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Structural and functional-group tuning in the design of neuraminidase inhibitors ☆

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Abstract

Analogues of the disaccharide α-NeuAc-(2 → 6)-β-D-Gal-OR have been made by modifications at C-1 and C-6 of the galactose and at C-4 of the NeuAc unit, for structure–activity relationship studies with influenza virus neuraminidase. These studies indicate that for the influenza neuraminidase, a larger aglycon at C-1 of galactose is less preferred, whereas the restriction of the rotamer orientation at C-6 of galactose in the "*tg*" mode favors enzyme binding. Substitution at C-4 of the NeuAc unit has the most profound effect in the influenza neuraminidase hydrolysis and inhibition. For example, azido and acetamido groups at C-4 of the NeuAc units render the sialosides resistant to neuraminidase hydrolysis. However, these derivatives are not inhibitors of the neuraminidase, indicating their lack of binding. On the other hand, a 4-amino substitution of the NeuAc unit not only renders the corresponding sialosides neuraminidase-resistant, but also makes them potent neuraminidase inhibitors. This potent inhibition indicates that the 4-amino groups in these sialosides may engage in favorable interaction with amino acids at the neuraminidase active-site. The conclusion is also supported by docking studies of the carbohydrate structures at the neuraminidase active-site.

Keywords: Neuraminidase inhibitors; Structure–activity relationship; Influenza virus neuraminidase

1. Introduction

Neuraminidases (NAs) are enzymes found on the surface of bacterial and viral outer membranes and are involved in the hydrolytic processing of terminal sialic acid (NeuAc)

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units of glycoproteins and glycolipids [1]. This hydrolytic processing is important in infectious processes and in the correct assembly of viral glycoproteins [2]. Accordingly, inhibitors of such neuraminidases should block viral and bacterial pathogenesis and should serve as drugs in undesired infectious processes. Influenza neuraminidase has been one such target for the design of anti influenza drugs. Based on a knowledge of the neuraminidase active-site, potent carbohydrate based inhibitors of the influenza neuraminidase have been made recently by von Itzstein et al. [3,4]. These are 2,3-dehydrosialic acid derivatives containing an amino or guanidino group at C-4.

Influenza virus has, in addition to neuraminidase, another sialic acid binding protein known as hemagglutinin (HA), which facilitates the virus to establish a foothold on the surface of the host cells [5]. The 2,3-dehydrosialic acid derivatives that are potent inhibitors of the influenza neuraminidase do not inhibit action of the hemagglutinins [6,7], as this protein requires sialic acid in the α-ketosidically bound form [8,9]. Typically, the HAs require an α-ketosidically linked NeuAc unit in a pyranose chair conformation [7,10,11], whereas the NAs preferably bind a 2,3-dehydro-NeuAc unit that resembles the transition-state pseudoboat structure of the hydrolytic process [12]. In addition, the HAs seem to bind to the sialosides in a cluster fashion by establishing multi-site attachment between the trimeric protein and the multi-antennary sialic acid units of the glycoprotein [13]. Such a requirement does not appear to be needed for the NAs. Due to these conflicting requirements, the design of sialoside inhibitors common to both HA and NA has not been successful to date. Many synthetic analogues, ranging from α-sialoside-containing polymers [14,15] to simple mono- [16] to bi-valent sialo-

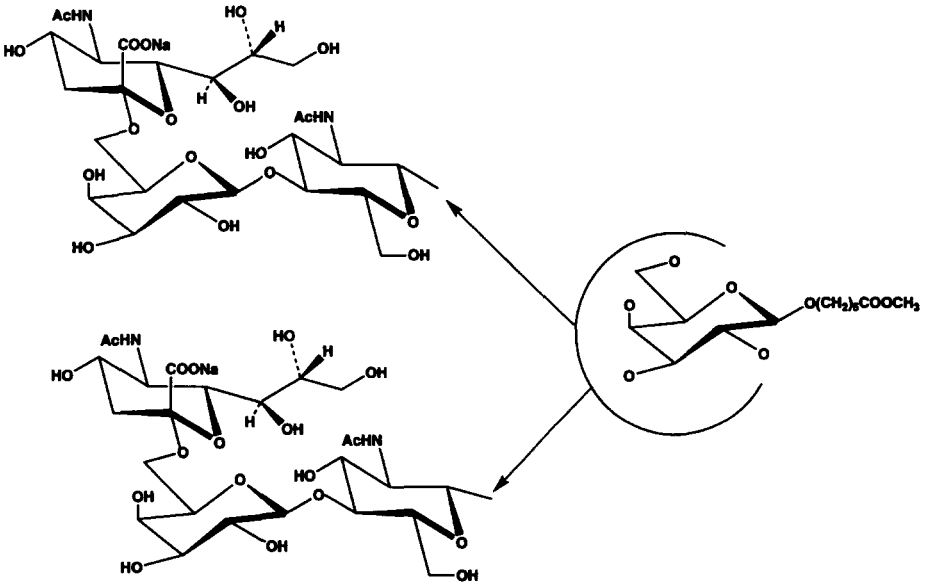
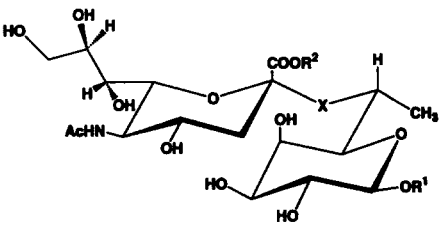


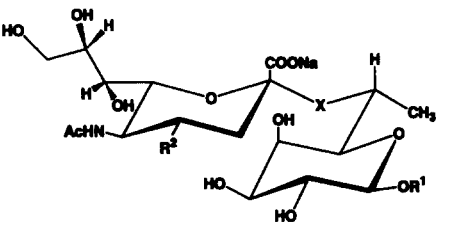
Fig. 1. The heptasaccharide containing two sialoside units for establishing multiple contact with the influenza virus hemagglutinin. The two trisaccharide fragments, α-NeuAc-(2 → 6)-β-D-Gal-(1 → 4)-β-D-GlcNAc are attached via the C-1 of the GlcNAc unit to any of the four hydroxyl groups of a galactose containing the 5-(methoxycarbonyl)pentyl unit.

sides [17–19] have been prepared and these inhibit the HA activity of the influenza virus better than a monovalent structure. To avoid the detrimental action of influenza neuraminidase on these analogues, modified derivatives such as C-linked sialosides have been made [20,21]. These are neuraminidase resistant, but are not inhibitors of the NAs as such.

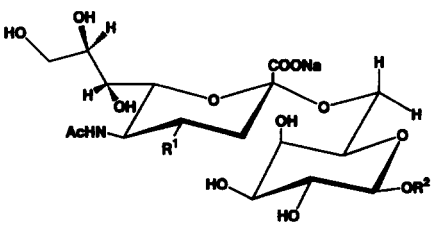
In recent years [19,22], our efforts have been to understand the key sialoside structural features required for both influenza hemagglutinin and neuraminidase binding, and to design common carbohydrate inhibitors for these two sialic acid-binding proteins. We earlier reported [19] that bivalent heptasaccharides containing α-NeuAc-(2 → 6)-β-D-Gal residues such as shown in Fig. 1, were able to bind to influenza virus HAs 10 times better than a monovalent sialoside. These are neuraminidase cleavable substrates. In an effort to convert these HA inhibitors into NA inhibitors, we undertook a sytematic study of the sialoside structural requirements for efficient recognition by the influenza NAs. These investigations concerned the role of (*1*) the aglycon that is linked to the NeuAc unit and in particular the rotameric preference around C-6 of galactose, (*2*) the steric bulk of the aglycon attached to C-1 of the galactose, (*3*) the contribution of the galactose hydroxyl groups in neuraminidase binding, (*4*) the effect of introducing azido, amino, and acetamido functionalities at C-4 of the NeuAc unit [23], and finally, (*5*) the effect of introducing a sulfur at C-2 of the NeuAc unit. Scheme 1 summarizes the list of



1 X = O, R^1 = H, R^2 = Na
2 X = O, R^1 = $CH_2CH_2SiMe_3$, R^2 = Na
3 X = O, R^1 = $(CH_2)_{17}CH_3$, R^2 = Et_3NH
4 X = S, R^1 = H, R^2 = Na
5 X = S, R^1 = $CH_2CH_2SiMe_3$, R^2 = Na
6 X = S, R^1 = $(CH_2)_{17}CH_3$, R^2 = Et_3NH



7 X = O, R^1 = H, R^2 = N_3
8 X = O, R^1 = $CH_2CH_2SiMe_3$, R^2 = NHAc
9 X = O, R^1 = H, R^2 = NH_2
10 X = S, R^1 = H, R^2 = N_3
11 X = S, R^1 = H, R^2 = NHAc
12 X = S, R^1 = H, R^2 = NH_2



13 R^1 = N_3, R^2 = H
14 R^1 = NHAc, R^2 = H
15 R^1 = NH_2, R^2 = H

Scheme 1. The analogues that were synthesized for biological evaluation with influenza neuraminidase.

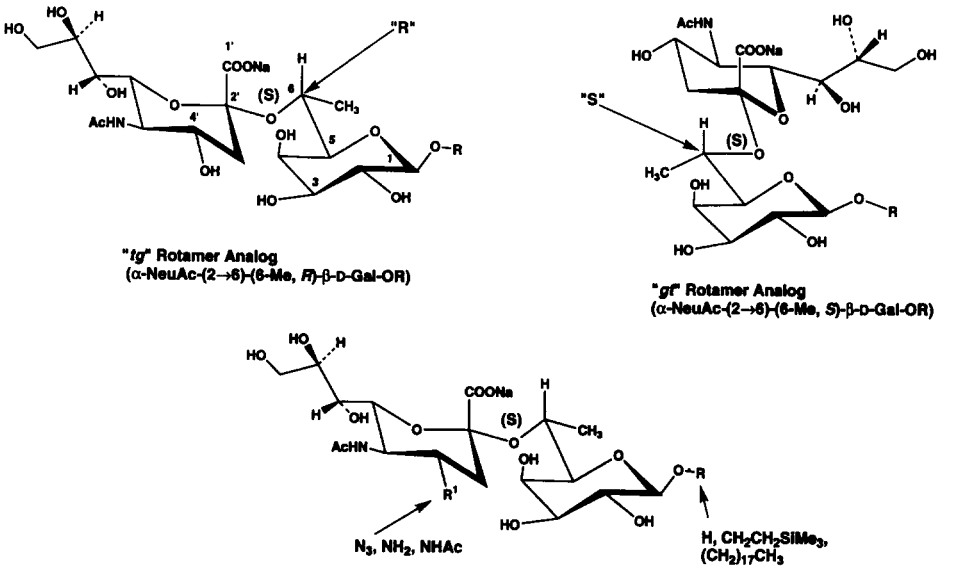


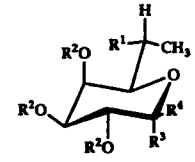
Fig. 2. The structural analogues of α-NeuAc-(2-6)-β-D-Gal-OR that were made to probe the structural requirements for efficient influenza neuraminidase binding. The arrows indicate the sites of modifications.

compounds that were made to carry out these investigations. Of these, the results of the influence of rotamer preference around C-6–O-6 of the galactose in influenza neuraminidase hydrolysis and inhibition have been reported by us recently [22]. These studies indicated that conformationally biased "*tg*" rotamer analogues, as shown in structures **2** and **4**, fit more favorably into the influenza neuraminidase binding-pocket than the "*gt*" rotamer analogues [22] (Fig. 2). Furthermore, the hydroxyl groups of the galactose residue linked to the NeuAc unit did not appear to have a major role in neuraminidase recognition. We examine in this report the effect of substitution at C-1 of the galactose and at C-4 of NeuAc unit [23] in neuraminidase hydrolysis and inhibition.

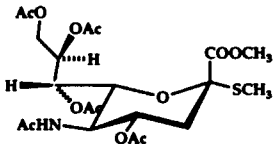
2. Results and discussion

Synthesis of sialoside analogues **2–15**.—The objective of our synthetic exercise was to generate many relevant structural analogues for evaluation with influenza neuraminidase. As shown in Scheme 1, these analogues can be classified into the following three groups. (*1*) Sialoside analogues **1–6** containing H, $CH_2CH_2SiMe_3$, and $(CH_2)_{17}CH_3$ at O-1 of the galactose for evaluation of the steric effect of the aglycon attached to the galactose carrying a NeuAc unit at its C-6 position; (*2*) analogues **7–12** containing azido, acetamido, and amino groups at C-4 of the NeuAc unit that is glycosidically linked to the 6-position of a rotamerically biased galactose; and (*3*) analogues **13–15** containing an azido, acetamido, or amino group at C-4 of the NeuAc unit that is glycosidically linked to the 6-position of a rotamerically flexible galactose.

Most of the synthetic transformations reported here proceeded in high yield (> 60%).



16 R^1 = O-allyl, R^2 = Ac, R^3 = Br, R^4 = H
17 R^1 = O-allyl, R^2 = Ac, R^3 = H, R^4 = $O(CH_2)_{17}CH_3$
18 R^1 = OH, R^2 = Bn, R^3 = H, R^4 = $O(CH_2)_{17}CH_3$



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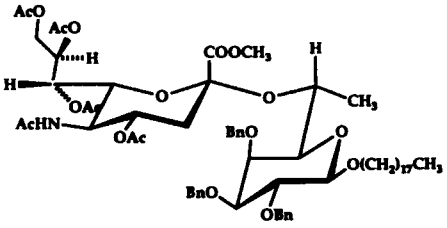

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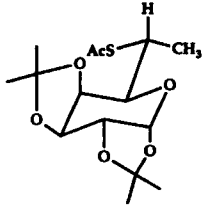

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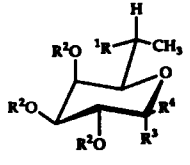
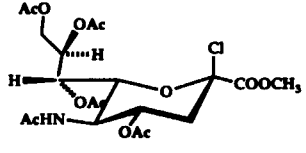


22 R^1 = SAc, R^2 = Ac, R^3 = H, R^4 = OAc or R^3 = OAc, R^4 = H
23 R^1 = SAc, R^2 = Ac, R^3 = Br, R^4 = H
24 R^1 = SAc, R^2 = Ac, R^3 = H, R^4 = $O(CH_2)_2SiMe_3$
25 R^1 = SAc, R^2 = Ac, R^3 = H, R^4 = $O(CH_2)_{17}CH_3$



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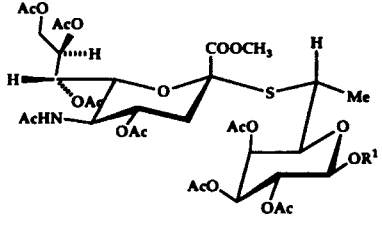
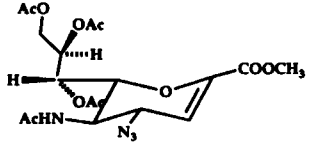


27 R^1 = $(CH_2)_2SiMe_3$
28 R^1 = $(CH_2)_{17}CH_3$



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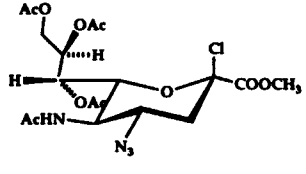

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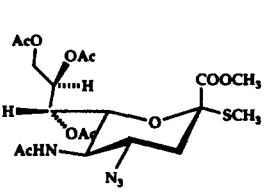
Scheme 2. The structures of the synthetic intermediates.

However, in those transformations where the yield was low, we did not attempt to optimize the reaction conditions, as enough material could be obtained for biochemical evaluations. The synthesis of derivatives **1**, **2**, and **4** has already been described [22]. To synthesize the octadecyl glycoside **3**, we started from the bromide [22] **16** (Scheme 2). Condensation of **16** with octadecanol catalyzed by silver carbonate–silver trifluoromethanesulfonate gave the β-glycoside **17**. This condensation reaction failed when only silver triflate in nitromethane (probably due to limited solubility of octadecanol in nitromethane at low temperature) or silver carbonate in CH_2Cl_2 was employed. Glycoside **17** was converted into alcohol **18** using well established methodologies [22]. Condensation of **18** with thioglycoside **19** under the conditions reported by Lönn and



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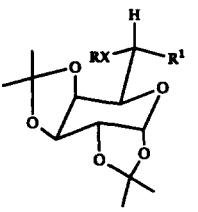
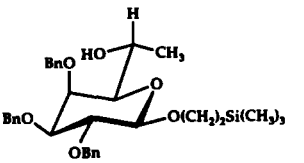


32 X = S, R = H, R^1 = Me
33 X = O, R = R^1 = H
34 X = O, R = H, R^1 = Me



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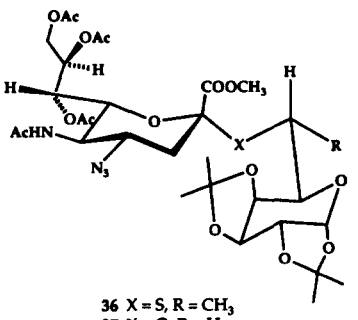
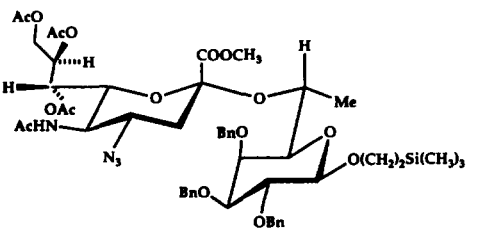


36 X = S, R = CH_3
37 X = O, R = H
38 X = O, R = CH_3



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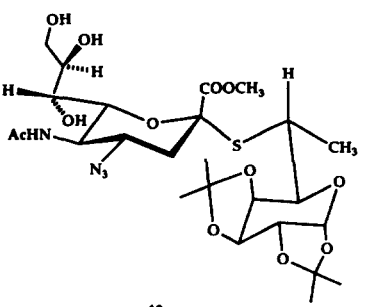

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Scheme 3. The structures of the synthetic intermediates.

Stenvall [24] gave the protected α-sialoside **20**, which upon deprotection gave the sialoside **3**.

To prepare the thiosialosides **5** and **6**, we required the bromide **23** for condensation with 2-(trimethylsilyl)ethanol or octadecanol. The bromide **23** was prepared [22] from the thioacetate derivative **21** by the removal of the isopropylidene group with aqueous 90% trifluroacetic acid, followed by acetylation to give **22**, then by the selective removal of the anomeric acetate [25], followed by reaction of the hemiacetal with Vilsmeier bromide. Condensation of **23** with 2-(trimethylsilyl)ethanol in the presence silver triflate–collidine gave the β-glycoside **24**. Condensation of **23** with octadecanol in the presence of silver carbonate–catalytic silver triflate, gave the octadecyl β-glycoside **25**. Selective removal of *S*-acetates in **24** and **25** with one equivalent of hydrazine gave the thiol, which upon condensation with the glycosyl chloride **26** according to our published method [22], gave the thioglycosides **27** and **28**. These were deprotected to **5** and **6**. The structures of these two thiosialosides were confirmed from their ^{1}H and ^{13}C NMR spectra and by comparison with the literature data [22].

To prepare the 4-azido, acetamido, and amino thio-sialosides **10–12**, and the O-linked sialosides **7–9** and **13–15**, we required the azido chloride **30** and the thioglycoside **31**, the synthesis of which were not known until our recent report [23]. We developed a short synthetic route to the chloride **30**, starting from the readily available azido glycal **29** [26,27], by reaction with hydrogen chloride in acetonitrile or acetic acid as the solvent (see Experimental). This reaction was unusual in that it succeeded only when the 4-substituent of **29** was an azide. It failed with glycals containing other allylic substituents such as acetamido, acetylthio, acetoxy groups and was only partially successful even with the 4-deoxyglycal derivative (< 20% conversion after 10 days). This azido chloride **30** which could be made in large quantities, was condensed with sodium methanethiolate to obtain **31** (Scheme 3) or with the sodium salt of **32** to furnish the protected thiodisaccharide **36**. Subsequent deprotection of this azido thiosialoside **36** to **10** was performed according to our earlier report [22]. The azide was reduced with hydrogen in presence of palladium on carbon to give the amino thiosialoside **12**. Acetylation of the amino group provided the acetamido-sialoside **11**. To synthesize the O-linked sialosides **7**, **8**, and **13**, we used the azido thiosialoside **31** as the glycosyl donor for condensation with alcohols **34** [28], **35** [22], and **33**. The condensation was carried out in presence of methylsulfenyl triflate in acetonitrile–propanonitrile mixture according to Lönn and Stenvall's procedure [24]. With alcohols **33** and **34**, yield greater than 45% of the α-sialosides **37** and **38** were obtained. The yield of **39** from alcohol **35** was low. The establishment of the α-sialosidic configuration in these sialosides **37**, **38**, and **39** was not straightforward, because of the absence of any reporter groups (typically H-4 of the NeuAc unit serves as the reporter group in natural sialosides). Even though, the H-3*ax* and H-3*eq* proton chemical shifts of the NeuAc unit in the protected sialosides **37–39** were in accordance with those expected for the α-sialosidic configuration [22], the final structural confirmation was obtained by the measurement of coupling constants between C-1 and H-3*ax* (*J* 5.6 Hz) [29,30] of the NeuAc unit in the completely deprotected disaccharides **7**, **8**, and **13**.

Biochemical evaluations with influenza neuraminidase.—Influence of the aglycon at C-1 of the galactose. Hydrolysis of the synthetic sialosides by the influenza neu-

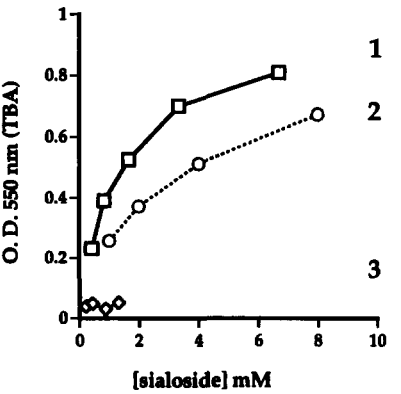

Fig. 3. Hydrolysis of **1**, **2**, and **3** by influenza virus neuraminidase.

raminidase was carried out as described in our recent report, [22,31] except [1] for the 4-azido-, amino-, and acetamido substituted sialosides (compounds **7–9** and **13–15**). Influenza neuraminidase efficiently hydrolyzed the sialosides **1** and **2**, but effected very little hydrolysis of the octadecyl glycoside **3** (Fig. 3). The Michaelis constants (K_m) for the hydrolysis of **1** and **2** were 1.35 and 2.3 mM, respectively (Table 1). On this basis, it can be concluded that the bulky group at C-1 of galactose has only a negative influence in the enzyme binding. This is in accordance with our observations that the K_m for the disaccharide α-NeuAc-(2 → 6)-β-D-Gal-$OCH_2CH_2SiMe_3$ and the trisaccharide α-NeuAc-(2 → 6)-β-D-Gal-(1 → 4)-D-GlcNAc are 1 and 6 mM, respectively. The reasons for the lack of hydrolysis of the octadecyl sialoside is not clear, especially in view of the fact that the corresponding thiosialoside **6** exhibited potent inhibition of the influenza neuraminidase (see later). On the basis of results seen with **1** and **2**, it was expected that the thio analogue **4** should be a better inhibitor than **5** and in fact, the K_i values for **4** and **5** were 0.3 and 3.2 mM, respectively (Table 1). Surprisingly, the thio analogue of the octadecyl glycoside (compound **6**) turned out to be a very potent inhibitor (K_i = 0.24 mM) of the influenza neuraminidase. This unexpected inhibition may indicate that the molecular forms of octadecyl sialosides **3** and **6** may be very different from those of **1**, **2**, **3**, and **4** and therefore a direct comparison may not be appropriate. Both **3** and **6** appear to exist as aggregates in water and in fact, may form micelles (no detergents were added in the enzyme reaction). This is borne out from the elution profile on Biogel P-2,

[1] The 4′-modified sialosides **7**, **8**, **13**, **14**, and **15** failed to give a color in the conventional periodate–resorcinol reaction. Therefore, the enzymatic hydrolysis of these O-sialosides (except for **15**) was monitored by thin-layer chromatography (TLC) on silica gel using 4:2:1 EtOAc–EtOH–water as eluant and the resulatant chromatogram was visualized by spraying with 5% H_2SO_4 in EtOH followed by heating. For comparison α-NeuAc-(2 → 6)-β-D-Gal-$OCH_2CH_2SiMe_3$ [22] was used as a standard. For **15**, the hydrolysis was monitored by TLC on silica gel using 2:2:1 EtOAc–EtOH–water as eluant and the resultant chromatograms were visualized by coating the TLC plates with 1% ninhydrin solution in EtOH followed by heating. In the case of **15**, all the starting material remained, even after incubating with influenza neuraminidase for 24 h. On this basis, a tentative conclusion about its resistance to hydrolysis was reached.

where the sialoside **6** eluted out unusually in the void volume itself and much faster than that seen for sialosides **1** and **2**. This molecular association for octadecyl sialosides was also evidenced from the proton NMR spectra of **3** and **6** in deuterium oxide, where hydrogen appeared as unusually broad signals.

Influence of the modifications at C-4 of the NeuAc unit.—The influenza neuraminidase hydrolysis of azido derivatives **7** and **13** and the acetamido sialosides **8** and **14** was monitored by TLC on silica gel. Even after 22 h, there was no evidence of hydrolysis and thus these analogues appear to be resistant to neuraminidase hydrolysis [23]. Accordingly, the thio analogues **10** and **11** were tested as inhibitors in the influenza neuraminidase hydrolysis reaction of α-NeuAc-(2 → 6)-β-LacNAc, which was ^{14}C-radio labeled in the *N*-acetyllactosamine (LacNAc) portion. As may be seen from Fig. 4, these two thio analogues did not show any inhibition.

Treatment of the amino sialoside **15** with influenza neuraminidase did not result in the hydrolysis of sialoside, as evidenced by the TLC of the mixture. However, when tested as an inhibitor with α-NeuAc-(2 → 6)-β-LacNAc as the substrate, potent inhibition of the neuraminidase was observed. From the Lineweaver–Burk plots (Fig. 5, panel B), the inhibition constant K_i was estimated to be 150 μM (Table 1). Accordingly, we tested the amino-thio-*tg*-sialoside analogue **12**. It turned out to be even a superior inhibitor (Fig. 5, panel A, $K_i = 51$ μM). Thus, the amino group in the O-linked sialoside **15** and in the thiosialoside **12** seems to enhance their binding potencies to influenza neuraminidase. Since the 4-amino group in a 2,3-dehydrosialic acid has been shown [4] to engage in salt bridge formation with the carboxyl group of Glu-119 at the neuraminidase active-site and facilitate tighter binding, it appears that such interactions may also take place in the sialosides **12** and **15**.

To probe this, we docked the minimum energy conformer of the *tg* sialoside analogue **9** into the published [32] crystal structure of the influenza neuraminidase–α-sialic acid complex (Fig. 6). The alignment of the *tg* analogue with the sialic acid was done through the glycerol side-chain. Also, a boat form of **9** (Fig. 7) was made from the

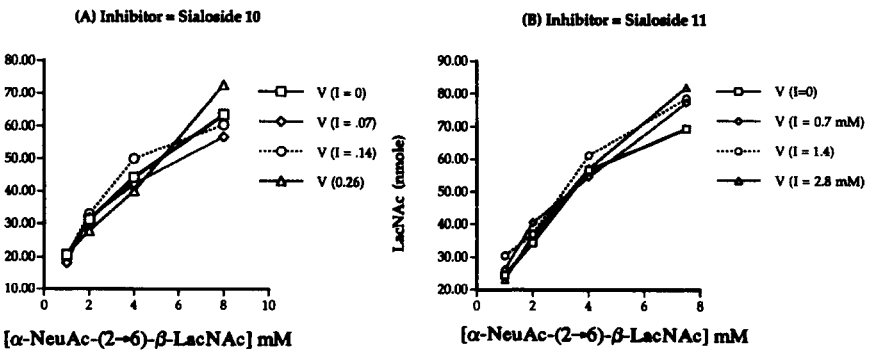

Fig. 4. The influenza neuraminidase hydrolysis of α-NeuAc-(2 → 6)-LacNAc in the presence of various concentration of the inhibitor **10** (0, 0.07, 0.14, and 0.26 mM, panel A) and inhibitor **11** (0, 0.7, 1.4, and 2.8 mM, panel B).

Table 1
The Michaelis or inhibition constants (K_m or K_i, respectively, mM) of the synthetic sialosides with influenza virus neuraminidase (see Experimental section for details)

Sialoside	Hydrolytic susceptibility [a]	K_m (mM)	K_i (mM)
α-NeuAc-(2 → 6)-(6-Me,*R*)-α,β-D-Gal (**1**)	(+)	1.4	
α-NeuAc-(2 → 6)-(6-Me,*R*)-β-D-Gal-O-$(CH_2)_2SiMe_3$ (**2**)	(+)	2.3	
α-NeuAc(2 → 6)-(6-Me,*R*)-β-D-Gal-O-$(CH_2)_{17}CH_3$ (**3**)	(−)	[b]	
α-NeuAc-(2 → 6)-(6-Me,*R*)-6-thio-α,β-D-Gal (**4**)			0.3
α-NeuAc(2 → 6)-(6-Me,*R*)-6-thio-β-D-Gal-O-$(CH_2)_2SiMe_3$ (**5**)			3.2
α-NeuAc-(2 → 6)-(6-Me,*R*)-6-thio-β-D-Gal-O$(CH_2)_{17}CH_3$ (**6**)			0.24
4-Azido-α-NeuAc-(2 → 6)-(6-Me,*R*)-α,β-D-Gal (**7**)	(−)		
4-Acetamido-α-NeuAc-(2 → 6)-(6-Me,*R*)-β-D-Gal-O$(CH_2)_2SiMe_3$ (**8**)	(−)		
4-Amino-α-NeuAc-(2 → 6)-(6-Me,*R*)-α,β-D-Gal (**9**)	[c]		
4-Azido-α-NeuAc-(2 → 6)-(6-Me,*R*)-6-thio-α,β-D-Gal (**10**)			NI [d]
4-Acetamido-a-NeuAc-(2 → 6)-(6-Me,R)-6-thio-α,β-D-Gal (**11**)			NI [d]
4-Amino-α-NeuAc-(2 → 6)-(6-Me,*R*)-6-thio-α,β-D-Gal (**12**)			0.05
4-Azido-α-NeuAc-(2 → 6)-α,β-D-Gal (**13**)	(−)		
4-Acetamido-α-NeuAc-(2 → 6)-α,β-D-Gal (**14**)	(−)		
4-Amino-α-NeuAc-(2 → 6)-α,β-D-Gal (**15**)	(−)		0.15

[a] (+) Indicates hydrolysis and (−) indicates lack of significant hydrolysis.
[b] K_m could not be determined because of lack of significant hydrolysis.
[c] Not tested.
[d] NI, no inhibition.

boat structure of the free sialic acid found in the crystal structure by adding to O2 of sialic acid unit, the C-6 of galactose and followed by energy minimization using semi-empirical methods [33,34]. From these modeling studies, we could indeed see that the the 4-amino group of the NeuAc unit of **9**, irrespective of whether the NeuAc was

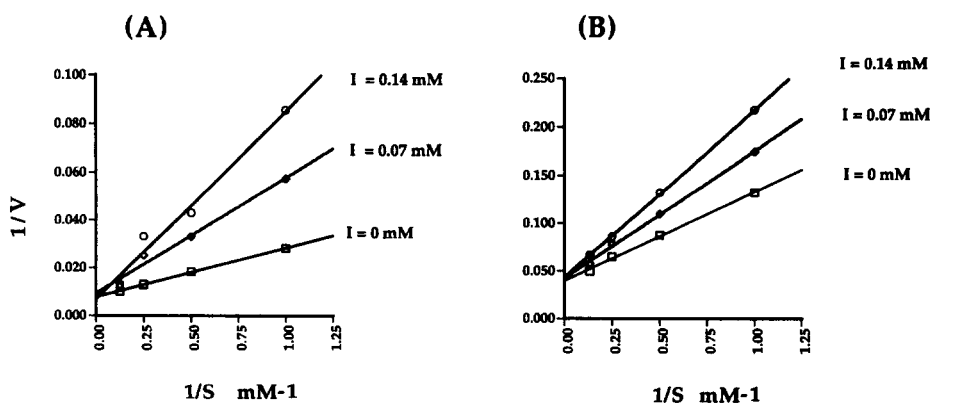

Fig. 5. Lineweaver–Burk plots for the inhibition to influenza neuraminidase hydrolysis of α-NeuAc(2-6)-β-LacNAc by sialoside inhibitors (I) **12** (panel A) and **15** (panel B).

maintained in the chair or boat conformation, was within 3.5 Å distance of the carboxyl oxygens of Glu-119 and Asp-151 (Figs. 6 and 7). This proximity should enable the establishment of salt bridges between the amino group and the carboxyl groups of these amino acids and should result in tighter binding. The K_i values (Table 1) measured for **12** and **15**, indeed reflect these additional interactions.

The lack of hydrolysis of the amino-O-sialoside **15** was, indeed, unexpected. A possible explanation for this can be provided on the basis of the above docking and the mechanistic studies of Taylor and von Itzstein [12] for the influenza neuraminidase catalyzed hydrolysis of sialosides. These authors proposed that the carboxyl group of Asp-151 and an adjacent water molecule found at the neuraminidase active-site may play a key role in the enzyme catalysis. Since the 4-amino group in the sialoside **9** is very close to Asp-151 and the water molecule, engaging these key groups by the amino function through hydrogen bonding may block the enzyme catalysis. By the same token, it can be proposed that the lack of binding of the azido and acetamido sialosides **10** and **11** may result from the unfavorable steric interaction of the bulky azido and acetamido functions with the carboxyl groups of Asp-151 and Glu-119 at the NA active-site.

3. Conclusions

These studies, taken together with our earlier results [22], indicate that tight-binding sialopyranoside inhibitors for influenza neuraminidase can be designed by proper sialoside tuning. Especially, the incorporation of a rotameric bias at C-6 of galactose and by the introduction of an amino group at C-4 of a NeuAc unit (as seen in **12**) substantially increases the inhibitory potencies by greater than 100 fold ($K_i = 51$ μM), as compared to many of the weakly binding thiosialosides made to date (compare the K_i of α-NeuAc-(2 → 6)-6-thio-β-D-Gal derivatives, which is greater than 5000 μM) [22]. The advantages of these kinds of sialo-α-pyranoside based inhibitors are that in addition to being inhibitors of the influenza NAs, these should also serve as inhibitors of the hemagglutinin. This proposal is made on the basis of the docking studies of the "*tg*" sialoside analogue **1** into the active-site of hemagglutinin complexed with 2,3-sialyllactose (unpublished). In contrast to NA, the influenza virus HA does not appear to have a rotameric preference around C-6–O-6 of the galactose, as both the "*gt*" and "*tg*" analogues can be fitted into the binding site. Now that a facile synthesis of the 4-amino sialosides is available, the preparation of the corresponding 4-guanidino sialosides should also be possible. Based on the work of Itzstein et. al., [4,35] we expect that such modifications should lead to enhancement in the binding efficiency of these inhibitors. The major drawback in all these sialopyranosides however, is that the axially oriented sialoside carboxyl group is not ideally placed for interaction with the three arginines at the influenza neuraminidase active-site. This results in a substantial loss of binding efficiency. We hope, however, that this loss can be compensated by the simultaneous inhibition of both the influenza HA and NAs by these analogues and thus may become useful antiviral drugs.

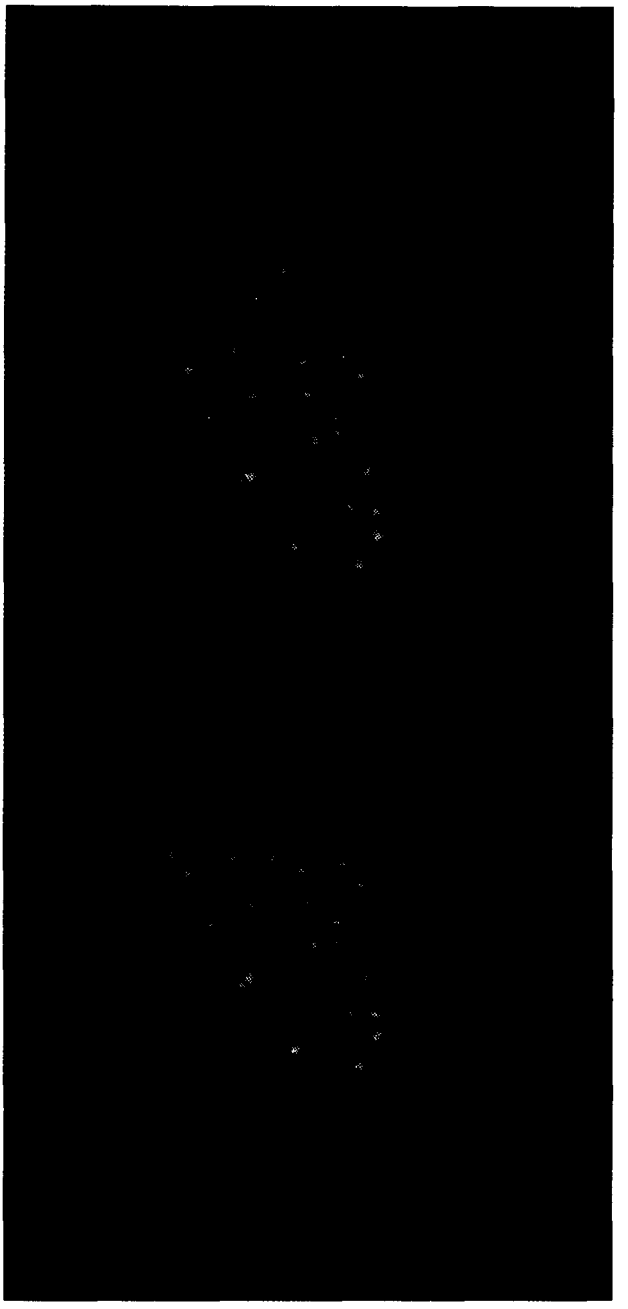

Fig. 6. The computer-docked structure of sialoside **9** at the influenza neuraminidase active site. The carbon atoms are colored green, the oxygen atoms are red, the hydrogen atoms are open circles and the nitrogen atoms are colored blue. The hydrogen bonds between the 4-amino hydrogens of the NeuAc unit of **9** and the Asp-151 of the neuraminidase are indicated by red dotted lines.

Fig. 7. The stereoview of the aminosialoside **9** with the NeuAc unit in the boat conformation, at the influenza neuraminidase active site. The NeuAc unit occupies the same position as the free sialic acid found in the crystal structure of the neuraminidase–sialic acid complex [32]. The C-6–O-6 bond of the galactose is maintained in the *gg* rotamer orientation. The hydrogen bonds between the 4-amino hydrogens of the NeuAc unit and the Asp-151 and Glu-119 of the neuraminidase are indicated by white dotted lines.

4. Experimental

Methods and materials.—Unless otherwise specified, all reagents were purchased from Aldrich Chemical Co (St. Louis, MO). Thin-layer chromatography was performed on precoated plates of Silica Gel 60 F_{254} (EM Science) and the spots were visualized with a spray containing 5% H_2SO_4 in EtOH, followed by heating. Column chromatography was done on Silica Gel 60 (230–400 mesh, EM Science). Optical rotations were measured with a Perkin–Elmer 241 polarimeter at ambient temperatures. ^1H NMR spectra were recorded at 300 or 500 MHz (GE Omega-300, GE Omega 500) and the ^{13}C NMR spectra were recorded with the same instruments operating at 75.48 or 125.74 MHz (300 and 500 MHz, respectively for protons). The hydrogen and carbon chemical shifts in organic solvents are expressed relative to tetramethylsilane (Me_4Si). For solutions of compounds in D_2O or CD_3OD, the hydrogen chemical shift values are expressed relative to the HOD signal (4.75 ppm at 296 K, internal acetone 2.23 ppm), and the carbon chemical shifts are expressed relative to external Me_4Si using the deuterium lock of the spectrometer, which set the chemical shifts of 1,4-dioxane at 66.9 ppm. The enzymatic assays with influenza neuraminidase/WSN/H1N1(virus suspension) were carried out as described earlier [22]. Elemental analyses were obtained for neutral products, but not for salt products; NMR data indicated consistent purity for all compounds.

Molecular docking studies.—The interaction of the aminosialoside **9** with the neuraminidase was probed by docking a model of the sialoside into the published influenza neuraminidase binding pocket [32]. A previously determined [22] minimum energy conformation of the disaccharide analogue **9** demonstrated a chair conformation for the NeuAc unit, whereas, in the crystal structure of the neuraminidase–sialic acid complex, the NeuAc unit was in a boat conformation. Therefore, in addition to maintaining the NeuAc unit in the disaccharide **9** in the chair conformation, we also constructed a boat form of **9**, so that the steric interaction with Tyr-406 would be avoided. The boat model was constructed as follows. To the crystal structure of sialic acid, the galactose residue from the minimum energy conformation of the *tg* sialoside **9** was superimposed along the four tetrahedrally coordinated bonds. Steric interfernce between the galactose ring and NeuAc side-chain was eliminated by manual rotation of the glycosidic bond. This structure was then energy minimized using semi-empirical methods [33,34], holding fixed the dihedral angle of the NeuAc unit.

Triethylammonium salt of octadecyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*nonulopyranylonic acid-(2 → 6)-7-deoxy*-D-glycero-β-D-galacto-*heptopyranoside* (**3**).—2,3,4-Tri-*O*-acetyl-6-*O*-allyl-7-deoxy-D-*glycero*-α-D-*galacto*-heptopyranosyl bromide [22] (**16**, 3.38 g) in CH_2Cl_2 (25 mL) was added to a suspension of octadecanol (4.16 g), Ag_2CO_3 (3.17 g), CF_3SO_3Ag (0.338 g) and 3A molecular sieves in CH_2Cl_2 (80 mL) over a period of 1 h. After 24 h, the mixture was filtered over a pad of Celite and rinsed with CH_2Cl_2. The filtrate was washed with water, dried ($MgSO_4$), and concentrated to a dry residue. This was dissolved in 60 mL of 1:1 benzene–nitromethane containing $HgBr_2$ (0.2 g) and heated for 24 h at 50°C. The mixture was cooled, diluted with CH_2Cl_2, and washed with aq 10% KI, water, and satd aq $NaHCO_3$, dried ($MgSO_4$), and concentrated. As the excess octadecanol had the same mobility as the product, the

mixture was acetylated with pyridine and Ac_2O and then purified by chromatography on silica gel using 1:6 EtOAc–hexane as eluant to give **17** (2.03 g, 41.4%); ^1H NMR ($CDCl_3$): δ 5.79 (m, CH = C), 5.62 (d, H-4), 5.25–5.10 (m, C = CH_2, H-2), 5.02 (dd, H-3), 4.41 (d, H-1), 4.02 and 3.75 (O-CH_2-C =), 3.87 and 3.46 (O-CH_2 of aglycon), 3.55 (H-6), 3.35 (d, H-5), 2.13, 2.04, 1.97 (3 × Ac), 1.58, 1.21, and 0.88 (hydrogens of octadecyl).

Compound **17** (2.0 g) was deacetylated with 40 mL of 2:1:1 MeOH–Et_3N–water at ice-bath temperature until TLC showed completion of the reaction. The mixture was evaporated to dryness to a residue (1.8 g) identified as octadecyl 6-*O*-allyl-7-deoxy-D-*glycero*-α-D-*galacto*-heptopyranoside. This (1.5 g) was dissolved in anhyd $HCONMe_2$ (50 mL) containing NaH (0.246 g) and benzyl bromide (2.0 g) and stirred for 9 days at room temperature. The reaction was quenched with MeOH, concentrated to a dry residue, redissolved in CH_2Cl_2, washed with water, ice-cold 1 M HCl, and satd aq $NaHCO_3$. Purification by chromatography on silica gel, using 19:1 toluene–hexane as eluant, gave octadecyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-7-deoxy-D-*glycero*-β-D-*galacto*-heptopyranoside (0.6 g); ^1H NMR ($CDCl_3$): δ 7.45–7.20 (aromatic hydrogens), 5.84 (m, CH = C), 5.25–4.58 (CH_2 = C, benzylic hydrogens), 4.32 (d, H-1), 4.12 (d, H-4), 4.04 and 3.62 (O-CH_2C =), 3.91 and 3.48 (O-CH_2 of aglycon), 3.80 (dd, H-2), 3.75 (m, H-6), 3.52 (dd, H-3), and 3.06 (d, H-5). This product was dissolved in dry THF (20 mL) containing Ir(COD)[$Ph_2PMe]_2PF_6$ (35 mg) and exposed to an atmosphere of H_2 for 5 min. The mixture was then stirred under N_2 for 18 h, concentrated to a dry residue, redissolved in CH_2Cl_2 (25 mL), cooled to ice-bath temperature, and aq 90% CF_3CO_2H (5 mL) added. After 10 min, the mixture was diluted with CH_2Cl_2 and washed with water and satd aq $NaHCO_3$. It was dried (MgSO4) and concentrated to a dry residue (556 mg, 98% yield). ^1H NMR indicated the product to be homogeneous and confirmed its structure as **18**; ^1H NMR ($CDCl_3$): δ 7.4–7.2 (aromatic hydrogens), 5.0–4.73 (benzylic hydrogens), 4.30 (d, H-1), 4.01 (d, H-4), 3.87 (H-6), 3.87 and 3.47 (O-CH_2 of aglycon), 3.52 (dd, H-3), and 2.89 (d, H-5).

Methylsulfenyl bromide solution in 1,2-dichloroethane (1 M, 1 mL) was added to a mixture of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*S*-methyl-2-thio-D-*glycero*-α-D-*galacto*-nonulopyranosylonate [22] (**19**, 0.132 g), **18** (0.160 g), CF_3SO_3Ag (0.07 g) in 1:3 CH_3CN–CH_2Cl_2 (20 mL) at −38°C. After 18 h, another portion of **19** (0.132 g), CF_3SO_3Ag (0.07 g) in CH_3CN (3 mL) followed by MeSBr solution (1 mL) were added. After 36 h, the mixture was worked-up and the product purified by chromatography on silica gel using 10:15:1 EtOAc–hexane–EtOH as eluant. Besides the starting material **18** (0.057 g), the desired α-glycoside **20** (37 mg) was obtained in 21.6% yield; ^1H NMR ($CDCl_3$): δ 5.30 (br s, H-7′ and H-8′), 5.12–4.58 (NH, benzylic hydrogen, H-4′), 3.47 (s, $COOCH_3$), 3.12 (d, H-5), 2.64 (dd, H-3′*eq*), 2.13, 2.10, 2.05, 2.00, and 1.87 (5 × Ac).

The *O*-acetyl and benzyl groups of **20** (37 mg) were removed as described earlier [22] and the methyl ester was hydrolyzed to the triethylammonium salt **3** (21 mg) with 2:1:1 MeOH–Et_3N–water (10 mL, 0°C, 22 h); $[\alpha]_D^{25}$ −3.2 ± 2° (*c* 1.03, MeOH); ^1H NMR (CD_3OD): δ 4.01 (m, H-6, H-1, $J_{1,2}$ 7.4 Hz), 3.07 (N-CH_2), 2.95 (d, *J* 8.5 Hz, H-5), 2.67 (m, H-3′*eq*), 1.87 (s, NAc), 1.52–1.45 (m, H-3′*ax*, CH_2 of aglycon), 1.25–1.1 (m, aglyconic hydrogens), and 0.76 (t, *J* 6.7 Hz, CH_3 of Et_3N); ^{13}C NMR (CD_3OD): δ

175.6, 175.1, 105.2, 103.5, 79.6, 74.8, 74.7, 73.2, 72.6, 70.8, 70.2, 69.4, 69.0, 64.6, 60.0, 54.1, 47.8, 42.6, 33.1, 30.9, 30.8, 30.6, 30.5, 27.1, 23.7, 22.6, 20.7, 14.4, 9.2, and 8.3.

Sodium salt of 2-(trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*nonulopyranylonic acid-(2 → 6)-7-deoxy-6-thio*-D-glycero-β-D-galacto-*heptopyranoside* (**5**).—6-*S*-Acetyl-1,2;3,4-di-*O*-isopropylidene-6-thio-D-*glycero*-α-D-*galacto*-heptopyranose (**21**, 5.3 g) was converted into the peracetate **22** (4.2 g) according to a published procedure [22]. A portion of **22** (1.0 g) was converted to the bromide **23** as described for **16**; ^{1}H NMR of **23** ($CDCl_3$): δ 6.76 (d, H-1), 5.70 (d, H-4), 5.34 (dd, H-3), 5.00 (dd, H-2), 4.11 (d, H-5), 3.89 (m, H-6), 2.27, 2.12, 2.00, and 1.97 (4 × Ac), and 1.35 (d, H-7). Crude **23** was condensed with 2-(trimethylsilyl)ethanol as described earlier to obtain the β-glycoside **24** (1.0 g, crude product). The *S*-acetyl group was selectively removed with hydrazine (0.055 g) and AcOH (0.103 g) in $HCONMe_2$ (30 mL, room temperature, 5 min) and the crude product obtained (800 mg) was condensed with the chloride **26** according to a published method [22]. Purification by chromatography on silica gel gave **27** (1.1 g) containing ca. 10% of the neuraminyl glycal. This impurity was subsequently removed by *O*-deacetylation of impure **27** with NaOMe–MeOH followed by gel permeation chromatography on Biogel P-2 (200–400 mesh) eluted and equilibrated with water to give the methyl ester of **5** (585 mg); ^{1}H NMR (D_2O): δ 4.32 (d, *J* 7.9 Hz, H-1), 4.07 (d, *J* 3.4 Hz, H-4), 3.93 and 3.72 (O-CH_2 of aglycon), 3.84 ($COOCH_3$), 3.8 (m, H-8′ and H-5′), 3.65 (dd, H-6′), 3.60 (m, H-4′), 3.61 (dd, *J* 6.6, 12.6 Hz, H-9′), 3.52 (m, H-3, H-9′), 3.52 (m, H-2, H-7′), 3.3 (m, H-6, H-5), 2.79 (dd, *J* 4.7, 13.1 Hz, H-3′*eq*), 1.99 (s, N*Ac*), 1.86 (dd, *J* 11.8, 12.9 Hz, H-3′*ax*), 1.44 (d, *J* 6.6 Hz, H-7), 1.02 and 0.93 (m, CH_2Si); ^{13}C NMR (D_2O): δ 175.3, 171.4, 102.4, 85.5, 76., 75.4, 73.6, 71.2, 70.9, 68.6, 68.5, 67.9, 63.4, 54.0, 51.9, 40.8, 39.8, 22.3, 21.0, 17.8, and −2.2. Treatment of a portion of the methyl ester product (100 mg) with Chelex resin [22] (3.5 g) in water (10 mL, 7 days) gave **5** (96 mg); $[\alpha]_D^{25}$ −2.8 ± 2° (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 4.30 (d, *J* 7.9 Hz, H-1), 4.12 (d, *J* 3.5 Hz, H-4), 3.91 and 3.70 (m, OCH_2 of aglycon), 3.80 (H-6′), 3.78 (H-8′), 3.75 (H-5′), 3.38 (dd, *J* 8.1, 9.9 Hz), 3.30 (m, H-5 and H-6), 2.73 (dd, *J* 4.8, 12.8 Hz, H-3′*eq*), 1.96 (NAc), 1.73 (dd, *J* 11.6, 12.7 Hz, H-3′*ax*), 1.41 (d, *J* 6.2 Hz, H-7), 1.00 and 0.90 (-CH_2Si); ^{13}C NMR (D_2O): δ 175.4, 174.9, 102.5, 87.3, 77.4, 75.3, 73.5, 72.4, 71.1, 68.9, 68.8, 68.4, 68.3, 62.9, 52.0, 41.5, 39.3, 22.3, 17.9, −2.2, and −3.4.

Triethylammonium salt of octadecyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*nonulopyranylonic acid-(2 → 6)-7-deoxy-6-thio*-D-glycero-β-D-galacto-*heptopyranoside* (**6**).—The bromide **23** (1.0 g, crude) was condensed with octadecanol (1.2 g) in the presence of Ag_2CO_3 (0.9 g) and CF_3SO_3Ag (40 mg) in CH_2Cl_2, as described above for **17**, to obtain the glycoside **25** (870 mg after purification by chromatography on silica gel using 1:4 EtOAc–hexane as eluant, 58% yield); ^{1}H NMR ($CDCl_3$): δ 5.57 (d, H-4), 5.18 (dd, H-2), 4.98 (dd, H-3), 4.42 (d, H-1), 3.90 (m, H-6, one of O-CH_2 of aglycon), 3.51 (m, H-5 and one of OCH_2 of aglycon), 2.28, 2.17, 2.08, and 1.98 (4 × Ac), 1.42 (d, H-7), 1.28 (hydrogens of octadecyl), 0.88 (CH_3 of the octadecyl). This was converted to the thiosialoside **28** (51% overall yield) as described above for **27**; ^{1}H NMR ($CDCl_3$): δ 5.51 (d, *J* 3.1 Hz, H-4), 5.29 (m, H-7′ and H-8′), 5.15 (dd, *J* 7.9, 10.4 Hz, H-2), 5.15 (d, *J* 9.8 Hz), 4.96 (dd, *J* 3.2, 10.4 Hz, H-3), 4.85 (m, H-4′), 4.46

(d, *J* 7.9 Hz, H-1), 4.30 (H-9′), 4.12 (H-9′), 3.99 (m, H-5′), 3.85 and 3.40 (O-CH_2 of aglycon), 3.79 ($COOCH_3$), 3.55 (d, *J* 7.3 Hz, H-5), 3.30 (m, H-6), 2.68 (dd, *J* 4.6, 12.8 Hz, H-3′*eq*), 2.15, 2.14, 2.03, 2.02, 1.95, and 1.88 (8 × Ac), 1.55 (m, 2 H), 1.45 (d, *J* 7.0 Hz, H-7), 1.24 (hydrogens of the octadecyl), and 0.87 (t, CH_3 of the octadecyl); ^{13}C NMR ($CDCl_3$): δ 170.9, 170.7, 170.3, 170.2, 170.1, 169.8, 169.6, 168.8, 101.3, 83.5, 75.4, 74.0, 71.4, 70.0, 69.3, 68.9, 68.1, 67.9, 66.9, 62.2, 53.0, 49.5, 39.9, 38.7, 31.9, 29.7, 29.6, 29.4, 29.3, 25.9, 23.2, 22.7, 21.1, 20.8, 20.7, 20.6, 20.2, and 14.1. Removal of the *O*-acetate groups (NaOMe–MeOH) and the hydrolysis of the methyl ester (2:1:1 MeOH–Et_3N–water, ice-bath temperature, 7 days) was done in quantitative yield to obtain **6**; $[\alpha]_D^{25}$ −9.0 ± 2° (*c* 1.0, H_2O); ^{1}H NMR (CD_3OD): δ 4.16 (d, *J* 2.8 Hz, H-4), 4.08 (d, *J* 7.3 Hz, H-1), 3.04 (d, *J* 10.1 Hz, H-5), 2.77 (dd, *J* 3.7, 12.2 Hz, H-3′*eq*), 1.93 (s, NAc), 1.65 (m, H-3′*ax*), 1.51 (m), 1.37 (d, *J* 7.0 Hz, H-7), 1.17 (hydrogens of the octadecyl), 0.79 (CH_3 of Et_3N); ^{13}C NMR (D_2O): δ 176.2, 175.7, 105.1, 87.8, 79.4, 76.8, 74.9, 73.0, 72.5, 70.9, 70.2, 69.6, 69.5, 64.4, 60.2, 53.9, 39.1, 33.0, 30.8, 30.7, 30.6, 30.4, 27.1, 23.7, 22.6, 21.9, 14.5, and 8.5.

*Methyl (5-acetamido-7,8,9-tri-*O*-acetyl-4-azido-3,4,5-trideoxy-*D-glycero-β-D-galacto-*nonulopyranosyl chloride)onate* (**30**).—Anhydrous HCl gas was bubbled for 20 min through an ice-cold solution of **29** (2.0 g) in CH_3CN (50 mL) containing 4A molecular sieves (5.0 g) and LiCl (1.0 g). The solution was then stirred at ambient temperature for 4 days. The mixture was cooled in an ice-bath and HCl gas bubbled through for an additional 10 min and the reaction continued for 2 more days. The mixture was then evaporated to dryness under reduced pressure and the residue was extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with ice-cold water (2 ×), satd aq $NaHCO_3$, dried ($MgSO_4$), and concentrated to a dry residue (1.6 g). ^{1}H NMR of the crude product showed that the product contained greater than 85% of the desired chloride **30** and ca. 10% of starting material **29**. An analytical sample was prepared by chromatographic purification on a column of silica gel using 20:20:1 EtOAc–hexane–acetone as eluant; IR ($CHCl_3$) cm^{-1}: 2103 (N_3), 1749 (ester) $[\alpha]_D^{25}$ −10.9 ± 2° (*c* 1.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.63 (d, 1 H, *J* 9.6 Hz, NH), 5.46 (dd, 1 H, *J* 2.8, 7.2 Hz, H-7), 5.19 (m, 1 H, H-8), 4.52 (dd, *J* 2.8, 11.0 Hz, H-6), 4.40 (dd, *J* 3.2, 12.8 Hz, H-9a), 4.26 (m, 1 H, H-4), 4.10 (dd, *J* 5.6, 12.8 Hz, H-9b), 3.88 (s, $COOCH_3$), 3.76 (m, H-5), 2.79 (dd, *J* 4.8, 14.3 Hz, H-3*eq*), 2.14, 2.07, 2.06, and 2.03 (4 × s, CH_3CO-); ^{13}C NMR ($CDCl_3$): δ 170.64, 170.60, 170.3, 169.6, 165.5, 96.3, 72.8, 69.6, 67.1, 61.9, 57.4, 53.8, 50.1, 40.8, 23.4, 20.9, 20.8, and 20.7. Anal. Calcd for $C_{18}ClH_{25}N_4O_{10}$: C, 43.85; H, 5.08; N, 11.37. Found: C, 43.99; H, 5.20; N, 10.93.

*Methyl (methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-azido-3,4,5-trideoxy-2-thio-*D-glycero-α-D-galacto-*nonulopyranosid)onate* (**31**).—Sodium thiomethoxide (1.13 g, 16.2 mmol) was added to a solution of the chloride **30** (1.6 g) in CH_3CN (20 mL) containing 4A molecular sieves (1.0 g) and stirred for 40 h under dry N_2. The mixture was then concentrated to dryness, the residue suspended in CH_2Cl_2 and poured over ice-cold HCl. The organic layer was separated, washed with satd aq $NaHCO_3$, dried (MgSO4), and concentrated to a dry residue (1.4 g). ^{1}H NMR of the crude product confirmed the major component to be **31** along with minor amounts of **29** that was present in the chloride **30**. The purity of the crude material was found to be sufficient for subsequent glycosylation reactions. Analytically pure **31** was obtained by chromatography on a

column of silica gel using 10:15:1 EtOAc–hexane–EtOH as eluant; IR ($CHCl_3$) cm^{-1}: 2103 (N_3), 1742 (ester); $[\alpha]_D^{25}$ $+28.7 \pm 2°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.60 (d, *J* 9.6 Hz, NH), 5.38 (m, 1 H, H-8), 5.30 (dd, *J* 1.8, 9.0 Hz, H-7), 4.30 (dd, *J* 2.7, 12.2 Hz, H-9a), 4.17 (dd, *J* 4.6, 12.2 Hz, H-6b), 4.07 (dd, *J* 1.9, 10.6 Hz, H-6), 4.0 (m, 1 H, H-4), 3.81 (s, 3 H, $COOCH_3$), 3.30 (m, 1 H, H-5), 2.75 (dd, 1 H, *J* 4.6, 12.9 Hz, H-3*eq*), 2.16, 2.15, 2.10, 2.04 and 1.99 (5 × s, 4 × CH_3CO- and *S*-CH_3), and 1.75 (dd, 1 H, *J* 11.8, 12.9 Hz, H-3*ax*). Anal. Calcd for $C_{19}H_{28}N_4O_{10}S$: C, 45.24; H, 5.56; N, 11.11. Found: C, 45.30; H, 5.60; N, 10.76.

Sodium salt of 5-acetamido-4-azido-3,4,5-trideoxy-D-glycero-α-D-galacto-*non-ulopyranosylonic acid-(2 → 6)-7-deoxy*-D-glycero-α,β-D-galacto-*heptopyranose* (**7**).— Methylsulfenyl bromide (1 M) in 1,2-dichloroethane (3 mL) was added to a solution of **31** (1.8 g), 7-deoxy-1,2;3,4-di-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (**34**, 1.1 g), and CF_3SO_3Ag (0.93 g) in 3:1 CH_3CN–C_2H_5CN (40 mL) at −30°C. The reaction was subsequently stirred for 16 h at −38°C. Satd aq $NaHCO_3$ (3 mL) was added and the mixture was stirred at room temperature for 10 min. It was then filtered over a Celite pad and the residue washed with CH_2Cl_2. The filtrate was washed with satd aq $NaHCO_3$, dried (MgSO4), and concentrated to a dry residue. Purification of this product by chromatography on silica gel using 10:15:1 EtOAc–hexane–EtOH gave the unreacted starting material **34** (586 mg) and a mixture of the disaccharide **38** and two other coeluting contaminants (1.51 g). This mixture was *O*-deacetylated with 0.5 M NaOMe (0.3 mL) in MeOH (20 mL, 2 days) and the resultant mixture was purified by gel-permeation chromatography on a column of Biogel P-2 (200–400 mesh, 1800 mL, the compound was loaded in three portions). This gave the disaccharide (methyl 5-acetamido-4-azido-3,4,5-trideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosylonate)-(2 → 6)-7-deoxy-1,2;3,4-di-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (533 mg); IR (cm^{-1}) ($CHCl_3$): 2103.7 (N_3); ^1H NMR (D_2O): δ 5.43 (d, *J* 4.9 Hz, H-1), 4.59 (dd, *J* 2.4, 7.8 Hz, H-3), 4.30 (m, H-4 and H-2), 4.15 (m, H-6), 3.92 (t, H-5′), 3.84 ($COOCH_3$), 2.72 (dd, *J* 4.6, 12.9 Hz, H-3′*eq*), 1.98 (s, NAc), 1.74 (t, *J* 12.7 Hz, H-3′*ax*), 1.48, 1.37, 1.34, and 1.31 (4 × isopropylidene methyls), AND 1.30 (d, *J* 6.1 Hz, H-7); ^{13}C NMR (D_2O): δ 175.3, 170.7, 111.1, 110.7, 101.9, 98.9, 75.9, 73.5, 73.0, 72.8, 72.5, 71.9, 70.7, 65.6, 60.7, 54.5, 52.2, 50.3, 50.2, 50.0, 49.8, 49.7, 49.5, 49.3, 39.8, 27.2, 26.0, 25.7, 23.6, and 21.4.

The foregoing disaccharide (402 mg) was dissolved in aq 50% CF_3COOH (11 mL) and kept at ice-bath temperature for 24 h. It was then processed by concentration to a dry residue and purification on Biogel P-2 to give (methyl 5-acetamido-4-azido-3,4,5-trideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosylonate)-(2 → 6)-7-deoxy-D-*glycero*-α-D-*galacto*-heptopyranose (250 mg). This was dissolved in water containing Chelex resin (sodium form, 4.5 g) and stirred at room temperature for 2 days, filtered and the filtrate was lyophilized to give sialoside **7** (259 mg); $[\alpha]_D^{25}$ $-32.2 \pm 2°$ (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 5.17 (d, *J* 3.7 Hz, H-1α), 4.50 (d, *J* 7.9 Hz, H-1β), 4.07 (m, H-6), 4.04 (d, *J* 3.7 Hz, H-4), 3.89 (t, *J* 10.4 Hz, H-5′), 3.40 (dd, *J* 7.9, 10.1 Hz, H-2), 3.29 (d, *J* 7.9 Hz, H-5), 2.70 (m, H-3′*eq*), 1.99 (N*Ac*), 1.70 (m, H-3′*ax*), 1.28 (d, *J* 6.4 Hz, H-7β), and 1.25 (d, *J* 6.1 Hz, H-1α) ^{13}C NMR (D_2O): δ 175.3, 173.6, 102.2, 102.1, 97.2, 92.7, 77.8, 73.5, 73.1, 73.0, 72.3, 70.5, 70.2, 69.6, 68.8, 68.3, 63.0, 59.9, 50.2, 37.5, 22.4, and 19.3.

Sodium salt of 2-(trimethylsilyl)ethyl 4,5-diacetamido-3,4,5-trideoxy-D-glycero-α-D-galacto-*nonulopyranosylonic acid-(2 → 6)-7-deoxy*-D-glycero-β-D-galacto-*heptopyranoside* (**8**).—Alcohol **35** (427 mg) was condensed with the azidothioglycoside **31** (525 mg) as described above for **37**. This provided, after purification by chromatography on silica gel, pure **39** (217 mg), in addition to the starting material **35** (197 mg); ^{1}H NMR ($CDCl_3$) of **39**: δ 7.4–7.2 (phenyl hydrogens), 5.53 (d, NH), 5.28 (m, H-7′ and H-8′), 5.15–4.60 (benzylic hydrogens), 4.32 (m, H-1 and H-9′), 4.21 (H-6′ and H-9′), 4.0 (m, H-6, H-4, H-4′) 3.78 (H-2), 3.48 (dd, H-3), 3.41 (s, $COOCH_3$), 3.26 (m, H-5′), 3.07 (d, H-5), 2.67 (dd, H-3′*eq*), 2.13, 2.12, 2.02, and 2.0 (4 × Ac), 1.71 (t, H-3′*ax*), 1.34 (d, H-7), and 1.05 (dd, CH_2Si). The acetate groups were then removed as already described, followed by the reduction of the azide with 20% palladium hydroxide on carbon. The product from this was acetylated with pyridine acetic anhydride. Repetition of hydrogenation with 20% palladium hydroxide on carbon followed by *O*-deacetylation and purification on a column of Bio gel gave the methyl ester of **8** (115 mg); ^{1}H NMR (D_2O): δ 4.34 (d, *J* 7.9 Hz, H-1), 4.22 (m, H-6), 4.02 (H-4 and H-4′), 3.84 (COOMe), 3.60 (dd, *J* 6.4, 11.9 Hz, H-9′), 3.50 (H-3, H-7′), 3.41 (dd, *J* 8.2, 10.1 Hz, H-2), 3.25 (d, *J* 8.5 Hz, H-5), 2.56 (dd, *J* 4.3, 13.1 Hz, H-3′*eq*), 1.918, 1.915 (2 × NAc), 1.28 (d, *J* 6.1 Hz, H-7), 1.02 and 0.95 (CH_2Si); ^{13}C NMR (D_2O): δ 175.8, 174.9, 170.5, 103.4, 101.0, 77.9, 74.7, 74.7, 74.6, 74.3, 71.9, 71.8, 70.0, 69.4, 69.2, 68.5, 64.2, 54.7, 50.4, 48.8, 36.9, 23.0, 20.1, 18.6, and −1.4.

A portion of the methyl ester (62 mg) was hydrolyzed with Chelex resin (700 mg) to afford the title compound **8** (65 mg); $[\alpha]_D^{25}$ −37.1 ± 2° (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 4.33 (d, *J* 8.1 Hz, H-1), 4.06 (m, H-6), 4.02 (d, *J* 3.4 Hz, H-4), 3.82 (t, *J* 10.0 Hz, H-5′), 3.39 (dd, *J* 7.8, 9.8 Hz, H-2), 3.24 (d, *J* 8.3 Hz, H-5), 2.54 (dd, *J* 4.2, 12.7 Hz, H-3′*eq*), 1.891, 1.886 (2 × NAc), 1.69 (t, *J* 12.7 Hz, H-3′*ax*), 1.28 (d, *J* 6.1 Hz, H-7), 0.99 and 0.90 (CH_2Si); ^{13}C NMR (D_2O): δ 175.0, 174.0 ($J_{C-1',H-3'ax}$ 5.6 Hz), 173.7, 102.5, 102.3, 77.6, 73.9, 73.2, 72.3, 71.2, 69.9, 68.5, 68.4, 68.0, 63.0, 50.0, 48.9, 37.8, 22.2, 19.4, 17.9, and −2.2.

Sodium salt of 5-acetamido-4-amino-3,4,5-trideoxy-D-glycero-α-D-galacto-*nonulopyranosylonic acid-(2 → 6)-7-deoxy*-D-glycero-α,β-D-galacto-*heptopyranose* (**9**).—A solution of compound **7** (25 mg) in 10 mL of aq 90% EtOH containing 10% Pd–C (27 mg) was stirred under H_2 for 2 h. The mixture was filtered over a pad of Celite and the filtrate was evaporated to dryness. The residue was redissolved in water and lyophilized to obtain **9** (19 mg); $[\alpha]_D^{25}$ +10.8 ± 2° (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 5.15 (d, *J* 3.4 Hz, H-1α), 4.47 (d, *J* 8.1 Hz, H-1β), 4.08 (d, *J* 2.9 Hz, H-4α), 4.06–4.0 (H-6, H-4β), 3.80 (H-6′), 3.76 (H-8′), 3.38 (dd, *J* 8.1, 10.0 Hz, H-2), 3.27 (d, *J* 7.8 Hz, H-5), 2.79 (m, H-4′), 2.58 (m, H-3′*eq*), 1.97 (s, NAcβ), 1.95 (NAcα), 1.59 (m, H-3′*ax*), 1.26 (d, *J* 6.4 Hz, H-7β), and 1.24 (d, *J* 6.1 Hz, H-7α); ^{13}C NMR (D_2O): δ 175.5, 174.0, 102.5, 102.4, 97.1, 92.6, 77.8, 74.1, 73.0, 72.3, 72.2, 70.4, 70.1, 69.5, 68.7, 68.4, 68.2, 62.9, 51.7, 50.0, 39.7, 39.6, 22.3, 19.3, and 19.2.

Sodium salt of 5-acetamido-4-azido-3,4,5-trideoxy-D-glycero-α-D-galacto-*nonulopyranosylonic acid-(2 → 6)-7-deoxy-6-thio*-D-glycero-α,β-D-galacto-*heptopyranose* (**10**).—Sodium hydride (23 mg) was added to a cold solution (−20°C) of **32** (290 mg) in anhyd $HCONMe_2$ (10 mL). After 5 min, a solution of crude **30** (492 mg) in $HCONMe_2$ (5 mL) was added and the mixture was stirred for 4 h at −20°C. It was then

evaporated to dryness under reduced pressure and the residue was extracted with CH_2Cl_2, washed with ice-cold HCl, and satd aq $NaHCO_3$. The mixture obtained after evaporation of the solvent was purified by chromatography on a column of silica gel using 10:15:1 EtOAc–hexane–EtOH as eluant to afford pure **36** (350 mg); ^{1}H NMR ($CDCl_3$): δ 5.57 (d, J 8.4 Hz, NH), 5.50 (d, 1 H, J 4.9 Hz, H-1), 5.32 (m, 1 H, H-8), 5.28 (dd, 1 H, J 2.2, 8.4 Hz, H-7), 4.55 (dd, J 2.7, 8.0 Hz, H-3), 4.40-4.17 (3 H, H-9′a,b, H-2, H-4), 4.13 (dd, 1 H, H-6′), 4.00 (m, 1 H, H-4′), 3.81 (s, 3 H, COOCH_3), 3.49 (dd, J 1.9, 8.0 Hz, H-5), 3.38–3.18 (H-6, H-5′), 2.78 (dd, J 4.3, 12.5 Hz, H-3′*eq*), 2.16, 2.13, 2.06 and 1.98 (4 × s, CH_3COO), 1.76 (t, 1 H, J 12.4 Hz, H-3′*ax*), 1.48, 1.44, 1.32, and 1.31 (4 s, 4-isopropylidene methyls), and 1.46 (d, H-7).

Sialoside **36** (370 mg) was dissolved in anhyd MeOH (15 mL), followed by the addition of 0.5 M NaOMe solution (0.2 mL). After 2 h, the solution was neutralized with H^+ resin, filtered, and evaporated to give (methyl 5-acetamido-4-azido-3,4,5-trideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosylonate)-(2 → 6)-7-deoxy-1,2;3,4-di-*O*-isopropylidene-6-thio-D-*glycero*-α-D-*galacto*-heptopyranose **40**. The residue was dissolved in aq 50% CF_3COOH (10 mL) and left at room temperature for 4 h and then for 16 h at 4°C. The solution was evaporated to dryness and purified on a column of Biogel P-2 (200–400 mesh, 1800 mL). This gave the methyl ester of **10** (196 mg). Hydrolysis of the methyl ester (185 mg) was carried out with Chelex resin (1.0 g, 40°C, 3 days) to obtain **10** (185 mg); $[\alpha]_D^{25}$ +15.9 ± 2° (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 5.16 (d, J 3.4 Hz, H-1α), 4.46 (d, J 8.1 Hz, H-1β), 4.15 (d, J 2.2 Hz, H-4α), 4.11 (d, J 3.2 Hz, H-4β), 3.89 (t, J 10.5 Hz, H-5′), 3.38 (dd, J 8.1, 10.0 Hz, H-2β), 3.3 (m, H-4′), 2.76 (m, H-3′*eq*), 1.97 (s, NAc), 1.76 (m, H-3′*ax*), 1.39 (d, J 6.1 Hz, H-7β), and 1.37 (d, J 7.1 Hz, H-7α); ^{13}C NMR (D_2O): δ 175.2, 174.4, 97.1, 92.7, 87.3, 87.2, 77.4, 75.4, 73.3, 72.6, 72.4, 72.2, 69.9, 69.6, 69.1, 68.6, 68.2, 62.8, 60.2, 50.1, 39.8, 39.5, 38.6, 22.3, 20.6, and 20.5.

Sodium salt of 4,5-diacetamido-3,4,5-trideoxy-D-glycero-α-D-galacto-*nonulopyranosylonic acid-(2 → 6)-7-deoxy-6-thio*-D-glycero-α,β-D-galacto-*heptopyranose* (**11**).—Compound **40** (290 mg) was dissolved in EtOH (20 mL) containing 10% Pd–C (75 mg). After 20 h, another portion of Pd–C (68 mg) was added. After 6 h, the catalyst was removed by filtration over a Celite pad and concentrated to a dry residue which was taken up in MeOH (10 mL) containing Ac_2O (120 mg). This was followed by the addition of Et_3N (101 mg). Due to incomplete reaction, the mixture was evaporated to dryness, redissolved in pyridine (10 mL) and Ac_2O (5 mL) and worked-up after 16 h. The residue (60 mg) was *O*-deacetylated with NaOMe in MeOH and the product was purified by Bio gel chromatography. This gave (methyl 4,5-diacetamido-3,4,5-trideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosylonate)-(2 → 6)-7-deoxy-1,2;3,4-di-*O*-isopropylidene-6-thio-D-*glycero*-α-D-*galacto*-heptopyranose (26 mg). Removal of the isopropylidene group with aq 50% CF_3COOH and hydrolysis of the methyl ester with Chelex resin as described above gave **11** (17 mg); $[\alpha]_D^{25}$ +31.0 ± 2° (*c* 1.05, H_2O); ^{1}H NMR (D_2O): δ 5.14 (d, J 3.2 Hz, H-1α), 4.45 (d, J 7.8 Hz, H-1β), 4.13 (d, J 2.0 Hz, H-4α), 4.08 (d, J 3.2 Hz, H-4β), 2.61 (m H-3′*eq*), 1.881 and 1.876 (2 × NAc), 1.75 (t, J 12.5 Hz, H-3′*ax*), 1.38 (d, J 6.4 Hz, H-7β), and 1.36 (d, J 6.8 Hz, H-7α); ^{13}C NMR (D_2O): δ 175.0, 174.5, 174.2, 97.1, 92.7, 77.4, 75.7, 73.3, 72.6, 72.4, 72.2, 69.9, 69.6, 69.2, 68.6, 68.3, 62.9, 49.9, 49.2, 39.8, 39.6, 38.9, 22.2, 20.7, and 20.5.

Sodium salt of 5-acetamido-4-amino-3,4,5-trideoxy-D-glycero-α-D-galacto-*non-ulopyranosylonic acid-(2 → 6)-7-deoxy-6-thio*-D-glycero-α,β-D-galacto-*heptopyranose* (**12**).—Sialoside **10** (30 mg) was dissolved in aq 90% EtOH (10 mL) containing 10% Pd–C (19 mg) and stirred under H_2 for 20 h. The mixture was filtered over a pad of Celite and the filtrate was concentrated to dryness, redissolved in water, and lyophilized (26 mg); $[\alpha]_D^{25}$ +38.1 ± 2° (*c* 1.02, H_2O); ^{1}H NMR (D_2O): δ 5.15 (d, *J* 3.3 Hz, H-1α), 4.45 (d, *J* 7.7 Hz, H-1β), 4.14 (d, *J* 2.2 Hz, H-4α), 4.09 (d, *J* 3.3 Hz, H-4β), 4.05 (t, *J* 10.6 Hz, H-5′), 3.8 (m, H-6′ and H-8′), 3.27 (m, H-4′), 2.83 (m, H-3′*eq*), 1.97 (s, NAc), 1.91 (m, H-3′*ax*), 1.39 (d, *J* 6.6 Hz, H-7β), and 1.37 (d, *J* 7.0 Hz, H-7α); ^{13}C NMR (D_2O): δ 175.4, 173.8, 97.1, 92.6, 87.0, 86.9, 77.3, 75.1, 73.3, 72.5, 72.3, 72.2, 72.1, 69.9, 69.5, 69.1, 68.6, 68.0, 67.9, 62.7, 51.1, 48.0, 39.9, 39.6, 37.4, 22.4, 20.6, and 20.4.

Sodium salt of 5-acetamido-4-azido-3,4,5-trideoxy-D-glycero-α-D-galacto-*non-ulopyranosylonic acid-(2 → 6)-α,β-D-galactopyranose* (**13**).—Methylsulfenyl bromide (1 M in 1,2-dichloroethane) was added to a solution of **31** (850 mg), 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose **33** (655 mg, Pfanstiehl Laboratories, Inc., Waukegan, IL), CF_3SO_3Ag (518 mg), and powdered 3A molecular sieves (1.5 g) in 4:1 CH_3CN–CH_2Cl_2 (25 mL) at −30°C. The reaction was subsequently stirred at −38°C for 16 h. Satd aq $NaHCO_3$ (3 mL) was added and the mixture was stirred at room temperature for 10 min. It was then filtered over a Celite pad and the residue washed with CH_2Cl_2. The filtrate was washed with satd aq $NaHCO_3$, dried (MgSO4), and concentrated to a dry residue. Purification of this product by chromatography on silica gel using 10:15:1 EtOAc–hexane–EtOH gave the unreacted 1,2;3,4-di-*O*-isopropylidene-α-D-galacto-pyranose **33** (400 mg), followed by pure **37** (216 mg), and a mixture of **37** (90% pure, 431 mg) contaminated with ca. 10% of glycal **29**. Data for **37**: $[\alpha]_D^{25}$ −30.4 ± 2° (*c* 0.9, $CHCl_3$); IR ($CHCl_3$) cm^{-1}: 2104 (N_3), 1747 (esters); ^{1}H NMR ($CDCl_3$): δ 5.51 (br d, 2 H, *J* 4.3 Hz, H-1, NH), 5.39 (m, 1 H, H-8′), 5.29 (dd, 1 H, *J* 1.9, 8.2 Hz, H-7′), 4.59 (dd, 1 H, *J* H-3), 4.35–4.20 (m, H-2, H-4, H-9′a,b, H-6′), 3.95–3.75 (H-4′, H-6, H-5, $COOCH_3$), 3.59 (m, H-6b), 3.37 (m, H-5′), 2.67 (dd, 1 H, *J* 4.4, 13.2 Hz, H-3′*eq*), 2.15, 2.13, 2.04 and 1.98 (4 × s, 4 × CH_3COO), 1.74 (t, 1 H, *J* 13.3 Hz, H-3′*ax*), 1.53, 1.42, 1.32, and 1.31 (4 × s, 4-isopropylidene methyls).

Sialoside **37** (90% pure, 425 mg) was dissolved in MeOH (25 mL) followed by the addition of 0.5 M NaOMe solution (0.2 mL). After 4 h, the mixture was neutralized with acidic resin, evaporated to dryness, redissolved in water (5 mL), and purified by gel permeation chromatography on Biogel P-2 to obtain pure (methyl 5-acetamido-4-azido-3,4,5-trideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosylonate)-(2 → 6)-1,2;3,4-di-*O*-isopropylidene-α-D-*galacto*-pyranose (270 mg); ^{1}H NMR (D_2O): δ 5.58 (d, *J* 4.9 Hz, H-1), 4.73 (dd, H-3), 4.50 (dd, H-2), 4.35 (dd, H-4), 3.65 (m, H-4′), 3.62 (dd, H-9′a), 3.59 (dd, *J* 6.4, 12.5 Hz, H-9′b), 3.52 (br d, *J* 9.5 Hz, H-7′), 2.69 (dd, *J* 4.6, 13.1 Hz, H-3′*eq*), 1.99 (s, NAc), 1.78 (t, *J* 12.8 Hz, H-3′*ax*), 1.51, 1.41, 1.34, and 1.33 (4 × isopropylidene methyls); ^{13}C NMR (D_2O): δ 175.2, 169.8, 110.5, 110.3, 98.9, 96.1, 73.4, 70.9, 70.8, 70.3, 70.2, 68.4, 67.3, 63.4, 58.6, 53.8, 50.2, 36.6, 25.2, 24.3, 23.6, and 22.4.

A portion of this (110 mg) was dissolved in aq 50% CF_3COOH (10 mL) and kept at ice-bath temperature for 1 h and then at room temperature for 4 h. It was then

evaporated to dryness, redissolved in water, and applied onto a column of Biogel P-2 (200–400 mesh, 1800 mL) equilibrated and eluted with water and the UV active (220 nm) fractions (7.5 mL, 119–126) were pooled and lyophilized (78 mg, methyl ester of **13**); ^{1}H NMR (D_2O): δ 5.18 (d, J 3.7 Hz, H-1α), 4.50 (d, J 7.9 Hz, H-1β), 3.84 (s, COOMe), 3.67 (m, H-4′), 3.52 (d, J 9.2 Hz, H-7′), 3.42 (dd, J 7.9, 10.1 Hz, H-2), 2.69 (dd, J 4.6, 13.1 Hz, H-3′*eq*), 1.99 (NAc), and 1.80 (m, H-3′*ax*).

Hydrolysis of the methyl ester (65 mg) with Chelex resin (760 mg) as described above gave the sialoside **13** (71 mg); $[\alpha]_D^{25}$ $-9.7 \pm 2°$ (c 1.04, H_2O); ^{1}H NMR (D_2O): δ 5.17 (d, J 4.0 Hz, H-1α), 4.50 (d, J 7.6 Hz, H-1β), 3.41 (dd, J 7.9, 10.1 Hz, H-2), 2.66 (dd, J 4.6, 12.8 Hz, H-3′*eq*), 1.98 (s, NAc), and 1.64 (H-3′*ax*); ^{13}C NMR (D_2O): δ 176.0, 174.3, 101.4, 97.5, 93.4, 74.6, 74.0, 73.7, 72.9, 72.8, 70.4, 70.1, 69.9, 69.4, 69.2, 65.1, 64.9, 63.7, 60.6, 51.1, 38.3, and 23.1.

Sodium salt of 4,5-diacetamido-3,4,5-trideoxy-D-glycero-α-D-galacto-*nonulopyranosylonic acid-(2 → 6)-*α,β-D-*galactopyranose* (**14**).—Compound **37** (162 mg) was dissolved in MeOH (15 mL) containing 10% Pd–C (48 mg) and left under H_2 for 18 h. It was then filtered over a Celite pad, concentrated to a dry residue, redissolved in CH_2Cl_2 (15 mL) containing pyridine (1 mL) and Ac_2O (0.5 mL). After 15 min, MeOH (0.5 mL) was added and the mixture was diluted with CH_2Cl_2 and washed with water, ice-cold HCl, and satd aq $NaHCO_3$. The residue from this was *O*-deacetylated with 0.5 M NaOMe in MeOH, neutralized with acidic resin, and concentrated and the residue from this was dissolved in aq 50% CF_3COOH and kept at ice-bath temperature for 16 h. It was then concentrated to a dry residue, redissolved in water, and applied onto a column of Biogel P-2 as described to obtain the methyl ester of **14** (75 mg); ^{13}C NMR (D_2O): δ 175.0, 174.2, 170.1, 170.0, 99.2, 96.8, 92.6, 73.8, 73.6, 73.0, 72.1, 70.8, 69.7, 69.4, 69.2, 68.7, 68.6, 64.3, 64.0, 53.9, 49.9, 47.7, 37.0, and 22.2.

A portion (65 mg) of this was hydrolyzed with Chelex resin (650 mg) to obtain **14** (74 mg); $[\alpha]_D^{25}$ $-10.4 \pm 2°$ (c 1.0, H_2O); ^{1}H NMR (D_2O): δ 5.18 (d, J 3.7 Hz, H-1α), 4.50 (d, J 7.9 Hz, H-1β), 3.41 (dd, J 7.9, 10.1 Hz, H-2β), 2.52 (H-3′*eq*), 1.902 and 1.895 (2 × NAc), and 1.66 (H-3′*ax*); ^{13}C NMR (D_2O): δ: 175.0, 174.1, 173.7, 100.7, 96.8, 92.7, 73.9, 73.6, 73.5, 73.0, 72.1, 69.7, 69.3, 69.2, 68.6, 64.3, 64.1, 63.0, 50.2, 48.8, 37.9, and 22.2.

Sodium salt of 5-acetamido-4-amino-3,4,5-trideoxy-D-glycero-α-D-galacto-*nonulopyranosylonic acid-(2 → 6)-*α,β-D-*galactopyranose* (**15**).—Sialoside **13** (26 mg) was dissolved in aq 90% EtOH (15 mL) containing 10% Pd–C (20 mg) and left under H_2 for 16 h. It was then filtered over a Celite pad, concentrated to a dry residue, redissolved in water, and lyophilized (12 mg); $[\alpha]_D^{25}$ $+10.5 \pm 2°$ (c 0.98, H_2O); ^{1}H NMR (D_2O): δ 5.19 (d, J 3.9 Hz, H-1α), 4.52 (d, J 8.1 Hz, H-1β), 3.93 (dd, H-4β), 3.43 (dd, J 7.8, 10.0 Hz, H-2), 2.94 (m, H-5′), 2.61 (dd, J 4.2, 12.7 Hz, H-3′*eq*), 2.00 (NAc-β), 1.86 (NAc-α), and 1.63 (m, H-3′*ax*); ^{13}C NMR (D_2O): δ 175.5, 173.7, 100.7, 96.8, 92.6, 73.9, 73.6, 72.9, 72.1, 72.0, 69.7, 69.4, 69.3, 69.3, 68.6, 64.2, 64.1, 62.9, 51.1, 50.1, 38.9, and 22.4.

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References

[1] T. Corfield, *Glycobiology*, 2 (1992) 509–521.
[2] P. Palese, K. Tobita, M. Ueda, and R.W. Compans, *Virology*, 61 (1974) 397–410.
[3] C.T. Holzer, M. von Itzstein, B. Jin, M.S. Pegg, W.P. Stewart, and W.Y. Wu, *Glycoconjugate J.*, 10 (1993) 40–44.
[4] M. von Itzstein, W.-Y. Wu, G.B. Kok, M.S. Pegg, J.C. Dyason, B. Jin, T.V. Phan, M.L. Smythe, H.F. White, S.W. Oliver, P.M. Colman, J.N. Varghese, D.M. Ryan, J.M. Woods, R.C. Bethell, V.J. Hotham, J.M. Cameron, and C.R. Penn, *Nature*, 363 (1993) 418–423.
[5] J.C. Paulson, in M. Conn (Ed.), *Interactions of Animal Viruses With Cell Surface Receptors*, Academic Press, New York, 1985, pp 131–219.
[6] P. Meindl, G. Bodo, P. Palese, J. Schulman, and H. Tuppy, *Virology*, 58 (1974) 457–463.
[7] M.N. Matrosovich, A.S. Gambaryan, A.B. Tuzikov, N.E. Byramova, L.V. Mochalova, A.A. Golbraikh, M.D. Shenderrovich, J. Finne, and N.V. Bovin, *Virology*, 196 (1993) 111–121.
[8] N.K. Sauter, M.D. Bednarski, B.A. Wurzburg, J.E. Hanson, G.M. Whitesides, J.J. Skehel, and D.C. Wiley, *Biochemistry*, 28 (1989) 8388–8396.
[9] W. Weis, J.H. Brown, S. Cusack, J.C. Paulson, J.J. Skehel, and D.C. Wiley, *Nature*, 333 (1988) 426–431.
[10] N.K. Sauter, J.E. Hanson, G.D. Glick, J.H. Brown, R.L. Crowther, S.-J. Park, J.J. Skehel, and D.C. Wiley, *Biochemistry*, 31 (1992) 9609–9621.
[11] S. Kelm, J.C. Paulson, U. Rose, R. Brossmer, W. Schmid, B.P. Bandgar, E. Schreiner, M. Hartmann, and E. Zbiral, *Eur. J. Biochem.*, 205 (1992) 147–153.
[12] N.R. Taylor and M. von Itzstein, *J. Med. Chem.*, 37 (1994) 616–624.
[13] T.J. Pritchett, R. Brossmer, U. Rose, and J.C. Paulson, *Virology*, 160 (1987) 502–506.
[14] A. Gamian, M. Chomik, C.A. Laferriere, and R. Roy, *Can. J. Microbiol.*, 37 (1991) 233–237.
[15] A. Spaltenstein and G.M. Whitesides, *J. Am. Chem. Soc.*, 113 (1991) 686–687.
[16] P.L. Toogood, P.K. Galliker, G.D. Glick, and J.R. Knowles, *J. Med. Chem.*, 34 (1991) 3138–3140.
[17] G.D. Glick and J.R. Knowles, *J. Am. Chem. Soc.*, 113 (1991) 4701–4703.
[18] S. Sabesan, J.Ø. Duus, P. Domaille, S. Kelm, and J.C. Paulson, *J. Am. Chem. Soc.*, 113 (1991) 5865–5866.
[19] S. Sabesan, J.Ø. Duus, S. Neira, P. Domaille, S. Kelm, J.C. Paulson, and K. Bock, *J. Am. Chem. Soc.*, 114 (1992) 8363–8375.
[20] M.A. Sparks, K.W. Williams, and G.M. Whitesides, *J. Med. Chem.*, 36 (1993) 778–783.
[21] W. Spevak, J.O. Nagy, D.H. Charych, M.E. Schaefer, J.H. Gilbert, and M.D. Bednarski, *J. Am. Chem. Soc.*, 115 (1993) 1146–1147.
[22] S. Sabesan, S. Neira, F. Davidson, J.Ø. Duus, and K. Bock, *J. Am. Chem. Soc.*, 116 (1994) 1616–1634.
[23] S. Sabesan, *Bioorg. Med. Chem. Lett.*, 4 (1994) 2457–2460.
[24] H. Lönn and K. Stenvall, *Tetrahedron Lett.*, 33 (1992) 115–116.
[25] G. Excoffier, D. Gagnaire, and J.P. Utille, *Carbohydr. Res.*, 39 (1975) 368–373.
[26] E. Schreiner, E. Zbiral, R.G. Kleineidam, and R. Schauer, *Liebigs Ann. Chem.*, (1991) 129–134.
[27] M. von Itzstein, B. Jin, W.Y. Wu, and M. Chandler, *Carbohydr. Res.*, 244 (1993) 181–185.
[28] R.U. Lemieux, T.C. Wong, and H. Thøgersen, *Can. J. Chem.*, 60 (1981) 81–86.
[29] J. Haverkamp, T. Spoormaker, L. Dorland, J.F.G. Vliegenthart, and R. Schauer, *J. Am. Chem. Soc.*, 101 (1979) 4851–4853.
[30] S. Prytulla, J. Lauterwein, M. Klessinger, and J. Thiem, *Carbohydr. Res.*, 215 (1991) 345–349.
[31] J. Montreuil, S. Bouquelet, H. Debray, B. Fournet, G. Spik, and G. Strecker, in M.F. Chaplin and J.F. Kennedy (Eds.), *Glycoproteins*, IRL Press, Washington DC, 1986, pp 190–193.
[32] J.N. Varghese, J.L. McKimm-Breschkin, J.B. Caldwell, A.A. Kortt, and P.M. Colman, *Proteins Struct. Funct. Genetics*, 14 (1992) 327–332.
[33] J.J.P. Stewart, MOPAC 6.0, QCPE Program No. 445.
[34] M.J.S. Dewar, *J. Mol. Struct.*, 100 (1983) 41.
[35] C.T. Holzer, M. von Itzstein, B. Jin, M.S. Pegg, W.P. Stewart, and W.Y. Wu, *Glycoconj. J.*, 10 (1993) 40–44.