Synthesis and Biological Evaluation of *N*-Acetylneuraminic Acid-Based Rotavirus Inhibitors

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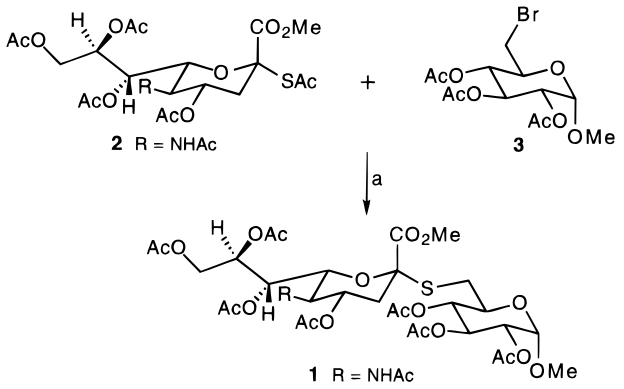
Rotavirus can cause severe gastrointestinal disease, especially in infants and young children, and is particularly prevalent in Third-World countries. Therefore, the development of potential inhibitors of this virus is of great interest. The present study describes the synthesis and *in vitro* biological evaluation of a number of *N*-acetylneuraminic acid-based compounds as potential rotavirus inhibitors. Our data suggests that it is indeed possible to inhibit adhesion of the virus, and hence *in vitro* replication, with carbohydrate-based molecules, although this inhibition does appear to be strain dependent.

Introduction

Rotaviruses are double-stranded RNA viruses and belong to the family *Reoviridae*.[1] It is well-recognized that this virus is the etiological pathogen of infantile and child gastroenteritis.[2] It has been estimated that more than 500 million people worldwide are affected by the disease annually and that in under-developed countries the disease results in a high mortality rate of an estimated 1 million people.[2] To the present time no chemotherapeutic agent to treat rotavirus infection has become available. Rotaviruses are highly host-cell specific and infect only mature epithelial cells in the small intestine.[3] Interestingly, virus receptors appear on the target epithelial cells as the cells mature and there is much evidence to support the notion that these receptors are indeed glycoproteins.[4−6] Much literature discusses the possible components of these glycoproteins and it would appear that *N*-acetylneuraminic acid, 2→6 α-ketosidically linked to a galactose residue, may play a key role in the viral adhesion process to target cells. Indeed biantennary 2→6-linked sialyloligosaccharides from egg yolk have been shown to be inhibitors of rotaviral infection.[6] The putative *N*-acetylneuraminic acid-recognizing lectin VP 4 (virus protein 4)[7] on the virus has not yet been fully characterized.

The fact that *N*-acetylneuraminic acid is thought to play a significant role in the viral adhesion process, led us to investigate the synthesis of possible inhibitors that mimicked the key elements of the naturally occurring receptors. Since the pharmacophore of the binding pocket is unknown, we felt it would be useful to prepare a range of α-linked sialosides as possible rotavirus inhibitors. One method of developing a simple model of this binding pocket is by using a variety of aglycon moieties, including galactose linked *via* positions other than C-6, and by varying the level of acetylation of these sialosides. Importantly, for sialosides to successfully act as potential rotavirus inhibitors they would need to be metabolically stable, and we felt this could be best achieved using sulfur-linked sialosides. We,[8] as well as others,[9] have shown that such thiosialosides are resistant to hydrolysis by influenza virus sialidase. The preparation of sulfur-linked sialosides has been achieved using a number of methods.[10−12] We, too, have previously reported[13−15] a mild and efficient synthesis of *S*-sialosides that circumvents some of the anticipated problems of the earlier reported syntheses.

Scheme 1[a]



[a] (a) DMF, Et_2NH, RT, 2 h, 82%.[13]

The present study describes our efforts toward the preparation and biological evaluation of a number of *S*-sialosylglycosides as useful biological probes and as potential inhibitors of rotavirus replication through the inhibition of virus adhesion.

Results and Discussion

(i) Synthesis of Thioglycosides of *N*-Acetylneuraminic Acid. Our previous efforts in preparing α-(2→6)-thioglycosides of *N*-acetylneuraminic acid (Neu5Ac-2-*S*-α-(2→6)-glycoside) (for example, **1**) utilized a coupling between 2-thioacetyl-*N*-acetylneuraminic acid (Neu5Ac2SAc, **2**) and a brominated glycosyl acceptor (for example **3**) in the presence of diethylamine (Scheme 1).[13] However, while we had found this method to be both mild and efficient, we had encountered problems when the glycosyl acceptor contained some steric encumbrance about the site of nucleophilic attack. Indeed, attempts at forming the sialoside of *N*-acetylneuraminic acid α-(2→6)-linked to galactose [Neu5Ac-2-*S*-α-(2→6)-Gal, **4**], a putative structural feature of glycoproteins which are believed to be intimately involved in viral adhesion processes of rotavirus, *via*

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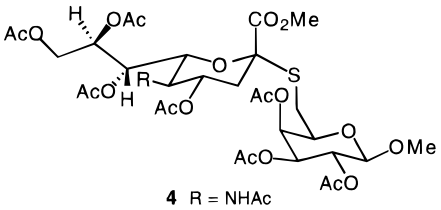
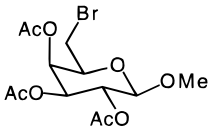
coupling between **2** and methyl 2,3,4-tri-*O*-acetyl-6-bromo-6-deoxy-β-D-galactopyranoside (**5**) under the conditions described[13] failed to furnish any of the desired coupled material **4**.



4 R = NHAc



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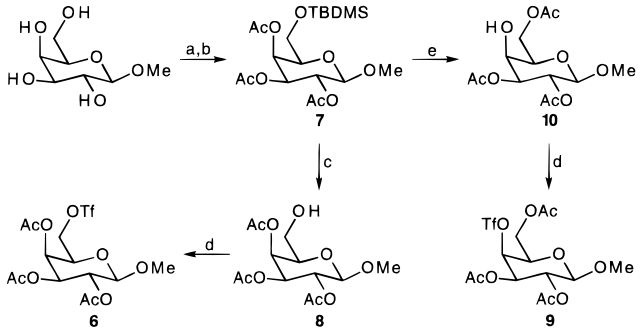
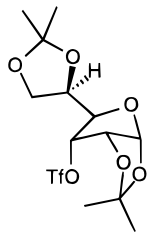
Since we were interested not only in preparing Neu5Ac-2-*S*-α-(2→6)-Gal (**4**) but also various α-(2→4)- and α-(2→3)-coupled thiosialosides, we sought a more reactive glycosyl acceptor, in order that sterically hindered sites could be successfully attacked by the sulfur nucleophile in **2**. An examination of the literature revealed several examples of thioglycoside formation *via* the displacement of secondary triflates with sulfur nucleophiles.[16−19] These reports differ from each other generally in the way in which the nucleophilic sulfur is generated. Therefore, we prepared the unstable 6-*O*-triflylgalactose derivative **6** from β-methyl galactoside using the sequence depicted in Scheme 2.[20] Similarly,

Scheme 2[a]



[a] (a) Imidazole, TBDMSCl, DMF, RT, 15 h; (b) Ac_2O, pyridine, RT, 15 h, 74%; (c) 80% aq AcOH, 50 °C, 2 h, 83%; (d) Tf_2O, pyridine −78 to 0 °C, 1 h; (e) 80% aq AcOH, 50 °C, 24 h, 63%.

the 4-*O*-triflylgalactose derivative **9** was prepared from the 6-*O*-silyl derivative **7** *via* the intermediate **10** (Scheme 2). The 3-*O*-triflylallose derivative **11** was



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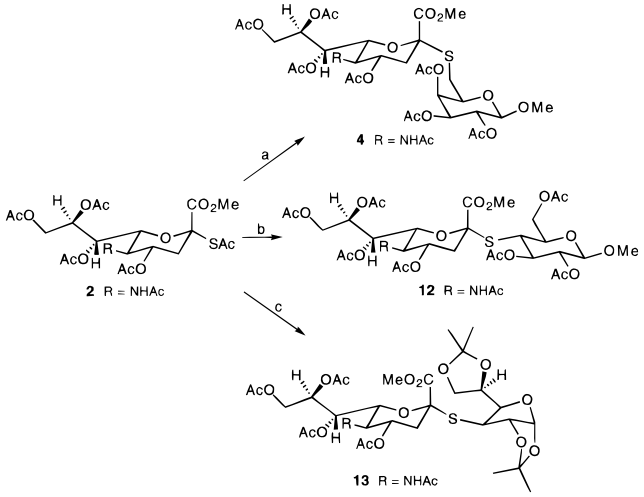
formed from commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose using the triflation conditions shown in Scheme 2.

To our delight, coupling between the triflate **6** and **2** in *N*,*N*-dimethylformamide (DMF) at 0 °C in the presence of diethylamine (Et_2NH) furnished the desired Neu5Ac-2-*S*-α-(2→6)-Gal (**4**) in high yield (77%) (Scheme 3). Similarly, coupling of the secondary triflates **9** and **11** with **2** under these conditions gave Neu5Ac-2-*S*-α-(2→4)-Glu (**12**) and Neu5Ac-2-*S*-α-(2→3)-Glu*f* (**13**), respectively (Scheme 3). Interestingly, these latter two results are in stark contrast to the preparation of thiosialosides reported by Hasegawa *et al.*[19] wherein reaction of the sodium salt of **2** with a secondary triflate provides the coupled thiosialoside in poor yield (<30%). While the secondary triflates employed by Hasegawa are not the same as those reported here, the improvement of yield of thiosialosides in our case is possibly due to the different way in which the sulfur nucleophile has been generated.

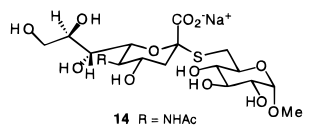
Scheme 3[a]



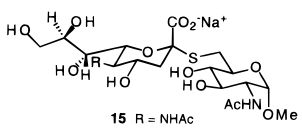
[a] (a) **6**, DMF, Et_2NH, 0 °C, 2 h, 77%; (b) **9**, DMF, Et_2NH, 0 °C, 2 h, 74%; (c) **11**, DMF, Et_2NH, 0 °C, 2 h, 84%.

The compounds described above, together with those reported previously,[13] are all in their fully protected form. In order to establish the activity, if any, of these compounds against rotavirus, it was first necessary to deprotect them. This was achieved simply by treatment of the corresponding protected compounds [e.g. **4**] with sodium methoxide in methanol followed by exposure to dilute sodium hydroxide solution. In this way, the thioglycosides **14**−**19** were isolated and purified by HPLC in good yields.

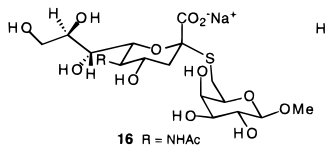
It is considered by some that partially acetylated *N*-acetylneuraminic acid derivatives are important in the inhibition of rotavirus.[21] We therefore felt that partially acetylated derivatives of compounds **14**−**19**



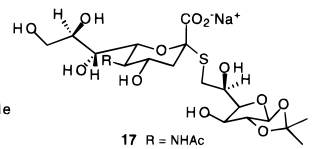
14 R = NHAc



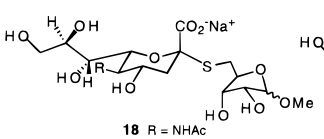
15 R = NHAc



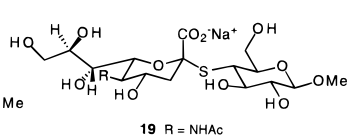
16 R = NHAc



17 R = NHAc

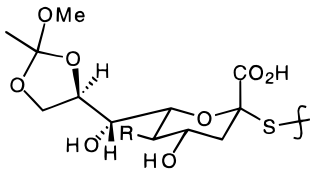


18 R = NHAc



19 R = NHAc

would help us to gain an insight into the role of partial acetylation on rotavirus inhibition. From a chemical point of view, the 9-hydroxyl group within Neu5Ac is the most reactive of the hydroxyls and is therefore the logical position at which to attempt selective monoacetylation. It must be remembered, however, that the remaining hydroxyl groups within *N*-acetylneuraminic acid as well as the hydroxyls in the other carbohydrate can also be acetylated, so any avenue into selective *mono*acetylation at the 9-position of Neu5Ac must take into account the potential reactivity of these other hydroxyls. In this regard, we felt that the best approach to 9-*O*-acetyl derivatives of *N*-acetylneuraminic acid would be by employing trimethyl orthoacetate, since such a method is known to proceed *via* the formation of an internal ortho ester such as **20**,[22,23] thereby precluding acetylation at any of the remaining hydroxyls.

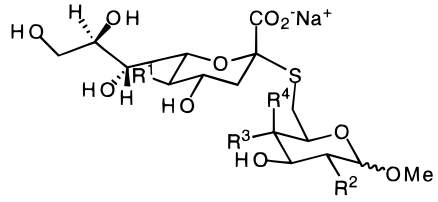


20 R = NHAc

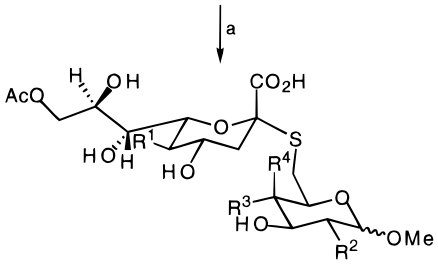
Accordingly, exposure of a dimethyl sulfoxide solution of Neu5Ac-2-*S*-α-(2→6)-Glc (**14**) to trimethyl orthoacetate in the presence of *p*-toluenesulfonic acid furnished the desired 9-*O*-acetyl derivative **21** in 74% yield after HPLC purification (Scheme 4). Similarly, treatment of Neu5Ac-2-*S*-α-(2→6)-GlcNAc (**15**) under the same conditions gave **22** in 78% yield (Scheme 4).

Interestingly, reaction of Neu5Ac-2-*S*-α-(2→6)-Gal (**16**) with trimethyl orthoacetate afforded a di-*O*-acetyl derivative in 63% yield. Careful examination of the spectroscopic data revealed that this material was Neu5,9Ac$_2$-2-*S*-α-(2→6)-4-*O*-AcGal (**23**), resulting from *in situ* internal ortho ester formation between the *cis* disposed 3- and 4-hydroxyl groups in the galactose unit

Scheme 4[a]



14 R^1 = NHAc, R^2 = R^3 = OH, R^4 = H
15 R^1 = R^2 = NHAc, R^3 = OH, R^4 = H
16 R^1 = NHAc, R^2 = R^4 = OH, R^3 = H



21 R^1 = NHAc, R^2 = R^3 = OH, R^4 = H
22 R^1 = R^2 = NHAc, R^3 = OH, R^4 = H
23 R^1 = NHAc, R^2 = OH, R^3 = H, R^4 = OAc

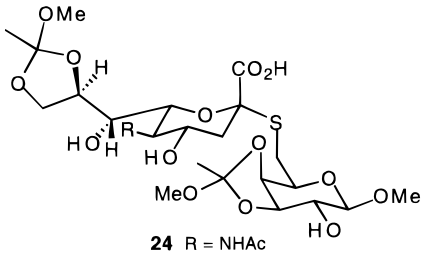
[a] (a) DMSO, MeC(OMe)$_3$, *p*-TsOH, RT, 2 h.

Table 1. Inhibition of Rotavirus Strains by Thioglycosides of Neu5Ac[a]

compound	NCDV (bovine)	SA11 (simian)	UK (bovine)	Wa (human)
14	6.25	25	>25	>25
15	6.25	25	>25	>25
16	6.25	12.5	>25	>25
17	6.25	6.25	25	>25
18	6.25	>25	>25	>25
19	6.25	25	>25	>25
21	0.75−1.5	>25	25	>25
22	0.35	>25	12.5	>25
23	0.75−1.5	25	12.5	>25

[a] Results are expressed as the concentration of the compound (mM) at which 50% infection of control infected monolayers occurred (IC_{50}).

leading to the intermediate **24**. It is well-reported that



24 R = NHAc

an ortho ester such as **24** can readily be formed between *cis* disposed hydroxyls[24] and that such a system will be hydrolyzed to leave the substituent in the axial position.[24]

(ii) Biological Evaluation of *S*-Sialosylglycosides as Inhibitors of Rotaviral Infection. The synthesized *N*-acetylneuraminic acid analogues were evaluated as potential inhibitors of rotaviral infection by a standard *in vitro* neutralization assay. It is clear from Table 1 that different serotypes of rotavirus have varying sensitivity to the *S*-sialosylglycosides **14**−**23**. Interestingly, bovine (NCDV) rotavirus appears to be the most sensitive serotype to these compounds. It is also worth noting that changing the position of attachment of the galactose moiety from α-(2→6) (compound **16**) to α-(2→4) (compound **19**) had no effect on virus inhibition across all serotypes of rotavirus. This result is consistent with the notion that the natural ligand for viral adhesion is *N*-acetylneuraminic acid α-(2→6)-linked to galactose.

The most revealing data from Table 1 is that for compounds **21**, **22**, and **23**, which all gave significant increases in the level of rotaviral (bovine, NCDV) inhibition. Compounds **21** and **22** are *O*-acetylated at the 9 position of the Neu5Ac moiety, and compound **23** is also *O*-acetylated at the 4-position in the galactoside aglycon unit. These *O*-acetylated compounds all showed approximately an order of magnitude increase in ability to reduce infection by 50% compared to control infected monolayers. The other serotypes of rotavirus evaluated in this study, that is SA11, bovine (UK), and human (Wa), appear to be marginally affected by these *S*-sialosylglycosides. While it has been reported by Willoughby *et al.*[21] that the SA11 serotype of rotavirus is specifically inhibited by an acetylated *N*-acetylneuraminic acid, their results suggest that it may not be exclusively 9-*O*-acetylated *N*-acetylneuraminic acids which are responsible for this inhibition. Our results lend support to this conclusion in that the selectively 9-*O*-acetylated thiosialosides **21**, **22**, and **23** have little effect on serotypes of rotavirus other than NCDV (bovine).

Studies aimed at the preparation of di- and tri-*O*-acetylated *S*-sialosylglycosides are in progress.

Experimental Section

Chemical Synthesis. General. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured at 25 °C using a Jasco DIP-370 polarimeter. Infrared spectra were recorded on a Hitachi 270−30 infrared spectrophotometer as KBr disks unless indicated otherwise. ^{1}H and ^{13}C spectra were recorded using a Brüker AM-300 spectrometer. Two dimensional correlation shift spectroscopy experiments were recorded using the following parameters: DQF-COSY−16 scans, 512 slices, relaxation delay 4.0 s, 2K data points transformed to 1K × 1K matrix, ssb 60° window, Qpol polynomial correction to fid prior to Fourier transformation; ^{1}H−^{13}C HMQC−48 scans, 256 slices, relaxation delay 2.5 s, 2K data points transformed to 1K × 1K matrix, ssb 90° window, Qpol polynomial correction to fid prior to Fourier transformation. Mass spectra were obtained using a JEOL JMS-DX 300 mass spectrometer. Microanalyses were performed by the Australian Microanalytical Service, Notting Hill, Australia, or by Chemical and Micro Analytical Services Pty. Ltd., Essendon North, Australia, and all analyses were within ±0.4% of the expected values. Column chromatography was performed using 230−400 mesh silica gel, and HPLC was carried out using a RP-18 column. Methyl β-D-galactopyranoside and 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose were purchased from Aldrich and used without purification. Triflic anhydride was purchased from Aldrich and distilled prior to use. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (**2**) was prepared according to the published procedure.[25]

(i) Preparation of Triflates 6, 9, and 11. Methyl 2,3,4-Tri-*O*-acetyl-6-(*tert*-butyldimethylsiloxy)-β-D-galactopyranoside (7). Imidazole (7.2 g, 106 mmol) and then *tert*-butylchlorodimethylsilane (7.8 g, 52 mmol) were added to a solution of methyl β-D-galactopyranoside (10.0 g, 52 mmol) in dry DMF (60 mL) at 0 °C under nitrogen. After stirring overnight at room temperature the mixture was poured into H_2O (100 mL) and extracted with EtOAc (3 × 100 mL). The combined extracts were washed with H_2O (2 × 100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was dissolved in pyridine (30 mL) and acetic anhydride (15 mL) and stirred overnight at room temperature. The mixture was concentrated and then dissolved in CH_2Cl_2 (100 mL) and washed with dilute HCl (1 N, 50 mL), H_2O (50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography (EtOAc:hexane, 1:2) afforded **7** (16.4 g, 74%) as an amorphous mass: mp 42−43 °C; $[\alpha]_D$ −18.6° (*c* 0.76, $CHCl_3$); IR 1754, 1368, 1245, 1220, 1074, 836 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ −0.01, 0.02 (2 × s, 2 × 3H, 2 × Si*Me*Bu^t), 0.85 (s, 9H, $SiMe_2Bu^t$), 1.96, 2.04, 2.12 (3 × s, 3 × 3H, 3 × OAc), 3.50 (s, 3H, OMe), 3.62 (dd, $J_{6,6'}$ = 11.0, $J_{6,5}$ = 9.8 Hz, 1H, H-6), 3.70−3.75 (m, 2H, H-5/H-6′), 4.37 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 5.02 (dd, $J_{3,2}$ = 10.4, $J_{3,4}$ = 3.3 Hz, 1H, H-3), 5.17 (dd, $J_{2,3}$ = 10.4, $J_{2,1}$ = 7.9 Hz, 1H, H-2), 5.46 (d, $J_{4,3}$ = 3.3 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ −5.8, −5.7 (2 × Si*Me*Bu^t), 18.0 ($SiMe_2CMe_3$), 20.5, 20.6, 20.7 (3 × OC(O)*Me*), 25.6 ($SiMe_2Bu^t$), 56.8 (OMe), 60.5 (C-6), 67.0, 69.1, 71.2, 73.4 (C-2/C-3/C-4/C-5), 102.1 (C-1), 169.5, 170.0, 170.1 (3 × O*C*(O)Me); FABMS 435 ((M + 1)$^+$, 4), 403 (31), 377 (16), 241 (79), 215 (38), 211 (57), 159 (56), 117 (100).

Methyl 2,3,4-Tri-*O*-acetyl-β-D-galactopyranoside (8). A solution of **7** (15.9 g, 36.6 mmol) in 80% aqueous acetic acid (100 mL) was stirred at 50 °C for 2 h and then concentrated under reduced pressure. Column chromatography (EtOAc:hexane, 3:2) afforded **8** (9.8 g, 83%) as a colorless crystalline solid: mp 109−112 °C (lit.[20] mp 125−126 °C; lit.[26] mp 108−109 °C); $[\alpha]_D$ +5.6° (*c* 0.98, $CHCl_3$) [lit.[20] $[\alpha]_D$ +5.2° (*c* 1.1, $CHCl_3$); lit.[26] $[\alpha]_D$ +5.0° (*c* 1.0, $CHCl_3$)]; IR 3530 (br), 1750, 1372, 1245, 1084, 1062 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.99, 2.06, 2.16 (3 × s, 3 × 3H, 3 × OAc), 3.52 (s, 3H, OMe), 3.55 (dd, $J_{5,6}$ = $J_{5,6'}$ = 7.2 Hz, 1H, H-5), 3.71−3.78 (m, 2H, H-6/H-6′), 4.41 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 5.05 (dd, $J_{3,2}$ = 10.4, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 5.22 (dd, $J_{2,3}$ = 10.4, $J_{2,1}$ = 7.8 Hz, 1H, H-2), 5.37 (d, $J_{4,3}$ = 3.4 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6 (3 × OC(O)*Me*), 56.8 (OMe), 60.4 (C-6), 67.8, 69.1, 71.0, 73.4 (C-2/C-3/C-4/C-5), 102.0 (C-1), 169.4, 170.0, 170.9 (3 × O*C*(O)-Me); FABMS 321 ((M + 1)$^+$, 5), 289 (79), 229 (30), 169 (67), 141 (27), 127 (80), 109 (100).

Methyl 2,3,6-Tri-*O*-acetyl-β-D-galactopyranoside (10). A solution of **7** (1.5 g, 3.5 mmol) in 80% aqueous acetic acid (20 mL) was stirred at 50 °C for 24 h and then concentrated under reduced pressure. Column chromatography (EtOAc:hexane, 3:2) afforded **8** (0.2 g, 18%), identical in all respects to the material described above, together with **10** (0.7 g, 63%) as an amorphous mass: mp 101−104 °C (lit.[20] mp 108−109 °C; lit.[27] mp 105−107 °C); $[\alpha]_D$ +5.4° (*c* 0.75, $CHCl_3$) [lit.[20] $[\alpha]_D$ +1° (*c* 1.2, $CHCl_3$); lit.[27] $[\alpha]_D$ +3.3° (*c* 0.7, $CHCl_3$)]; IR 3565 (br), 1744, 1728, 1370, 1256, 1230, 1046 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05, 2.08, 2.09 (3 × s, 3 × 3H, 3 × OAc), 2.42 (brs, 1H, O*H*), 3.49 (s, 3H, OMe), 3.75 (dd, $J_{5,6}$ = 6.4, $J_{5,6'}$ = 1.3 Hz, 1H, H-5), 4.04 (brs, 1H, H-4), 4.32 (dd, $J_{6,5}$ = 6.4, $J_{6',5}$ = 1.3 Hz, 2H, H-6/H-6′), 4.38 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 4.94 (dd, $J_{3,2}$ = 10.2, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 5.22 (dd, $J_{2,3}$ = 10.2, $J_{2,1}$ = 7.8 Hz, 1H, H-2); ^{13}C NMR ($CDCl_3$) δ 20.6, 20.7 (2 ×) (3 × OC(O)*Me*), 56.6 (OMe), 62.4 (C-6), 67.0, 69.0, 72.0, 73.3 (C-2/C-3/C-4/C-5), 101.8 (C-1), 169.6, 170.3, 170.8 (3 × O*C*(O)Me); FABMS 321 ((M + 1)$^+$, 7), 289 (100), 169 (49), 103 (83).

General Method for the Preparation of Triflates. Methyl 2,3,4-Tri-*O*-acetyl-6-*O*-triflyl-β-D-galactopyranoside (6). To a solution of **8** (2.2 g, 6.9 mmol) in dry CH_2Cl_2 (30 mL) at −78 °C under nitrogen was added pyridine (1.76 mL, 21.8 mmol) and then triflic anhydride (1.63 mL, 9.7 mmol) dropwise. After 10 min at −78 °C the reaction was placed in an ice bath and stirred for 1 h at 0 °C. The mixture was washed with dilute HCl (1 N, 20 mL) and H_2O (2 × 20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was passed through a short column (5 × 2 cm) of silica gel (EtOAc:hexane, 2:3) to afford **6** (2.50 g, 80%) as an unstable colorless oil. This material was used immediately in the coupling reaction with **2** to provide the thiosialoside **4** (see below).

The following were prepared in a similar manner.

Methyl 2,3,6-Tri-*O*-acetyl-4-*O*-triflyl-β-D-galactopyranoside (9) was prepared from **10** in 63% yield as colorless needles: mp 112−113 °C; $[\alpha]_D$ −31.5° (*c* 0.61, $CHCl_3$); IR 1770, 1740, 1410, 1377, 1255, 1062 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.07, 2.08, 2.11 (3 × s, 3 × 3H, 3 × OAc), 3.51 (s, 3H, OMe), 3.99 (dd, $J_{5,6}$ = 7.8, $J_{5,6'}$ = 5.6 Hz, 1H, H-5), 4.06 (dd, $J_{6,6'}$ = 10.8, $J_{6,5}$ = 7.8 Hz, 1H, H-6), 4.39 (dd, $J_{6',6}$ = 10.8, $J_{6',5}$ = 5.6 Hz, 1H, H-6′), 4.45 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 5.10 (dd, $J_{3,2}$ = 8.9, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 5.22 (dd, $J_{2,3}$ = 8.9, $J_{2,1}$ = 7.9 Hz, 1H, H-2), 5.23 (d, $J_{4,3}$ = 3.0 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.6 (2 ×) (3 × OC(O)*Me*), 56.9 (OMe), 60.6 (C-6), 67.9, 69.7, 69.9 (C-2/C-3/C-5), 80.6 (C-4), 102.0 (C-1), 118.3 (*C*F_3), 168.9, 170.0 (2 ×) (3 × O*C*(O)Me); FABMS 421 ((M − CH_3O)$^+$, 46), 333 (11), 199 (23), 183 (30), 169 (100). Anal. ($C_{14}H_{19}O_{11}SF_3$) C, H.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-triflyl-α-D-allofuranose (11) was prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose in 83% yield as a colorless oil: $[\alpha]_D$ +69.1° (*c* 1.12, $CHCl_3$); IR (neat) 3000, 1418, 1382, 1214, 1142, 1014, 872, 850, 610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.37, 1.40, 1.46, 1.60 (4 × s, 4 × 3H, 4 × $(RO)_2CMe$), 3.91 (dd, $J_{5,6'}$ = 8.7, $J_{5,6}$ = 4.5 Hz, 1H, H-5), 4.11−4.22 (m, 3H, H-4/H-6/H-6′), 4.78 (dd, $J_{2,3}$ = 5.3, $J_{2,1}$ = 4.0 Hz, 1H, H-2), 4.91 (dd, $J_{3,4}$ = 6.6, $J_{3,2}$ = 5.3 Hz, 1H, H-3), 5.84 (d, $J_{1,2}$ = 4.0 Hz, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 24.7, 26.2, 26.4, 26.8 (4 × $(RO)_2CMe$), 66.3 (C-6), 75.2, 77.7, 77.9 (C-2/C-4/C-5), 83.0 (C-3), 104.2 (C-1), 110.2, 114.3 (2 × $(RO)_2CMe_2$), 118.4 (*C*F_3); FABMS 393 ((M + 1)$^+$, 8), 377 (30), 335 (51), 317 (90), 277 (35), 259 (62), 243 (27), 203 (37), 185 (100).

(ii) Preparation of Thioglycosides of *N*-Acetylneuraminic Acid *via* Coupling with Triflates. Methyl *S*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-*O*-acetyl-6-thio-β-D-galactopyranoside (4). To a solution of **2** (1.5 g, 2.7 mmol) and **6** (1.8 g, 4.0 mmol) in dry DMF (10 mL) at 0 °C under a nitrogen atmosphere was added diethylamine (5 mL). After stirring for 2 h at 0 °C the diethylamine was removed *in vacuo*, and the residue was

diluted with ethyl acetate (30 mL), washed with dilute HCl (1 N, 20 mL), H_2O (2 × 20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography (Et_2O:EtOAc, 3:1) gave **4** (1.7 g, 77%) as an amorphous mass: mp 104−105 °C; $[\alpha]_D$ −9.5° (*c* 0.69, $CHCl_3$); IR 1749, 1708, 1371, 1221, 1047 cm^{-1}; ^1H NMR[28] ($CDCl_3$) Neu5Ac unit−δ 1.89 (s, 3H, AcN), 2.72 (dd, $J_{3e,3a}$ = 12.8, $J_{3e,4}$ = 4.6 Hz, 1H, H-3e), 3.82 (s, 3H, CO_2*Me*), 3.85−3.89 (m, 1H, H-6), 3.94 (ddd, $J_{5,NH}$ = $J_{5,4}$ = $J_{5,6}$ = 9.8 Hz, 1H, H-5), 4.13 (dd, $J_{9,9'}$ = 12.7, $J_{9,8}$ = 4.0 Hz, 1H, H-9), 4.29 (dd, $J_{9',9}$ = 12.7, $J_{9',8}$ = 2.1 Hz, 1H, H-9′), 4.92 (ddd, $J_{4,3a}$ = $J_{4,5}$ = 9.8, $J_{4,3e}$ = 4.6 Hz, 1H, H-4), 5.24 (d, $J_{NH,5}$ = 9.8 Hz, 1H, N*H*), 5.26−5.29 (m, 2H, H-7/H-8); Gal unit−δ 2.66 (dd, $J_{6,6'}$ = 14.4, $J_{6,5}$ = 7.2 Hz, 1H, H-6), 2.92 (dd, $J_{6',6}$ = 14.4, $J_{6',5}$ = 7.2 Hz, 1H, H-6′), 3.54 (s, 3H, OMe), 3.85−3.89 (m, 1H, H-5), 4.55 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1), 5.07 (dd, $J_{3,2}$ = 10.4, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 5.16 (dd, $J_{2,3}$ = 10.4, $J_{2,1}$ = 7.7 Hz, 1H, H-2), 5.53 (d, $J_{4,3}$ = 3.2 Hz, 1H, H-4); others−δ 1.96, 2.02, 2.03, 2.05, 2.13, 2.15, 2.18 (7 × s, 7 × 3H, 7 × OAc); ^{13}C NMR[29] ($CDCl_3$) Neu5Ac unit−δ 23.1 (NC(O)*Me*), 38.0 (C-3), 49.4 (C-5), 53.0 (CO_2*Me*), 62.3 (C-9), 67.9 (C-7), 68.9 (C-8), 71.2 (C-4), 73.8 (C-6), 83.6 (C-2), 168.2 (C-1); Gal unit−δ 29.6 (C-6), 56.8 (OMe), 66.8 (C-2), 68.2, 69.1 (C-3/C-4), 72.3 (C-5), 101.5 (C-1); others−δ 20.5 (2 ×), 20.7 (3 ×), 21.0 (7 × OC(O)*Me*), 169.5, 169.6, 169.8, 170.1 (2 ×), 170.3, 170.5, 170.7 (7 × O*C*(O)Me/N*C*(O)Me); FABMS 810 ((M + 1)$^+$, 7), 750 (8), 474 (44), 416 (29), 414 (100). Anal. ($C_{33}H_{47}NO_{20}S$) C, H, N.

The following were prepared in a similar manner.

Methyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→4)-2,3,6-tri-*O*-acetyl-4-thio-*β*-D-glucopyranoside (12) was prepared by coupling between **2** and **9** in 74% yield after chromatography (Et_2O:EtOAc, 3:1) as colorless plates: mp 107−109 °C; $[\alpha]_D$ +35.6° (*c* 0.57, $CHCl_3$); IR 1742, 1660, 1370, 1228, 1032 cm^{-1}; ^1H NMR[28] ($CDCl_3$) Neu5Ac unit−δ 1.90 (s, 3H, AcN), 2.79 (dd, $J_{3e,3a}$ = 12.7, $J_{3e,4}$ = 4.7 Hz, 1H, H-3e), 3.78−3.83 (m, 2H, H-5/H-6), 3.85 (s, 3H, CO_2*Me*), 4.22 (dd, $J_{9,9'}$ = 12.8, $J_{9,8}$ = 4.6 Hz, 1H, H-9), 4.34 (dd, $J_{9',9}$ = 12.8, $J_{9',8}$ = 2.5 Hz, 1H, H-9′), 4.95−5.03 (m, 1H, H-4), 5.25−5.29 (m, 2H, H-7/N*H*), 5.42−5.46 (m, 1H, H-8); Glc unit−δ 3.21 (dd, $J_{4,5}$ = $J_{4,3}$ = 11.0 Hz, 1H, H-4), 3.49 (s, 3H, OMe), 3.59 (dd, $J_{5,4}$ = 11.0, $J_{5,6}$ = 7.9 Hz, 1H, H-5), 4.29 (dd, $J_{6,6'}$ = 11.4, $J_{6,5}$ = 7.9 Hz, 1H, H-6), 4.39 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1), 4.82 (d, $J_{6',6}$ = 11.4 Hz, 1H, H-6′), 4.89 (dd, $J_{2,3}$ = 9.5, $J_{2,1}$ = 7.6 Hz, 1H, H-2), 4.95−5.03 (m, 1H, H-3); others−δ 2.02 (2 ×), 2.04 (2 ×), 2.07, 2.13, 2.15 (5 × s, 21H, 7 × OAc); ^{13}C NMR[29] ($CDCl_3$) Neu5Ac unit−δ 24.3 (NC(O)*Me*), 39.5 (C-3), 51.0 (C-5), 54.3 (CO_2*Me*), 63.1 (C-9), 67.9 (C-7), 69.5 (C-8), 73.4 (C-4), 75.4 (C-6), 84.8 (C-2), 169.4 (C-1); Glc unit−δ 45.2 (C-4), 57.5 (OMe), 66.3 (C-6), 69.8 (C-3), 73.7 (C-2), 75.4 (C-5), 102.1 (C-1); others−δ 21.6 (2 ×), 21.8 (4 ×), 22.2 (7 × OC(O)-*Me*), 170.6 (2 ×), 171.0, 171.3 (3 ×), 171.7 (2 ×) (7 × O*C*(O)-Me/N*C*(O)Me); FABMS 750 ((M$^+$ − CO_2Me)$^+$, 15), 476 (10), 474 (24), 416 (28), 414 (100). Anal. ($C_{33}H_{47}NO_{20}S$) C, H, N.

Methyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (13) was prepared by coupling between **2** and **11** in 84% yield after chromatography (Et_2O) as colorless plates: mp 88−90 °C; $[\alpha]_D$ −10.6° (*c* 0.66, $CHCl_3$); IR 1743, 1668, 1548, 1440, 1374, 1224, 1062 cm^{-1}; ^1H NMR[28] ($CDCl_3$) Neu5Ac unit−δ 1.89 (s, 3H, AcN), 2.81 (dd, $J_{3e,3a}$ = 12.7, $J_{3e,4}$ = 4.7 Hz, 1H, H-3e), 3.81 (s, 3H, CO_2*Me*), 3.83 (dd, $J_{6,5}$ = 9.8, $J_{6,7}$ = 1.5 Hz, 1H, H-6), 3.96 (ddd, $J_{5,6}$ = $J_{5,NH}$ = $J_{5,4}$ = 9.8 Hz, 1H, H-5), 4.21 (dd, $J_{9,9'}$ = 12.5, $J_{9,8}$ = 5.2 Hz, 1H, H-9), 4.33 (dd, $J_{9',9}$ = 12.5, $J_{9',8}$ = 2.6 Hz, 1H, H-9′), 4.90 (ddd, $J_{4,3a}$ = 11.3, $J_{4,5}$ = 9.8, $J_{4,3e}$ = 4.7 Hz, 1H, H-4), 5.26 (d, $J_{NH,5}$ = 9.8 Hz, 1H, N*H*), 5.31 (dd, $J_{7,8}$ = 7.6, $J_{7,6}$ = 1.5 Hz, 1H, H-7), 5.43 (ddd, $J_{8,7}$ = 7.6, $J_{8,9}$ = 5.2, $J_{8,9'}$ = 2.6 Hz, 1H, H-8); Glc*f* unit−δ 3.77 (d, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 3.91 (dd, $J_{5,6'}$ = 8.5, $J_{5,6}$ = 4.2 Hz, 1H, H-5), 4.03 (dd, $J_{6,6'}$ = 11.1, $J_{6,5}$ = 4.2 Hz, 1H, H-6), 4.13−4.20 (m, 2H, H-4/H-6′), 4.87 (d, $J_{2,1}$ = 3.4 Hz, 1H, H-2), 5.79 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1); others−δ 1.30, 1.31, 1.40, 1.49 (4 × s, 4 × 3H, 4 × (RO)$_2$C*Me*), 2.02, 2.03, 2.12, 2.13 (4 × s, 4 × 3H, 4 × OAc); ^{13}C NMR[29] ($CDCl_3$) Neu5Ac unit−δ 23.0 (NC(O)*Me*), 38.4 (C-3), 49.9 (C-5), 53.0 (CO_2*Me*), 61.6 (C-9), 69.4, 69.7 (C-7/C-8), 74.3 (C-4), 74.7 (C-6), 82.9 (C-2), 168.1 (C-1); Glc*f* unit−δ 49.5 (C-3), 67.5 (C-6), 67.6 (C-4), 79.1 (C-2), 87.6 (C-5), 104.7 (C-1); others−δ 20.7, 20.9, 21.0, 21.3 (4 × OC(O)-*Me*), 25.4, 26.0, 26.5, 27.0 (4 × (RO)$_2$C*Me*), 109.2, 112.1 (2 × (RO)$_2$C*Me*$_2$), 169.7, 169.8, 170.1, 170.4, 170.7 (4 × O*C*(O)Me/N*C*(O)Me); FABMS 690 ((M$^+$ − CO_2Me)$^+$, 12), 476 (20), 474 (55) 414 (100). Anal. ($C_{32}H_{47}NO_{17}S$) C, H, N.

(iii) Synthesis of Fully Deprotected Derivatives of α-Thioglycosides of *N*-Acetylneuraminic Acid. Methyl *S*-(Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-6-thio-α-D-glucopyranoside (14). Sodium (100 mg, 4.3 mmol) was added to a solution of **1** (2.8 g, 3.5 mmol) in dry MeOH (40 mL) at 0 °C under nitrogen. After stirring for 2 h at room temperature, the MeOH was removed under reduced pressure, the residue redissolved in MeOH (40 mL), NaOH (2 N, *ca*. 5 mL) added until pH 13, and the mixture stirred overnight at room temperature. The solution was neutralized with acidic resin (Amberlite IR-120H), the resin was removed by filtration and washed with MeOH (3 × 50 mL), and the combined MeOH fractions were concentrated under reduced pressure. HPLC (H_2O) afforded **14** (1.35 g, 75%) as a white solid: mp 152−157 °C; $[\alpha]_D$ +228.4° (*c* 0.78, H_2O); IR 3415 (br), 1710, 1641, 1563, 1377, 1110, 1056 cm^{-1}; ^1H NMR[28] (D_2O) Neu5Ac unit−δ 1.77 (dd, $J_{3a,3e}$ = 12.6, $J_{3a,4}$ = 11.7 Hz, 1H, H-3a), 1.96 (s, 3H, AcN), 2.73 (dd, $J_{3e,3a}$ = 12.6, $J_{3e,4}$ = 4.5 Hz, 1H, H-3e), 3.45−3.52 (m, 1H, H-7), 3.57 (dd, $J_{9,9'}$ = 12.6, $J_{9,8}$ = 6.5 Hz, 1H, H-9), 3.57−3.63 (m, 1H, H-6), 3.64 (ddd, $J_{4,3a}$ = 11.7, $J_{4,5}$ = 10.4, $J_{4,3e}$ = 4.5 Hz, 1H, H-4), 3.79 (dd, $J_{9',9}$ = 12.6, $J_{9',8}$ = 2.4 Hz, 1H, H-9′), 3.76−3.84 (m, 2H, H-5/H-8); Glc unit−δ 2.80 (dd, $J_{6,6'}$ = 13.7, $J_{6,5}$ = 8.9 Hz, 1H, H-6), 3.22 (dd, $J_{4,3}$ = $J_{4,5}$ = 9.1 Hz, 1H, H-4), 3.25 (dd, $J_{6',6}$ = 13.7, $J_{6',5}$ = 2.3 Hz, 1H, H-6′), 3.34 (s, 3H, OMe), 3.45−3.52 (m, 2H, H-2/H-3), 3.60−3.65 (m, 1H, H-5), 4.67 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1); ^{13}C NMR[29] (D_2O) Neu5Ac unit−δ 24.3 (NC(O)*Me*), 42.6 (C-3), 54.0 (C-5), 65.1 (C-9), 70.3 (C-4), 70.6 (C-7), 73.5 (C-8), 77.0 (C-6), 80.9 (C-2), 173.8 (C-1), 177.2 (N*C*(O)Me); Glc unit−δ 32.3 (C-6), 57.3 (OMe), 72.4 (C-5), 73.6 (C-2), 75.0 (C-4), 75.1 (C-3), 101.4 (C-1); FABMS 524 ((M + 1)$^+$, 70), 314 (23), 292 (35), 279 (25), 274 (41), 242 (95), 207 (59), 185 (68), 131 (59), 115 (100). Anal. ($C_{18}H_{30}NO_{13}SNa$) C, H, N.

The following were prepared in a similar manner.

Methyl *S*-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-6-thio-α-D-glucopyranoside (15) was prepared in 78% yield after HPLC (H_2O) as an amorphous white solid: mp 168−174 °C; $[\alpha]_D$ +205.7° (*c* 0.67, H_2O); IR 3425 (br), 1710, 1638, 1552, 1374, 1120, 1046 cm^{-1}; ^1H NMR[28] (D_2O) Neu5Ac unit−δ 1.77 (dd, $J_{3a,3e}$ = 12.7, $J_{3a,4}$ = 11.3 Hz, 1H, H-3a), 2.70 (dd, $J_{3e,3a}$ = 12.7, $J_{3e,4}$ = 4.7 Hz, 1H, H-3e), 3.45 (dd, $J_{6,5}$ = 9.8, $J_{6,7}$ = 1.2 Hz, 1H, H-6), 3.57 (dd, $J_{9,9'}$ = 10.6, $J_{9,8}$ = 4.9 Hz, 1H, H-9), 3.59−3.62 (m, 1H, H-7), 3.63 (ddd, $J_{4,3a}$ = 11.3, $J_{4,5}$ = 9.8, $J_{4,3e}$ = 4.7 Hz, 1H, H-4), 3.75 (dd, $J_{9',9}$ = 10.6, $J_{9',8}$ = 1.3 Hz, 1H, H-9′), 3.77 (dd, $J_{5,6}$ = $J_{5,4}$ = 9.8 Hz, 1H, H-5), 3.76−3.80 (m, 1H, H-8); GlcNAc unit−δ 2.81 (dd, $J_{6,6'}$ = 13.8, $J_{6,5}$ = 8.9 Hz, 1H, H-6), 3.24 (dd, $J_{6',6}$ = 13.8, $J_{6',5}$ = 2.5 Hz, 1H, H-6′), 3.28 (s, 3H, OMe), 3.30 (dd, $J_{4,3}$ = $J_{4,5}$ = 9.1 Hz, 1H, H-4), 3.50−3.55 (m, 1H, H-3), 3.60−3.64 (m, 1H, H-5), 3.82 (dd, $J_{2,3}$ = 10.4, $J_{2,1}$ = 3.7 Hz, 1H, H-2), 4.59 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1); others−δ 1.92, 1.93 (2 × s, 2 × 3H, 2 × AcN); ^{13}C NMR[29] (D_2O) Neu5Ac unit−δ 43.8 (C-3), 55.4 (C-5), 66.7 (C-9), 71.6 (C-4), 72.1 (C-6), 74.9 (C-8), 78.5 (C-7), 86.1 (C-2), 176.2 (C-1); GlcNAc unit−δ 33.6 (C-6), 57.3 (C-2), 58.8 (OMe), 73.9 (C-5), 74.7 (C-3), 76.8 (C-4), 101.6 (C-1); others−δ 25.6, 25.7 (2 × NC(O)-*Me*), 178.8, 178.9 (2 × N*C*(O)Me); FABMS 565 ((M + 1)$^+$, 23), 543 (54), 512 (20), 292 (57), 274 (100). Anal. ($C_{20}H_{33}N_2O_{13}$-SNa) C, H, N.

Methyl *S*-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-6-thio-*β*-D-galactopyranoside (16) was prepared in 78% yield after HPLC (H_2O) as an amorphous white solid: mp 103−106 °C; $[\alpha]_D$ +31.0° (*c* 0.64, H_2O); IR 3420 (br), 1707, 1641, 1554, 1377, 1056 cm^{-1}; ^1H NMR[28] (D_2O) Neu5Ac unit−δ 1.73 (dd, $J_{3a,3e}$ = 12.6, $J_{3a,4}$ = 11.4 Hz, 1H, H-3a), 1.92 (s, 3H, AcN), 2.70 (dd, $J_{3e,3a}$ = 12.6, $J_{3e,4}$ = 4.6 Hz, 1H, H-3e), 3.43−3.47 (m, 1H, H-6), 3.52−3.57 (m, 1H, H-8), 3.54 (dd, $J_{9,9'}$ = 11.9, $J_{9,8}$ = 5.2 Hz, 1H, H-9), 3.62−3.66 (m, 1H, H-4), 3.67−3.70 (m, 1H, H-7), 3.73 (dd, $J_{5,6}$ = $J_{5,4}$ = 9.8 Hz, 1H, H-5), 3.78 (dd, $J_{9',9}$ = 11.9, $J_{9',8}$ = 2.6 Hz, 1H, H-9′); Gal unit−δ 2.81 (dd, $J_{6,6'}$ = 13.9, $J_{6,5}$ = 6.6

Hz, 1H, H-6), 2.90 (dd, $J_{6',6}$ = 13.9, $J_{6',5}$ = 7.3 Hz, 1H, H-6′), 3.35 (dd, $J_{2,3}$ = 9.8, $J_{2,1}$ = 7.9 Hz, 1H, H-2), 3.44 (s, 3H, OMe), 3.48 (dd, $J_{3,2}$ = 9.8, $J_{3,4}$ = 3.3 Hz, 1H, H-3), 3.62−3.66 (m, 1H, H-5), 3.87 (d, $J_{4,3}$ = 3.3 Hz, 1H, H-4), 4.15 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1); ^{13}C NMR[29] (D_2O) Neu5Ac unit−δ 25.2 (NC(O)*Me*), 43.3 (C-3), 54.7 (C-5), 65.9 (C-9), 70.9 (C-4), 71.1 (C-6), 74.6 (C-7), 78.1 (C-8), 86.8 (C-2), 175.6 (C-1), 178.1 (N*C*(O)Me); Gal unit−δ 32.3 (C-6), 60.3 (OMe), 72.2 (C-4), 73.5 (C-2), 75.9 (C-3), 76.9 (C-5), 107.0 (C-1); FABMS 524 ((M + 1)$^+$, 9), 502 (32), 469 (12), 292 (50), 277 (33), 274 (51), 257 (25), 201 (100). Anal. ($C_{18}H_{30}NO_{13}SNa$) C, H, N.

Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→6)-1,2-*O*-isopropylidene-6-thio-α-D-glucofuranose (17) was prepared in 72% yield after HPLC (H_2O) as an amorphous mass: mp 120−124 °C; [α]$_D$ +10.3° (*c* 0.77, H_2O); IR 3460 (br), 1712, 1620, 1378, 1214, 1070 cm^{-1}; ^{1}H NMR (D_2O) Neu5Ac unit−δ 1.75 (dd, $J_{3a,3e}$ = 12.7, $J_{3a,4}$ = 11.2 Hz, 1H, H-3a), 1.90 (s, 3H, AcN), 2.68 (dd, $J_{3e,3a}$ = 12.7, $J_{3e,4}$ = 4.5 Hz, 1H, H-3e), 3.42 (d, $J_{6,5}$ = 9.8 Hz, 1H, H-6), 3.52 (dd, $J_{9,9'}$ = 11.5, $J_{9,8}$ = 5.8 Hz, 1H, H-9), 3.56 (dd, $J_{5,6}$ = $J_{5,4}$ = 9.8 Hz, 1H, H-5), 3.63 (ddd, $J_{4,3a}$ = 11.2, $J_{4,5}$ = 9.8, $J_{4,3e}$ = 4.5 Hz, 1H, H-4), 3.73 (dd, $J_{9',9}$ = 11.5, $J_{9',8}$ = 2.7 Hz, 1H, H-9′), 3.83−3.89 (m, 2H, H-7/H-8); Glc unit−δ 1.22, 1.39, (2 × s, 2 × 3H, 2 × (RO)$_2$C*Me*), 2.79 (dd, $J_{6,6'}$ = 13.3, $J_{6,5}$ = 6.9 Hz, 1H, H-6), 3.11 (dd, $J_{6',6}$ = 13.3, $J_{6',5}$ = 2.1 Hz, 1H, H-6′), 3.71−3.79 (m, 2H, H-4/H-5), 4.17 (d, $J_{3,4}$ = 2.3 Hz, 1H, H-3), 4.55 (d, $J_{2,1}$ = 3.6 Hz, 1H, H-2), 5.88 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1); ^{13}C NMR (D_2O) Neu5Ac unit−δ 24.6 (NC(O)*Me*), 44.6 (C-3), 55.5 (C-5), 66.3 (C-9), 70.8 (C-4), 72.3 (C-8), 75.5 (C-6), 78.4 (C-7), 88.6 (C-2), 176.3 (C-1), 177.7 (N*C*(O)Me); Glc unit−δ 28.9, 29.3 (2 × (RO)$_2$C*Me*), 37.3 (C-6), 71.9 (C-4), 77.3 (C-3), 85.6 (C-5), 88.2 (C-2), 108.3 (C-1), 116.3 ((RO)$_2$*C*Me$_2$); FABMS 572 ((M + Na$^+$, 8), 550 (16), 223 (25), 131 (60), 115 (100).

Methyl *S*-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→5)-5-thio-D-ribofuranoside (18) was prepared in 74% yield after HPLC (H_2O) as an amorphous white solid: mp 120−125 °C; [α]$_D$ +14.7° (*c* 0.97, H_2O); IR 3385 (br), 1705, 1632, 1440, 1374, 1122, 1024 cm^{-1}; ^{1}H NMR (D_2O) Neu5Ac unit−δ 1.78 (dd, $J_{3a,3e}$ = 12.7, $J_{3a,4}$ = 11.9 Hz, 1H, H-3a), 1.94 (s, 3H, AcN), 2.72 (dd, $J_{3e,3a}$ = 12.7, $J_{3e,4}$ = 4.2 Hz, 1H, H-3e), 3.47 (d, $J_{6,5}$ = 9.6 Hz, 1H, H-6), 3.52−3.59 (m, 2H, H-5/H-9), 3.65 (ddd, $J_{4,3a}$ = 11.9, $J_{4,5}$ = 10.0, $J_{4,3e}$ = 4.2 Hz, 1H, H-4), 3.74−3.83 (m, 3H, H-7/H-8/H-9′); Rib unit−δ 2.91 (dd, $J_{5,5'}$ = 13.7, $J_{5,4}$ = 6.6 Hz, 1H, H-5), 3.00 (dd, $J_{5',5}$ = 13.7, $J_{5',4}$ = 5.1 Hz, 1H, H-5′), 3.30 (s, 3H, OMe), 3.97 (d, $J_{2,3}$ = 4.6 Hz, 1H, H-2), 4.02 (ddd, $J_{4,5}$ = 6.6, $J_{4,3}$ = 6.0, $J_{4,5'}$ = 5.1 Hz, 1H, H-4), 4.13 (dd, $J_{3,4}$ = 6.0, $J_{3,2}$ = 4.6 Hz, 1H, H-3), 4.78 (s, 1H, H-1). ^{13}C NMR (D_2O) Neu5Ac unit−δ 24.7 (NC(O)*Me*), 43.1 (C-3), 54.3 (C-5), 65.4 (C-9), 74.1, 76.1, 77.0, 77.5 (C-4/C-6/C-7/C-8), 86.8 (C-2), 175.8 (C-1), 177.6 (N*C*(O)Me); Rib unit−δ 35.3 (C-5), 57.8 (OMe), 70.8, 70.9 (C-2/C-3), 83.8 (C-4), 110.6 (C-1); FABMS 472 ((M − Na)$^+$, 5), 292 (10), 277 (34), 257 (20), 215 (27), 201 (100).

Methyl *S*-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→4)-4-thio-β-D-glucopyranoside (19) was prepared in 77% yield after HPLC (H_2O) as an amorphous white solid: mp 140−145 °C; [α]$_D$ +189.6° (*c* 1.10, H_2O); IR 3430 (br), 1710, 1640, 1552, 1376, 1275, 1058 cm^{-1}; ^{1}H NMR[28] (D_2O) Neu5Ac unit−δ 1.83 (dd, $J_{3a,3e}$ = 12.1, $J_{3a,4}$ = 11.9 Hz, 1H, H-3a), 1.97 (s, 3H, AcN), 2.80 (dd, $J_{3e,3a}$ = 12.1, $J_{3e,4}$ = 4.2 Hz, 1H, H-3e), 3.48−3.53 (m, 1H, H-7), 3.55 (dd, $J_{9,9'}$ = 12.3, $J_{9,8}$ = 5.8 Hz, 1H, H-9), 3.62 (d, $J_{6,5}$ = 10.2 Hz, 1H, H-6), 3.68 (ddd, $J_{4,3a}$ = 11.9, $J_{4,5}$ = 10.2, $J_{4,3e}$ = 4.2 Hz, 1H, H-4), 3.79−3.86 (m, 3H, H-5/H-8/H-9); Gal unit−δ 2.82 (dd, $J_{4,3}$ = $J_{4,5}$ = 10.2 Hz, 1H, H-4), 3.22 (dd, $J_{2,3}$ = 9.2, $J_{2,1}$ = 8.0 Hz, 1H, H-2), 3.47 (dd, $J_{3,4}$ = 10.2, $J_{3,2}$ = 9.2 Hz, 1H, H-3), 3.49−3.53 (m, 1H, H-5), 3.50 (s, 3H, OMe), 3.73 (dd, $J_{6,6'}$ = 12.2, $J_{6,5}$ = 6.5 Hz, 1H, H-6), 4.15 (dd, $J_{6',6}$ = 12.2, $J_{6',5}$ = 1.8 Hz, 1H, H-6′), 4.27 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1); ^{13}C NMR[29] (D_2O) Neu5Ac unit−δ 24.6 (NC(O)*Me*), 43.1 (C-3), 54.1 (C-5), 65.5 (C-9), 70.4 (C-4), 70.8 (C-7), 73.4 (C-6), 77.5 (C-8), 86.5 (C-2), 174.7 (C-1), 177.5 (N*C*(O)Me); Glc unit−δ 49.5 (C-4), 59.5 (OMe), 64.5 (C-6), 76.6 (2 ×) (C-2/C-3), 78.3 (C-5), 105.5 (C-1); FABMS 524 ((M + 1)$^+$, 4), 502 (25), 470 (13), 292 (74), 274 (73), 201 (32), 185 (100). Anal. ($C_{18}H_{30}NO_{13}SNa$) C, H, N.

(iv) Synthesis of 9-*O*-Acetyl Derivatives of α-(2→6)-Thioglycosides of *N*-Acetylneuraminic Acid. Methyl *S*-(5-Acetamido-9-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-6-thio-α-D-glucopyranoside (21). To a solution of **14** (300 mg, 0.57 mmol) in DMSO (2 mL) at room temperature under nitrogen was added trimethyl orthoacetate (750 μL, 5.8 mmol) and *p*-TsOH·H_2O (10 mg). The solution was stirred at room temperature for 2 h and then applied to a column (7 × 1 cm) of Dowex 1-X4 (HCO_2^-) ion exchange resin (100−200 mesh). The column was washed with water (50 mL) and then eluted with formic acid (1 N, 100 mL). The eluant was concentrated under reduced pressure and then purified by HPLC (H_2O) to give **21** (230 mg, 74%) as a white solid: mp 151−154 °C; [α]$_D$ +112.9° (*c* 0.72, H_2O); IR 3420 (br), 1728, 1620, 1578, 1377, 1119, 1038 cm^{-1}; ^{1}H NMR[28] (D_2O) Neu5Ac unit−δ 1.74 (dd, $J_{3a,3e}$ = 12.4, $J_{3a,4}$ = 11.7 Hz, 1H, H-3a), 1.97 (s, 3H, AcN), 2.07 (s, 3H, OAc), 2.74 (dd, $J_{3e,3a}$ = 12.4, $J_{3e,4}$ = 4.3 Hz, 1H, H-3e), 3.53−3.58 (m, 1H, H-7), 3.61−3.69 (m, 1H, H-4), 3.77 (dd, $J_{5,6}$ = $J_{5,4}$ = 10.1 Hz, 1H, H-5), 3.78 (dd, $J_{6,5}$ = 10.1, $J_{6,7}$ = 2.4 Hz, 1H, H-6), 4.01 (ddd, $J_{8,7}$ = 8.7, $J_{8,9}$ = 6.0, $J_{8,9'}$ = 1.8 Hz, 1H, H-8), 4.12 (dd, $J_{9,9'}$ = 11.7, $J_{9,8}$ = 6.0 Hz, 1H, H-9), 4.35 (dd, $J_{9',9}$ = 11.7, $J_{9',8}$ = 1.8 Hz, 1H, H-9′); Glc unit−δ 2.80 (dd, $J_{6,6'}$ = 13.6, $J_{6,5}$ = 8.7 Hz, 1H, H-6), 3.20 (dd, $J_{4,3}$ = 10.7, $J_{4,5}$ = 3.4 Hz, 1H, H-4), 3.26 (dd, $J_{6',6}$ = 13.6, $J_{6',5}$ = 3.1 Hz, 1H, H-6′), 3.35 (s, 3H, OMe), 3.50 (dd, $J_{2,3}$ = 9.7, $J_{2,1}$ = 3.7 Hz, 1H, H-2), 3.53−3.58 (m, 1H, H-3), 3.61−3.69 (m, 1H, H-5), 4.68 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1); ^{13}C NMR[29] (D_2O) Neu5Ac unit−δ 23.0 (OC(O)-*Me*), 24.8 (NC(O)*Me*), 43.5 (C-3), 54.4 (C-5), 68.3 (C-9), 71.0 (C-4), 72.1 (C-8), 73.1 (C-6), 77.1 (C-7), 87.2 (C-2), 177.1 (C-1), 177.7 (N*C*(O)Me/O*C*(O)Me); Glc unit−δ 32.9 (C-6), 57.8 (OMe), 71.2 (C-5), 74.0 (C-2), 75.5 (C-4), 75.6 (C-3), 101.8 (C-1); FABMS 582 ((M + K)$^+$, 18), 566 ((M + Na)$^+$, 25), 315 (15), 283 (47), 239 (30), 223 (98), 207 (95), 185 (100). Anal. ($C_{20}H_{33}NO_{14}S·2H_2O$) C, H, N.

The following were prepared in a similar manner.

Methyl *S*-(5-acetamido-9-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-6-thio-α-D-glucopyranoside (22) was prepared in 78% yield after HPLC (H_2O) as an amorphous white solid: mp 166−170 °C; [α]$_D$ +101.6° (*c* 0.52, H_2O); IR 3395 (br), 1720, 1640, 1548, 1438, 1378, 1266, 1126, 1040 cm^{-1}; ^{1}H NMR[28] (D_2O) Neu5Ac unit−δ 1.83 (dd, $J_{3a,3e}$ = 12.4, $J_{3a,4}$ = 11.6 Hz, 1H, H-3a), 2.10 (s, 3H, OAc), 2.77 (dd, $J_{3e,3a}$ = 12.4, $J_{3e,4}$ = 4.5 Hz, 1H, H-3e), 3.57 (dd, $J_{7,8}$ = 8.7, $J_{7,6}$ = 1.4 Hz, 1H, H-7), 3.62−3.72 (m, 2H, H-4/H-6), 3.83 (dd, $J_{5,6}$ = $J_{5,4}$ = 10.1 Hz, 1H, H-5), 4.05 (ddd, $J_{8,7}$ = 8.7, $J_{8,9}$ = 6.0, $J_{8,9'}$ = 2.0 Hz, 1H, H-8), 4.15 (dd, $J_{9,9'}$ = 11.6, $J_{9,8}$ = 6.0 Hz, 1H, H-9), 4.38 (dd, $J_{9',9}$ = 11.6, $J_{9',8}$ = 2.0 Hz, 1H, H-9′); GlcNAc unit−δ 2.87 (dd, $J_{6,6'}$ = 13.8, $J_{6,5}$ = 8.8 Hz, 1H, H-6), 3.29 (dd, $J_{6',6}$ = 13.8, $J_{6',5}$ = 2.6 Hz, 1H, H-6′), 3.33−3.37 (m, 1H, H-4), 3.34 (s, 3H, OMe), 3.58−3.72 (m, 2H, H-3/H-5), 3.89 (dd, $J_{2,3}$ = 10.4, $J_{2,1}$ = 3.6 Hz, 1H, H-2), 4.66 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1); others−δ 1.99, 2.00 (2 × s, 2 × 3H, 2 × AcN); ^{13}C NMR[29] (D_2O) Neu5Ac unit−δ 22.8 (OC(O)*Me*), 42.6 (C-3), 54.1 (C-5), 68.5 (C-9), 70.3 (C-4), 70.9 (C-7), 71.1 (C-8), 77.0 (C-6), 84.5 (C-2), 174.8 (C-1), 177.3 (O*C*(O)Me); GlcNAc unit−δ 32.4 (C-6), 56.1 (C-2), 57.6 (OMe), 72.6 (C-5), 73.4 (C-3), 75.5 (C-4), 100.4 (C-1); others−δ 24.4, 24.6 (2 × NC(O)*Me*), 176.8 (2 × N*C*(O)-Me); FABMS 585 ((M + 1)$^+$, 75), 334 (47), 316 (68), 252 (51), 220 (73), 201 (68), 185 (100). Anal. ($C_{22}H_{35}N_2O_{14}SNa·2H_2O$) C, H, N.

Methyl *S*-(5-acetamido-9-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-4-*O*-acetyl-6-thio-β-D-galactopyranoside (23) was prepared in 63% yield after HPLC (H_2O) as an amorphous white solid: mp 164−167 °C; [α]$_D$ +57.5° (*c* 0.71, H_2O); IR 3455 (br), 1726, 1640, 1552, 1376, 1246, 1062 cm^{-1}; ^{1}H NMR[28] (D_2O) Neu5Ac unit−δ 1.80 (dd, $J_{3a,3e}$ = 12.1, $J_{3a,4}$ = 11.4 Hz, 1H, H-3a), 2.00 (s, 3H, AcN), 2.76 (dd, $J_{3e,3a}$ = 12.1, $J_{3e,4}$ = 4.3 Hz, 1H, H-3e), 3.59 (dd, $J_{7,8}$ = 8.2, $J_{7,6}$ = 1.5 Hz, 1H, H-7), 3.62 (dd, $J_{6,5}$ = 10.2, $J_{6,7}$ = 1.5 Hz, 1H, H-6), 3.72 (ddd, $J_{4,3a}$ = 11.4, $J_{4,5}$ = 10.2, $J_{4,3e}$ = 4.3 Hz, 1H, H-4), 3.80−3.84 (m, 1H, H-5), 3.96 (ddd, $J_{8,7}$ = 8.2, $J_{8,9}$ = 6.5, $J_{8,9'}$ = 2.1 Hz, 1H, H-8), 4.14 (dd, $J_{9,9'}$ = 11.7, $J_{9,8}$ = 6.5 Hz, 1H, H-9), 4.39 (dd, $J_{9',9}$ = 11.7, $J_{9',8}$ = 2.1 Hz, 1H, H-9′); Gal unit−δ 2.78 (dd, $J_{6,6'}$ = 14.0, $J_{6,5}$ = 6.2 Hz, 1H, H-6), 2.88 (dd, $J_{6',6}$ = 14.0, $J_{6',5}$ = 7.2 Hz, 1H, H-6′), 3.46 (dd, $J_{2,3}$ = 9.7, $J_{2,1}$ = 7.9 Hz, 1H, H-2), 3.54 (s, 3H, OMe), 3.80−3.84 (m, 1H, H-3), 3.90 (dd, $J_{5,6'}$ = 7.2, $J_{5,6}$ = 6.2 Hz, 1H, H-5),

4.32 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 5.32 (d, $J_{4,3}$ = 3.2 Hz, 1H, H-4); others−δ 2.11, 2.16 (2 × s, 2 × 3H, 2 × OAc); ^{13}C NMR[29] (D_2O) Neu5Ac unit−δ 24.6 (NC(O)*Me*), 42.7 (C-3), 54.2 (C-5), 68.7 (C-9), 70.5 (C-4), 70.9 (C-7), 71.6 (C-8), 77.3 (C-6), 86.3 (C-2), 175.0 (C-1); Gal unit−δ 31.6 (C-6), 59.8 (OMe), 73.2 (C-2), 73.9, 74.1 (C-3/C-4), 75.1 (C-5), 106.3 (C-1); others−δ 22.7, 22.9 (2 × OC(O)*Me*), 176.1, 176.9, 177.5 (2 × O*C*(O)Me/ N*C*(O)Me); FABMS 608 ((M + Na)$^+$, 30), 586 ((M + 1)$^+$, 75), 335 (70), 334 (70), 316 (62), 292 (27), 274 (29), 201 (68), 185 (100). Anal. ($C_{22}H_{35}NO_{15}S\cdot 2H_2O$) C, H, N.

Biological Evaluation. Cells. MA104 cells, a monkey kidney cell line, were grown at 37 °C in RPMI supplemented with 10% fetal calf serum, 20 mM HEPES, 2 mM L-glutamine, 2 mM pyruvate, 26.6 μg/mL gentamicin, and 2 μg/mL fungizone.

Virus. All viruses were cultivated in MA104 cells. Virus was preactivated with 10 μg/mL porcine trypsin (type IX; Sigma) for 30 min at 37 °C before inoculating onto confluent cell monolayers grown in 200 mL flat surface glass bottles at a multiplicity of inection of 10 plaque-forming units/cell. After 60 min at 37 °C, the infected cells were incubated in Eagles minimal medium containing 1 μg/mL porcine trypsin until extensive cytopathic effect (cpe) was evident. Cell lysates were frozen, thawed twice, and centrifuged (3000*g*, 10 min), and the supernatants were stored at −70 °C. Virus stocks were activated prior to use by incubation of cell lysates with 10 μg/mL porcine trypsin for 30 min at 37 °C. The reaction was stopped by the addition of fetal calf serum (final concentration of 2%). For use in neutralization assays, the virus was titrated to determine a dilution which gave ∼200 fluorescent focus forming units/well of the 96 well-microtiter tray.

Neutralization Assays. *N*-Acetylneuraminic acid analogues (starting concentration, 50 mM) were serially diluted (2-fold) in virus diluent supplemented with 20 mM HEPES and incubated with equal volumes of rotavirus for 1 h at 37 °C. The cell−virus mixture was then added to confluent monolayers of MA104 cells grown in 96-well microtiter trays and left for 1 h at 37 °C. The inoculum was then removed and replaced with maintenance medium and left for 16 h at 37 °C in a 5% CO_2 environment. Neutralization was determined by indirect immunofluorescent staining.

Indirect Immunofluorescence. Rotavirus-infected cell monolayers were incubated in 80% acetone for 10 min. The cells were then washed three times with PBS and covered with hyperimmune rabbit anti-SA11 serum and incubated at 37 °C for 30 min. Following this, cells were stained with fluorescein isothiocyanate-conjugated anti-rabbit immunoglobulin. After 30 min at 37 °C cells were washed three times with PBS and viewed through a fluorescent microscope. Results were expressed as the concentration of the compound at which 50% infection of control infected monolayers occurred.

Conclusion. The synthesis of a number of novel thiosialosides is reported. These compounds have been evaluated for biological activity against four strains of rotavirus and the results suggest that *O*-acetylation is an important factor for rotavirus recognition. Equally as important is the conclusion, from these data, that there are considerable differences in receptor specificity between the rotaviral strains. We are presently investigating the synthesis of alternatively linked thiosialosides which have varying degrees of *O*-acetylation and therefore may provide further information about the receptor specificity of rotaviruses.

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