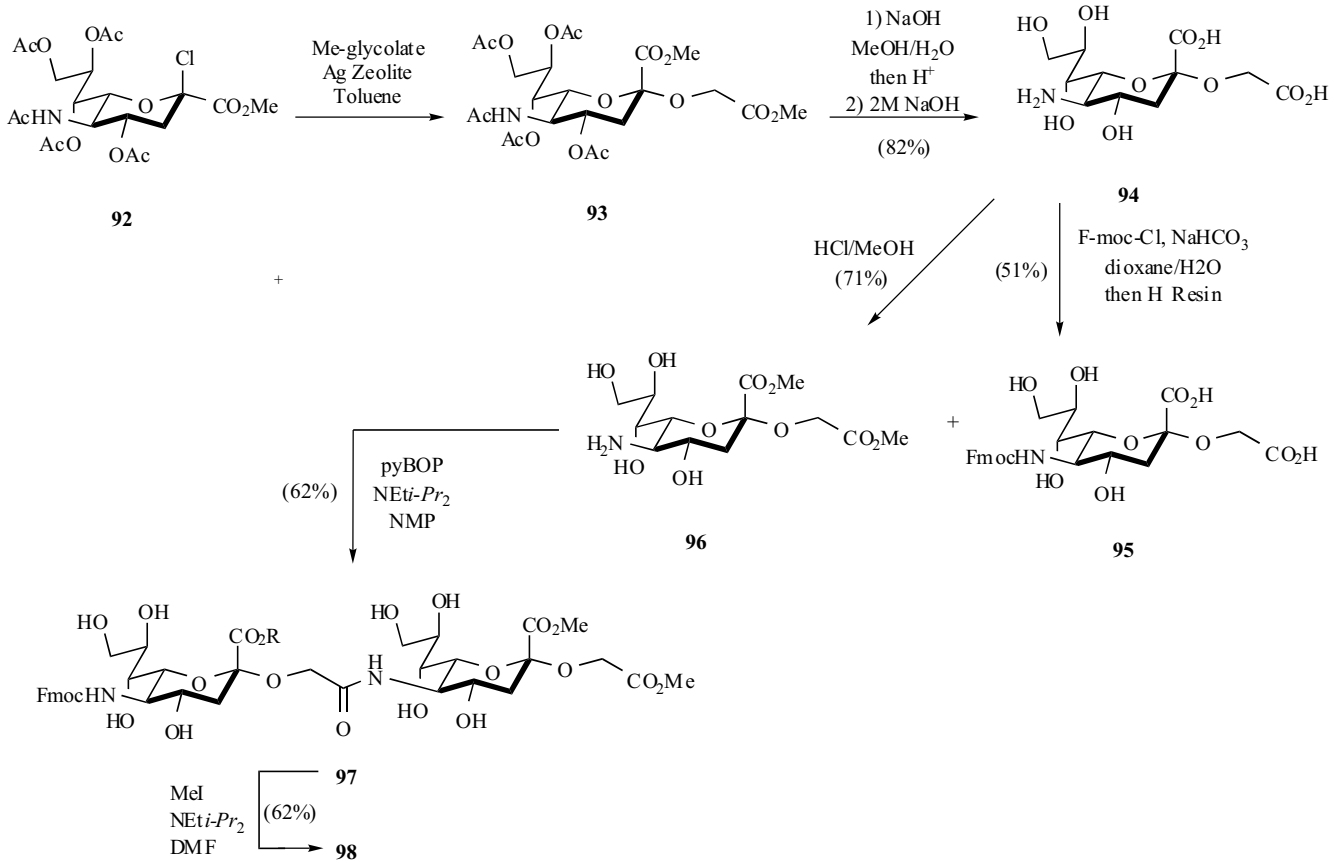
89 **90** **91**

Scheme 22. Application of 5-azido neuraminyl donor in glycosylation.

Another approach to prepare glycosides of sialic acids involves the functional transformation of the C-5 position from *N*-acetyl to azide. Several research groups have employed this methodology [58, 59]. The approach put forth by Wong and Lin is perhaps, the most expeditious (Scheme 21) [60]. Peracetylated chloride donor **86** was treated with thiocresol in CH_2Cl_2 in the presence of Hunigs base to yield donor **87** (85%). Subsequent deacetylation of **87**, catalytic diazo transfer, and reacetylation gave **89** with an overall yield of 66%. Glycosylation studies were performed to examine the 5-azido containing donor, **89**. The representative results of these studies are illustrated in Scheme 22. Under standard glycosylation protocols (NIS (3 equiv to **89**)/TfOH (3-10 mol % to NIS) 3A mol. sieves, MeCN, -40°C) 5-azido donor **89** and acceptor **90** (2:1 mol. equiv. ratio) gave modified α(2→9) Neu5Ac disaccharide **91** in 65% yield with excellent α-stereoselectivity.

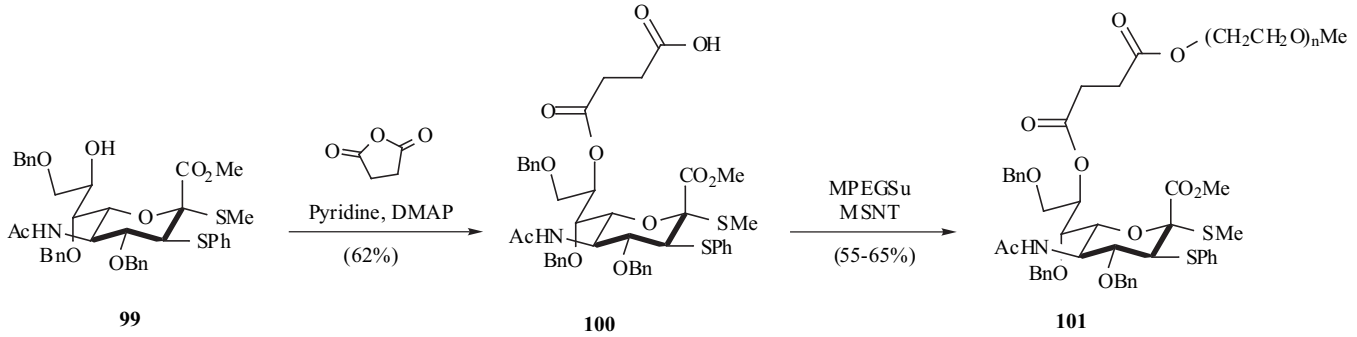
Unconventional Sialic Acid Linkages

Recent research efforts have also been aimed at the synthesis of unusual Neu5Ac α(2→5) Neu5Gc oligosaccharides [61-63]. Considerable effort is required to



92 **93** **94** **95** **96** **97** **98**

Scheme 23. Synthesis of unusual Neu5Ac α(2→5)Neu5Gc oligosaccharides.



99 **100** **101**

Scheme 24. Synthesis of a Neu5Ac donor attached to PEG.

construct this linkage. Hindsgaul and co-workers devised a clever method to engineer a sialic acid disaccharide using an atypical glycosylation sequence in which the key assembly of the donor and acceptor fragments was performed without protecting groups (Scheme 23) [62]. Acetyl protected Neu5Ac chloride **92** was converted to methyl glycolate sialoside **93** and subsequently *O,N*-deacetylated and deesterified to give **94**, which was used to prepare both donor **95** and acceptor **96**. F-moc-ylation of **94** gave **95**, while treatment with acidified methanol produced **96**. Treatment with peptide coupling reagent pyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) in the presence of diisopropylethyl amine and NMP (*N*-methylpyrrolidine) to give disaccharide **97** in 62% yield. Disaccharide **97** was methyl esterified to give **98** and after the removal of F-moc protecting group the glycosylation process could be repeated to afford higher ordered oligosaccharides.

Sialic Acid Donors on PEG

The application of solid phase chemistry for carbohydrate synthesis is of growing interest [64]. One of the few published reports of polymer-based chemistry using a sialyl donor is by Ito and Ogawa. Their synthesis utilizes a polyethylene glycol (PEG) polymer supported sialic acid glycosyl donor [65]. PEG resin was chosen as the polymeric support to facilitate glycosylation in the solution phase, eliminating many of the problems inherent in solid phase synthesis. The sialyl donor chosen employed a stereocontrolling C-3 thioaryl moiety. Donor precursor **99** was prepared in several steps from the corresponding appropriately protected Neu5Ac glycal (Scheme 24). **99** was succinylated in the presence of DMAP and pyridine to give **100** for subsequent attachment to PEG using 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT). Glycosylation was performed with various donors and afforded good yields and stereoselectivities. While the application of such methodologies is hampered due to the unnecessary and extensive chemical manipulations required to arrive at the donor, the present example illustrates a unique and promising area to explore.

CONCLUDING REMARKS

The technology for high yielding, regioselective and stereoselective glycosylation with sialic acid is steadily improving. There remains a need for continued efforts by synthetic carbohydrate chemists to be innovative in their approaches. Improvements made in donor design and more thorough investigations into the alteration of the molecular landscape affecting glycosylation are required. The field of promoter development also needs to test the limits of these reactions. Future approaches need to apply solid phase chemistry, combinatorial libraries and automated approaches to the synthesis of sialic acid containing oligosaccharides.

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