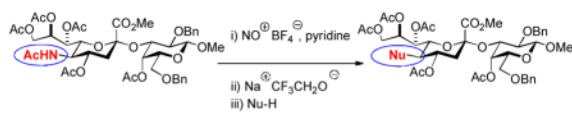# Chemical Diversification of Sialic Acid Glycosides by Stereospecific, Chemoselective Deamination

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Abstract

Nitrosation of peracetylated NeuAc glycosides followed by treatment with sodium trifluoroethoxide and then a suitable nucleophile enables late stage modification of sialic acid glycosides with stereospecific replacement of the acetamido functionality.

Keywords

sialic acids; deamination; diversification; glycosylation

The sialic acids are nine carbon sugars based on the 2-keto-3-deoxy-D-glycero-D-galactononulosonic acids whose α-glycosides adorn the non-reducing termini of many *N*-glycoproteins and gangliosides and are the monomeric units of the α-(2→8)- and α-(2→9)-oligosialic acids.[1] The recognition of these sialyl glycoconjugates by various lectins, and the trimming of sialyl residues by sialidase enzymes, play important roles in many human disease states and have stimulated interest in the synthesis of libraries of modified sialyl glycoconjugates and their deployment in the search for improved diagnostics and therapeutics.[1–2] The difficulties inherent in sialic acid chemistry and particularly in the stereocontrolled synthesis of α-sialosides[3] have, however, restricted the preparation of such libraries to enzymatic approaches which are limited by the range of substrates accepted.[4] We describe here a method for the chemical synthesis of sialyl glycoside libraries that combines recent progress in stereocontrolled α-sialoside synthesis[5] with a mild oxidative deamination process to enable late-stage modification of pre-assembled glycosides, thereby extending the range of accessible diversity.

Focused libraries of specific classes of oligosaccharides and/or glycoconjugates are arguably best accessed by the late stage modification of pre-assembled substances combining synthetic efficiency with the ability to introduce targeted diversity with the minimum of synthetic effort. This strategy, which differs from the current enzymatic cascade approach to

sialyl glycoside libraries which demands synthesis of a different precursor for every eventual member,[4] requires reliable, robust methodology for glycoside synthesis and mild efficient methodology for their subsequent modification. The introduction of the 4-*O*,5-*N*-oxazoldinone-protected sialyl donors and their more readily deprotected *N*-acetyl derivatives has removed many of the obstacles to chemical α-sialoside synthesis (Scheme 1, line 1),[5] thereby opening the door to the modification of sialyl glycosides as a means of entry into libraries, provided suitably mild chemistry for subsequent modification can be identified. We reasoned that the modified Schreiner and Zbiral oxidative deamination of neuraminic acid glycosides would be a suitable reaction for such modifications if conditions could be found to extend the range of nucleophiles beyond the acetic and and hydrazoic acid-based systems previously employed (Scheme 1, line 2).[6]

Oxidative deamination of peractyl *N*-acetylneuraminic acid (NeuAc) methyl ester was achieved by Schreiner and Zbiral with nitrosyl acetate giving the *N*-nitroso adduct,[6a] whereas in our laboratory we prefer the more convenient, commercial nitrosyl tetrafluoroborate for this purpose.[6b] Subsequent steps involve selective removal of the acetyl group from the *N*-nitrosoacetamide with sodium trifluoroethoxide to give a diazo derivative of NeuAc that is then substituted by the incoming nucleophile. Participation by the 4-*O*-acetate is invoked to explain both the regio- and stereoselectivity of the process.[6a] As nitrosyl tetrafluoroborate is known to activate disarmed thioglycosides toward glycosylation,[7] we tested the compatibility of the oxidative deamination thioglycosides. In the event, treatment of a dichloromethane solution of thioglycoside **6** in the presence of pyridine with NOBF$_4$ at −10 °C gave a solution of the presumed *N*-nitroso derivative **7** that was briefly exposed to trifluoroethanol (TFE) and sodium isopropoxide in isopropanol at the same temperature before addition of either acetic acid, thioacetic acid, or the hydrogen fluoride-pyridine complex resulting in the formation of the 5-desacetamido acetoxy, acetylthio, and fluoro NeuAc derivatives **8**, **9**, and **10**, respectively, in good yield. albeit the fluoride **10** was accompanied by 11% of the isopropoxy analogue **11** (Scheme 2).[8] The survival of the thioglycoside moiety is due to the use of pyridine, which captures the nitrosonium ion as the *N*-nitrosopyridinium complex[9] **12** (Scheme 2) and moderates its reactivity. While the formation of the 2-keto-3-deoxy-D-glycero-D-galactononulosonic acid (KDN) derivative **8** recalls the earlier studies[6] with acetic acid as nucleophile, the formation of the acetylthio and fluoro derivatives demonstrate the ability to incorporate a wider range of nucleophiles. The formation of the isopropyl ether **11** as byproduct alongside fluoride **10** could be avoided by using preformed sodium trifluoroethoxide as base in conjunction with 18-crown-6 and excluding isopropanol from the reaction mixture, when 63% of the fluoride **10** was obtained. The isolation of **11** as byproduct also suggested that conditions for the use of simple alcohols as nucleophiles in the oxidative deamination protocol. Thus, after some experimentation it was found that deacetylation with sodium trifluoroethoxide, followed by the addition of propargyl alcohol and tetrafluoroboric acid, to protonate the intermediate diazo compound, yielded the propargyl ether **13** in 66% yield (Scheme 2). Next a series of α-sialosides was prepared from the donor **1** on activation with NIS/TfOH in a mixture of dichloromethane and acetonitrile at −78 °C[5c] as reported in Table 1, entries 1–5. Alternatively, for more complex acceptors based on the thioglycoside motif the Wong sialyl phosphate type donor[5e] **14** was employed with activation by trimethylsilyl trifluoromethanesulfonate at −78 °C in dichloromethane and acetonitrile (Table 1, entries 6 and 7). In each case the oxazolidinone then was removed under Zemplen conditions and any acetate esters reinstalled with acetic anhydride and pyridine (Scheme 1, and Table 1). Nitrosylation was achieved with nitrosyl tetrafluoroborate in the presence of pyridine and the intermediate *N*-nitrosoamides substituted under the conditions reported in Table 1.

Entries 1 – 3 of Table 1 illustrate the application of the protocol to the synthesis of KDN glycosides incorporating α(1→6)-galactopyranoside, α(1→6)-glucopyranoside, and α(1→3)-galactopyranoside linkages and demonstrate the compatibility of the protocol with glycosidic bonds and with benzyl ethers. Entry 4 of Table 1 employs thioacetic acid as nucleophile and provides the novel disaccharide **28**, which may be viewed either as a 5-acetylthio-5-desacetamido derivative of a NeuAc glycoside or as a 5-acetylthio-5-deoxy analogue of a KDN glycoside. As selective deacetylation of thioacetates in the presence of acetates is facile, thioacetate derivatives such as **28** enable further regioselective diversification at the 5-position by alkylation of thiols and disulfide formation, or by the thiol-ene and yne click[10] and other processes.[11] As 5-mercapto analogues of NeuAc and/ or KDN have not previously been accessible through the use of sialyl transferases,[4b, 4c] this example extends the range of accessible α-sialoside derivatives modified at the 5-position and demonstrates the versatility of the chemical approach. Entry 5 (Table 1) illustrates the formation of a 5-fluoro derivative of NeuAc and/or KDN. Entries 6 and 7 (Table 1) reiterate the applicability of the method to thioglycoside containing substrates, whether of the arylthio or alkylthio classes. Table 1, entry 6 introduces the possibility of diversification at the 5-position of the sTn antigen, and is especially noteworthy as it has been previously demonstrated that modification of this antigen at the amide can lead to analogues that display improved antigenicity.[12] Entry 7 (Table 1) demonstrates how the oxidative deamination process may be applied concomitantly to two sialic acid residues in an α(2→9)-linked disaccharide and represents the first chemical synthesis of a KDN disaccharide of this class. Such modifications of polysialic acids are of interest in view of recent approaches to α(2→9)-linked polysialic acids because of their potential as antibacterial agents and synthetic vaccines.[13]

Application of the oxidative deamination protocol to a GM3 trisaccharide and a branched tetrasaccharide is demonstrated beginning from trisaccharide **32**, acetylation of which gave the derivative **33**, while glucosylation afforded the tetrasaccharide **34** (Scheme 3). Both substances were treated with nitrosyl tetrafluoroborate and pyridine and then exposed to sodium trifluoroethoxide followed by acetic acid giving the KDN analogues **35** and **36**, respectively, in good yield.

The combination of *N*-acetyloxazolidinone-directed α-sialidation with oxidative deamination provides rapid entry into NeuAc and/or KDN analogues modified at the 5-position. Suitable nucleophiles for this process include acetate, thioacetate, fluoride and simple alcohols. The ability to chemically synthesize sialic acid glycosides in this manner and then affect late stage modification is potentially very flexible and should enable access to many derivatives with a minimum of synthetic effort. Modifications are not limited to those incorporated in substrates for sialyl transferases.

Experimental Section

General procedure for nitrosation of sialosides

A stirred 0.1 M solution of sialoside in anhydrous CH_2Cl_2 under Ar was treated with anhydrous pyridine (10 equiv) and cooled to −10 °C. After stirring for 10 min, powdered nitrosyl tetrafluoroborate (4 equiv) was added in one portion to the mixture. The resultant light green solution was stirred at −10 °C for 3 h then was diluted with CH_2Cl_2 and washed sequentially with cold 1N HCl, cold saturated aqueous NaHCO3, and brine. Drying (Na_2SO_4) and concentration below 10 °C gave the nitrosated sialosides which were carried forward without further purification.

General procedure for deamination with acetic or thioacetic acid as nucleophile

A 0.1 M solution of the nitrosyl sialoside and trifluoroethanol (1.5 equiv) in anhydrous CH_2Cl_2 under Ar was treated at -10 °C with freshly prepared 0.2 N sodium isopropoxide in isopropanol (1.2 equiv). The resulting mixture was stirred for exactly 2 min then was treated with a cold solution of glacial acetic acid (20 equiv) or thioacetic acid (20 equiv) in CH_2Cl_2 (0.1M). After stirring for 5 min the reaction mixture was warmed to 0 °C and was quenched with saturated aqueous $NaHCO_3$. The organic layer was washed with cold brine, dried (Na_2SO_4), concentrated and chromatographed over silica gel to afford the deaminated sialosides.

General procedure for deamination with HF and alcohol nucleophiles

18-Crown-6 (0.22 mmol) and sodium 2,2,2-trifluoroethoxide (54 mg, 0.2 mmol) were dissolved in anhydrous CH_2Cl_2 (0.5 mL) under Ar, cooled to -10 °C, and added to the nitrosyl sialoside (0.1 mmol) in anhydrous CH_2Cl_2 (1 mL) at -10 °C under Ar. After 2 min, either the alcohol (2 mmol in 2 mL CH_2Cl_2) immediately followed by $HBF_4.Et_2O$ (0.4 mmol) or HF.pyridine (2 mmol) was added to the reddish reaction mixture. The mixture was stirred for 5 min, diluted with CH_2Cl_2 (5 mL) then quenched with saturated aqueous $NaHCO_3$ (5 mL). The organic layer was washed with brine (5 mL), dried, concentrated and purified by chromatography over silica gel to afford the deaminated sialosides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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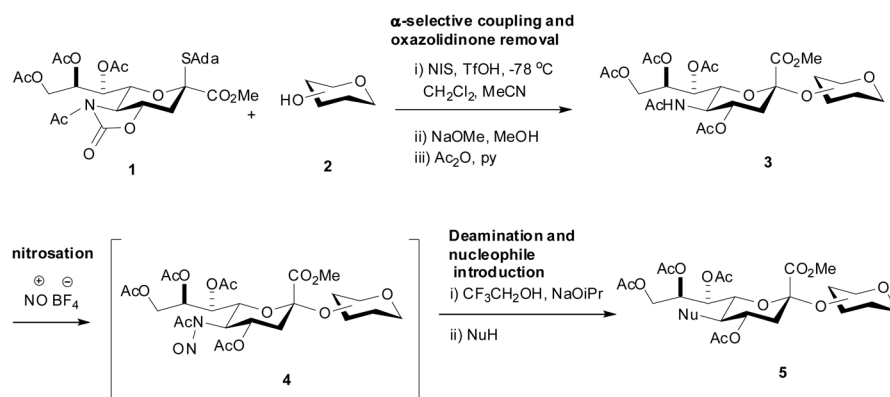
Scheme 1.
Chemical synthesis of sialoside libraries with late stage modification of preassembled glycosides.
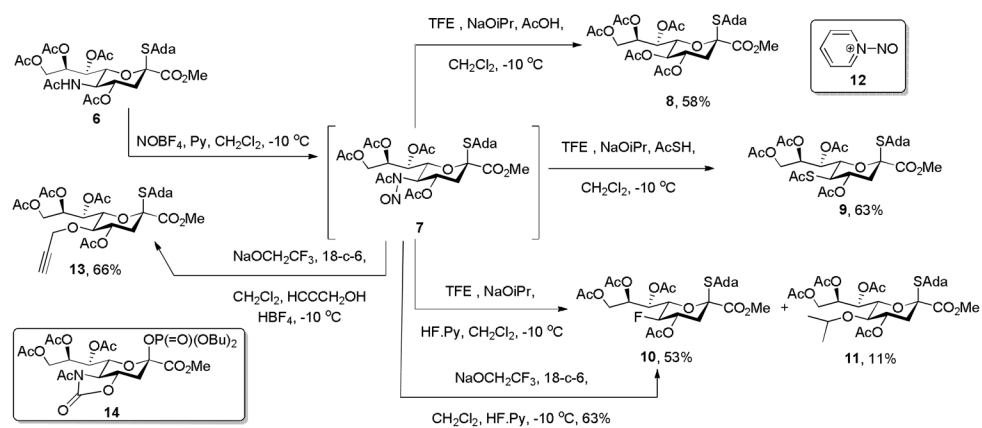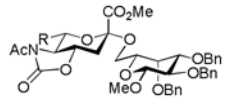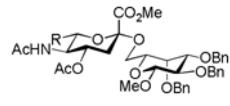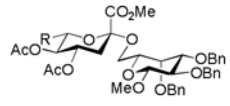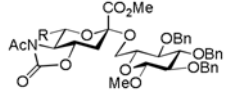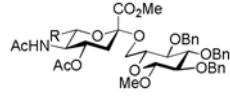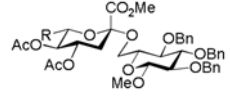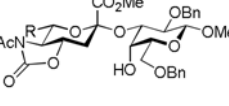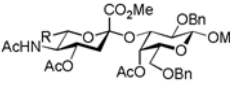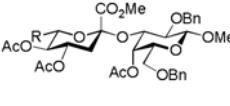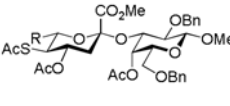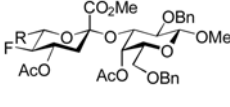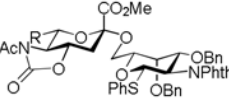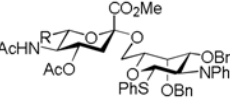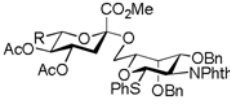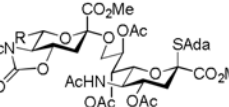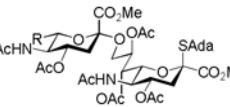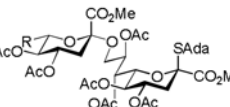
Scheme 2.
Compatibility with a thioglycoside and introduction of acetoxy, acetylthio, and fluoride groups.
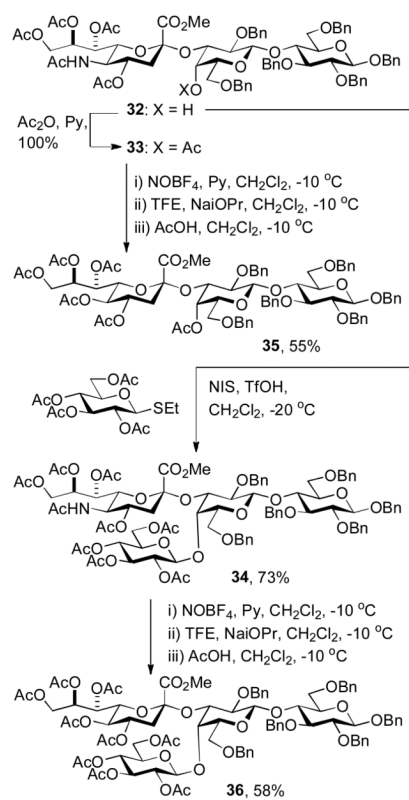
Scheme 3.
Application of the oxidative deamination reaction to a GM3 trisaccharide and a branched tetrasaccharide.

Table 1

Stereoselective α-sialoside synthesis and oxidative deamination.

	Glycosylation[a]	Oxazolidinone[a] Removal	Nucleophile, conditions	Substitution Products[a]
1	**15**, 81%, α- only from **1**	**20**, 77%	AcOH, TFE, NaOiPr	**25**, 51%
2	**16**, 84%, α- only from **1**	**21**, 88%	AcOH, TFE, NaOiPr	**26**, 48%
3	**17**, 83%, 10:1 β:α, from **1**	**22**, 76%	AcOH, TFE, NaOiPr	**27**, 54%
4	**17**	**22**	AcSH, TFE, NaOiPr	**28**, 67%
5	**17**	**22**	HF.NEt$_3$, NaTFE, 18-c-6,	**29**, 63%
6	**18**, 86%, α- only, from **14**	**23**, 91%	AcOH, TFE, NaOiPr	**30**, 49%
7	**19**, _[b], from **14**	**24**, 74%, α- only[c]	AcOH, Bu$_4$NOAc, TFE, NaOiPr	**31**, 42%

[a] R = CH(OAc)CH(OAc)CH$_2$OAc;

[b] not isolated;

[c] overall yield from **14**.