Syntheses of C-3-Modified Sialylglycosides as Selective Inhibitors of Influenza Hemagglutinin and Neuraminidase

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In an effort to develop new structures as inhibitors of both influenza virus proteins hemagglutinin and neuraminidase, a series of sialic acid derivatives, including those with one of the hydrogen atoms at the C-3 position replaced by either OH or F, were synthesized. The sialic acid derivative with a 3-*eq*-OH group was first synthesized by means of a new process and used as the key intermediate for further derivatization at the C-3 position. The stability of these compounds under acid- and sialidase-catalyzed hydrolysis conditions was studied, and the results showed that these compounds exhibit stronger resistance towards both conditions than their parent *p*-nitrophenyl α-sialoside. Further inhibition assay indicated that the 3-*ax*-OH or F derivatives **4**, **5**, and **24**, the 4-epimer of **4**, are effective specific inhibitors of the sialidases from *Clostridium perfringens*, among other bacterial sialidases tested. The 3-*eq*-OH derivative **3**, however, showed little inhibition. The same tendency was observed for the inhibition of human influenza sialidases N1 and N2. Compounds **3**–**5** and sialic acid were then converted into the distealoylphosphatidylethanolamine conjugates. Of these liposome-like compounds, the ones from **4** and **5** showed potent and selective inhibitory activities against the hemagglutinin H3 subtype, but displayed resistance to the influenza virus neuraminidases N1 and N2.

Introduction

The influenza virus has two major membrane-associated surface proteins: hemagglutinin (HA) and neuraminidase (NA) (Figure 1). The trimeric HA receptor is used for the attachment of viral particles to the host cell through a multivalent specific receptor−ligand interaction.[1] This binding interaction is destroyed by the NA-catalyzed cleavage of the sialoside ligand to allow the virus to escape from the host during the budding process.[2] The ligands or substrates for these two proteins are sialylated oligosaccharides expressed on the host cell surface as part of the glycoconjugates. It is believed that infection of the virus can be inhibited by disturbing either one of the above processes. Recently, several potent inhibitors of NA have been synthesized based on the mechanism and structure of this enzyme.[3] However, development of high-affinity inhibitors of HA is limited to the use of sialic acid-containing polymers as multivalent inhibitors.[4] The H1 serotype of HA, especially, strictly recognizes an α-Neu5Ac(2−3)Gal residue where any modification on the Neu5Ac residue fails to possess inhibitory activity.[1d,5] Also, it was shown that changing the oxygen atom in the glycosidic linkage of Neu5Ac to a sulfur atom is not tolerated by HA (H1) despite the fact that they are both inhibitors of NA.[5b,5c] The polymers containing sialyl lactose have been shown to possess strong inhibitory activities against H1-type HA.[6,7] Also, polymeric compounds carrying Neu5Ac either as *C*- or *S*-glycosidic linkages show strong inhibition of H3-type HA.[8]

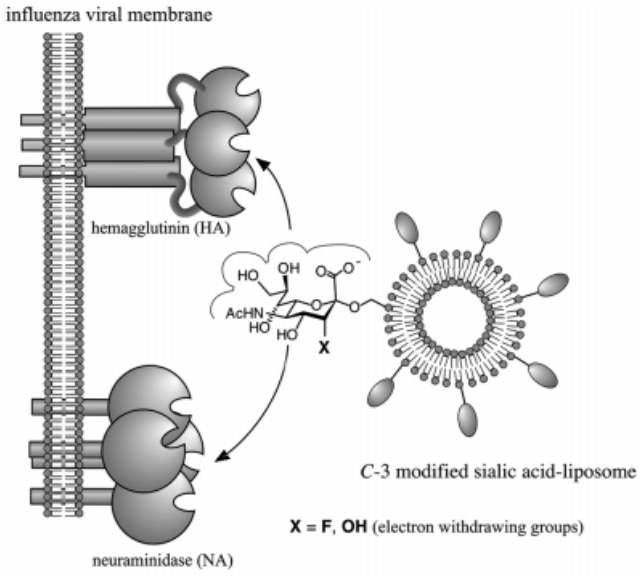

Figure 1. Bifunctional inhibitor of HA and NA

Our aim was to find derivatives or analogs of sialic acid in the form of α-*O*-glycosides which act as inhibitors of HA and NA. These glycosides could be easily prepared and serve as new building blocks for the development of multivalent inhibitors.[9]

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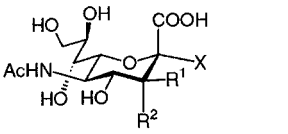
As part of our initial effort directed towards this goal, we describe a useful strategy for the syntheses of a series of sialic acid derivatives as *p*-nitrophenyl α-glycosides **3**−**5**. In each case, the C-3 position was modified with substituents such as OH and F. Preparation of conjugates of such sialosides **6**−**9**, with phosphatidylethanolamine as the liposome, is also described (Figure 2).



	X	R^1	R^2
1	O-4-MU	H	H
2	O-PNP	H	H
3	O-PNP	OH	H
4	O-PNP	H	OH
5	O-PNP	H	F
6	O-DSPE	H	H
7	O-DSPE	OH	H
8	O-DSPE	H	OH
9	O-DSPE	H	F

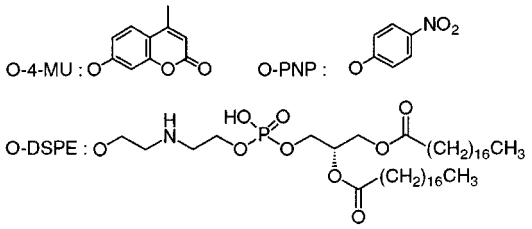
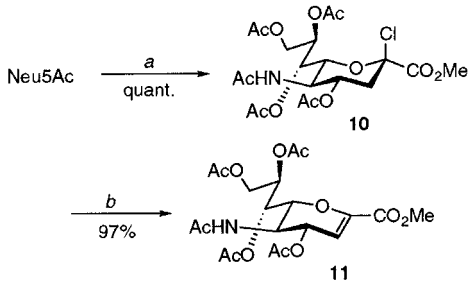


Figure 2. C-3-modified sialosides and liposomes

Results and Discussion

Our decision to make this series of derivatives is based on the crystallographic analysis of HA[10] which revealed that the sialic acid molecule modified at C-3 (axial) or C-7 may be tolerated by the receptor. In addition, based on the crystal structure of NA,[11] the introduction of axially oriented functional groups at C-3 is acceptable. Incorporation of electron-withdrawing substituents at C-3, a position next to the anomeric center, was thus envisioned as such compounds will become resistant to NA owing to the destabilization of the oxocarbonium ion-like transition state during the hydrolysis of glycoside (Figure 1).[12] The *p*-nitrophenyl group was chosen as a chromogenic aglycon as it is useful for enzymatic analysis. Furthermore, in order to enhance anti-influenza activities, a multivalent display of the sialoside on liposomes was prepared by conjugation with phosphatidylethanolamine. Sialyl glycal **11** was used as the starting material for the synthesis of the α-glycosides of 3-*eq*-hydroxyl *N*-acetyl neuraminic acid **14** and **27**, which were then converted into other C-3-modified sialosides.[13]

Before we started the synthesis of C-3-modified sialic acid derivatives, we realized that the synthesis of glycal **11** (Neu5Ac2,3en) required a tedious chromatographic purification after each reaction even though overall yields of up to 86% were reported.[13b,13d,13e,14] We therefore first investigated a more practical approach to the preparation of **11** (Scheme 1). The best result was obtained in three steps starting from sialic acid. Briefly, the methyl ester of sialic acid (Neu5Ac) obtained by acid catalyzed esterification[14a] in almost quantitative yield was treated with acetyl chloride. Concentration of the mixture gave the chloride **10** in more than 98% yield according to ^{1}H-NMR analysis. The crude product **10** was treated with pyridine at 50 °C followed by concentration and trituration to remove pyridine hydrochloride, giving the glycal **11**. Based on the ^{1}H-NMR analysis, compound **11** thus obtained was more than 97% pure and was suitable for the following chemical transformations without further purification. This material could be purified by silica gel column chromatography to yield pure **11** in 96% overall yield, and it could also be obtained as prisms by trituration.

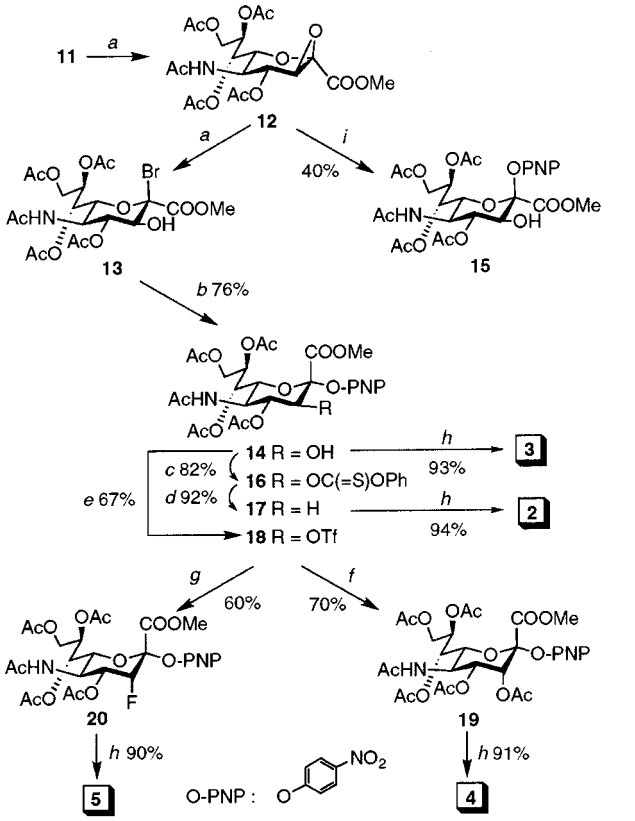


Scheme 1. Reaction conditions: *a*: i) Dowex-50(H^+)−MeOH, ii) AcCl; *b*: Py, 50 °C

Glycosyl bromide **13** with a hydroxyl group at C-3 was prepared from **11** via epoxide **12**.[13b] The α-glycoside **14** was obtained in 76% yield when **13** was treated with sodium *p*-nitrophenoxide in DMF. On the other hand, an attempt using 2,3-epoxide **12** and *p*-nitrophenol in the presence of TMSOTf resulted in the formation of β-glycoside **15** in 40% yield, together with an unidentified by-product (Scheme 2).

The anomeric configuration of **14** was determined by ^{1}H NMR spectroscopy. The coupling constant ($J_{7,8}$) of α-glycoside **14** is 8.5 Hz, and the difference in chemical shifts between 9-H and 9′-H ($\Delta = |\delta(9\text{-H}) - \delta(9'\text{-H})|$) is 0.19 ppm.[13b,13d] Compound **14** was converted into the known α-glycoside **2** through radical-mediated reduction of phenylcarbonylthioate **16** and was identical to an authentic sample.[15]

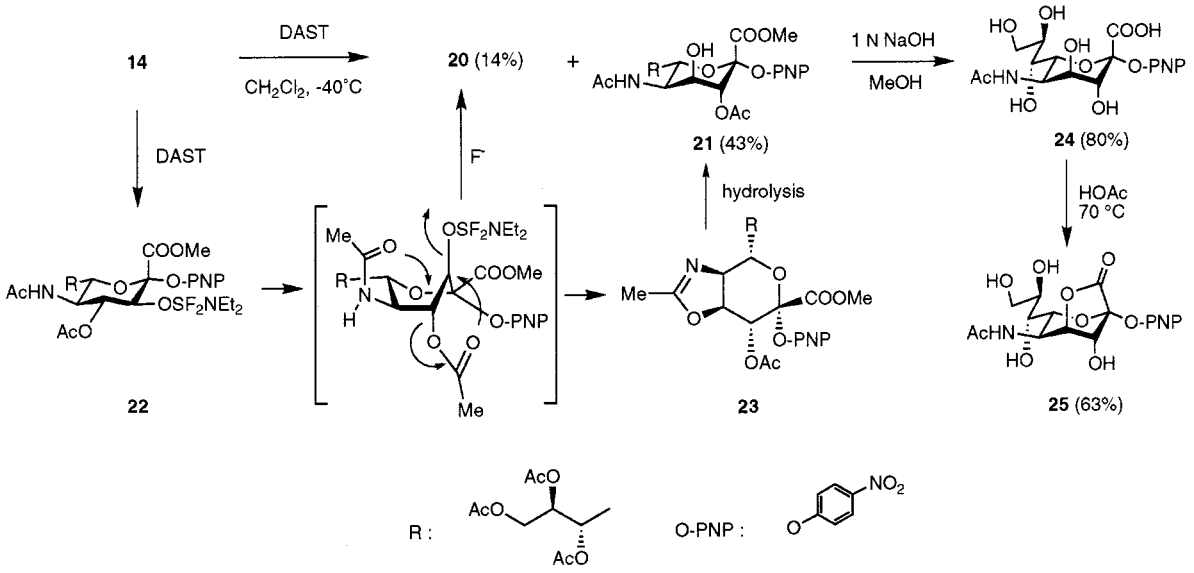
A series of transformations was then carried out using **14**. The trifluoromethanesulfonate **18** was prepared as the common intermediate, which could be isolated by column chromatography and was also found to be stable at room temperature for several weeks. The reaction of **18** with cesium acetate in the presence of 18-crown-6 in refluxing benzene affords **19** (70%). Tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF)[16] was treated with the triflate **18** to afford fluoride **20** in moderate yield. The structure of **20** was supported by ^{1}H- and ^{13}C-NMR analyses which exhibit typical H−F coupling ($J_{3,F}$ = 49.2 Hz) and C−F coupling ($J_{3,F}$ = 191.4 Hz). An attempt to introduce the fluorine atom directly to **14** using diethylaminosulfur trifluoride (DAST) did not give a satisfactory result. Instead of the expected fluoride **20** (14%), compound **21** was obtained as the major product (43%). (Scheme 3) Com-



Scheme 2. Reaction conditions: *a*: ref. 13b; *b*: *p*-$NO_2C_6H_4ONa$/DMF; *c*: PhOC(=S)Cl−DMAP/MeCN, *d*: nBu_3SnH−AIBN/toluene, 110 °C; *e*: Tf_2O−Py/CH_2Cl_2, −5 °C; *f*: CsOAc−18-Crown-6/benzene, reflux; *g*: TASF/THF, reflux; *h*: LiOH, MeOH/H_2O; *i*: *p*-$NO_2C_6H_4OH$, TMSOTf/CH_2Cl_2

pound **21** was considered to be formed by hydrolysis of the oxazoline **23**, which was perhaps generated from **22** through double neighboring-group participation. In fact, the same reaction in which dry methanol was used to quench excess DAST, gave the intermediate **22** (17%), 4,5-oxazoline **23** (51%), and traces of **20** after silica gel column chromatography, although compounds with structure $ROSF_2NEt_2$ were reported to be unstable and could not be isolated.[17] The structure of **23** was evident from both ^{1}H-NMR spectroscopy and FAB-MS. The pyranose ring was assigned the $B_{3,6}$ conformation based on analysis of the coupling constants of the ring protons. Hydrolysis of **23** with acetic acid afforded the 4-*epi*-hydroxy derivative **21**. The axial configuration of the 3-substituent in these compounds was confirmed by ^{1}H NMR spectroscopy. For instance, the coupling constants ($J_{3,4}$) observed for compounds **19**, **20**, and **21** are 3.2, 2.7, and 4.5 Hz, respectively. Finally, deprotection of **14**, **17**, **19**, and **20** with LiOH in a mixture of water and methanol yields the desired compounds **2**−**5**, respectively. However, **21** was deprotected with NaOH in MeOH/water to give compound **24**, since deprotection with LiOH was unsuccessful. Also, **24** tends to form lactone **25** during mass spectrometric analysis. Thus, **24** was converted into **25** by treatment with absolute AcOH to see the difference in enzymatic activity.

In order to understand the effect of C-3 substituents on the chemical stability of ketosidic linkages, acid hydrolysis of these synthetic sialosides was examined.[18] The course of the reaction was monitored by ^{1}H-NMR spectroscopy, in which the amount of *p*-nitrophenol released was determined. The conditions used in the experiment were 1.0 N D_2SO_4/D_2O (pD 1.37) at 50 °C in 600 μL in a NMR tube. The signals for the aromatic ring protons of each parent compound and those of the *p*-nitrophenol formed were clearly distinguishable. (Table 1) The hydrolysis of the synthetic *p*-nitrophenyl α-glycoside of Neu5Ac (**2**) and C-3-modified sialic acid (**3**−**5**, and **24**) was thus performed under the same reaction conditions. As summarized in Table 2, it is clear that the hydrolysis reactions are slower than that of the authentic compound **2**. The observed half-times for the hydrolysis of compounds **3**−**5**, and **24** are 23, 30, 48, and 62 h, respectively, while that for the parent compound **2** is just 0.15 h. This result is believed to be due to



Scheme 3

Table 1. Characteristics of compounds **2**−**5** and **24**

Compound	Substituent at C-3	^{1}H NMR (δ/ppm) 3-H*ax*	3-H*eq*	2′,6′-H	3′,5′-H	UV λ_{max} (nm)[a]	*pK*a 25 °C
2	none (H)	2.76	2.05	8.19	7.33	300	3.97
3	OH (*equiv.*)	4.32	−	8.21	7.28	298	3.22
4	OH (*ax*)	−	5.02	8.21	7.20	312	3.22
5	F (*ax*)	−	5.33	8.24	7.35	298	3.20
24	3,4-dixial OH	−	5.14	8.22	7.20	312	3.18
p-nitrophenol		−	−	8.05	6.74	318	−

[a] λ max corresponding to $\pi \rightarrow \pi^*$ transition.

Table 2. Stability against acid- and enzyme-catalyzed hydrolysis and neuraminidase inhibition

Compound	Hydrolysis $T_{1/2}$ (h) 1 N H_2SO_4 50 °C	Hydrolysis (%) NAs [a] 24 h	*Ki* (M) NA (*C. perfringens*)[b]
2	0.15	100	−
3	23	nd[c]	very weak
4	30	nd	$1.1 \cdot 10^{-6}$
5	48	nd	$2.2 \cdot 10^{-6}$
24	62	nd	$4.4 \cdot 10^{-6}$

[a] NA: neuraminidase; NAs used in the assay were from Arthrobacter ureafaciens, Clostridium perfringens, Vibrio cholerae, influenza virus A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2). − [b] *K*m = 2.3×10^{-4} for compound **1**. − [c] Not detected.

the effect of the electron-withdrawing substituent at the 3-position, which could destabilize the oxocarbonium ion-like transition state during the hydrolysis of the glycoside.[12]

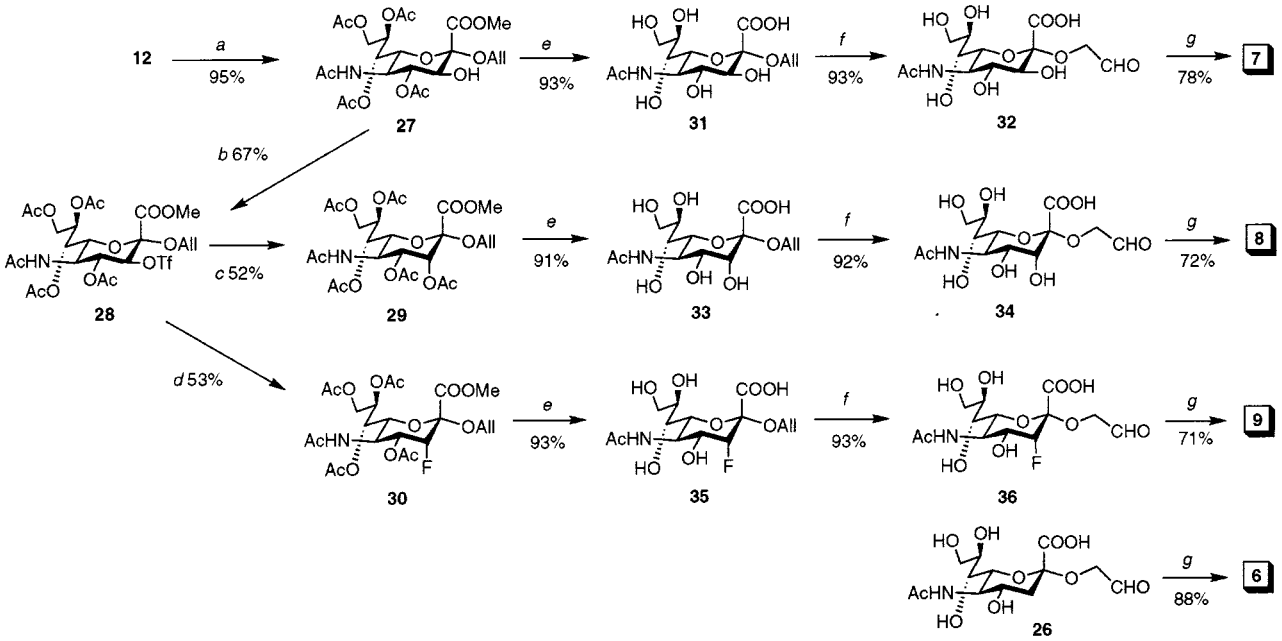
The enzymatic hydrolysis of **3**−**5**, and **24** with sialidases (EC 3.2.1.18) from *Arthrobacter ureafaciens*, *Clostridium perfringens*, *Vibrio cholerae*, influenza A/PR/8/34 and A/Aichi/2/68 were then examined. Incubation of **2** with any of these sialidases resulted in the complete hydrolysis in ca 30 min. On the other hand, there was no apparent hydrolysis of any *p*-nitrophenyl α-glycoside of C-3-modified Nue5Ac even after 24 h.

An inhibition study based on capillary electrophoresis[19] was also carried out using **3**−**5** and **24** as inhibitors of **1** against the sialidases from *A. ureafaciens*, *C. perfringens*, and *V. cholerae*. Compounds **4**, **5**, and **24** were found to be potent inhibitors of sialidase from *C. perfringens* with *K*i values of $1.1 \cdot 10^{-6}$, $2.2 \cdot 10^{-6}$, and $4.4 \cdot 10^{-6}$M, respectively, while compound **3** showed only weak inhibition. This suggests that the equatorial substituent might interact with some amino acid side chain in the catalytic site. Interestingly, none of these compounds showed inhibitory activities against two other sialidases. In addition, compounds **4** and **24** (the 4-epimer of **4**) showed approximately the same inhibitory activity. The observed inhibitory activity of compound **24** was compared with lactone **25**, which showed no inhibition against the enzyme. The same tendency was also observed in the preliminary inhibition study of influenza sialidases (N1 and N2), where compounds **4** and **5** were shown to be potent inhibitors of these enzymes. Compound **3** showed no inhibition.

Encouraged by the result that the designed sialic acid derivatives are selective inhibitors of NA and resistant to both acid- and neuraminidase-catalyzed hydrolysis, we turned our attention to the synthesis of such sialic acid derivatives as phospholipid liposomes[9b] to study their inhibition against HA.

The epoxide **12** was treated with allyl alcohol in the presence of Amberlyst to afford α-allyl sialoside **27** with an *eq*-OH group at C-3, which was then used to synthesize **7**−**9**. In order to perform the inversion reaction at C-3, the same strategy was used as described for the synthesis of PNP sialosides. Thus, **27** was converted into the triflate **28** and allowed to react with CsOAc in the presence of crown ether to give **29** (52%), and with TASF to give **30** (53%). Saponification of compounds **27**, **29**, and **30** followed by ozonolysis and quenching with dimethylsulfide[20] afforded the aldehydes **32**, **34**, and **36** in high yields. Each aldehyde is coupled with distearoylphosphatidylethanolamine (DSPE) under reductive amination conditions[9b] to afford the conjugates **7**−**9**, respectively. The parent NeuAc-DSPE conjugate **6** was prepared from the known aldehyde **26**[20] using the same reductive amination (Scheme 4).

Our preliminary binding and inhibition assay of compounds **6**, **7**, **8**, and **9** using human influenza viruses A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2) (Table 2) indicated that the C-3-modified sialic acid derivatives are tolerated by the H3 subtype hemagglutinin as was also anticipated from crystallographic analysis. The virus-binding assay revealed that the binding affinity of each compound was as potent as the parent liposome structure **6** (144% enhancement in the case of **7**) against influenza virus A/Aichi/2/68 (H3). A series of compounds **7**−**9** was also shown to be resistant to the NA of both virus strains (data not shown). Although compound **6** was also shown to be more stable than the gangliosides used as standards against NAs,[9b] this was considered to be due to the steric bulk of liposome. Our preliminary assay result showed that the DSPE conjugates **8** and **9** were not inhibitors of influenza sialidases (N1 and



Scheme 4. Reaction conditions: *a*: allyl alcohol/Amberlyst; *b*: Tf_2O-Py/CH_2Cl_2; *c*: CsOAc−18-Crown-6/benzene; *d*: TASF/THF; *e*: LiOH/MeOH−H_2O; *f*: O_3/MeOH then Me_2S; *g*: distearoylphosphatidylethanolamine−$NaBH_3CN$ in $CHCl_3$/MeOH (1:1)

Table 3. Binding and inhibition assay of compounds **6−9** against Influenza viruses A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2)

Compound	Substituent at C-3	Binding assay (%)[a]		HAI (M)[b]	
		H1N1	H3N2	H1N1	H3N2
6	none (H)	nd[c]	25	$1\cdot10^{-3}$	$6.3\cdot10^{-5}$
7	OH (*equiv.*)	nd	36	$1\cdot10^{-3}$	$3.1\cdot10^{-5}$
8	OH (*ax*)	nd	32	$>1\cdot10^{-3}$	$6.3\cdot10^{-5}$
9	F (*ax*)	nd	33	$1\cdot10^{-3}$	$3.1\cdot10^{-5}$
IV^3Neu5AcnLac4Cer		100	100	–	–
IV^6Neu5AcnLac4Cer		nd	>200	–	–

[a] Binding activities of Neu5Ac-DSPE derivatives (**6−9**) to influenza virus A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2) compared to the binding activity of IV^3Neu5AcnLac4Cer as a control. − [b] Hemagglutination inhibition assays were carried out using viruses (2^4 HA unit) and each compound at 4 °C for 1 h. − [c] nd: Not detected.

N2), which may seem to be inconsistent with the inhibitory activity observed for PNP-sialosides (**4** and **5**). However, this might again be explained by the influence of the steric factor of liposome. The C-3-modified compounds did not show any significant binding affinity with H1-type viruses, which is consistent with the previous study.[4,5,8,9b] The hemagglutinin inhibitory activities of these compounds against H1 and H3 types were also investigated and the results are shown in Table 3. Consistent with the virus binding assay, compounds **7−9** are also selective for the H3 type, and approximately 1000-fold increase in the binding affinity was observed when compared to monomeric sialic acid.

Conclusion

A series of *p*-nitrophenyl α-*O*-glycosides of C-3-modified sialic acid **3−5** was synthesized from peracetylated Neu5Ac2en methyl ester (**11**), which was prepared by an improved method. These compounds are resistant to acidic and enzymatic hydrolysis. Interestingly, these C-3 axially modified sialic acid derivatives specifically inhibited sialidase from *C. perfringens* (with a *K*i value of 1.1−4.4 μM) and influenza sialidases (N1 and N2) but no inhibition activity was observed against the other sialidases tested. The C-3-modified sialosides of phosphatidylethanolamine (**7−9**) were also synthesized to examine their anti-influenza activity, in the hope that a multivalent effect would be observed. Preliminary binding and inhibition analyses showed that compounds **7**, **8**, and **9** effectively inhibited human influenza viruses A/Aichi/2/68 (H3N2). Work is in progress using these C-3-modified sialic acids as new building blocks for the development of multivalent inhibitors targeting both the influenza neuraminidase and the receptor hemagglutinin.

Experimental Section

General Methods: TLC was performed on Silica Gel 60-F254 (Merck) and detected by fluorescence quenching or by sulfuric acid. Column chromatography was performed on Silica Gel (Merck, Kieselgel 60). − Optical rotations were determined with a HORIBA SEPA-200 polarimeter. − ^1H NMR spectra were re-

corded at 270 MHz (JEOL EX-270) in $CDCl_3$, CD_3OD (internal Me_4Si, δ = 0), or D_2O (internal 3-trimethylsilyl-[2,2,3,3-D_4]propionic acid sodium salt, δ = 0). − High-resolution fast atom bombardment mass spectrometry (HRFAB-MS) was measured with a JEOL JMS-HX-110 mass spectrometer. Electrospray ionization mass spectrometry (ESI-MS) was measured with a Finigan MAT TSQ 700 mass spectrometer. − Double-deionized water was prepared with a Milli-Q system (Millipore Corp.). − Capillary electrophoresis enzyme assays were performed with a Waters Quanta 4000E capillary electrophoresis system, which was equipped with a 53 cm × 75 μm i.d. fused silica capillary. The capillary used was pretreated or regenerated with 0.1 M KOH (2 min) and elution buffer before each injection. Samples were loaded by hydrostatic pressure at a 10 cm height for 30 s (38.4 nL). Electrophoresis was performed at 20 kV using 50 mM sodium borate (pH 10.2) as electrolyte at a constant temperature of 37 °C. Detections were carried out by on-column measurement of UV absorption at 405 nm at 7.5 cm from the cathode. − Pherograms were recorded with Millenium 2010 system (Millipore Corp). − For the kinetic investigation of PNP-sialosides, Beckman P/ACE System 5010 (USA), which was equipped with a 57 cm × 75 μm i.d. fused silica capillary, was used. The LIF detection using He-Cd Laser from Kimmon Electro Co. Ltd. (Japan) was carried out by on-column measurement at 7.0 cm from the cathode with excitation at 325 nm and a 375 nm band-pass filter was used. The capillary used was pretreated as described above. Samples were loaded automatically by pressure injection for 5.0 sec (300 nL). − Electrophoresis was performed at 15 kV using 50 mM sodium borate as electrolyte at a constant temperature of 35 °C.

Materials: The epoxide **12** and bromide **13** were prepared from **11** according to the procedure described by Okamoto et al.,[13b] aldehyde **26** was synthesized as described by Roy et al.[20] Sialidases (EC 3.2.1.18) from *Arthrobacter ureafaciens*, *Clostridium perfringens*, and *Vibrio cholerae* were commercial products from Boehringer Mannheim Gmb. Millex-GV syringe filters (0.22 mm × 4 mm i.d.) were from Nihon Milliopore Ltd.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (11): A solution of Neu5Ac (10.00 g, 32.6 mmol) and Dowex-50 (H^+) resin (10.00 g) in dry methanol (150 mL) was stirred for 18 h at room temperature, then filtered and washed with methanol. The filtrate and washings were neutralized with Dowex-1 (OH^-) (10.00 g). The solution was filtered and evaporated to dryness to give the crude methyl ester (10.38 g), which was used for the next reaction without further purification. A reaction vessel containing the crude methyl ester in acetyl chloride (100 mL) was stoppered and left for 48 h at room temperature. The solution was azeotroped with toluene three times to give the crude chloride **10** (16.27 g). The crude **10** was dissolved in pyridine (200 mL), and the reaction mixture was stirred for 1 h at 50 °C. The solvent was evaporated to dryness, the residue was treated with ethyl acetate to remove the precipitated pyridine hydrochloride, and then concentrated to give the desired product **11** as a slightly yellow solid in 99% yield (14.94 g). The material obtained was more than 97% pure without any further purification over three steps. Finally, it was purified on a silica gel column with *n*-hexane/acetone (4:3) to afford **11** (14.64 g, 97%). Recrystallization from ethyl acetate/*n*-hexane gave **11** as colorless prisms; m.p. 129−131 °C; the ^1H NMR spectrum was identical to that published.[13b] Each intermediate was also directly crystallized and the physical data were in good agreement with reported values.[13a]

Methyl (*p*-Nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-α-D-*erythro*-L-*gluco*-2-nonulopyranosid)onate (14): A solution of freshly prepared glycosyl bromide **13**[13b] (500 mg, 0.90 mmol) and sodium *p*-nitrophenoxide (520 mg, 2.7 mmol, 3 equiv.) in dry *N*,*N*-dimethylformamide (DMF, 50 mL) was stirred for 12 h at room temperature. The solution was concentrated and xylene was evaporated repeatedly from the residue to remove traces of DMF. The residue was purified on a silica gel column (*n*-hexane/acetone 4:3) to give **14** (430 mg, 76%). $[\alpha]_D^{25}$ = +51.5 (*c* = 0.63, $CHCl_3$). − ^1H NMR ($CDCl_3$): δ = 8.21, 7.16 (AX, each 2 H, *J* = 9.2 Hz), 5.70 (d, 1 H, *J* = 10.0 Hz), 5.34 (ddd, 1 H, *J* = 2.5, 5.8, 8.9 Hz), 5.27 (dd, 1 H, *J* = 1.3, 8.9 Hz), 4.88 (dd, 1 H, *J* = 1.3, 10.0 Hz), 5.23 (t, 1 H, *J* = 8.9 Hz), 4.25 (dt, 1 H, *J* = 8.9, 10.0 Hz), 4.24 (dd, 1 H, *J* = 2.5, 12.3 Hz), 4.12 (dd, 1 H, *J* = 4.9, 8.9 Hz), 4.06 (dd, 1 H, *J* = 5.8, 12.3 Hz), 3.70 (s, 3 H), 3.20 (d, 1 H, *J* = 4.9 Hz), 2.16, 2.11, 2.07, 2.05, 1.94 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 171.57, 170.60, 170.37, 170.11, 170.04, 168.03, 158.97, 143.34, 125.42, 118.61, 101.09, 73.96, 73.56, 72.99, 68.77, 67.10, 62.25, 53.21, 48.12, 23.13, 20.90, 20.81, 20.72, 20.66. − $C_{26}H_{32}N_2O_{16}$ (628.54): calcd. C 49.68, H 5.13, N 4.46; found C 49.63, H 5.09, N 4.42.

Methyl (*p*-Nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-β-D-*erythro*-L-*gluco*-2-nonulopyranosid)onate (15): To a solution of epoxide **12** (135 mg, 0.28 mmol) in dichloromethane (5 mL) containing *p*-nitrophenol (77 mg, 0.55 mmol) and 4 Å molecular sieves (300 mg) was added trimethylsilyl triflate (54 μL, 0.28 mmol) at −78 °C under an argon atmosphere. The mixture was stirred for 1 h, diluted with dichloromethane, filtered and the filtrate was washed with 5% $NaHCO_3$ and brine, dried with anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified on a silica gel column (benzen-acetone 3:1) to afford **15** (70 mg, 40%). $[\alpha]_D^{25}$ = −41.1 (*c* = 0.83, $CHCl_3$). − ^1H NMR ($CDCl_3$): δ = 8.17, 7.14 (AX, each 2 H, *J* = 9.2 Hz), 5.59 (d, 1 H, *J* = 9.9 Hz), 5.44 (dd, 1 H, *J* = 8.9, 10.2 Hz), 5.32 (dd, 1 H, *J* = 2.3, 4.0 Hz), 4.90 (ddd, 1 H, *J* = 2.3, 4.0, 7.0 Hz), 4.64 (dd, 1 H, 2.3, 12.9 Hz), 4.25 (ddd, 1 H, *J* = 9.9, 10.2, 10.9 Hz), 4.08 (dd, 1 H, *J* = 7.0, 12.9 Hz), 4.06 (dd, 1 H, *J* = 2.3, 10.9 Hz), 3.94 (t, 1 H, *J* = 8.9 Hz), 3.83 (s, 3 H), 2.89 (d, 1 H, *J* = 8.9 Hz), 2.18, 2.17, 2.10, 2.08, 1.87 (each s, 3 H). − FABMS *m*/*z*: 628.1 $[M + 1]^+$.

Methyl (*p*-Nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-3-*O*-(phenoxythiocarbonyl)-α-D-*erythro*-L-*gluco*-2-nonulopyranosid)onate (16): A solution of compound **14** (20 mg, 0.03 mmol), 4-(dimethylamino)pyridine (10 mg), and phenyl chlorocarbonylthioate (8.6 μL, 0.06 mmol, 2 equiv.) in anhydrous acetonitrile (3 mL) was stirred for 2 h at room temperature under an argon atmosphere. The mixture was evaporated to dryness. The residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water and brine, dried with anhydrous Na_2SO_4, and evaporated in vacuo to give the crude product, which was purified by chromatography on a silica gel column (*n*-hexane/acetone 4:3) to give **16** (20 mg, 82%). $[\alpha]_D^{25}$ = +5.7 (*c* = 0.80, $CHCl_3$). − ^1H NMR ($CDCl_3$): δ = 7.02−8.22 (m, 9 H), 6.13 (d, 1 H, *J* = 8.5 Hz), 5.55 (d, 1 H, *J* = 9.6 Hz), 5.46 (t, 1 H, *J* = 10.0 Hz), 5.33 (dd, 1 H, *J* = 1.0, 4.2 Hz), 5.31 (ddd, 1 H, *J* = 3.1, 4.2, 5.8 Hz), 5.00 (dd, 1 H, *J* = 1.0, 11.6 Hz), 4.48 (ddd, 1 H, *J* = 9.6, 10.0, 11.6 Hz), 4.23 (dd, 1 H, *J* = 3.1, 12.0 Hz), 4.06 (dd, 1 H, *J* = 5.8, 12.0 Hz), 3.70 (s, 3 H), 2.18, 2.17, 2.07, 2.06, 1.95 (each s, 3 H).

Methyl (*p*-Nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (17): To a solution of **16** (15 mg, 0.02 mmol) in toluene (3 mL) was added tributyltin hydride (10 mL, 0.04 mmol, 2 equiv.) and AIBN (5 mg) under an argon atmosphere. The mixture was heated at 110 °C for 1 h, evaporated to dryness, and the residue was purified by chromato-

graphy on a silica gel column (*n*-hexane/acetone 4:3) to give **17** (14 mg, 92%). The ^{1}H NMR spectrum was identical to published data.[15]

Methyl (*p*-Nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-3-*O*-trifluoromethylsulfonyl-α-D-*erythro*-L-*gluco*-2-nonulopyranosid)-onate (18): To a cold (ice/NaCl bath) solution of **14** (200 mg, 0.32 mmol) in dichloromethane (6 mL) and pyridine (0.152 mL, 1.91 mmol, 6 equiv.), triflic anhydride (Tf_2O) (0.206 mL, 1.28 mmol, 4 equiv.) was added dropwise over 2 min. The reaction solution was removed from the cooling bath and monitored by TLC (*n*-hexane/acetone 2:1). After 20 min, the reaction solution was washed sequentially with ice-cold saturated $NaHCO_3$ solution and water, then dried with anhydrous Na_2SO_4, evaporated and dried under vacuum to give the crude product, which was purified on a silica gel column (*n*-hexane/acetone 2:1) to give **18** (163 mg, 67%). $[\alpha]_D^{25}$ = +35.3 (*c* = 0.39, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 8.23, 7.14 (AX, each 2 H, *J* = 9.2 Hz), 5.64 (d, 1 H, *J* = 8.5 Hz), 5.35 (t, 1 H, *J* = 10.4 Hz), 5.32 (m, 2 H), 5.15 (d, 1 H, *J* = 10.4 Hz), 5.08 (d, 1 H, *J* = 10.8 Hz), 4.39 (ddd, 1 H, *J* = 8.5, 10.4, 10.8 Hz), 4.18 (dd, 1 H, *J*= 2.3, 12.3 Hz), 4.05 (dd, 1 H, *J* = 3.9, 12.3 Hz), 3.67 (s, 3 H), 1.95, 2.06, 2.12, 2.13, 2.18 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 170.51, 170.48, 170.12, 169.97, 169.81, 166.41, 158.30, 143.56, 125.73, 117.83, 98.08, 82.79, 74.07, 69.44, 67.94, 66.33, 62.05, 53.55, 48.52, 22.97, 20.79, 20.63, 20.27. − FABMS *m*/*z* 761.2 $[M + 1]^+$.

Methyl (*p*-Nitrophenyl 5-acetamido-3,4,7,8,9-penta-*O*-acetyl-5-deoxy-α-D-*erythro*-L-*manno*-2-nonulopyranosid)onate (19): To a solution of **18** (65 mg, 0.09 mmol) in dry benzene (10 mL) containing 18-crown-6 (11 mg, 0.04 mmol) was added cesium acetate (50 mg, 0.26 mmol, 3 equiv.). The reaction solution was then heated at reflux for 36 h under an argon atmosphere. The reaction mixture was evaporated and dried under vacuum to give the crude product, which was purified on a silica gel column (*n*-hexane/acetone 4:3) to give **19** (40 mg, 70%). $[\alpha]_D^{25}$ = +21.8 (*c* = 0.41, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 8.17, 7.13 (AX, each 2 H, *J* = 9.2 Hz), 5.88 (d, 1 H, *J* = 3.2 Hz), 5.44 (ddd, 1 H, *J* = 3.4, 5.4, 8.9 Hz), 5.33 (dd, 1 H, *J* = 1.6, 8.9 Hz), 5.22 (d, 1 H, *J* = 9.9 Hz), 5.13 (dd, 1 H, *J* = 3.2, 10.8 Hz), 4.64 (dd, 1 H, *J* = 1.6, 10.8 Hz), 4.36 (dt, 1 H, *J* = 9.9, 10.8 Hz), 4.24 (dd, 1 H, *J* = 3.4, 12.6 Hz), 4.13 (dd, 1 H, *J* = 5.4, 12.6 Hz), 3.70 (s, 3 H), 2.26, 2.20, 2.17, 2.04, 2.06, 1.95 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 170.62, 170.48, 170.19, 170.06, 169.85, 165.97, 158.54, 143.72, 125.55, 119.10, 99.17, 72.09, 69.56, 68.54, 68.03, 67.06, 62.14, 53.75, 44.80, 23.29, 20.99, 20.78, 20.72, 20.58. − HRMS (FAB) *m*/*z* calcd. for $C_{28}H_{35}N_2O_{17}$ $[M + H]^+$: 671.1936; found 671.1943.

Methyl (*p*-Nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-3-fluoro-α-D-*erythro*-L-*manno*-2-nonulopyranosid)onate (20). − Method A: Tris(dimethylamino)sulfonium difluorotrimetylsilicate (TASF) (86 mg, 0.31 mmol) was loaded into a two-necked flask which was capped with a rubber septum. The flask was fitted with a reflux condenser and a positive pressure of argon was applied. To this mixture, a solution of **18** (80 mg, 0.105 mmol) in anhydrous tetrahydrofuran (5 mL) was added with a syringe. The reaction solution was then heated at reflux for 24 h, poured into water and extracted with ethyl acetate (3 × 20 mL). The extract was washed with brine, then dried with anhydrous Na_2SO_4, evaporated and dried under vacuum. The product was purified on a silica gel column (*n*-hexane/acetone 2:1) to give **20** (40 mg, 60%).

Method B: To a solution of **14** (35 mg, 0.06 mmol) in anhydrous dichloroethane at −40 °C was added DAST (41 μL, 0.33 mmol, 6 equiv.). The cooling bath was removed, and the solution was stirred for 48 h at room temperature. The reaction solution was cooled to −30 °C and methanol (4 mL) was added to quench the excess DAST. The solution was then concentrated under reduced pressure to dryness. The residue was purified on a silica gel column (*n*-hexane/acetone 2:1) to give **20** (5 mg, 14%) and methyl (*p*-nitrophenyl 5-acetamido-3,7,8,9-tetra-*O*-acetyl-5-deoxy-α-D-*erythro*-L-*altro*-2-nonulopyranosid)onate (**21**) (15 mg, 43%). The same reaction, in which dry methanol was used to quench the excess DAST, gave methyl [*p*-nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-3-*O*-(diethylamino)(difluoro)sulfanyl-α-D-*erythro*-L-*ugluco*-2-nonlopyranosid]onate (**22**) (7 mg, 17%), methyl (*p*-nitrophenyl 3,7,8,9-tetra-*O*-acetyl-4,5-dideoxy-4′,5′-dihydro-2′-methyloxazolo[5,4-*d*]-α-D-*erythro*-L-*altro*-2-nonulopyranosid)onate (**23**) (20 mg, 51%) and **20** (5 mg, 14%) as an amorphous powder.

Data for 20: $[\alpha]_D^{25}$ = +20.7 (*c* = 0.34, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 8.19, 7.22 (AX, each 2 H, *J* = 9.2 Hz), 5.43 (d, 1 H, *J* = 8.7 Hz), 5.41 (ddd, 1 H, *J* = 2.4, 4.5, 7.9 Hz), 5.22 (dd, 1 H, *J* = 2.7, 49.2 Hz), 4.75 (dd, 1 H, *J* = 1.2, 11.1 Hz), 4.25 (dd, 1 H, *J* = 2.4, 12.1 Hz), 4.17 (dd, 1 H, *J* = 4.5, 12.1 Hz), 4.11 (ddd, 1 H, *J* = 8.7, 9.5, 11.1 Hz), 3.74 (s, 3 H), 2.20, 2.16, 2.14, 2.06, 1.97 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 170.51, 170.40, 170.20, 169.40, 165.91, 158.31, 143.90, 125.63, 118.83, 99.30 ($J_{C,F}$ = 15.9 Hz), 87.91 ($J_{C,F}$ = 191.4 Hz), 72.12, 68.46 (d, *J* = 16.0 Hz), 68.04, 67.08, 62.04, 53.90, 45.21 ($J_{C,F}$ = 4.6 Hz), 23.50, 20.95, 20.74, 20.72, 20.70. − HRMS (FAB) *m*/*z* calcd. for $C_{26}H_{32}FN_2O_{15}$ $[M + H]^+$: 631.1819; found 631.1790.

Data for 21: $[\alpha]_D^{25}$ = +10.3 (*c* = 0.35, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 8.16, 7.15 (AX, each 2 H, *J* = 9.2 Hz), 6.11 (d, 1 H, *J* = 9.5 Hz), 5.72 (d, 1 H, *J* = 4.5 Hz), 5.49 (ddd, 1 H, *J* = 2.3, 4.5, 8.1 Hz), 5.28 (dd, 1 H, *J* = 1.2, 8.1 Hz), 4.96 (dd, 1 H, *J* = 1.2, 9.5 Hz), 4.70 (br, 1 H), 4.27 (dd, 1 H, *J* = 2.3, 11.7 Hz), 4.20 (dd, 1 H, *J* = 4.5, 11.7 Hz), 4.11 (br, 1 H, 4.03 (dt, 1 H, *J* = 3.2, 9.5 Hz), 3.61 (s, 3 H), 2.24, 2.22, 2.16, 2.07, 2.04 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 171.59, 171.46, 171.07, 170.69, 170.03, 166.09, 159.26, 144.04, 125.88, 119.84, 98.62, 71.59, 69.96, 69.13, 68.59, 68.37, 62.63, 53.41, 46.72, 24.03, 21.57, 21.37, 21.30, 21.19. − FABMS *m*/*z*: 628.2 $[M + 1]^+$.

Data for 22: $[\alpha]_D^{25}$ = +21.9 (*c* = 0.55, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 8.23, 7.12 (AX, each 2 H, *J* = 9.2 Hz), 5.39 (d, 1 H, *J* = 9.9 Hz), 5.34 (ddd, 1 H, *J* = 3.1, 5.5, 7.2 Hz), 5.26 (d, 1 H, *J* =7.2 Hz), 5.24 (t, 1 H, *J* = 9.9 Hz), 4.91 (dd, 1 H, *J* = 1.8 Hz), 4.47 (d, 1 H, *J* = 9.9 Hz), 4.33 (q, 1 H, *J* = 9.9 Hz), 4.18 (dd, 1 H, *J* = 3.1, 12.2 Hz), 4.04 (dd, 1 H, *J* =12.2, 5.5 Hz), 3.66 (s, 3 H), 3.18 (q, 4 H, *J* = 6.3 Hz), 2.17 (each s, 3 H), 2.14, 2.10, 2.06, 1.94, 1.06 (t, 6 H, *J* = 6.3 Hz). − ^{13}C NMR ($CDCl_3$): δ = 171.18, 170.00, 167.73, 159.20, 143.00, 125.62, 117.81, 100.27, 75.85, 73.98, 71.02, 68.30, 66.83, 62.27, 52.89, 47.98, 36.44, 23.11, 20.85, 20.70, 13.71.

Data for 23: $[\alpha]_D^{25}$ = +116.1 (*c* = 0.55, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 8.19, 7.13 (AX, each 2 H, *J* = 9.2 Hz), 5.54 (dd, 1 H, *J* = 2.3, 7.4 Hz), 5.53 (d, 1 H, *J* = 8.2 Hz), 5.37 (ddd, 1 H, *J* = 2.6, 6.1, 7.4 Hz), 4.95 (dd, 1 H, *J* = 8.2, 8.4 Hz), 4.46 (dd, 1 H, *J* = 2.6, 12.3 Hz), 4.23 (dd, 1 H, *J* = 8.4, 11.4 Hz), 4.21 (dd, 1 H, *J* = 6.1, 12.3 Hz), 4.02 (dd, 1 H, *J* = 2.3, 11.4 Hz), 3.70 (s, 3 H), 2.17, 2.14, 2.06, 2.02, 1.78 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 170.62, 169.79, 169.72, 169.20, 167.44, 165.39, 159.21, 142.98, 142.98, 118.35, 98.15, 77.20, 73.39, 70.03, 69.17, 68.56, 64.17, 61.83, 53.71, 29.24, 20.97, 20.72, 20.56, 20.13. − FABMS *m*/*z*: 611.2 $[M + 1]^+$.

(*p*-Nitrophenyl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic) Acid (**2**) To a solution of **17** (10 mg,

0.02 mmol) in methanol (5 mL) was added LiOH·H_2O (5 mg, 0.10 mg, 6 equiv.) dissolved in water (1 mL). After stirring for 1 h at room temperature, the reaction mixture was acidified to pH 4 by adding the cation exchange resin Dowex-50 (H^+). The resins were filtered off, and the filtrate was evaporated to dryness. Chromatography of the residue on Sephadex G-25 in water afforded **2** (6 mg, 94%). The ^{1}H-NMR spectrum was identical to the published data.[15]

(*p*-Nitrophenyl 5-acetamido-5-deoxy-α-D-*erythro*-L-*gluco*-2-nonulopyranosidonic) Acid (3): To a solution of methanol (5 mL) containing **14** (30 mg, 0.05 mmol) was added LiOH·H_2O (12 mg, 0.29 mg, 6 equiv.) dissolved in water (1 mL). After stirring for 1 h at room temperature, the reaction mixture was treated as described for the preparation of **2** to give **3** (20 mg, 93%) as amorphous material after lyophilization. $[\alpha]_D^{25}$ = +40.0 (*c* = 0.23, H_2O). − ^{1}H NMR (D_2O): δ = 8.21, 7.28 (AX, each 2 H, *J* = 9.2 Hz), 4.32 (d, 1 H, *J* = 10.8 Hz), 4.11 (t, 1 H, *J* = 8.6 Hz), 3.77−3.87 (m, 4 H), 3.61 (dd, 1 H, *J* = 6.4, 12.3 Hz), 3.55 (d, 1 H, *J* = 8.6 Hz), 2.04 (s, 3 H). − ESIMS *m*/*z*: 445.0 [M − H]$^-$ (in H_2O/MeOH, 1:1).

(*p*-Nitrophenyl 5-acetamido-5-deoxy-α-D-*erythro*-L-*manno*-2-nonulopyranosidonic) Acid (4): To a solution of methanol (5 mL) containing **19** (20 mg, 0.03 mmol) was added LiOH·H_2O (9 mg, 0.19 mg, 6 equiv.) dissolved in water (1 mL). After stirring for 1 h at room temperature, the reaction mixture was treated as described for the preparation of **2** to give **4** (12 mg, 91%) as amorphous material after lyophilization. $[\alpha]_D^{25}$ = +9.2 (*c* = 0.13, H_2O). − ^{1}H NMR (D_2O): δ = 8.21, 7.20 (AX, each 2 H, *J* = 9.2 Hz), 5.02 (d, 1 H, *J* = 2.4 Hz), 4.41 (t, 1 H, *J* = 9.5 Hz), 4.32 (dd, 1 H, *J* = 2.4, 9.5 Hz), 4.09 (d, 1 H, *J* = 9.5 Hz), 3.92 (ddd, 1 H, *J* = 2.2, 6.8, 8.7 Hz), 3.89 (dd, 1 H, *J* = 2.2, 12.4 Hz), 3.67 (dd, 1 H, *J* = 6.8, 12.4 Hz), 3.55 (d, 1 H, *J* = 8.7 Hz), 2.04 (s, 3 H). − ESIMS *m*/*z*: 445.0 [M − H]$^-$ (in H_2O/MeOH, 1:1).

(*p*-Nitrophenyl 5-acetamido-3-fluoro-3,5-dideoxy-α-D-*erythro*-L-*manno*-2-nonulopyranosidonic) Acid (5): To a solution of methanol (5 mL) containing **20** (14 mg, 0.02 mmol) was added LiOH·H_2O (6 mg, 0.13 mg, 6 equiv.) dissolved in water (1 mL). After stirring for 1 h at room temperature, the reaction mixture was treated as described for the preparation of **2** to give product **5** (9 mg, 90%) as amorphous material after lyophilization. $[\alpha]_D^{25}$ = +13.7 (*c* = 0.18, H_2O). − ^{1}H NMR (D_2O): δ = 8.24, 7.35 (AX, each 2 H, *J* = 9.2 Hz), 5.33 (dd, 1 H, *J* = 2.2, 49.5 Hz), 4.26 (t, 1 H, *J* = 10.2 Hz), 4.18 (d, 1 H, *J* = 10.2 Hz), 3.97 (dd, 1 H, *J* = 2.2, 10.2 Hz), 3.86 (m, 2 H), 3.64 (dd, 1 H, *J* = 6.2, 11.2 Hz), 3.62 (d, 1 H, *J* = 8.7 Hz), 2.04 (s, 3 H). − ESIMS *m*/*z*: 447.0 [M − H]$^-$ (in H_2O/MeOH, 1:1).

(*p*-Nitrophenyl 5-acetamido-5-deoxy-α-D-*erythro*-L-*altro*-2-nonulopyranosidonic) Acid (24): To a solution of methanol (5 mL) containing **21** (15 mg. 0.02 mmol) was added 1 N NaOH (1 mL). After stirring for 1 h at room temperature, the reaction mixture was acidified to pH 4 by adding cation exchange resin Dowex-50 (H^+) at 0 °C. The resins were filtered off, and the filtrate was evaporated to dryness. Chromatography of the residue on Sephadex LH-20 in MeOH afforded **24** (9 mg, 80%) as amorphous material after lyophilization. $[\alpha]_D^{25}$ = +13.3 (*c* = 0.27, H_2O). − ^{1}H NMR (D_2O): δ = 7.20, 8.22 (AX, each 2 H, *J* = 8.7 Hz), 5.14 (d, 1 H, *J* = 2.0 Hz), 4.38 (d, 1 H, *J* = 9.3 Hz), 4.35 (d, 1 H, *J* = 2.0 Hz), 4.17 (d, 1 H, *J* = 9.3 Hz), 3.87 (dd, 1 H, *J* = 2.8, 11.2 Hz), 3.85 (ddd, 1 H, *J* = 5.6, 9.3, 11.2 Hz), 3.66 (dd, 1 H, *J* = 5.6, 11.2 Hz), 3.61 (d, 1 H, *J* = 9.3 Hz), 2.03 (s, 3 H). − ESIMS *m*/*z*: 445.0 [M − 1]$^-$ (in H_2O/MeOH, 1:1).

***p*-Nitrophenyl 5-Acetamido-5-deoxy-α-D-*erythro*-L-*altro*-2-nonulopyranosidono-1,4-lactone (25):** Compound **24** (5.0 mg. 0.01 mmol) was dissolved in acetic acid (5 mL). The solution was stirred for 12 h at 70 °C, then concentrated to dryness. Chromatography of the residue on preparative TLC using $CHCl_3$/MeOH/H_2O (4:3:0.5, R_f: 0.68) afforded **25** (3.1 mg, 63%) as amorphous material after lyophilization. $[\alpha]_D^{25}$ = +21.3 (*c* = 0.30, MeOH). − ^{1}H NMR (CD_3OD): δ = 7.23, 8.21 (AX, each 2 H, *J* = 9.2 Hz), 5.36 (d, 1 H, *J*= 2.9 Hz), 4.58 (dd, 1 H, *J* = 2.9, 9.3 Hz), 4.12 (d, 1 H, *J* = 9.3 Hz), 3.80−3.57 (m, 3 H), 3.67 (br s, 1 H), 3.44 (d, 1 H, *J* = 7.8 Hz), 2.95 (s, 3 H). − ESIMS *m*/*z*: 428.0 [M + 1]$^+$ (in H_2O/MeOH, 1:1).

Methyl (Allyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-α-D-*erythro*-L-*gluco*-2-nonulopyranosid)onate (27): A solution of freshly prepared epoxide **12**[13b] (500 mg, 1.03 mmol) and Amberlyst (50 mg) in dry allyl alcohol (50 mL) was stirred for 1 h under an argon atmosphere at room temperature until **12** had been consumed (based on TLC). The mixture was filtered and concentrated, and the residue was purified on a silica gel column (*n*-hexane/acetone 4:3) to give **27** (515 mg, 95%). $[\alpha]_D^{23}$ = −25.0 (*c* = 0.66, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 5.91 (m, 1 H), 5.69 (d, 1 H, *J* = 11.2 Hz), 5.64 (ddd, 1 H, *J* = 4.3, 5.6, 8.1 Hz), 5.56 (dd, 1 H, *J* = 3.9, 10.9 Hz), 5.47 (dd, 1 H, *J* = 2.2, 8.1 Hz), 5.34 (dd, 1 H, *J* = 2.3, 10.9 Hz), 5.24 (t, 1 H, *J* = 13.5 Hz), 4.51 (dd, 1 H, *J* = 2.2, 10.9 Hz), 4.30 (m, 1 H), 4.26 (dd, 1 H, *J* = 4.3, 11.6, Hz), 4.22 (ddd, 1 H, *J* = 10.9, 11.2, 13.5 Hz), 4.07 (m, 1 H), 4.04 (dd, 1 H, *J* = 5.6, 11.6 Hz), 3.83 (dd, 1 H, *J* = 5.7, 13.5 Hz), 3.78 (s, 3 H), 3.08 (d, 1 H, *J* = 5.7 Hz), 2.18, 2.14, 2.08, 2.07, 1.89 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 171.70, 170.58, 170.17, 169.79, 169.14, 133.89, 117.22, 100.07, 74.16, 73.44, 72.49, 68.84, 67.26, 66.20, 62.65, 52.67, 48.19, 23.04, 21.08, 20.88, 20.79, 20.73. − $C_{23}H_{33}NO_{14}$ (547.51): calcd. C 50.46, H 6.08, N 2.56; found C 50.09, H 6.05, N 2.51.

Methyl (Allyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-3-*O*-trifluoromethylsulfonyl-α-D-*erythro*-L-*gluco*-2-nonulopyranosid)onate (28): To a cold (ice/NaCl bath) solution of **27** (200 mg, 0.37 mmol) in dichloromethane (6 mL) and pyridine (0.182 mL, 2.28 mmol, 6 equiv.), triflic anhydride (Tf_2O) (0.254 mL, 1.52 mmol, 4 equiv.) was added dropwise over 2 min. The reaction solution was removed from the cooling bath and monitored by TLC (*n*-hexane/acetone 4:3). After 20 min, the reaction solution was washed sequentially with ice-cold saturated $NaHCO_3$ solution and water, then dried with anhydrous Na_2SO_4, evaporated, and purified on a silica gel column (*n*-hexane/acetone 3:2) to give **28** (170 mg, 67%). $[\alpha]_D^{23}$ = −30.1 (*c* = 0.56, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 5.88 (m, 1 H), 5.75 (d, 1 H, *J* = 10.8 Hz), 5.40−5.26 (m, 4 H), 5.20 (dd, 1 H, *J* = 2.0, 11.2 Hz), 4.92 (d, 1 H, *J* = 11.1 Hz), 4.79 (dd, 1 H, *J* = 3.0, 10.8 Hz), 4.68 (dt, 1 H, *J* = 10.8, 11.1 Hz), 4.58 (m, 1 H), 4.52 (dd, 1 H, *J* = 3.5, 12.5 Hz), 4.20 (m, 1 H), 4.15 (dd, 1 H, *J* = 5.8, 12.5 Hz), 3.83 (s, 3 H), 2.15, 2.10, 2.09, 2.03, 1.90 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 170.74, 170.61, 170.25, 170.06, 169.73, 167.40, 133.10, 117.23, 97.54, 83,41, 72.63, 70.15, 68.28, 66.76, 62.35, 53.02, 48.87, 30.96, 22.93, 21.04, 20.93, 20.79, 20.74.

Methyl (Allyl 5-acetamido-3,4,7,8,9-penta-*O*-acetyl-5-deoxy-α-D-*erythro*-L-*manno*-2-nonulopyranosid)onate (29): To a solution of **28** (112 mg, 0.17 mmol) in dry benzene (6 mL) containing 18-crown-6 (23 mg, 0.09 mmol) was added cesium acetate (100 mg, 0.52 mmol, 3 equiv.). The mixture was then heated at reflux for 60 h under argon atmosphere. The reaction mixture was evaporated and purified on a silica gel column (*n*-hexane/acetone 3:2) to give **29** (50 mg, 52%). $[\alpha]_D^{23}$ = +6.3 (*c* = 0.49, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 5.77 (m, 1 H), 5.71 (d, 1 H, *J* = 2.7 Hz), 5.34 (dd, 1 H, *J* = 2.4, 7.1 Hz), 5.30 (m, 1 H), 5.22 (m, 1 H), 5.12 (m, 1 H), 5.00 (dd, 1 H, *J* = 2.7, 10.8 Hz), 4.38 (m, 1 H), 4.34 (dd, 1 H, *J* = 3.2, 12.3 Hz),

4.13 (dd, 1 H, J = 2.4, 10.8 Hz), 4.12 (dd, 1 H, J = 4.9, 12.3 Hz), 3.83 (s, 3 H), 2.11, 2.08, 1.97, 1.96, 1.95 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 170.62, 170.13, 169.72, 169.58, 169.47, 132.78, 115.98, 97.21, 71.84, 68.93, 68.56, 67.55, 66.74, 65.26, 61.91, 52,44, 44.48, 22.73, 20.61, 20.34, 20.29, 20.09. − HRMS (FAB) *m*/*z* calcd. for $C_{25}H_{36}NO_{15}$ $[M + H]^+$: 590.2085; found 589.2070.

Methyl (Allyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-3-fluoro-α-D-*erythro*-I-*manno*-2-nonulopyranosid)onate (30): Tris(dimethylamino)sulfonium difluorotrimetylsilicate (TASF) (100 mg, 0.36 mmol) was loaded into a two-necked flask which was capped with a rubber septum. A solution of **28** (80 mg, 0.12 mmol) in anhydrous tetrahydrofuran (5 mL) was added with a syringe. The flask was fitted with a reflux condenser and a positive pressure of argon was applied. The reaction solution was then heated at reflux for 24 h. The mixture was poured into water and extracted with ethyl acetate (3 × 20 mL). The extract was washed with brine, then dried with anhydrous Na_2SO_4, evaporated, and purified on a silica gel column (*n*-hexane/acetone 3:2) to give **30** (36 mg, 54%). $[\alpha]_D^{23}$ = +3.6 (*c* = 0.41, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 5.83 (m, 1 H), 5.44 (m, 2 H), 5.30 (m, 1 H), 5.28 (dd, 1 H, J = 2.1, 9.2, Hz), 5.21 (dd, 1 H, J = 1.7, 12.4 Hz), 5.10 (dd, 1 H, J = 1.5, 8.3 Hz), 5.03 (dd, 1 H, J = 2.1, 48.6 Hz), 4.32 (m, 1 H), 4.27 (dd, 1 H, J = 2.8, 12.5 Hz), 4.21 (dd, 1 H, J = 1.5, 10.4 Hz), 4.13 (dd, 1 H, J = 5.1, 12.5 Hz), 4.11 (m, 1 H), 3.96 (dd, 1 H, J = 6.2, 12.7 Hz), 3.79 (s, 3 H), 2.15, 2.13, 2.08, 2.02, 1.88 (each s, 3 H). − ^{13}C NMR ($CDCl_3$): δ = 171.09, 170.99, 170.65, 170.48, 166.50, 133.66, 118.01, 97.95 ($J_{C,F}$ = 15.6 Hz), 87.99 ($J_{C,F}$ = 192.8 Hz), 71.74, 69.83 ($J_{C,F}$ = 16.9 Hz), 68.54, 67.78, 66.83, 62.75, 53.46, 45.93 ($J_{C,F}$ = 4.6 Hz), 23.79, 21.57, 21.31, 21.18, 21.13. − HRMS (FAB) *m*/*z* calcd. for $C_{23}H_{33}FNO_{13}$ $[M + H]^+$: 550.1936; found 550.1975.

(Allyl 5-acetamido-5-deoxy-α-D-*erythro*-L-*gluco*-2-nonulopyranosidonic) Acid (31): To a solution of **27** (100 mg, 0.18 mmol) in methanol (5 mL) was added $LiOH·H_2O$ (45 mg, 1.08 mmol, 6 equiv.) dissolved in water (2 mL). After stirring for 1 h at room temperature, the reaction mixture was acidified to pH 4 by adding cation exchange resin (Dowex-50 (H^+) at 0 °C. The resin was filtered off, and the filtrate was evaporated to dryness. Chromatography of the residue on Sephadex LH-20 with methanol afforded **31** (61 mg, 93%). $[\alpha]_D^{23}$ = −11.0 (*c* = 0.42, MeOH). − ^{1}H NMR (D_2O): δ = 5.99 (m, 1 H), 5.37 (dd, 1 H, J = 17.6, 2.6 Hz), 5.26 (dd, 1 H, J = 10.8 Hz), 4.28 (m, 2 H), 4.10 (d, 1 H, J = 10.3 Hz), 4.05 (t, 1 H, J = 10.4 Hz), 3.90−3.80 (m, 3 H), 3.68−3.60 (m, 2 H), 3.54 (dd, 1 H, J = 1.5, 8.8 Hz), 2.04 (s, 3 H). − HRMS (FAB) *m*/*z* calcd. for $C_{14}H_{22}NO_{10}$ $[M - H]^-$: 364.1244; found 364.1248.

(2-Oxoethyl 5-acetamido-5-deoxy-α-D-*erythro*-L-*gluco*-2-nonulopyranosidonic) Acid (32): The allyl glycoside **31** (50 mg, 0.14 mmol) was dissolved in methanol (10 mL). The solution was cooled to −78 °C, purged with oxygen for 5 min and then treated with ozone by bubbling till the solution remained blue. After 10 min, the ozone was evacuated with nitrogen for 15 min, and while at −78 °C, dimethyl sulfide (0.5 mL) was added. The resulting solution was allowed to warm to room temperature over 1 h. The clear solution was then evaporated and purified on Sephadex LH-20 with methanol to afford **32** (48 mg, 93%). $[\alpha]_D^{23}$ = −13.2 (*c* = 0.24, MeOH). − ^{1}H NMR (D_2O): δ = 5.11 (m, 1 H), 4.00 (m, 1 H), 3.98 (d, 1 H, J = 10.9 Hz), 3.89−3.80 (m, 3 H), 3.62 (dd, 1 H, J = 4.7, 10.1 Hz), 3.48 (dd, 1 H, J = 6.8, 12.2 Hz), 3.44 (dd, 1 H, J = 1.2, 7.0 Hz), 3.43 (dd, 1 H, J = 3.0, 10.1 Hz), 2.04 (s, 3 H). − HRMS (FAB) *m*/*z* calcd. for $C_{13}H_{20}NO_{11}$ $[M - H]^-$: 366.1036; found 366.1042.

(Allyl 5-acetamido-5-deoxy-α-D-*erythro*-L-*manno*-2-nonulopyranosidonic) Acid (33): To a solution of **29** (50 mg, 0.07 mmol) in methanol (5 mL) was added $LiOH·H_2O$ (17 mg, 0.41 mg, 6 equiv.) dissolved in water (2 mL). After stirring for 1 h at room temperature, the reaction mixture was treated as described for the preparation of **31** to give **33** (23 mg, 91%) as amorphous material after lyophilization. $[\alpha]_D^{23}$ = +9.7 (*c* = 0.17, MeOH). − ^{1}H NMR (D_2O): δ = 5.99 (m, 1 H), 5.35 (dd, 1 H, J = 2.3, 17.6 Hz), 5.23 (dd, 1 H, J = 10.6 Hz), 4.40 (d, 1 H, J = 3.0 Hz), 4.27 (m, 1 H), 4.23 (t, 1 H, J = 11.7 Hz), 4.08 (m, 1 H), 3.95 (m, 1 H), 3.92 (dd, 1 H, J = 2.8, 12.9 Hz), 3.77 (dd, 1 H, J = 1.9, 11.7 Hz), 3.70 (dd, 1 H, J = 3.0, 11.7 Hz), 3.67 (dd, 1 H, J = 7.0, 12.9 Hz), 3.61 (dd, 1 H, J = 1.9, 9.4 Hz), 2.04 (s, 3 H). − FABMS *m*/*z*: 364 $[M - H]^-$.

(2-Oxoethyl 5-acetamido-5-deoxy-α-D-*erythro*-L-*manno*-2-nonulopyranosonic) Acid (34): The allyl glycoside **33** (12 mg, 0.03 mmol) was dissolved in methanol (10 mL), and the reaction mixture was treated as described for the preparation of **32**, to give **34** (11 mg, 92%) as amorphous material after lyophilization. $[\alpha]_D^{23}$ = +15.6 (*c* = 0.13, MeOH). − ^{1}H NMR (D_2O): δ = 5.05 (m, 1 H), 4.49 (d, 1 H, J = 2.9 Hz), 4.28 (t, 1 H, J = 11.0 Hz), 3.96−3.85 (m, 3 H), 3.79 (dd, 1 H, J = 1.9, 9.3 Hz), 3.68 (dd, 1 H, J = 2.9, 11.0 Hz), 3.65 (dd, 1 H, J = 5.9, 10.9 Hz), 3.60−3.54 (m, 2 H), 2.05 (s, 3 H). − HRMS (FAB) *m*/*z* calcd. for $C_{13}H_{20}NO_{11}$ $[M - H]^-$: 366.1036; found 366.1041.

(Allyl 5-acetamido-3-fluoro-3,5-dideoxy-α-D-*erythro*-L-*manno*-2-nonulopyranosidonic) Acid (35): To a solution of **30** (28 mg, 0.05 mmol) in methanol (5 mL) was added $LiOH·H_2O$ (13 mg, 0.3 mg, 6 equiv.) dissolved in water (1 mL). After stirring for 1 h at room temperature, the reaction mixture was treated as described for the preparation of **31** to give product **35** (16 mg, 93%) as amorphous material after lyophilization. $[\alpha]_D^{23}$ = +8.5 (*c* = 0.21, MeOH). − ^{1}H NMR (D_2O): δ = 5.96 (m, 1 H), 5.36 (dd, 1 H, J = 1.7, 16.9 Hz), 5.25 (dd, 1 H, J = 1.7, 10.1 Hz), 5.17 (dd, 1 H, J = 2.3, 51.7 Hz), 4.27 (m, 1 H), 4.22 (t, 1 H, J = 10.9 Hz), 4.13 (dd, 1 H, J = 6.3, 11.8 Hz), 3.95−3.88 (m, 2 H), 3.84 (dd, 1 H, J = 2.3, 10.9 Hz), 3.80 (d, 1 H, J = 1.8 Hz), 3.66 (dd, 1 H, J = 6.3, 12.2 Hz), 3.60 (d, 1 H, J = 8.8 Hz), 2.05 (s, 3 H). − FABMS *m*/*z*: 366 $[M - H]^-$.

(2-Oxoethyl 5-acetamido-3-fluoro-3,5-dideoxy-α-D-*erythro*-L-*manno*-2-nonulopyranosidonic) Acid (36): The allyl glycoside **35** (12 mg, 0.03 mmol) was dissolved in methanol (10 mL). The reaction mixture was treated as described for the preparation of **32**, to give **36** (11 mg, 93%) as amorphous material after lyophilization. $[\alpha]_D^{23}$ = +4.8 (*c* = 0.12, MeOH). − ^{1}H NMR (D_2O): δ = 5.08 (m, 1 H), 4.10 (d, 1 H, J = 2.7, 51.6 Hz), 4.14 (t, 1 H, J = 11.3 Hz), 3.83−3.75 (m, 2 H), 3.80 (dd, 1 H, J = 2.7, 12.8 Hz), 3.70 (dd, 1 H, J = 1.4, 11.3 Hz), 3.66 (dd, 1 H, J = 5.2, 9.8 Hz), 3.59 (dd, 1 H, J = 5.9, 13.1 Hz), 3.52 (d, 1 H, J = 9.2 Hz), 3.44 (dd, 1 H, J = 5.2, 11.1 Hz), 1.97 (s, 3 H). − FABMS *m*/*z* 368 $[M - H]^-$.

α-(3-β-Hydroxy-Neu5Ac)-ethyl-DSPE (7): Under an argon atmosphere, sodium cyanoborohydride (27 mg, 0.04 mmol) was added in its solid form to a solution of distearoylphosphatidylethanolamine (30 mg, 0.04 mmol) in methanol and chloroform (5 mL, 1:1 v/v). The mixture was stirred at room temperature for 15 min. A solution of **32** (10 mg, 0.03 mmol) in methanol and chloroform (2 mL, 1:1 v/v) was then added dropwise. The clear solution was stirred for another 2 h, until TLC analysis (methanol/chloroform/H_2O, 10:5:1, v/v/v; visualization by molybdenum) indicated the complete consumption of **32** and the formation of a new compound. The reaction mixture was evaporated and purified a on silica gel column (methanol/chloroform/H_2O; 10:5:1, v/v/v) and followed by a Sephadex LH-20 column (MeOH) to give **7** (23 mg, 78%). − ^{1}H NMR ($CDCl_3$/CD_3OD, 1:1, v/v): δ = 5.21 (m, 1 H), 4.39 (dd, 1 H,

J = 3.4, 12.0 Hz), 4.17 (dd, 1 H, J = 6.9, 12.0 Hz), 3.98 (m, 2 H), 3.79 (m, 2 H), 3.60−3.20 (m, 11 H), 2.30 (m, 4 H), 2.04 (s, 3 H), 1.58 (m, 4 H), 1.23 (m, 52 H), 0.84 (t, 6 H, J = 7.2 Hz). − ^{13}C NMR ($CDCl_3$/CD_3OD, 1:1, v/v): δ = 173.47, 173.26, 172.90, 172.70, 99.57, 72.54, 70.64, 69.85, 69.72, 68.14, 66.77, 63.11, 61.88, 50.82, 33.84, 33.32, 31.21, 28.97, 28.84, 28.64, 24.22, 24.19, 21.92, 12.99. − MALDITOFMS m/z: 1119.9 $[M + Na]^+$.

α-(3-α-Hydroxy-Neu5Ac)-ethyl-DSPE (8): Under an argon atmosphere, sodium cyanoborohydride (14 mg, 0.02 mmol) was added in its solid form to a solution of distearoylphosphatidylethanolamine (15 mg, 0.02 mmol) in methanol and chloroform (5 mL, 1:1 v/v). The mixture was stirred at room temperature for 15 min. A solution of **34** (5 mg, 0.01 mmol) in methanol and chloroform (2 mL, 1:1 v/v) was then added dropwise. The clear solution was treated as described for the preparation of **7** to give **8** (11 mg, 72%). − ^{1}H NMR ($CDCl_3$/CD_3OD, 1:1, v/v): δ = 5.23 (m, 1 H), 4.44 (d, 1 H, J = 2.4 Hz), 4.40 (dd, 1 H, J = 2.9, 12.5 Hz), 4.18 (dd, 1 H, J = 6.7, 12.5 Hz), 4.12−4.02 (m, 3 H), 3.84 (m, 3 H), 3.69−3.26 (m, 10 H), 2.37 (m, 4 H), 2.04 (s, 3 H), 1.64 (m, 4 H), 1.30 (m, 52 H), 0.88 (t, 6 H, J = 6.9 Hz). − ^{13}C NMR ($CDCl_3$): δ = 174.19, 173.97, 173.63, 171.19, 121.15, 100.04, 73.18, 71.67, 70.59, 70.49, 69.07, 63.79, 63.55, 62.58, 58.01, 57.21, 34.19, 34.05, 31.94, 29.70, 29.57, 29.35, 29.16, 24.95, 24.90, 22.66, 22.03, 13.69. − MALDITOFMS m/z: 1099.6 $[M + H]^+$.

α-(3-α-Fluoro-Neu5Ac)-ethyl-DSPE (9): Under an argon atmosphere, sodium cyanoborohydride (27 mg, 0.04 mmol) was added in its solid form to a solution of distearoylphosphatidylethanolamine (30 mg, 0.04 mmol) in methanol and chloroform (5 mL, 1:1 v/v). The mixture was stirred at room temperature for 15 min. A solution of **36** (10 mg, 0.03 mmol) in methanol and chloroform (2 mL, 1:1 v/v) was then added dropwise to it. The clear solution was treated as described for the preparation of **7** to give **9** (22 mg, 71%). − ^{1}H NMR ($CDCl_3$/CD_3OD, 1:1, v/v): δ = 5.26 (m, 1 H), 5.17 (d, 1 H, J = 2.4, 52.3 Hz), 4.44 (dd, 1 H, J = 3.2, 12.3 Hz), 4.18 (dd, 1 H, J = 6.5, 12.3 Hz), 4.16−3.55 (m, 15 H), 2.32 (m, 4 H), 2.06 (s, 3 H), 1.61 (m, 4 H), 1.28 (m, 52 H), 0.87 (t, 6 H, J = 6.9 Hz). − ^{13}C NMR ($CDCl_3$/CD_3OD, 1:1, v/v): δ = 173.6 7, 173.51, 173.46, 173.15, 99.92 ($J_{C,F}$ = 16.9 Hz), 91.27 ($J_{C,F}$ = 181.4 Hz), 72.56, 70.85, 69.88, 69.77, 68.03, 63.20, 62.84, 62.50, 59.53, 56.75, 33.55, 33.41, 31.25, 29.01, 28.88, 28.66, 28.45, 24.28, 24.22, 21.96, 21.48, 13.14. − MALDITOFMS m/z: 1123.0 $[M + Na]^+$.

Acid Hydrolysis of 2−5 and 24: The hydrolyses were performed with **2−5** and **24** (4−10 mM) in D_2SO_4/D_2O (pD 1.37, 0.6 mL, 1.0 N), and were monitored by ^{1}H NMR over time at 270 MHz on a JEOL EX-270 spectrometer at 50 °C. Spectral data were collected at 3 h intervals using an acquisition time of 2 s and 20 scans, until the hydrolysis had proceeded to 50%. The reaction yields were calculated from the relative integrals of the signals corresponding to aromatic protons (3′,5′-H and 2′,6′-H) of the substrates to those of *p*-nitrophenol.

Sialidase-Catalyzed Hydrolysis of 2−5 and 24: The reaction mixture (200 μL) containing sodium acetate buffer (50 mM, pH 5.5 for *Anthrobacter ureafaciens* and *Vibrio cholerae*, and pH 5.0 for *Clostridium perfringens*), calcium chloride (5 mM), αNeu5Ac-*O*-PNP (**2**, 0.5 mM) or synthetic substrates (αNeu5Ac^{3}R-*O*-PNP, **3−5** and **24**, 0.5 mM) was incubated with 20 mU of sialidase from *A. ureafaciens*, 15 mU of sialidase from *C. perfringens* or 50 mU of sialidase from *V. cholerae*. No calcium chloride was added in the assays using sialidase from *A. ureafaciens* and *C. perfringens*. The reaction was started by the addition of sialidase and the reaction mixture was incubated at 37 °C. After each time interval, 10 μL of the reaction mixture was removed and the reaction was terminated by the addition of sodium carbonate (10 μL, 200 mM). Assays were performed on a Waters Quanta 4000E capillary electrophoresis system as described in the general procedure section.

Kinetic Analysis of 2−5, 24 and 25 against Neuraminidases: Kinetic analyses of synthetic inhibitors were carried out after initial IC_{50} evaluations. Assays were performed using the buffer systems used for the hydrolysis test. The reaction mixtures containing 4-methylumbelliferyl α-D-*N*-acetylneuraminic acid (αNeu5Ac-OMU, 0.4 mM), various amounts of synthetic compounds (**2−5**, **24** and **25**), and neuraminidase [*Arthrobacter* sialidase (1.25 mU), *Clostridium* sialidase (0.1 mU), or *Vibrio* sialidase (2.0 mU)] in a total volume of 30 μL were incubated for 10 min at 37 °C. The reactions were terminated by adding sodium carbonate (30 μL, mM).

Enzyme-Linked Immunosorbent Assay (ELISA)/Virus-Binding Assay

Influenza Virus: Influenza virus A/PR/8/34 (H1N1) and A/Aichi/2/6 (H3N2) strains were propagated in the allantoic cavity of 11-day-old chicken eggs at 35 °C for 48 h and purified by sucrose density gradient centrifugation.[21] Viral hemagglutination (HA) units were determined using 0.5% chicken erythrocytes.

Anti-Influenza Virus Antibodies: Rabbit anti-influenza virus antibodies were raised against whole A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2) viruses grown in eggs.[22]

Thin-Layer Chromatography (TLC)/Virus-Binding Assay of 6−9: Silica gel plastic plates (Polygram Sil G; Macherey−Nagel, Germany) were developed with glycolipids by using a solvent containing chloroform, methanol and 12 mM aqueous $MgCI_2$ (5:4:1, v/v/v). Immunochemical detection of virions on these thin-layer plates was performed as described previously.[22] Briefly, the TLC plates were put into a covered container and blocked by PBS supplemented by 1% egg albumin (crystallized, Taiyo Kagaku Company, Ltd., Yokkaichi, Japan) and 1% polyvinylpyrrolidone (solution A) at room temperature for 2 h. After removal of the blocking solution by suction, the plates were washed with PBS three times and incubated with purified intact virus suspension (PBS containing A/PR/8/34 or A/Aichi/2/68) at a concentration of 2^8 HA units at 4 °C for a maximum of 12 h. As a negative control, test plates were incubated without virus. After washing with PBS five times, the test plates were blocked by solution A at 4 ????C for 30 min, washed with PBS five times and incubated with the solution of antibody (diluted ascites) at 4 °C for 2 h. Antibody was diluted with each anti-influenza virus antibody diluted 1:1000 with PBS containing 3% polyvinylpyrrolidone (solution B). After washing with PBS the plates were blocked by solution A at 4 °C for 30 min. After removal of the blocking solution, the plates were washed with PBS and incubated at 4 °C for 2 h with horseradish peroxidase-conjugated protein A (Organon Teknika N.V. Cappel Products) for the rabbit anti-influenza virus antibodies, diluted 1:1000 with solution B. After washing with PBS, the plates were incubated with the substrate solution [10 mM Tris-HCl buffer (pH 7.2): 3% 4 chloro-1-naphthol in methanol/3% aqueous H_2O_2 (5:1:0.02, v/v/v)] at room temperature for 20 min. The binding activities of the virus to each compound (**6−9**) were determined by scanning the stained chromatogram at 620 nm (reference 430 nm) with a dual-wavelength flying-spot scanner (CR-910, Shimadzu Kyoto). As a control, the sialylphospholipids were revealed by spraying the plate with Dittmer's or resorcinol/HCl reagent.

Hemagglutination Inhibition Activity of Synthetic Sialylphospholipids 6−9: The hemagglutination inhibition activity (HAI) assay was

carried out by using 96-well-type microtest plates essentially as described previously.[23] Phosphate buffer saline (PBS), pH 6.5, containing 0.01% gelatin was used as the dilution buffer. Human erythrocytes were used as indicator cells. Virus suspension (2^4 HA units in 0.025 mL of PBS) was added to each well containing synthetic sialylphospholipids (**6–9**) in twofold serial dilutions (starting concentration, 2 mM) with PBS containing 0.01% gelatin. After incubated for 1 h at 4 °C, 0.5% (v/v) human erythrocytes in PBS (0.05 mL/well) were added to the plates, which were then kept at 4 °C for 1 h. The maximum dilution of the samples showing complete inhibition of the hemagglutination was defined as the hemagglutination inhibition titre.

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