Sialic Acids

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

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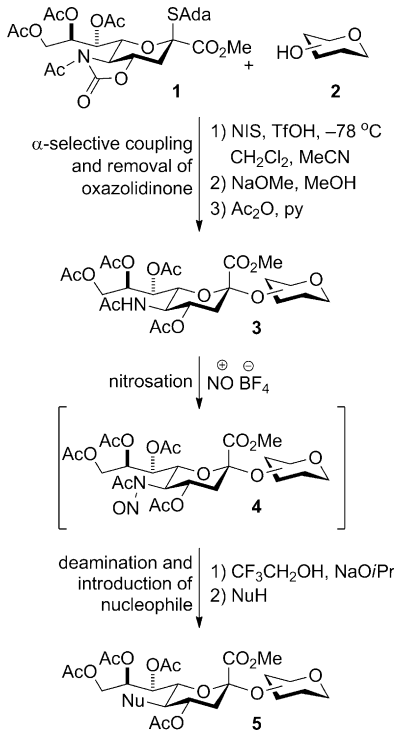
The sialic acids are nine-carbon sugars based on the 2-keto-3-deoxy-D-glycero-D-galactononulosonic acids, whose α-glycosides adorn the nonreducing termini of many *N*-glycoproteins and gangliosides and are the monomeric units of the α-(2→8)- and α-(2→9)-oligosialic acids.[1] The recognition of these 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 preassembled 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 preassembled substances, combining synthetic efficiency with the ability to introduce targeted diversity with a minimum of synthetic effort. This strategy requires a reliable, robust methodology for glycoside synthesis and a mild, efficient methodology for their subsequent modification, thus differing from the current enzymatic cascade approach to sialyl glycoside libraries, which demands the synthesis of a different precursor for every eventual member.[4] The introduction of the 4-*O*,5-*N*-oxazolidinone-protected sialyl donors and their more readily deprotected *N*-acetyl derivatives has removed many of the obstacles of chemical α-sialoside synthesis (Scheme 1, upper part),[5] thereby opening the door to the modification of sialyl glycosides as a means of entry into libraries, provided that suitably mild conditions for the subsequent modification can be identified. We reasoned that the modified oxidative deamination of neuraminic acid glycosides described by Schreiner and Zbiral would be a suitable reaction for such modifications if conditions could be found to extend the range



Scheme 1. Chemical synthesis of sialoside libraries with late-stage modification of preassembled glycosides.

of nucleophiles (NuH) beyond the previously employed systems based on acetic and hydrazoic acid (Scheme 1, lower part).[6]

Oxidative deamination of the peracetylated *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 to use the more convenient, commercial nitrosyl tetrafluoroborate ($NOBF_4$) 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 $NOBF_4$ is known to activate thioglycosides toward glycosylation,[7] we tested the compatibility of the oxidative deamination conditions with thioglycosides. In the event, treatment of a solution of thioglycoside **6** in dichloromethane with $NOBF_4$ in the presence of pyridine (Py) at −10 °C gave a solution of the presumed *N*-nitroso derivative **7**, which was briefly exposed to trifluoroethanol (TFE) and sodium isopropoxide in isopropanol at the same temperature before

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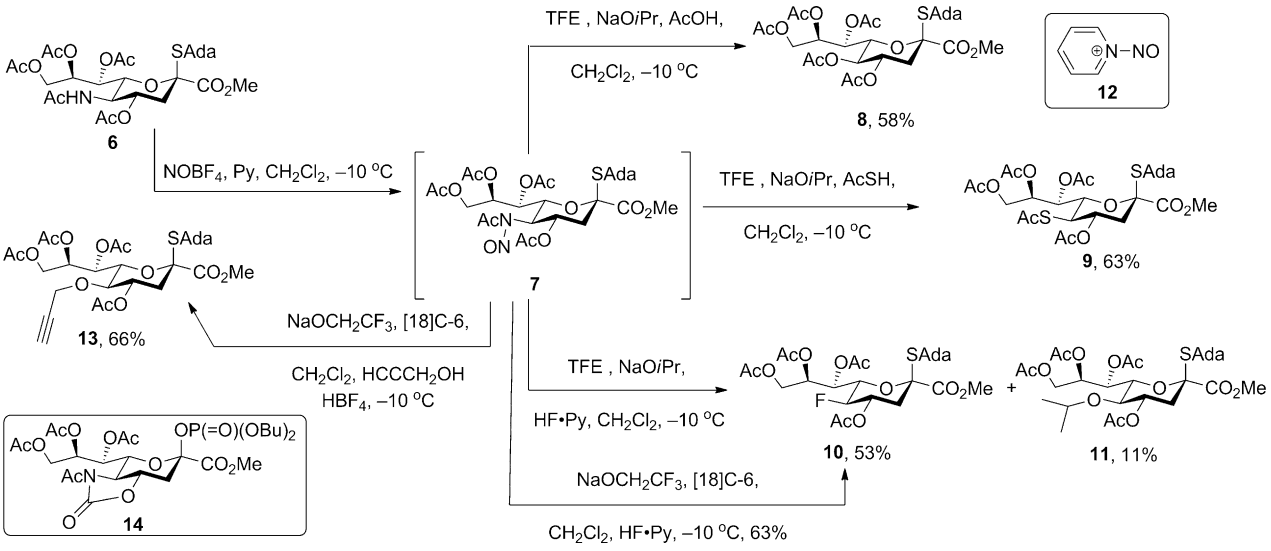
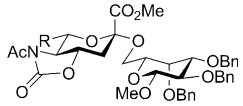
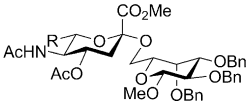
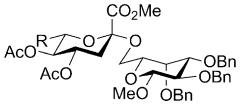
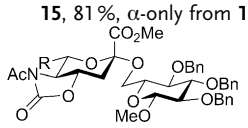
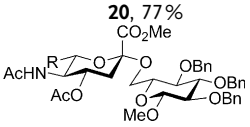
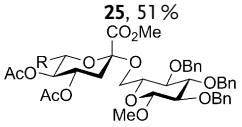
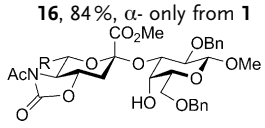
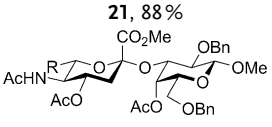
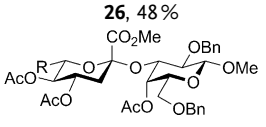
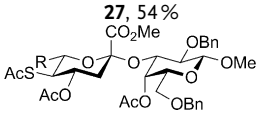
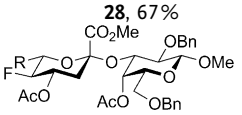
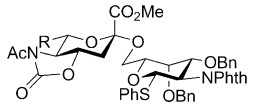
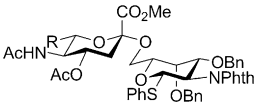
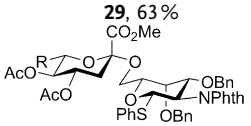
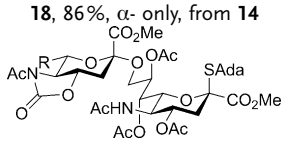
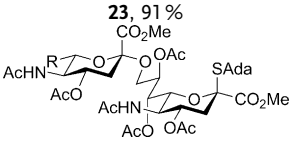
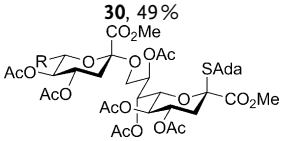
addition of either acetic acid, thioacetic acid, or the hydrogen fluoride/pyridine complex, resulting in the formation of the 5-desacetamido acetoxy, acetylthio, and fluoro NeuAc derivatives **8**, **9**, and **10**, respectively, in good yield. Fluoride **10** was however accompanied by 11% of the isopropoxy analogue **11** (Scheme 2).[8] The survival of the thioglycoside moiety is a result of 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 earlier studies[6] with acetic acid as nucleophile, the formation of the acetylthio and fluoro derivatives demonstrates the ability of the method to incorporate a wider range of nucleophiles. The formation of the isopropyl ether **11** as by-product alongside fluoride **10** could be avoided by using preformed sodium trifluoroethoxide as base in conjunction with 18-crown-6 ([18]C-6) and excluding isopropanol from the reaction mixture, giving fluoride **10** in 63% yield. The formation of **11** as by-product also suggested that conditions could be found for the use of simple alcohols as nucleophiles in the oxidative deamination protocol. Thus, after some experimentation we found that deacetylation with sodium trifluoroethoxide, followed by the addition of propargyl alcohol and tetrafluoroboric acid to protonate the intermediate diazo compound, gave the propargyl ether **13** in 66% yield (Scheme 2). Next, a series of α-sialosides was prepared from the donor **1** on activation with *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) in a mixture of dichloromethane and acetonitrile at −78 °C[5c] (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 $NOBF_4$ in the presence of pyridine and the intermediate *N*-nitrosoamides were substituted under the conditions reported in Table 1.

We applied the protocol to the synthesis of KDN glycosides incorporating α(1→6)-galactopyranoside, α(1→6)-glucopyranoside, and α(1→3)-galactopyranoside linkages, and demonstrated the compatibility of the protocol with glycosidic bonds and benzyl ethers (Table 1, entries 1–3). The employment of thioacetic acid as nucleophile provided the novel disaccharide **28** (Table 1, entry 4), 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 formation of disulfides, 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,c] this example extends the range of accessible α-sialoside derivatives modified at the 5-position and demonstrates the versatility of the chemical approach. The formation of a 5-fluoro derivative of NeuAc and/or KDN is illustrated in entry 5 (Table 1). The applicability of the method to thioglycoside-containing substrates, whether of the arylthio or alkylthio classes, is reiterated in entries 6 and 7 (Table 1). The possibility of diversification at the 5-position of the sTn antigen (Table 1, entry 6) 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] The oxidative deamination may be applied concomitantly to two sialic acid residues in an α(2→9)-linked disaccharide (Table 1, entry 7), representing 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]



Scheme 2. Compatibility with a thioglycoside and introduction of acetoxy, acetylthio, and fluoride groups.

Table 1: Stereoselective synthesis of α-sialosides and oxidative deamination.

	Product of glycosylation	Product of oxazolidinone removal	Nucleophile (conditions)	Product of substitution
1			AcOH (TFE, NaO*i*Pr)	
	15, 81%, α-only from **1**	**20**, 77%		**25**, 51%
2			AcOH (TFE, NaO*i*Pr)	
	16, 84%, α- only from **1**	**21**, 88%		**26**, 48%
3			AcOH (TFE, NaO*i*Pr)	
	17, 83%, 10:1 β:α, from **1**	**22**, 76%		**27**, 54%
4	**17**	**22**	AcSH (TFE, NaO*i*Pr)	
5	**17**	**22**	$HF{\cdot}NEt_3$ (NaTFE, [18]C-6)	**28**, 67% 
6			AcOH (TFE, NaO*i*Pr)	**29**, 63% 
	18, 86%, α- only, from **14**	**23**, 91%		**30**, 49%
7			AcOH (Bu_4NOAc, TFE, NaO*i*Pr)	
	19, –,[a] from **14**	**24**, 74%, α-only[b]		**31**, 42%

[a] Not isolated. [b] Overall yield from **14**. Ada = adamantyl, Bn = benzyl, Phth = phthaloyl, R = CH(OAc)CH(OAc)CH_2OAc.

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 $NOBF_4$ and pyridine and then exposed to sodium trifluoroethoxide followed by acetic acid, giving the KDN analogues **35** and **36**, respectively, in good yield.

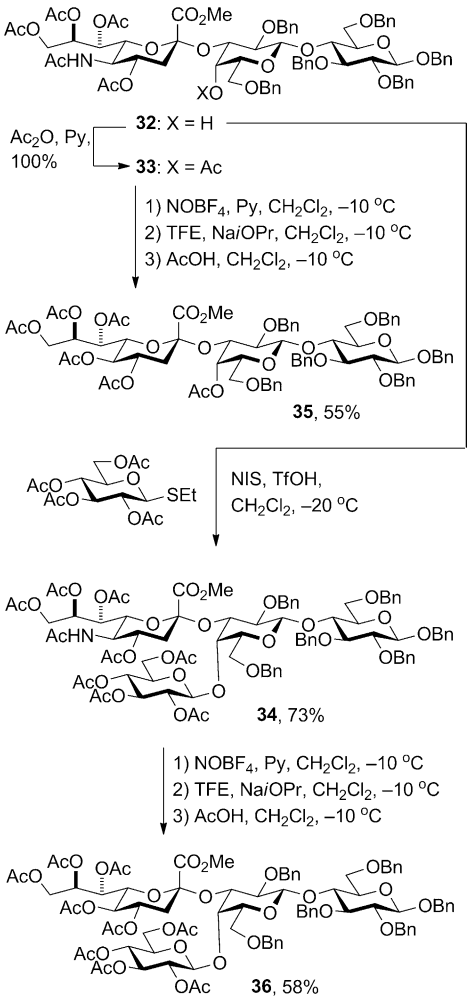
The combination of *N*-acetyloxazolidinone-directed α-sialidation with oxidative deamination provides rapid entry to NeuAc and/or KDN analogues modified at the 5-position. Suitable nucleophiles for this process include acetate, thioacetate, fluoride, and simple alcohols. The ability to chemically synthesize 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 solution (0.1M) 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 $NOBF_4$ (4 equiv) was added in one portion to the mixture. The resultant light-green solution was stirred at −10°C for 3 h, then diluted with CH_2Cl_2, and washed sequentially with cold HCl (1N), cold saturated aqueous $NaHCO_3$, 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 solution (0.1M) of the nitrosyl sialoside and trifluoroethanol (1.5 equiv) in anhydrous CH_2Cl_2 under Ar was treated at −10°C with freshly prepared sodium isopropoxide in isopropanol (0.2N, 1.2 equiv). The resulting mixture was stirred for exactly 2 min, then treated with a cold solution of glacial acetic acid (20 equiv) or thioacetic acid (20 equiv) in CH_2Cl_2 (1M). After stirring for 5 min, the reaction mixture was warmed to 0°C and quenched with saturated aqueous $NaHCO_3$. The organic layer was washed with cold brine, dried (Na_2SO_4), concentrated, and purified by column chromatography on silica gel (eluent: hexane/ethyl acetate = 1:1) 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 $HBF_4{\cdot}Et_2O$ (0.4 mmol) or HF·pyridine (2 mmol) was



Scheme 3. Application of the oxidative deamination reaction to a GM3 trisaccharide and a branched tetrasaccharide.

added to the reddish reaction mixture. The mixture was stirred for 5 min, diluted with CH_2Cl_2 (5 mL), and then quenched with saturated aqueous $NaHCO_3$ (5 mL). The organic layer was washed with brine (5 mL), dried (Na_2SO_4), concentrated, and purified by column chromatography on silica gel (eluent: hexane/ethyl acetate = 1:1) to afford the deaminated sialosides.

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