Chapter 3

Synthesis of Neu5Ac, KDN, and KDO C-Glycosides

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Ulosonic acids are unique monosaccharides that are often found at the non-reducing terminus of glycoconjugates. Since glycoconjugates are typically catabolized by the stepwise enzymatic removal of their monosaccharide units, modification of the ulosonic acid component represents an intriguing target for blocking this transformation. Glycoconjugates containing a modified ulosonic acid would be expected to be resistant to catabolism and glycan maturation and thus, might have significant therapeutic potential. The possible applications of such derivatives would include: 1. inhibitors of enzymes, such as neuraminidases, acting on ulosonic acid containing molecules; 2. therapeutic glycoconjugate containing agents, resistant to catabolism and glycan maturation, having increased biological half-lives, such as stable analogs of the glycolipids GM4 and GM3; 3. as immunogens for the preparation of anti-carbohydrate, antibody-based therapeutics; or 4. as active vaccine agents in the treatment of diseases involving glycoconjugates, such as bacterial infections, viral infections and cancer. This review describes the design and synthesis of glycoconjugates containing ulosonic acid C-oligosaccharides.
Introduction

Ulsonic acids are a diverse family of unique, complex monosaccharides that serve many important biological functions (Figure 1) (1-4). The most common ulsonic acid, Neu5Ac, is a constituent of many glycoconjugates, occupying the non-reducing end of oligosaccharide chains. Glycoconjugates containing Neu5Ac (and other ulsonic acids) mediate a number of important biological events. The most significant function may be associated with their negative charge. Many Neu5Ac residues are found in the glycoconjugates bound to human cells (5). This affords a charged shell covering cells that can prevent their aggregation by electrostatic repulsion and facilitate aggregation through calcium binding (6). The viscosity of many biological fluids is regulated by the introduction or release of Neu5Ac in the oligosaccharide portion of glycoprotein components (7). Neu5Ac also represents an important biological receptor domain (1,2,8-14). Cell surfaces containing this ligand interact with biomolecules such as receptors, hormones, enzymes, toxins and viruses. Cell-cell recognition between circulating leukocytes in blood vessels and endothelial cells is believed to occur, in part, through the interaction between mammalian lectins (selelctins) and Neu5Ac containing oligosaccharide ligands (9). Neu5Ac, present in glycolipids such as GM4, interacts with adhesion molecules important in cell growth and tissue regeneration (15). Many pathogens also use Neu5Ac to localize on the surface of cells they infect (16,17). The capsular polysaccharide of a variety of pathogenic bacteria (16,18,19) as well as the N-linked oligosaccharide sequences of virus envelope glycoproteins, such as gp120 of the human immunodeficiency virus (HIV), contain high levels of Neu5Ac (20).

![Figure 1. The α-anomeric forms of ulsonic acids, Neu5Ac (1), KDN (2), and KDO (3).]}

Other ulsonic acids, structurally related to Neu5Ac have been the focus of active scientific investigation due to their important biological functions. KDN (3-deoxy-D-glycero-D-galacto-2-nonulosonic acid) 2 bears a hydroxyl group in place of the acetamido group found at C-5 of Neu5Ac 1. KDN is linked to galactosamine and galactose as the terminal unit of polysialoglycoproteins found in the membranes of mammalian tissues (21). KDO (3-deoxy-D-glycero-manno-2-octulosonic acid) 3 is a ketosidic component in all lipopolysaccharides (LPS) of gram-negative bacteria (22). It has also been identified in several bacterial exopolysaccharides (K-antigens) (23-25). While the precise biological function of KDN and KDO is still undetermined, they also appear to play a role in cellular interactions with biomolecules.

The glycan component of glycoconjugates (i.e., glycoproteins/peptides, glycolipids) are catabolized in both the extracellular environment and within cells. The turnover of endogenous glycoconjugates is initiated in extracellular spaces where they are found, through the action of both exo- and endolitics enzymes (26-28). Exo-glycosidases act sequentially to remove the glycan residue at a time from the non-reducing end of a glycan chain. A 4-deoxybiantennary glycan chain of a glycoprotein, for example must first be desialylated, through the action of a neuraminidase, prior to removal of the galactose residue, galactose. The resulting asiaglycoproteins have higher clearance due to the galactose-binding lectin in liver (29,30). In addition to their sequential breakdown by exol glycutes the glycan chains of a glycoconjugate can be removed through the action of endolitic glycosidases (26-28). For example, endoglycanses can remove intact glycans from glycoproteins/peptides and ceraminidases can remove the intact glycans from glycolipids (31). Intracellular catabolism of glycoconjugates occurs in lysosomal compartments primarily through the sequential action of exol glycutes (32-34).

Neuraminidase action, important in most but not all glycoconjugate catabolism and maturation, represents a uniquely important therapeutic target. Neuraminidases, obtained from diverse species and tissues, interact with Neu5Ac present on the outer layer of cell membranes and result in its removal (17). This has a dramatic consequence for infection, adhesion and recognition events. Neuraminidases belong to a class of enzymes called glycosidases. Modified glycosides of Neu5Ac such as thioglycloses (35-37), initially synthesized to serve as powerful glycosylating reagents (38), are not neuraminidase substrates but instead act as inhibitors. Glycoconjugates terminated with other ulsonic acids such as KDN, are also catabolized through the action of hydrolates (39).

Recent developments in carbohydrate synthesis (40) opened up the possibility of forming the crucial C-glycosydic bond in Neu5Ac and other ulsonic acid containing oligosaccharides. Despite major advances in the glycosylation chemistry (40), glycosylation reactions of Neu5Ac (and other ulsonic acids) proved particularly complex, often resulting in the 2,3-dehydro derivatives (41). These difficulties can be attributed to three factors inherent to the Neu5Ac molecule. First, the carboxyl group attached at C2 electronically disfavors oxocarbenium ion formation, an intermediate for almost all glycosidation reactions. Second, the carboxyl group sterically restricts glycosidation
formation. Third, the lack of a substituent at the adjacent C3 precludes assisting and directing effects.

Elegant methods for direct carbon-carbon (C–C) bond formation at the anomic center in aldoses and ketoses (42-45), afforded only trivial alkyl and hydroxymethyl C-glycosides of Neu5Ac and other ulosonic acids (46-49). The anomeric outcome of the C-glycosylation may also be determined by the geometry of the enolate. The major problem confounding the synthesis of sialic acid C-glycosides appeared to be the requirement that the C–C bond being formed results in a new tertiary C-atom center. The driving force for developing new chemistry was driven by the importance of these targets. The biological significance of the C-glycosides of Neu5Ac and related ulosonic acids is obvious as they represent an important new class of hydrolytically stable analogs and mimetics of the natural O-glycosides. The use of Neu5Ac, KDN, and KDO C-glycosides (Figure 2) 4, 5, and 6 as potential stable ligands for carbohydrate receptors may have a wide variety of potential therapeutic applications. This new class of compounds might play a role as glycoenzyme regulators. These “C”-glycosides might find use as potent inhibitors of neuraminidase (KDOase or KDNase) (39) and they might also be used as antiviral agents against neuraminidase-containing influenza (50,51) or as agents to block the removal of ulosonic acid containing glycoconjugates (52).

Figure 2. Structure of α-“C”-glycosides of ulosonic acids, \( R^1 = H \) or \( OH \); \( R^2 = H \) or alkyl/aryl; \( R^3 = alkyl/aryl/C-linked saccharide. \)

The half-life of many therapeutic glycoproteins, such as tissue plasminogen activator (tPA), is controlled by their neuraminidase catalyzed de-sialylation followed by their removal in the liver through galactose binding lectins (53-55). This clearance might be effectively blocked by inhibiting neuraminidase activity. C-glycosides might also be useful as receptor agonists/antagonists. The development of vaccines against carbohydrates is of crucial importance in the fields of therapeutic glycobiology and immunology (56-57). A significant portion of the anti-tumor response against cancers involves carbohydrate tumor antigens (58). Microorganisms also often express carbohydrate antigens and the immune response of the host to these antigens is an important mechanism of defense (44). Despite considerable interest, there remain many questions about host response to carbohydrate antigens. At present it is not known whether lymphocytes are able to recognize carbohydrate-defined epitopes and very little is understood about the fate of carbohydrates during antigen processing (59). It is also unclear why antigens show little T-cell dependent class switching from IgM to IgG and affinity maturation (60). It is clear that antibody affinity maturation can be significantly increased (61) through one or more of the following means: (1) the selection of potent adjuvants; (2) the use of high antigenic carrier proteins (such as KLH) (62,63); and (3) increased carbohydrate valency (62,63). Interestingly, the clustering of carbohydrate antigens on the carrier maximizes immunoreactivity (62,63). One approach for improving carbohydrate antigens that has not been thoroughly explored is vaccination over an extended length of time (63). It is here that a catabolically stable C-glycoside containing vaccine might play an important role. The increased conformational flexibility of ulosonic acid C-glycosides (58,64-69) may affect their antigenicity (70) and also may result in altered binding affinity or cross-reactivity (71). Complexes of the synthesis and testing of these C-glycoside targets as immunogens and flexible antigenic determinants can address these issues. Anti-carbohydrate IgG-type antibodies might be used therapeutically in the application of targeted endogenous glycans, such as sLe\(^a\) containing ligands for the treatment of reperfusion injury (72,73). Alternatively, these agents might be useful in preparing immunogens for active immunization against ulosonic acid containing glycoconjugates in the design and preparation of anti-viral (20,74-77), anti-bacterial (16,18,23-25) and anti-cancer vaccines (56,57,60,62-64-78).

**Synthesis of Neu5Ac α-C-Glycosides 4 by Samarium-mediated Reduction**

Samarium iodide, a powerful one electron reductant, has been used for C–C bond formation in carbohydrate synthesis by only a few research laboratories (80). Encouraged by the reports of Sinaï (79) and Wong (80) on the samarium mediated coupling of α-alkoxy sulfones with ketones, and glycosyl phosphates with ketones or aldehydes, we undertook a new approach for the synthesis of Neu5Ac α-C-glycosides relying on aryl sulfone donors (7) (Scheme 1). We initially selected Neu5Ac 2-pyridyl sulfone as our donor, minimizing the energy of one-electron transfer, to react with an aldehyde acceptor and afford the desired α-C-glycoside (81). Samarium mediated C-glycosylation was achieved in good yield with excellent stereoselectivity. Two competing mechanisms were advanced to provide a rational understanding of the mechanism of the Samarium mediated union of the sulfone donor with aldehyde and keto-based acceptors.
When SmI₂ was initially used to mediate the C-glycosylation of Neu5Ac pyridyl sulfone 7 donor (81) and sugar aldehyde 8 (Scheme 1), hexamethylphosphoric (HMPA) was used. HMPA reportedly accelerates radical SmI₂ reductions, and it is usually used as a ligand in samarium-mediated reactions (82). Neu5Ac α-C-glycoside 7 could also be formed in the absence of HMPA (81, 83), with the characteristic blue color of SmI₂ fading to a clear yellow as the reaction proceeded to completion within a few minutes. Furthermore, the Neu5Ac phenyl sulfone donor gave yields identical to Neu5Ac pyridyl sulfone donor in both the presence and absence of HMPA (Scheme 3). From these studies, it appeared that SmI₂ alone was sufficient for the formation of a relatively stable tertiary intermediate (Scheme 4) at the anomeric center of Neu5Ac. The use of the added HMPA has been conveniently avoided in all further reactions and Neu5Ac phenyl sulfone was used as donor.

Scheme 1.

A simple radical mechanism cannot explain the stereoselectivity of this reaction and an ionic mechanism does not take into account the propensity of samarium to form radical species. The stereoselective formation of C-glycoside is proposed to occur through two one-electron transfer steps (7 to 8, 8 to 9) yielding a samarium(III) glycoside intermediate (9) (Scheme 2). The resulting nucleophile is then believed to take advantage of the inherent oxophilic nature of the equatorial samarium to coordinate the stereoselective delivery of the incoming aldehyde or ketone electrophile to the bottom face (10). Hydrolysis of the samarium complexed product 11 affords C-glycosides 12. This mechanism rationalizes α-stereoselectivity by invoking a syn-type of addition with the bulky samarium substituent is situated in the thermodynamically formed equatorial position (Scheme 2).

Scheme 2.

Scheme 3 i. 4 equiv of SmI₂ (0.1 M in THF); ii. 4 equiv of SmI₂ and 4 equiv HMPA in THF.
When a fully protected sugar ketone 17 (84) was subjected to conditions for samarium mediated C-glycosylation, no trace of the C-disaccharide was observed. Instead, the α-2-deoxy derivative 18, corresponding to an inhibitor of X-31 HA hemagglutinin, was obtained after deacetylation (85). The steric hindrance of sugar ketone 17 might be responsible for the failure of this C-glycosylation reaction. The stereochemical outcome of this reaction suggested that the reaction might proceed through an intermediate samarium enolate derivative 21 (Scheme 4). Further mechanistic studies will be required to distinguish between samariated intermediates 10 and 21 (Schemes 2 and 4).

In conclusion, neuraminic acid 2-pyridyl sulfone, 2-phenyl sulfone and chloro derivatives react with ketones or aldehydes in THF in the presence of HMPA resulting in the near instantaneous and stereospecific formation of Neu5Ac α-C-glycosides.

KDN

KDN, 3-deoxy-D-glycero-D-galacto-2-nonulopyranosylonic acid 2, is a novel type of sialic acid in which the acetamido group at C-5 of acetylneuraminic acid is replaced by a hydroxyl group. This ulosonic acid was first isolated from rainbow trout eggs (86). In the past 20 years, a number of KDN-glycoconjugates, exhibiting structural determinants related to human tumor-associated antigens, have been reported in mammals (87-89). In addition, oligo/poly-KDN and KDN-glycoprotein play an important role in the binding of calcium ions (90).

We reported the first stereocontrolled synthesis of KDN containing glycosides via glycosyl samarium (III) intermediates (91-95). KDN 22 was prepared according to a previously described method (96), and the Neu5Ac phenylsulfone 22 was synthesized using a similar procedure to that for preparing Neu5Ac phenylsulfone 7 (97). Treatment of a neat mixture of KDN phenylsulfone 22 and ketones 13, 15, 23, 25 and 27 (1.2 equiv) in an atmosphere with 4 equiv of freshly prepared 0.1 M SmI₂ solution in THF at room temperature, gave a nearly instantaneous conversion to the KDN glycosides 14, 16, 24, 26 and 28 in excellent yields (Scheme 5).

Next, the scope of this C-glycosylation reaction was investigated. The Neu5Ac chloride was found to serve as a donor in samarium mediated C-glycosylation, unfortunately, purification of C-glycoside from excess chloride donor is often problematic, making the Neu5Ac phenyl sulfone the donor of choice. A variety of acceptors were also evaluated including alkenes, epoxides, vinyl esters, aldehydes and ketones. Only the aldehydes and ketones afforded the desired C-glycoside products.
KDN(α2-3)Gal and KDN(α2-6)GalNAC are core structures in the naturally occurring KDN oligosaccharides. Thus, samarium mediated C-glycosylation was applied to the synthesis of C-linked KDN(α2-3)Gal 29 and KDN(α2-6)Gal 30 derivatives. Coupling of phenylsulfone 22 with sugar electrophile 8 (98) under Barbier conditions diastereoselectively generated (2-3) linked C-disaccharide 29, while a similar reaction with 19 (99) gave diastereomers at newly formed chiral center (C-6) (Scheme 6).

Scheme 6. i. 4 equiv of SmI₂ (0.1 M in THF).

In summary, synthesis of the KDN α-C glycosides proceeds from chloride, phenyl sulfone and pyridylsulfone donors, through the same organosamarium intermediate 10 with ketones or aldehydes as observed for the synthesis of Neu5Ac α-C glycosides (Scheme 7). This reaction stereospecifically affords tertiary C-C bonds. This chemistry has advantages over previously reported methodologies, employing alkyl lithium species (100), since it tolerates acetyl groups and utilizes mild reaction conditions.

Scheme 7. Synthesis of “C”-glycosides of Neu5Ac and KDN using a ketone or aldehyde containing electrophile. Where R¹ and R² are H, alkyl/aryl or saccharide moieties.

KDO

KDO (3-deoxy-D-mannno-2-octulosonic acid) is a key component of cell wall lipopolysaccharide (LPS) of Gram-negative bacteria. KDO forms the critical linkage between the polysaccharide and lipid A regions of LPS (101-104). KDO, like Neu5Ac and KDN, is well known to form glycosidic 2-derivatives in studies that paralleled those for the successful syntheses of the α-C-glycoside of Neu5Ac and KDN (Scheme 7). Unfortunately, this chemistry afforded only undesired β-C-glycoside 33 (Scheme 8).

Scheme 8. Scope of samarium chemistry in the synthesis of “C”-glycosides of KDO. Where R¹ and R² are H, alkyl or saccharide moieties.

To successfully act as mimics, KDO C-glycosides are expected to have the same configuration as the corresponding O-glycosides. Unfortunately, unlike Neu5Ac (1) and KDN (2) which resides primarily in the 2C₂ conformation, KDO (3) prefers the 5C₂ conformation (Figure 1). Thus, a new strategy was necessary to stereoselectively synthesize the α-C-disaccharide of KDO. The reaction of 4,5,7,8-tetra-O-acetyl-3-deoxy-α-D-manno-2-octulospyranosyl chloride donor, 36, with the 6-formylgalactopyranoside acceptor, 19, in the presence of SmI₂ was expected to form the α-C-disaccharide of KDO. The ammonium salt of KDO 34 was prepared according to previously described methods (105-108). The synthesis of t-butyl (4,5,7,8-tetra-O-acetyl-3-deoxy-D-manno-2-octulospyranosyl chloride)onate 36 was carried out in two steps. Reaction using t-butyl trichloroacetimidate (109-111) requires a nonpolar solvent such as cyclohexane, which is unable to dissolve 34. The acetylation and chlorination of 34 afforded 35, which was freely soluble in cyclohexane. Esterification of 35 with t-butyl trichloroacetimidate in the presence of
catalytic amount of BF₃, gave 36 in 91% yield (2 steps overall). Glycosylation of the 6-formylgalactopyranoside acceptor 19 with the KDO donor 36 in the presence of freshly prepared samarium(II) iodide, afforded the corresponding C-disaccharide 37 in 77% yield (Scheme 9a).

\[ \text{Scheme 9a.} \]

It was rationalized that the KDO C-glycoside is generated only in the α-configuration because the α-face of the samarium enolate intermediate 38 is much less sterically hindered than β-face (Scheme 9b).

\[ \text{Scheme 9b.} \]

Properties of O- and C-Glycosides

Sialyl Tn (sTn) C-Glycosides

Glycoproteins are major components on the surface of mammalian cells. Many carry O-linked oligosaccharides (O-glycans), which are conjugated through serine or threonine residues. Others carry N-linked oligosaccharides (N-glycans), conjugated through an asparagine residue. The recognition of these O- and N-glycoconjugates play key roles in the transmission of biological information at the cellular level (112-114). Their numerous biological functions include roles in cellular recognition, adhesion, cell-growth regulation, cancer metastasis, and inflammation. Cell-surface glycans also serve as attachment sites for infectious bacteria, viruses, and toxins, resulting in pathogenesis (115). Anomalies in cell-surface carbohydrates are often closely associated with transformation, malignancy and other various pathological conditions, including immunodeficiency syndromes, cancer and inflammation (117).

Sialyl Tn (sTn) is a carbohydrate antigen associated with many different types of tumors (118-120) as well as viral pathogens, such as HIV. Danishefsky and coworkers successfully synthesized and evaluated sTn glycoside and its trimer as cancer vaccines (112-124).

Fully protected sTn -C- glycoside analog 39 was prepared by glycosylation of the neuraminic acid sulfone donor 7 with an aldehyde acceptor 40 (Scheme 10). The donor 7 was prepared from neuraminic acid in four steps as previously reported (125,126). The critical intermediate, aldehyde acceptor 41 was prepared in 14 steps. Starting from the commercially available diisopropylidene galactose derivative 41, the corresponding 6-iodo derivative was prepared followed by displacement with cyanide nucleophile to yield 39. The modest yield (30%) of this reaction might be due to unfavorable electronic and steric effects arising from the ring oxygen atom and the axially oriented oxygen at C-4 respectively. Reduction of the 6-cyano derivative 43 with DIBAL-H afforded aldehyde 44 in moderate overall yield (127). Quantitative reduction of aldehyde 44 with NaBH₄ in MeOH afforded the corresponding alcohol 45. De-isopropylation of 45 accomplished by treatment with amberlite IR-120 (H⁺) resin in water at 80°C for 3 h, provided the 6-deoxy galacto-heptopyranose 46 in quantitative yield. The one carbon extension 47 was obtained in good yield from 46 using a one pot, three step procedure consisting of: 1. peracetylation with acetic anhydride and pyridine/ HBr/HOAc; 2. conversion of the anomeric acetate to the corresponding bromide with excess HBr/HOAc; and 3. reductive elimination of the 1-bromo acetoxy groups with Zn/Cu (128). Azidonitrilation (129) of 47 with excess ammonium nitrate (CAN) and sodium azide in dry acetonitrile afforded...
primarily the 2-azido-1-nitrate addition product having the desired galacto configuration. Treatment of the crude product with LiBr (129) in dry acetonitrile under ionic conditions afforded 48.

Glycosylation of 48 with the N'-benzoyl carbonyl protected OBN ester of L-Serine 49, prepared as previously reported (130), in the presence of silver perchlorate afforded the glycopeptide 50. Separation of glycopeptide 50 from unreacted starting material, Z-Ser-OBN, was cumbersome since both compounds migrated with similar Rf values on silica. However, conversion of 50 to 51 by treatment with thiaocetic acid/pyridine (131), resulted in a large change in the glycopeptide polarity, permitting the removal of the unreacted aminoacid acceptor from the desired glycopeptide product 51. Efforts next focused on the selective deprotection of the C-7 primary acetyl group in 51. Selective enzymatic deacetylation using an esterase from Rhodospirillum toruloides has been previously reported to regioselectively deprotect primary acetates in the presence of secondary acetates (132-133). Treatment of 51 with this esterase at pH 5 using a sodium phosphate-sodium citrate buffer afforded 52 in quantitative yield. The site of the enzymatic deacetylation was unequivocally established as the Swern oxidation (134) of 52 afforded aldehyde acceptor 40. Glycosylation of neuraminic acid sulfone donor 7 with aldehyde acceptor 40 in the presence of freshly prepared SmI₂ (135-141) afforded the fully protected sTn α-C-glycoside 53 as a diastereomic mixture. Efforts to deoxygenate the bridge hydroxymethylene group by Barton deoxygenation (142) failed. Chemical resolution was achieved by oxidizing the bridge hydroxyl group to ketone which was then stereoselectively reduced (142). The pure sTn-α-C-glycoside 53 is currently being conjugated to KLH carrier protein for evaluation of efficacy as a carbohydrate vaccine.

**Serine-based Neuraminic Acid**

The synthesis of serine-based C-glycosides has only been carried out in a few laboratories and has relied on cross-metathesis of oxazoline silyl ether (143-145); or the Ramberg Bäcklund rearrangement (146). Using a method for the preparation of C-glycosides of neuraminic acids using SmI₂ (147), we have synthesized a serine-based C-glycoside of neuraminic acid.

![Scheme 10. Synthesis of the C-glycoside analog of sTn.](image)

To prepare a C-glycosidic linkage, it was first necessary to synthesize neuraminic acid donor 7 (148) and a serine-based acceptor 54 (Scheme 11). Homoserine was chosen as the substitute of serine since during C-glycosylation an additional carbon is required to replace the interglycosidic O-linkage. A strategy to prepare the acceptor involved the orthogonal protection of amino acid carboxyl groups in homoserine and the oxidation of its hydroxyl group to aldehyde acceptor for C-glycosylation. Cbz protected homoserine 55 was converted to corresponding allyl ester 57e and benzyl ester 57f, respectively (Scheme 11). Both 57e and 57f were smoothly oxidized to aldehyde acceptors 54e and 54f (149). C-glycosylation of these two acceptors afforded C-glycoside 58 with concomitant loss of carboxyl protection and subsequent lactonization.
Only a small amount of desired allyl protected homoserine C-glycoside 59 was obtained. This intramolecular cyclization product is favorable due to the formation of a stable, five-membered ring, commonly observed in homoserine-based syntheses (Figure 3) (150).

![Chemical structures](image)

**Figure 3. Two C-glycosylation products formed.**

Lactone 58 could be opened under hydrogenolysis conditions using acetic acid and water as reaction media, affording the unprotected serine-based neuraminic acid C-glycoside (151-152). Attempts to protect the resulting free amino acid with FmocCl (153-154) only afforded the N-Fmoc protected, lactonization product.

![Chemical reactions](image)

**Scheme 12a. Reagents: (i) TBDMSI, Pyridine, (ii) LiBH₄, THF, (iii) BnBr, 1.2 eq NaH, DMF, 0°C**

It was clear that the protection of amino group and the presence of an α-COOH group in homoserine complicated samarium-based C-glycosylation.

![Chemical reactions](image)

**Scheme 12b. Reagents: (i) dihydropyran, PPTS, DCM, (ii) TBAF, THF, (iii) Dess-Martin periodinane, DCM, (iv) 2 Sml₂, THF, (v) Ac₂O, Pyridine, (vi) PPTS, EtOH, 50°C, (vii) RuCl₃, NaO₄, CCl₄, CH₂CN, H₂O, 0°C.**
Reduction of the carboxyl group at the beginning of the synthesis and its subsequent oxidation following C-glycosylation was next examined to avoid lactonization and improve C-glycosylation yield.

The hydroxyl group in Cbz-L-homoserine allyl ester 57e was protected as the silyl ether 60 (155), and the allyl ether was reduced to afford alcohol 61 (Scheme 12a) (156). Surprisingly, benzylaion of the newly generated hydroxyl group in 61 with benzyl bromide and sodium hydride in DMF at 0 °C afforded aziridine 62 as a major product. Reducing the reaction temperature and the amount of NaH failed to prevent the formation of the undesired aziridine. Benzylaion under acidic conditions using silver oxide and benzyl bromide, however, prevented the formation of aziridine 62 but afforded the desired 63 in very low yield (157).

Failure of benzyl protection led us to examine tetrahydropyranyl ether (THP) protection, which is easy to install, stable to most nonacidic reagents, and easy to remove. THP protection of 61 went smoothly (158) and affording 64 in 83% yield (Scheme 12b). Quantitative tetrabutylammonium fluoride (TBAF) removal of silyl ether afforded 65, which was readily oxidized to the C-glycosylation aldehyde acceptor 66. C-glycosylation of 66 afforded α-C-glycoside 67 at 45% isolated yield. Acetylation of newly generated bridge hydroxyl group afforded fully protected C-glycoside 68. Removal of THP using pyridinium p-toluenesulfonate (PPTS), followed by oxidation of resulting hydroxyl group, generated our target, L-homoserine based C-glycoside 70 (159-161).

GM4 and GM3

Ganglioside GM4 is an important cell adhesion molecule (15). It promotes neuronal adhesion through its interaction with myelin-associated glycoprotein (MAG). This adhesion is important in the regeneration of neuronal tissues (15). The C-disaccharide of GM4 71 is currently being synthesized in our laboratory by standard O-glycosylation of C-disaccharide 72 (Scheme 13) with protected ceramic acid 74 prepared by the method previously described by Hasegawa (162). The C-disaccharide donor 72 has been successfully synthesized (Scheme 14) from perbenzylated 3-formyl galactose acceptor 73 and Neu5Ac phenyl sulfone donor 7. Once the ceramic acid has been attached the ganglioside analog will be deprotected to afford GM4 C-glycoside 71. The ceramic side chain of the C-glycosides of GM4 71 will be oxidized with ozone to afford an aldehyde group that was coupled to KLH by reductive amination (163). This conjugate will be used by our laboratory to prepare antibodies in mice against C-GM4 71 as a control for cross-reactivity with the natural GM4 (O-glycoside). The solution conformation of C-GM4 will also be compared to GM4 using NMR spectroscopy (111).

GM3, a related trisaccharide containing ganglioside, plays an important role in cell growth and differentiation. GM3 inhibits epidermal growth factor (EGF) receptor mediated signal transduction (164). Tumors, such as those involving brain cancer, overexpress EGF receptor. Effective new therapeutic agents need to modulate the mitogenic effect of EGF on such tumors. The synthesis of oligosaccharide GM3 93 is currently underway starting from lactose 94 (Scheme 15). The planned chemical synthesis of C-GM3 93 follows a parallel route to the one currently being successfully used in C-GM4 synthesis. Once synthesized the conformational flexibility and biological activity of C-GM3 93 will be evaluated.
Conclusions

In conclusion, samarium mediated C-glycosylation is an effective method of synthesizing α-C-glycosides of Neu5Ac, KDN, and KDO. These glycosides can be incorporated into structures corresponding to natural products of biological interest affording catabolically stable analogues of a number of potentially important biological activities. For example, recent evaluations of Neu5Ac with hydrophobic aglycones demonstrate reasonably favorable activities as neuraminidase inhibitors (165-166). Moreover, conformational evaluations indicate Neu5Ac C-glycosides show that they can occupy similar conformational spaces to the O-glycoside natural products (167). Thus, these C-glycosides should resemble the natural products and provide analogs with excellent stability. Future biological testing will provide the results needed to confirm the value of these analogs.

Experimental

General procedure for Neu5Ac, KDN or KDO C-glycosidation.

An appropriately hydroxyl (i.e., acetylated) and carboxyl (i.e., methoxymethyl esterified) protected Neu5Ac, KDN or KDO phenyl sulfone glycoside (2 to 4 mg) and 1.2 to 2.0 equiv of electrophile (i.e., a ketone or aldehyde containing molecule) are dried together under high vacuum for 4 h, then dissolved in degassed anhydrous THF (0.5 to 1 mL). SmI₂ (4 equiv, freshly prepared from I₂ and CH₂CH₂I, 0.1M in THF) is added in one portion at room temperature with vigorous stir-ring. After approximately 10 min, the reaction mixture is diluted with ether, filtered, and the filtrate is concentrated under reduced pressure and then purified by silica gel column with the appropriate eluent such as EtOAc.

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