

Synthesis of *N*-Glycolyl-8-*O*-sulfoneuraminic Acid

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N-Glycolyl-8-*O*-sulfoneuraminic acid (**1**) was synthesized for the first time in good yield. The key intermediates for synthesis of **1**, neuraminic acid *O*-benzyl α -glycoside (**3**) and neuraminic acid *S*-methyl α -glucoside (**10**) were prepared from readily available *N*-acetylneuraminic acid. The structures of these compounds were confirmed by analysis of their NMR spectra.

Key words *N*-glycolyl-8-*O*-sulfoneuraminic acid; sialic acid; sulfur trioxide trimethylamine complex; sulfation; saponification

Sialic acids are biologically important compounds widely distributed in nature in various forms. Sialic acid residues are located at the non-reducing ends of oligosaccharides, glycoproteins and glycolipids.^{1,2)} A sialic acid containing a sulfate group, *N*-glycolyl-8-*O*-sulfoneuraminic acid (**1**, Neu5Gc8S), was found in a component of sialosphingolipid (Neu5Gc8S α 2 $\rightarrow$ 6Glc β 1 $\rightarrow$ 1Cer) of gonads and eggs of sea urchin by Kochetkov *et al.*³⁾ and Kubo *et al.*⁴⁾

As a part of our studies on the partial *O*-sulfation of sialic acid, we report here the first synthesis of **1**. Our investigation began by evaluating two synthetic strategies, one starting from methyl [benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosid]onate (**2**)^{5,6)} and the other from benzyl 2,7-anhydro-4,9-di-*O*-benzoyl-5-*N*-(*O*-benzyl-*O*-glycolyl)-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosonate (**10**).⁷⁾

The structures of the synthesized derivatives were elucidated mainly on the basis of the 300 MHz proton nuclear magnetic resonance (¹H-NMR) spectra. The

proton assignments were based on published data⁸⁾ and spin-decoupling experiments.

Results and Discussion

The starting material **2** was prepared according to the reported procedure.^{5,6)} There have been several reports on hydrolysis methods for the 5-acetamido group of *N*-acetylneuraminic acid derivatives.^{7,9–12)} We have developed a facile method for preparation of the 5-*N*-deacetyl derivative, benzyl 5-amino-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosidonic acid (**3**).¹²⁾

Compound **2** was hydrolyzed by heating it in an aqueous barium hydroxide solution to afford the free amine **3** in

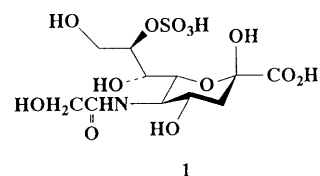


Fig. 1

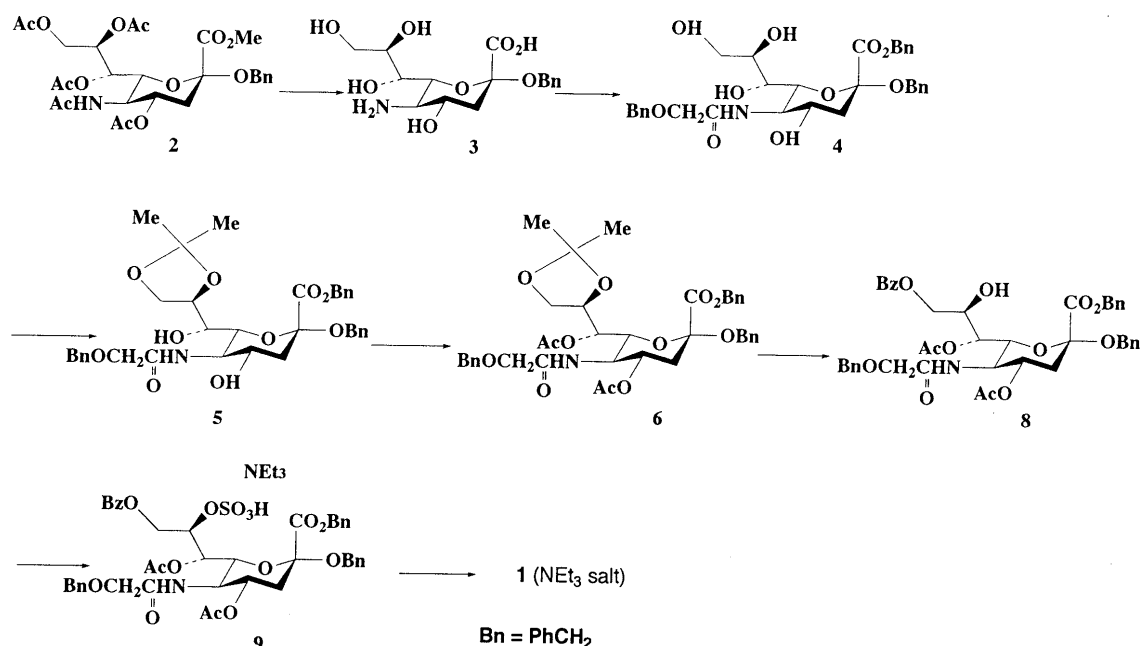


Chart 1

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60% yield after purification by recrystallization from isopropyl alcohol. After the treatment of **3** with *N*-succinimidyl *O*-benzylglycolate, the product was submitted to the following esterification step without isolation. The cesium salt of the product was treated with benzyl bromide in *N,N*-dimethylformamide (DMF) to give benzyl (benzyl *N*-(*O*-benzylglycolyl)-3,5-dideoxy-2- α -D-glycero-D-galacto-2-nonulopyranosid)onate (**4**) in 80% yield. The structure of **4** was deduced from a comparison of the $^1\text{H-NMR}$ data of **3** and **4**. Signals of six methylene protons on three benzyl groups in **4** (methylene on ester group, methylene on aglycon, and methylene on ether) appeared at δ 4.29 and 4.74, δ 4.52 and 4.56, and δ 5.13 and 5.18, respectively. The treatment of **4** with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid in acetone gave the crystalline 8,9-*O*-isopropylidene derivative **5**, which was acetylated with acetic anhydride in pyridine to give the fully protected intermediate **6** in good yield. The structures were elucidated on the basis of the $^1\text{H-NMR}$ spectra. The *O*-acetylated positions of **6** were elucidated to be C-4 and C-7 on the basis of the fact that two deshielded signals due to 4-H and 7-H appeared at δ 4.98 and δ 5.42, respectively.

After removal of the isopropylidene group in **6** with 80% $\text{AcOH-H}_2\text{O}$, the product **7** was treated with benzoic anhydride in pyridine to give regioselectively the 9-*O*-benzoate (**8**) in good yield. The sterically hindered 8-OH of **7** was not benzoated owing to the low reactivity of

benzoic anhydride.¹³⁾ The structure of **8** was unambiguously assigned from the $^1\text{H-NMR}$ spectral data as shown in Table 1, with characteristic signals of 3- H_{ax} at δ 2.09 (dd, $J=13.0, 12.0\text{ Hz}$), 3- H_{eq} at δ 2.88 (dd, $J=12.0, 5.0\text{ Hz}$), and 8-H at δ 4.26 (br dt, $J=9.0, 7.0\text{ Hz}$).

The sulfation of **8** was carried out with an excess of sulfur trioxide trimethylamine complex in dry DMF in the presence of molecular sieves 4A powder to give the trimethylammonium salt, whose cationic moiety was easily exchanged during purification by silica gel chromatography using a triethylamine-containing eluent,¹⁴⁾ to give **9** in 92% yield. The structure of **9** was deduced from a comparison of the $^1\text{H-NMR}$ spectral data of **8** and **9** as shown in Table 1, the chemical shift at δ 5.23 for 8-H being strongly indicative of the sulfated position.

In the deprotection of **9**, the acetyl groups and benzyl ester were hydrolyzed with 1 *N* aqueous sodium hydroxide at room temperature, and the benzyl ether was cleaved by catalytic hydrogenation over palladium-on-charcoal to give **1** as an amorphous powder in 48% overall yield from **2**. The structure of **1** was confirmed from a comparison of the spectral data of *N*-glycolylneuraminic acid and **1**. The sulfated position of **1** was elucidated to be C-8 on the basis of the fact that a downfield-shifted signal for 8-H appeared at δ 4.36 in the $^1\text{H-NMR}$ spectrum, owing to caused by the deshielding effect of the 8-sulfate group. In the $^{13}\text{C-NMR}$ spectrum of **1**, a downfield-shifted signal at δ 80.47 is observed for C-8 as compared with that of

Table 1. $^1\text{H-NMR}$ Spectral Data for **1**,^{a)} **8**, **9**, **11**, and **12**^{a)} in CDCl_3 or D_2O ^{a)}

Compd.	Chemical shifts (δ)											
	3- H_{ax}	3- H_{eq}	4-H	5-H	6-H	Sialic acid moiety		9-H	9-H'	NH	NCOCH	NCOCH'
						7-H	8-H					
1	1.77	2.17	4.06	3.94	3.97	3.81	4.36	3.80	3.99	—	4.06	4.10
8	2.09	2.88	4.89	4.26	4.00	5.22	4.26	4.47	4.33	6.29	3.83	3.87
9	1.99	2.75	5.08	4.16	4.45	5.73	5.23	4.38	4.87	6.51	3.81	3.88
11	2.35	2.29	5.14	4.41	5.13	4.91	4.62	4.52	4.92	7.00		3.98
12	2.13	1.97	3.90	3.97	4.62	4.63	4.10	3.70	3.76	—		4.30

Compd.	Spin couplings (Hz)											
						Sialic acid moiety						
	$J_{3\text{ax},3\text{eq}}$	$J_{3\text{ax},4}$	$J_{3\text{eq},4}$	$J_{4,5}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$	$J_{5,\text{NH}}$	$J_{\text{NCOCHH}'}$
1	13.0	12.0	5.0	10.0	9.0	0—	9.5	6.2	2.7	12.0	—	5
8	13.0	12.0	5.0	10.5	10.5	2.5	9.0	9.0	7.0	12.0	10.5	15.0
9	12.5	12.5	5.0	10.0	10.5	2.0	4.0	6.0	3.0	12.0	10.0	15.0
11	15.0	5.0	0—	0—	0—	0—	9.5	2.5	2.0	12.0	10.0	15.0
12	15.0	6.5	0—	0—	0—	0—	8.5	3.0	2.5	12.5	—	0

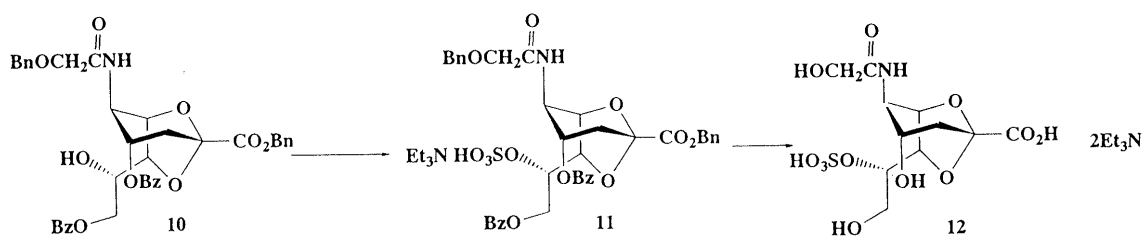


Chart 2

N-glycolylneuraminic acid triethylamine salt, and the resonances of the adjacent C-7 and C-9 atoms are displaced upfield by 1.9 and 3.2 ppm, respectively. ^{13}C -NMR spectral data of *N*-glycolylneuraminic acid triethylamine salt are given in the experimental section. These results are in agreement with general observations on the influence of a sulfate group on ^{13}C -NMR chemical shifts.¹⁵⁾

In the same manner, treatment of **10** in DMF with sulfur trioxide trimethylamine complex gave the sulfate **11** in 92% yield. Compound **12** was obtained by saponification and catalytic hydrogenolysis of **11**. The structures were unambiguously assigned from the ^1H -NMR spectral data. However, the 2,7-anhydro bond of **12** could not be hydrolyzed under acidic conditions, and the sulfate group remained intact.

In conclusion, we have developed a convenient method for preparation of the free amine derivative of sialic acid, α -benzyl glycoside of neuraminic acid (**3**), and we utilized this derivative to synthesize **1**. Furthermore, we found that the sulfate group of **12** was more easily hydrolyzed than its 2,7-anhydro bond under acidic conditions.

Experimental

Melting points were measured with a Yamato melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Thin layer chromatography (TLC) was performed on silica gel (Merck) plates, and spots were detected by spraying with 5% sulfuric acid solution. Infrared (IR) spectra were recorded with a JASCO A-2 spectrometer. The NMR spectra were taken in CDCl_3 or D_2O with tetramethylsilane (TMS) as an internal standard, with a Varian VXR-300 spectrometer. Column chromatography was conducted on Silica gel 60 (70–230 mesh, Merck).

Benzyl 5-Amino-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosidonic Acid (3) A solution of methyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosid)-onate (**2**, 2.0 g, 3.45 mmol) in 5% aqueous barium hydroxide (40 ml) was heated for 10 h at 90 °C. The mixture was brought to pH 6.0 by addition of 1 N sulfuric acid at 0 °C and filtered through Celite. The filtrate was brought to pH 10 by addition of 1 N sodium hydroxide and evaporated to dryness *in vacuo* at 60 °C. The residue was dissolved in water (10 ml) and the precipitate was removed by filtration. The filtrate was filtered through IRC-50 (H^+) resin (20 ml) which was washed with water (40 ml), and the combined filtrates were lyophilized to give crude **3** (1.13 g, 92%) as a powder. It was recrystallized from isopropyl alcohol to give pure **3** (0.74 g, 60%) as colorless needles. mp 186–187 °C $[\alpha]_{\text{D}}^{18} -42^\circ$ ($c=1$, H_2O). (ref.¹²⁾ $[\alpha]_{\text{D}}^{20} -41^\circ$ ($c=0.5$, H_2O). Anal. Calcd for $\text{C}_{16}\text{H}_{23}\text{NO}_8$: C, 53.77; H, 6.49; N, 3.92. Found: C, 53.55; H, 6.53; N, 3.88. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3380, 2960, 1610, 1550. ^1H -NMR (300 MHz, CDCl_3) δ : 1.61 (1H, t, $J=12.0$ Hz, 3- H_{ax}), 2.72 (1H, dd, $J=4.5$, 12.0 Hz, 3- H_{eq}), 3.64 (1H, ddd, $J=12.0$, 10.0, 5.0 Hz, 4-H), 3.14 (1H, t, $J=10.0$ Hz, 5H), 3.90 (1H, dd, $J=10.0$, 2.0 Hz, 6-H), 3.70 (1H, dd, $J=9.0$, 2.0 Hz, 7-H), 3.76 (1H, ddd, $J=2.0$, 5.5, 2.5 Hz, 8-H), 3.62 (1H, dd, $J=11.5$, 5.5 Hz, 9-H), 3.78 (1H, dd, $J=11.5$, 2.5 Hz, 9-H'), 4.44 (1H, d, $J=11.0$ Hz, $-\text{CH}-\text{Ph}$), 4.64 (1H, d, $J=11.0$ Hz, $-\text{CH}'-\text{Ph}$), 7.72 (5H, m, phenyl group).

Benzyl [Benzyl 5-*N*-(*O*-Benzylglycolyl)-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosid]onate (4) *N*-Succinimidyl *O*-benzylglycolate (0.3 g, 1.5 mmol) was added to a solution of **3** (0.36 g, 1 mmol) in acetonitrile–water (4:1, 10 ml), and the mixture was kept for 16 h at room temperature, and then evaporated to dryness *in vacuo*. The residue was dissolved in ethyl acetate (50 ml) and filtered. The filtrate was evaporated to dryness. The residue was dissolved in DMF (5 ml), and to this solution benzyl bromide (1.0 g, 5.8 mmol) was added. The mixture was stirred for 5 h at room temperature and evaporated at 60 °C under reduced pressure. The residual syrup was purified on a column of silica gel with chloroform–ethanol (1:1) to give **4** (0.48 g, 80%) as a colorless powder. $[\alpha]_{\text{D}}^{23} -12.3^\circ$ ($c=0.73$, CHCl_3). Anal. Calcd for $\text{C}_{32}\text{H}_{37}\text{NO}_{16}$: C, 64.52; H, 6.26; N, 2.35. Found: C, 64.36; H, 6.31; N, 2.32. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3390, 1735, 1665, 1550. ^1H -NMR (300 MHz, CDCl_3) δ : 1.95 (1H, t, $J=12.5$ Hz, 3- H_{ax}), 2.86 (1H, dd, $J=12.5$, 5.0 Hz, 3- H_{eq}), 3.60 (1H,

ddd, $J=12.0$, 10.0, 4.5 Hz, 4-H), 3.94 (1H, brt, $J=10.0$, 8.0 Hz, 5-H), 3.54 (1H, brd, $J=10.5$ Hz, 6-H), 3.58 (1H, brd, $J=10.0$ Hz, 7-H), 3.95 (1H, ddd, $J=10.0$, 4.5, 3.0 Hz, 8-H), 3.75 (1H, dd, $J=11.0$, 4.5 Hz, 9-H), 3.88 (1H, dd, $J=11.0$, 3.0 Hz, 9-H'), 4.60 (3H, brs, 3 $\times$ $-\text{OH}$), 6.73 (1H, d, $J=8.0$ Hz, 5-NH), 4.29 (1H, d, $J=11.5$ Hz, 1- OCHPh), 4.74 (1H, d, $J=11.5$ Hz, 1- $\text{OCH}'\text{Ph}$), 4.52 (1H, d, $J=12.0$ Hz, 2- OCHPh), 4.56 (1H, d, $J=12.0$ Hz, 2- $\text{OCH}'\text{Ph}$), 4.01 (2H, s, 5- NCOCH_2O), 5.13 (1H, d, $J=12.0$ Hz, 5- $\text{NCOCH}_2\text{OCHPh}$), 5.18 (1H, d, $J=12.0$ Hz, 5- $\text{NCOCH}_2\text{OCH}'\text{Ph}$), 7.12–7.43 (15H, phenyl groups). ^{13}C -NMR (75 MHz, CDCl_3) δ : 176.37 (C1), 98.69 (C2), 40.60 (C3), 68.27 (C4), 52.30 (C5), 74.00 (C6), 69.36 (C7), 70.87 (C8), 64.12 (C9), 69.04 (5- NHCOCH_2), 172.82 (5- NHCO), 68.27 (1- OCH_2), 73.80 (2- OCH_2), 66.37 (5- NHCOCH_2), 127.84, 127.93, 128.23, 128.30, 128.50, 128.63, 128.74, 128.80, 1128.91, 134.46, 136.35, 136.66 (phenyl groups).

Benzyl [Benzyl 4,7-Di-*O*-acetyl-5-*N*-(*O*-benzylglycolyl)-3,5-dideoxy-8,9-*O*-isopropylidene- α -D-glycero-D-galacto-2-nonulopyranosid]onate (6) 2,2-Dimethoxypropane (0.5 ml) and *p*-toluenesulfonic acid (50 mg) were added to a solution of **4** (298 mg, 0.5 mmol) in acetone (10 ml). The mixture was stirred for 1 h at room temperature, and then treated with Dowex-1 (OH^-) anion-exchange resin (0.5 g) to remove the acid. The resin was filtered off and washed with acetone. The combined filtrate and washings were evaporated under reduced pressure. The residue was crystallized from ethyl acetate–isopropyl ether to give benzyl [benzyl 5-*N*-(*O*-benzylglycolyl)-3,5-dideoxy-8,9-*O*-isopropylidene- α -D-glycero-D-galacto-2-nonulopyranosid]onate (**5**, 311 mg, 98%) as colorless needles. **5**: mp 120–121 °C. ^1H -NMR (300 MHz, CDCl_3) δ : 1.91 (1H, dt, $J=11.0$, 12.0 Hz, 3- H_{ax}), 2.79 (1H, dd, $J=4.5$, 12.0 Hz, 3- H_{eq}), 3.66 (1H, dt, $J=11.0$, 4.5 Hz, 4-H), 3.96 (1H, ddd, $J=8.0$, 11.0, 10.0 Hz, 5-H), 3.56–3.66 (2H, m, 6-H, 7-H), 4.91 (1H, q, $J=6.0$ Hz, 8-H), 4.08 (1H, dd, $J=5.5$, 8.5 Hz, 9-H), 4.11 (1H, dd, $J=5.5$, 8.5 Hz, 9-H'), 6.52 (1H, d, $J=8.0$ Hz, 5-NH), 4.01 (2H, s, 5- NHCOCH_2), 1.33 (3H, s, isopropylidene methyl), 1.38 (3H, s, isopropylidene methyl), 2.40 (1H, brs, 4-OH), 1.65 (1H, brs, 7-OH), 5.91 (2H, s, 1- OCH_2Ph), 4.44 (1H, d, $J=11.5$ Hz, 2- OCHPh), 4.57 (1H, d, $J=11.5$ Hz, 2- $\text{OCH}'\text{Ph}$), 4.45 (1H, d, $J=11.5$ Hz, $\text{NCOCH}_2\text{OCHPh}$), 4.78 (1H, d, $J=11.5$ Hz, $\text{NCOCH}_2\text{OCH}'\text{Ph}$), 7.19–7.42 (15H, phenyl groups).

Acetic anhydride (1 ml) was added to a solution of **5** (191 mg, 0.3 mmol) in pyridine (2 ml). The mixture was stirred for 14 h at room temperature, poured into ice-water, and extracted twice with chloroform (5 ml). The extract was washed with water, dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue was purified by recrystallization from isopropyl ether to give **6** (194 mg, 90%) as colorless needles. mp 105–106 °C. $[\alpha]_{\text{D}}^{23} -5.0^\circ$ ($c=1$, CHCl_3). Anal. Calcd for $\text{C}_{39}\text{H}_{45}\text{NO}_{12}$: C, 65.08; H, 6.30; N, 1.95. Found: C, 65.12; H, 6.24; N, 1.86. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3000, 1755, 1695, 1610, 1595, 1530. ^1H -NMR (300 MHz, CDCl_3) δ : 2.00 (1H, dd, $J=13.0$, 12.0 Hz, 3- H_{ax}), 2.81 (1H, dd, $J=13.0$, 5.0 Hz, 3- H_{eq}), 4.98 (1H, ddd, $J=12.0$, 10.5, 5.0 Hz, 4-H), 4.12 (1H, q, $J=10.0$ Hz, 5-H), 3.92 (1H, dd, $J=10.5$, 2.0 Hz, 6-H), 5.42 (1H, 1H, dd, $J=3.0$, 2.0 Hz, 7-H), 4.27 (1H, dt, $J=6.5$, 3.0 Hz, 8-H), 4.04 (1H, t, $J=6.5$ Hz, 9-H), 4.07 (1H, t, $J=6.5$ Hz, 9-H'), 2.01 (3H, s, OAc), 2.13 (3H, s, OAc), 6.32 (1H, d, $J=10.0$ Hz, 5-NH), 5.18 (1H, d, $J=11.5$ Hz, 1- $\text{O}-\text{CHPh}$), 5.25 (1H, d, $J=11.5$ Hz, 1- COCHPh), 4.53 (1H, d, $J=11.5$ Hz, 2- OCHPh), 4.58 (1H, d, $J=11.5$ Hz, 2- $\text{OCH}'\text{Ph}$), 1.36 (6H, s, isopropylidene methyl), 3.85 (1H, d, $J=15.0$ Hz, 5- NCOCHO), 3.92 (1H, d, $J=15.0$ Hz, 5- $\text{NCOCH}'\text{O}$), 4.39 (1H, d, $J=11.5$ Hz, 5- NHCOCH_2), 4.74 (1H, d, $J=11.5$ Hz, 5- $\text{NCOCH}_2\text{OCHPh}$), 7.29–7.42 (15H, phenyl groups).

Benzyl [Benzyl 4,7-Di-*O*-acetyl-9-*O*-benzoyl-5-*N*-(*O*-benzylglycolyl)-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosid]onate (8) A solution of **6** (144 mg, 0.2 mmol) in 90% acetic acid was stirred for 5 h at 50 °C. The mixture was evaporated *in vacuo* to yield an amorphous powder, which was purified on a column of silica gel with dichloromethane–methanol (100:1) to give the de-isopropylidene derivative (**7**, 117 mg, 90%) as a colorless powder. ^1H -NMR (300 MHz, CDCl_3) δ : 2.07 (1H, dd, $J=12.0$, 13.0 Hz, 3- H_{ax}), 2.87 (1H, dd, $J=5.0$, 13.0 Hz, 3- H_{eq}), 4.89 (1H, ddd, $J=12.0$, 10.5, 5.0 Hz, 4-H), 4.28 (1H, q, $J=10.5$ Hz, 5-H), 3.93 (1H, dd, $J=10.5$, 2.0 Hz, 6-H), 4.99 (1H, dd, $J=2.0$, 9.5 Hz, 7-H), 3.96 (1H, m, 8-H), 3.51 (1H, dt, $J=4.5$, 12.0 Hz, 9-H), 3.68 (1H, ddd, $J=2.0$, 9.5, 12.0 Hz, 9-H'), 6.30 (1H, d, $J=10.5$ Hz, 5-NH), 3.82 (1H, d, $J=4.5$ Hz, 8-OH), 2.51 (1H, dd, $J=4.5$, 9.5 Hz, 9-OH), 2.01 (3H, s, OAc), 2.14 (3H, s, OAc), 5.22 (1H, d, $J=11.5$ Hz, 1- OCHPh), 5.25 (1H, d, $J=11.5$ Hz, 1- $\text{OCH}'\text{Ph}$), 4.52 (1H, d, $J=11.5$ Hz, 2- OCHPh), 4.58 (1H, d, $J=11.5$ Hz, 2- $\text{OCH}'\text{Ph}$), 3.84 (1H, d, $J=15.0$ Hz, 5- NHCOCHO), 3.89 (1H, d, $J=15.0$ Hz, 5- $\text{NHCOCH}'\text{O}$), 7.15–7.45

(15H, phenyl groups).

A solution of **7** (194 mg, 0.3 mmol) in dry pyridine (2 ml) containing benzoic anhydride (0.3 g, 1.3 mmol) was stirred for 4 h at 40 °C. The mixture was poured into 3% sodium hydrogen carbonate and extracted with chloroform. The extract was evaporated to dryness. The residue was purified on a column of silica gel with dichloromethane–methanol (200:1) to give **8** (223 mg, 95%) as a colorless powder. $[\alpha]_D^{23} -2.5^\circ$ ($c=0.72$, CHCl_3). *Anal.* Calcd for $\text{C}_{43}\text{H}_{35}\text{NO}_{13}$: C, 66.75; H, 4.56; N, 1.81. Found: C, 66.46; H, 4.72; N, 1.79. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 2960, 1755, 1700, 1610, 1695, 1545. $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 2.01 (3H, s, OAc), 2.12 (3H, s, OAc), 5.22 (1H, d, $J=11.5$ Hz, 1-CHPh), 5.25 (1H, d, $J=11.5$ Hz, 1-OCH'), 4.50 (1H, d, $J=11.5$ Hz, 2-OCHPh), 4.57 (1H, d, $J=11.5$ Hz, 2-OCHPh), 4.57 (1H, d, $J=11.5$ Hz, 2-OCH'), 4.34 (1H, d, $J=11.5$ Hz, $\text{NHCOCH}_2\text{OCHPh}$), 4.77 (1H, d, $J=11.5$ Hz, $\text{NHCOCH}_2\text{OCHPh}$), 8.02 (2H, d, $J=7.0$ Hz, benzoyl group), 7.52 (1H, t, $J=7.0$ Hz, benzoyl group), 3.97 (1H, br d, $J=4.5$ Hz, 8-OH), 7.18–7.42 (16H, phenyl groups). $^1\text{H-NMR}$ Spectral data for the sialic acid moiety are given in Table 1.

Benzyl [Benzyl 4,7-Di-O-acetyl-9-O-benzoyl-5-N-(O-benzyl-glycolyl)-3,5-dideoxy-8-O-sulfo- α -D-glycero-D-galacto-2-nonulopyranosid]onate, Triethylammonium Salt (9) Molecular sieves 4A powder (500 mg) and an excess of sulfur trioxide trimethylamine complex (0.15 g, 1.1 mmol) were added to a solution of **8** (78 mg, 0.1 mmol) in dry DMF (0.5 ml) under an argon atmosphere. The mixture was stirred for 36 h at room temperature. After the addition of triethylamine (1 ml), the mixture was filtered and the filtrate was evaporated under reduced pressure at 60 °C. The residue was subjected to TLC on silica gel, being developed with toluene–ethanol–triethylamine (3:1:0.5) to give **9** (89 mg, 92%) as a colorless powder. $[\alpha]_D^{23} +10.1^\circ$ ($c=1.5$, CHCl_3). *Anal.* Calcd for $\text{C}_{43}\text{H}_{45}\text{NO}_{16}\text{S}\cdot\text{C}_6\text{H}_5\text{N}$: C, 60.98; H, 6.27; N, 1.45. Found: C, 60.88; H, 6.34; N, 1.38. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1760, 1730, 1700, 1610, 1595, 1540, 1510, 1240, 815. $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 1.97 (3H, s, OAc), 2.09 (3H, s, OAc), 5.31 (1H, d, $J=11.5$ Hz, 1-OCHPh), 5.37 (1H, d, $J=11.5$ Hz, 1-OCHPh), 4.55 (1H, d, $J=11.5$ Hz, 2-OCHPh), 4.60 (1H, d, $J=11.5$ Hz, 2-OCHPh), 4.46 (1H, d, $J=11.5$ Hz, $\text{NHCOCH}_2\text{OCHPh}$), 4.86 (1H, d, $J=11.5$ Hz, $\text{NHCOCH}_2\text{OCHPh}$), 7.50 (1H, t, $J=7.0$ Hz, benzoyl group), 8.08 (2H, d, $J=7.0$ Hz, benzoyl group), 7.10–7.47 (16H, phenyl group), 10.07 (1H, brs, $-\text{OSO}_3\text{HNEt}_3$), 1.27 (9H, t, $J=7.5$ Hz, $2\times\text{NEt}_3$), 3.05 (6H, q, $J=7.0$ Hz, $2\times\text{NEt}_3$). $^1\text{H-NMR}$ spectral data for the sialic acid moiety are given in Table 1.

N-Glycolyl-8-sulfoneuraminic Acid, Triethylammonium Salt ($1\cdot 2\text{NEt}_3$) A solution of **9** (48 mg, 0.05 mmol) in 1 N aqueous sodium hydroxide (0.5 ml) was kept at room temperature for 14 h, then diluted with water (10 ml), and acidified to pH 3.0 with Dowex-50 (H^+) resin at 0 °C. The resin was filtered off and washed with water (10 ml). The filtrate and washing were combined and lyophilized. The residue was dissolved in methanol–water (19:1, 5 ml) and hydrogenated over 10% palladium on charcoal (50 mg) for 5 h at room temperature. The reaction mixture was filtered through Celite, and the filtrate was evaporated in order to remove methanol *in vacuo* and lyophilized. The residue was dissolved in a small amount of water, and precipitated with dioxane to give **1** (25.8 mg, 85%) as a colorless powder. $[\alpha]_D^{23} -10.8^\circ$ ($c=1$, H_2O). *Anal.* Calcd for $\text{C}_{11}\text{H}_{19}\text{NO}_{13}\text{S}\cdot 2\text{NEt}_3$: C, 45.46; H, 8.13; N, 6.91. Found: C, 45.45; H, 8.23; N, 6.84. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3410, 2980, 2950, 1640, 1560, 1240, 810. $^{13}\text{C-NMR}$ (75 MHz, D_2O) δ : 178.35 (C1), 98.32 (C2), 40.93 (C3), 68.74 (C4), 53.68 (C5), 71.64 (C6), 68.18 (C7), 80.47 (C8), 61.68 (C9), 61.84 (5- NCOCH_2), 177.47 (5- NHCO), 9.08 and 47.50 (NEt_3). $^1\text{H-NMR}$ spectral data are given in Table 1.

N-Glycolylneuraminic Acid Triethylamine Salt $^{13}\text{C-NMR}$ (75 MHz, D_2O) δ : 178.34 (C1), 98.12 (C2), 41.08 (C3), 68.63 (C4), 53.63 (C5), 71.67 (C6), 70.07 (C7), 72.09 (C8), 64.84 (C9), 62.66 (5- NCOCH_2), 177.18 (5- NHCO), 9.89, 48.31 (NEt_3).

Benzyl 2,7-Anhydro-4,9-di-O-benzoyl-5-N-(O-benzylglycolyl)-3,5-

dideoxy-8-O-sulfo- α -D-glycero-D-galacto-2-nonulopyranosonate, Triethylammonium Salt (11) Molecular sieves 3A powder (0.5 g) and an excess of sulfur trioxide trimethylamine complex (0.6 g, 4.3 mmol) were added to a solution of benzyl 2,7-anhydro-4,9-di-O-benzoyl-5-N-(O-benzylglycolyl)-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosonate (**10**, 160 mg, 0.23 mmol) in dry DMF (2 ml) under an argon atmosphere. The mixture was stirred for 36 h at room temperature. After the addition of triethylamine (0.5 ml), the mixture was filtered and evaporated to dryness under reduced pressure. The residue was subjected to TLC on silica gel [toluene–ethanol–triethylamine (3:1:0.5)] to give **11** (186 mg, 92%) as colorless powder. $[\alpha]_D^{23} +50.4^\circ$ ($c=1$, CHCl_3). *Anal.* Calcd for $\text{C}_{39}\text{H}_{37}\text{NO}_{14}\text{S}\cdot\text{NEt}_3$: C, 61.62; H, 5.98; N, 3.20. Found: C, 61.51; H, 6.22; N, 3.16. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450, 3050, 2840, 2750, 1735, 1700, 1610, 1600, 1540 1230, 815. $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 5.19 (1H, d, $J=11.5$ Hz, 1-OCHPh), 5.27 (1H, d, $J=11.5$ Hz, 1-OCHPh), 4.58 (1H, d, $J=11.5$ Hz, $\text{NHCOCH}_2\text{OCHPh}$), 4.62 (1H, d, $J=11.5$ Hz, $\text{NHCOCH}_2\text{OCHPh}$), 7.3–8.1 (20H, phenyl group), 1.30 (9H, t, $J=7.5$ Hz, NEt_3), 3.10 (6H, q, $J=7.5$ Hz, NEt_3).

2,7-Anhydro-5-N-glycolyl-8-sulfo- α -D-glycero-D-galacto-2-nonulopyranosonic Acid, Triethylammonium Salt (12) A solution of **11** (100 mg, 0.11 mmol) in 1 N aqueous sodium hydroxide (0.5 ml) was kept at room temperature for 14 h, then diluted with water (10 ml), and acidified to pH 3.0 with Dowex-50 (H^+) resin at 0 °C. The resin was filtered off and washed with water (10 ml). The filtrate and washings were combined and lyophilized. The residue was dissolved in methanol–water (19:1, 5 ml) and treated with hydrogen over 10% palladium-on-charcoal (50 ml) for 5 h at room temperature. The solution was filtered through Celite and triethylamine (0.1 ml) was added to the filtrate, which was then concentrated under reduced pressure, and lyophilized. The residue was dissolved in a small amount of water, and precipitated with dioxane to give **12** (58 mg, 90%) as a colorless powder. $[\alpha]_D^{23} +19.8^\circ$ ($c=0.8$, H_2O). *Anal.* Calcd for $\text{C}_{11}\text{H}_{17}\text{NO}_{12}\text{S}\cdot 2\text{NEt}_3$: C, 46.84; H, 8.04; N, 7.13. Found: C, 46.86; H, 8.10; N, 7.08. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3410, 2890, 2950, 1650, 1560, 1240, 810. $^1\text{H-NMR}$ spectral data are given in Table 1.

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