Chem. Pharm Bull. **37**(8) 2037—2040 (1989)

Studies on Sialic Acids. XIX. Syntheses of Partially *O*-Acetylated 4-Methylcoumarin-7-yl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acids

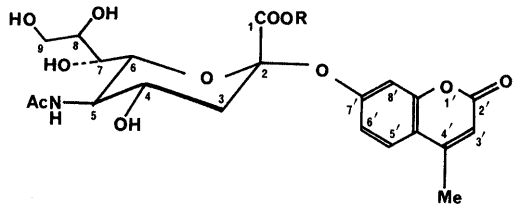
Kimio FURUHATA and Haruo OGURA*

School of Pharmaceutical Sciences, Kitasato University, Shirokane, Minato-ku, Tokyo 108, Japan. *Received January 26, 1989*

Benzyl (4-methylcoumarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate was prepared as a starting compound for the syntheses of the title compounds. The 9-*O*-, 4-*O*-, 7-*O*-, and 7,8-di-*O*-acetylated 4-methylcoumarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acids were prepared without migration of the *O*-acetyl groups. The structures of these compounds were confirmed by analysis of the proton nuclear magnetic resonance (^{1}H-NMR) spectra.

Keywords *N*-acetylneuraminic acid; *O*-acetylation; ^{1}H-NMR; fluorogenic substrate

Partially *O*-acetylated derivatives of naturally occurring *N*-acetylneuraminic acid have been investigated by Corfield *et al.*[1] and Shukla *et al.*[2] *O*-Acetyl groups on the exocyclic side chain and at C-4 have significant effects on enzyme functions.[3,4] 4-Methylcoumarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**1**) is a well-known artificial, fluorogenic substrate for neuramidase (EC 3.2. 1.18).[5,6] As a part of our research on the syntheses of partially *O*-acetylated derivatives of *N*-acetylneuraminic acid,[7] we now report the syntheses of various partially *O*-acetylated derivatives of **1** as new fluorogenic substrate for neuramidase. The structure of these synthesized derivatives was elucidated mainly on the basis of the 300 and 400 proton nuclear magnetic resonance (^{1}H-NMR) spectra. The proton assignments were based on published data[7-10] and by spin-decoupling experiments.



1: R = H
2: R = benzyl (Bn)

Chart 1

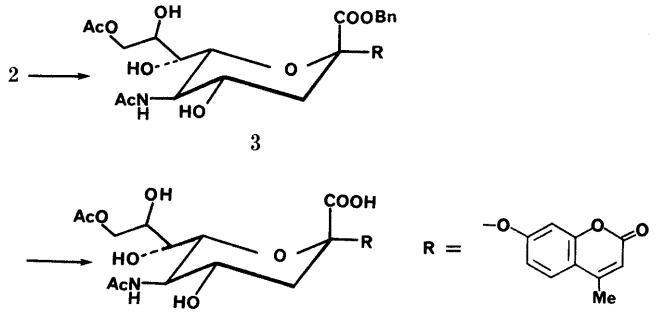


Chart 2

There have been a few reports on synthetic methods for **1**.[5,6,9,11] We prepared **1** by utilizing the conditions reported by Myers *et al.*[11]

Benzyl esterification of **1** was carried out by treating the cesium salt of **1** with benzyl bromide in *N*,*N*-dimethylformamide (DMF) to give crystalline benzyl (4-methylcoumarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**2**) in 90% yield.

The highly regioselective acetylation at OH-9 of **2** was carried out by the treatment of **2** with trimethyl orthoacetate[7] in methyl acetate in the presence of a catalytic amount of *p*-toluenesulfonic acid to give benzyl (4-methylcoumarin-7-yl 5-acetamido-9-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**3**). The benzyl ester group of **3** was hydrogenolyzed to afford 4-methylcoumarin-7-yl 5-acetamido-9-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**4**) in 90% yield. This structure was ascertained by analysis of the ^{1}H-NMR spectra, the chemical shifts at δ 4.07 for 9-H and at δ 4.38 for 9-H′ being strongly indicative of the position of mono-*O*-acetylated.

Treatment of **2** with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid gave benzyl (4-methylcoumarin-7-yl 5-acetamido-8,9-*O*-isopropylidene-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**5**) in good yield. Acetylation at OH-4 was regioselectively carried out by the treatment of **5** with acetic anhydride and excess pyridine at 20 °C for 1 h to give benzyl (4-methylcoumarin-7-yl 5-acetamido-4-*O*-acetyl-8,9-*O*-isopropylidene-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**6**) in 82% yield. Removal of the isopropylidene group was carried out by treatment with 80% acetic acid to give

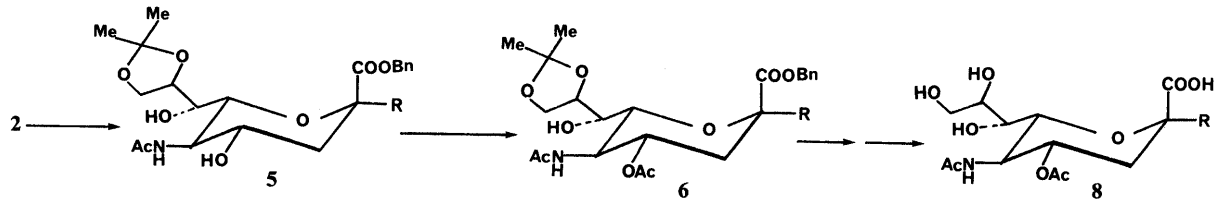


Chart 3

TABLE I. ^{1}H-NMR Spectral Data

Proton	Compound				
	1	**4**	**8**	**13**	**17**
3-H_{ax}	1.93 dd (12.6, 11.8)	1.95 t (13.0)	2.00 t (12.5)	1.96 dd (13.0, 10.0)	1.96 dd (12.5, 10.0)
3-H_{eq}	2.80 dd (12.6, 4.6)	2.87 dd (12.5, 4.5)	2.77 dd (12.5, 5.2)	2.78 dd (13.0, 5.4)	2.67 dd (12.5, 4.5)
4-H	3.72 ddd (9.5, 11.6, 4.5)	3.79 ddd (10.0, 12.5, 4.5)	4.94 ddd (10.0, 12.5, 5.2)	3.66 dt (5.4, 10.0)	3.68 dt (4.5, 10.0)
5-H	3.88 t (10.2)	3.86 t (10.0)	4.12 t (10.0)	3.86 t (10.0)	3.76 t (10.0)
6-H	4.00 dd (1.6, 10.4)	4.04 dd (2.0, 9.5)	4.16 br d (10.0)	4.19 dd (10.4, 2.0)	4.69 dd (10.0, 1.3)
7-H	3.53 dd (9.0, 1.6)	3.53 dd (9.0, 2.0)	3.75—3.85 (2H)	4.96 dd (2.0, 9.0)	5.10 dd (1.3, 7.5)
8-H	3.78—3.84 m	4.01 m		3.93 ddd (6.0, 3.0, 9.0)	5.14 ddd (5.0, 3.2, 7.5)
9-H	3.58 (12.5, 6.7)	4.07 br d (11.0)	3.45—3.60 (2H)	3.38 dd (6.0, 12.0)	3.79 dd (12.5, 3.2)
9-H′	3.78—3.82	4.38 br d (11.0)		3.56 dd (12.0, 3.0)	3.64 dd (12.5, 5.0)
NAc	1.97 s	2.01 s	1.88 s	1.86 s	1.90 s
OAc	—	2.05 s	1.98 s	1.93 s	1.99 × 2 s
3′-H	6.18 d (1.2)	6.21 d (1.2)	6.10 br s	6.15 br s	6.11 br s
4′-Me	2.31 d (1.2)	2.44 d (1.2)	2.28 br s	2.23 br s	2.32 br s
5′-H	7.60 d (8.6)	7.67 d (9.0)	7.56 d (9.0)	7.55 d (9.0)	7.56 d (9.0)
6′-H	7.07 dd (8.6, 2.4)	7.28 dd (9.0, 2.0)	7.04 br d (9.0)	7.07 dd (2.0, 9.0)	7.05 br d (9.0)
8′-H	7.05 d (2.4)	7.24 d (2.0)	7.02 br s	7.05 d (2.0)	7.02 br s

δ values in CD_3OD for **4** and in D_2O for **1**, **8**, **13**, and **17** at 19 °C, and coupling constants in Hz.

the intermediate **7**, the benzyl group of which was hydrogenolyzed to afford 4-methylcoumarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**8**) in 80% yield. The structure of **8** was assigned on the basis of the 400 MHz ^{1}H-NMR spectra as shown in Table I. The *O*-acetylated position of **8** was confirmed to be at C-4 by the downfield shift of the signal for H-4 as compared with that of **1**.[9]

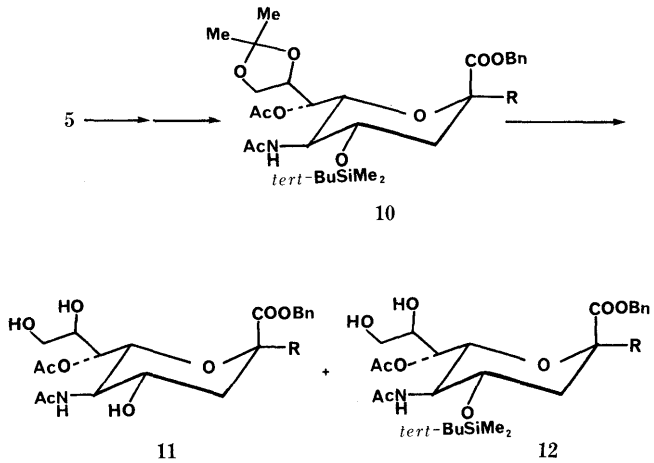


Chart 4

The 4-*O*-*tert*-butyldimethylsilyl derivative of **5** is a suitable starting material for acetylation of the sterically hindered 7-OH group. Protection of the 4-OH group was achieved by the treatment of **5** with *tert*-butyldimethylchlorosilane and imidazole in acetonitrile to give **9**, which was used for the next reaction without purification. The treatment of **9** with acetic anhydride and pyridine gave benzyl (4-methylcoumarin-7-yl 5-acetamido-7-*O*-acetyl-4-*tert*-butyldimethylsilyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**10**) in 60% yield. In the deprotection of **10**, the treatment of **10** with 80% acetic acid gave a mixture of two major components as determined by thin layer chromatography (TLC). The rection mixture was separated by column chromatography on silica gel to obtain benzyl (4-methylcoumarin-7-yl 5-acetamido-7-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**11**) in 48% yield and the corresponding 4-*O*-*tert*-butyldimethylsilyl ether (**12**) in 21% yield. Removal of the benzyl group by catalytic hydrogenolysis of **11** gave **13** in 85% yield. The migration of the *O*-acetyl group during these deprotection procedures was not observed by ^{1}H-NMR spectroscopy. The *O*-acetylated position of **13** was confirmed to be at C-7 by assignment of the chemical shift δ 4.96 (7-H).

The di-*O*-acetyl derivative (**17**) of **1**, 4-methylcoumarin-7-yl 5-acetamido-7,8-di-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-galacto-2-nonulopyranosidonic acid was synthesized from benzyl (4-methylcoumarin-7-yl 5-acetamido-4,9-di-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**14**). 4-OH and 9-OH were protected by forming the silyl ether, which was achieved by treating **2** with *tert*-butyldimethylchlorosilane and imid-

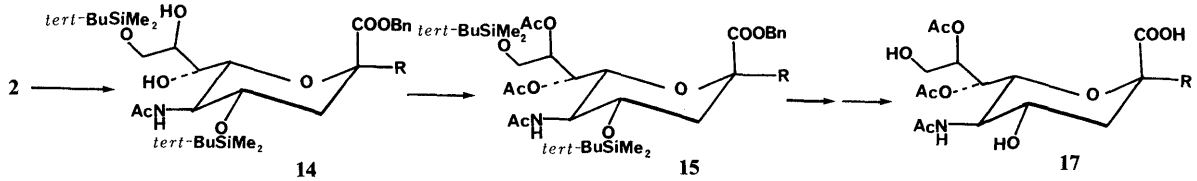


Chart 5

azole in acetonitrile to give **14** in 45% yield.

Acetylation of 7-OH and 8-OH was carried out by the treatment of **14** with acetic anhydride and pyridine to give benzyl (4-methylcoumarin-7-yl 5-acetamide-7,8-di-*O*-acetyl-4,9-di-*O*-*tert*-butyldimethylsily-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**15**) in 78% yield. Removal of the *O*-*tert*-butyldimethylsilyl groups gave the intermediate, **16**, the benzyl group of which was hydrogenolyzed to afford **17** in 44% yield.

The structure of **17** was assigned on the basis of the 300 MHz ^{1}H-NMR spectra. The *O*-acetylated position of **17** were confirmed to be at C-7 and C-8 by the downfield shift of the signals for 7-H and 8-H owing to *O*-acetylation effects.[10]

In conclusion, partially *O*-acetylated 4-methylcoumarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acids were prepared without migration of the *O*-acetyl groups during the synthetic processes, as be confirmed by the ^{1}H-NMR spectra.

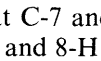
Experimental

General Methods Melting points were measured with a Yamato melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO-DIP-181 digital polarimeter. TLC was performed on silica gel (Merk GF-254) plates, and spots were detected under ultraviolet (UV) light or by spraying 5% sulfuric acid solution. Infrared (IR) spectra were measured with a JASCO IR-A2 instrument. The ^{1}H-NMR spectra were measured with Varian EM-390, VXR-300, and XL-400 MHz spectrometer at room temperature. Tetramethylsilane (TMS) in chloroform-*d* ($CDCl_3$) and methanol-d_4 (CD_3OD) or sodium 3-(trimethylsilyl)-1-propane-sulfonate (DSS) in deuterium oxide (D_2O) was used as an internal standard. Column chromatography was conducted on Silica gel 60 (70—230 mesh, Merk).

Benzyl (4-Methylcoumarin-7-yl 5-Acetamido-3,5-dideoxy-α-D-glycero-D-*galacto*-2-nonulopyranosid)onate (2) A mixture of **1** (0.4 g) in DMF (2 ml), Cs_2CO_3 (0.6 g) and BnBr (0.7 g) was stirred for 16 h at room temperature, then filtered through Celite. Ether was added to the filtrate, and ether-soluble materials were removed. The ether-insoluble residue was dissolved in acetone (20 ml). The acetone solution was filtered, and the filtrate was evaporated to a syrup which was purified by crystallization from 2-propanol to give **2** (444 mg, 70%) as colorless needles. mp 90—95 °C, $[\alpha]_D^{16}$ +7.0° (c=1, in MeOH). *Anal.* Calcd for $C_{28}H_{31}NO_{11}$: C, 60.32; H, 5.60; N, 2.51. Found: C, 60.26; H, 5.88; N, 2.78. IR ν_{max}^{KBr} cm^{-1}: 1730, 1650, 1610, 1560. ^{1}H-NMR (90 MHz, $CDCl_3$): 1.90 (3H, s, NAc), 1.95 (1H, t, J=12.0 Hz, 3-H_{ax}), 2.15 (3H, s, 4′-Me), 2.68 (1H, dd, J=12.5, 4.5 Hz, 3-H_{eq}), 4.95 (1H, d, J=12.0 Hz, –CHPh), 5.15 (1H, d, J=12.0 Hz, –CHPh), 6.17 (1H, d, J=1.5 Hz, 3′-H).

Benzyl (4-Methylcoumarin-7-yl 5-Acetamido-9-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (3) Trimethyl orthoacetate and *p*-TsOH were added to a solution of **2** (166 mg) in methyl acetate. The mixture was stirred for 20 min at room temperature, poured into water (20 ml) and extracted with chloroform. The extract was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was purified by silica-gel column chromatography with chloroform–methanol (10 : 1) to give **3** (146 mg, 88%) as a white powder. $[\alpha]_D^{16}$ +22.4° (c=0.5, in MeOH). *Anal.* Calcd for $C_{30}H_{33}NO_{12}$: C, 60.10; H, 5.55; N, 2.24. Found: C, 60.01; H, 5.51; N, 2.33. IR ν_{max}^{KBr} cm^{-1}: 1750, 1675, 1630, 1580. ^{1}H-NMR (300 Hz, $CDCl_3$): 2.03 (3H, s, NAc), 2.08 (3H, s, OAc), 2.15 (1H, t, J=13.0 Hz, 3-H_{ax}), 2.35 (3H, br s, 4′-Me), 2.91 (1H, dd, J=13.0, 4.5 Hz, 3-H_{eq}), 3.54 (1H, br d, J=10.0 Hz, 7-H), 3.74 (1H, m, 4-H), 3.90—3.98 (2H, m, 5-H and 6-H), 4.07 (1H, br t, J=8.0 Hz, 8-H), 4.19 (1H, dd, J=7.0, 11.5 Hz, 9-H), 4.46 (1H, dd, J=2.5, 11.5 Hz, 9-H′), 5.03 (1H, d, J=12.0 Hz, –CHPh), 5.19 (1H, d, J=12.0 Hz, –CHPh), 6.17 (1H, d, J=1.2 Hz, 3′-H), 6.67 (1H, br s, NH), 6.95—7.40 (8H, aromatic protons).

4-Methylcoumarin-7-yl 5-Acetamido-9-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (4) A solution of **3** (100 mg) in ethanol was treated with hydrogen over 10% palladium-on-charcol for 1 h at room temperature. The solution was filtered through Celite and evaporated to dryness at 20 °C. The residue was dissolved in a small amount of ethanol, and precipitated with ether to give **4** (76 mg, 90%) as colorless powder. $[\alpha]_D^{16}$ +35.0° (c=2, H_2O). *Anal.* Calcd for $C_{23}H_{27}NO_{12}$: C, 54.22; H, 5.34; N, 2.74. Found: C, 54.18; H, 5.00; N, 2.51. IR ν_{max}^{KBr} cm^{-1}: 1745, 1610, 1560. ^{1}H-NMR (400 MHz) data are given in Table I.

Benzyl (4-Methylcoumarin-7-yl 5-Acetamido-8,9-*O*-isopropylidene-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (5) 2,2-Dimethoxypropane (0.2 ml) and *p*-TsOH (1 ml) were added to a solution of **2** (112 mg) in acetone (10 ml). The mixture wes 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 the filtrate was washed with acetone. The combined filtrate and washings were evaporated under reduced pressure to give **5** (108 mg, 98%) as a colorless powder. $[\alpha]_D^{16}$ −6.4° (c=1, in MeOH). *Anal.* Calcd for $C_{31}H_{35}NO_{11}$: C, 62.30; H, 5.90; N, 2.34. Found: C, 62.15; H, 6.05; N, 2.90. IR ν_{max}^{KBr}: 1735, 1660, 1615, 1550. ^{1}H-NMR (90MHz, $CDCl_3$): 1.25 (3H, s, ), 1.30 (3H, s, ), 1.92 (3H, s, NAc), 1.97 (1H, t, J=12.5 Hz, 3H_{ax}), 2.21 (3H, br s, 4′-Me), 2.68 (1H, dd, J=13.0, 4.5 Hz, 3-H_{eq}), 5.15 (1H, d, J=12.0 Hz, –CHPh), 5.20 (1H, d, J=12.0 Hz, –CHPh), 6.22 (1H, br s, 3′-H), 6.93 (1H, d, J=8.5 Hz, NH).

Benzyl (4-Methylcoumarin-7-yl 5-Acetamido-4-*O*-acetyl-8,9-*O*-isopropylidene-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonuropyranosid)onate (6) Acetic anhydride (0.5 ml) was added to a solution of **5** (100 mg) in pyridine (0.5 ml). The mixture was stirred for 1 h at 18—22 °C, 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. The residue was purified by silica-gel column chromatography with chloroform–methanol (40 : 1) to give **6** (92 mg, 82%). $[\alpha]_D^{16}$ −26.6° (c=1, in MeOH). *Anal.* Calcd for $C_{33}H_{37}NO_{12}$: C, 61.23; H, 5.93; N, 2.23. Found: C, 60.98; H, 5.87; N, 2.36. IR ν_{max}^{KBr} cm^{-1}: 1740, 1665, 1610, 1550. ^{1}H-NMR (90 MHz, $CDCl_3$): 1.35 (3H, s, ), 1.40 (3H, s, ), 1.98 (3H, s, NAc), 2.08 (3H, s, OAc), 2.20 (1H, t, J=13.0 Hz, 3-H_{ax}), 2.36 (3H, br s, 4′-Me), 2.71 (1H, dd, J=13.0, 4.5 Hz, 3-H_{eq}), 4.13 (1H, q, J=10.0 Hz, 5-H), 4.45 (1H, d, J=9.5 Hz, 6-H), 5.10 (2H, s, –CHPh), 5.20 (1H, ddd, J=12.8, 4.5, 9.5 Hz, 4-H), 6.12 (1H, d, NH), 6.15 (1H, br s, 3′-H).

Benzyl (4-Methylcoumarin-7-yl 5-Acetamido-4-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonuropyranosid)onate (7) A solution of **6** (30 mg) in 80% acetic acid was stirred for 20 h at room temperature. The mixture was evaporated to yield an amorphous powder, which was purified by silica gel column chromatography with chloroform–methanol (5 : 1) to give **7** (24 mg, 85%) as a colorless powder. $[\alpha]_D^{16}$ −7.2° (c=0.5, in MeOH). *Anal.* Calcd for $C_{30}H_{33}NO_{12}$: C, 60.10; H, 5.55; N, 2.37. Found: C, 59.90; H, 5.23; N, 2.20. IR ν_{max}^{KBr} cm^{-1}: 1735, 1600, 1615, 1560. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.03 (3H, s, NAc), 2.15 (3H, s, OAc), 2.25 (1H, t, J=12.5 Hz, 3-H_{ax}), 2.45 (3H, s, 4′-Me), 2.89 (1H, dd, J=12.5, 4.8 Hz, 3-H_{eq}), 3.66 (1H, dd, J=9.0, 1.5 Hz, 7-H), 3.76 (1H, dd, J=6.5, 12.0 Hz, 9-H), 3.88—3.96 (2H, m, 8-H and 9-H′), 4.34 (1H, t, J=10.0 Hz, 5-H), 4.53 (1H, dd, J=1.5, 10.0 Hz, 6-H), 5.13 (1H, ddd, J=10.0, 3.0, 4.8 Hz, 4-H), 5.18 (1H, d, J=12.0, –CHPh), 5.33 (1H, d, J=12.0 Hz, –CHPh), 6.32 (1H, d, J=1.2 Hz, 3′-H), 7.05 (1H, d, J=1.0 Hz, 8′-H), 7.15 (1H, dd, J=1.0, 9.0 Hz, 6′-H), 7.60 (1H, d, J=9.0 Hz, 5′-H).

4-Methylcoumarin-7-yl 5-Acetamido-4-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (8) A solution of **7** (15 mg) in ethanol was treated with hydrogen over 10% palladium-on-charcoal for 2 h at room temperature. This solution was filtered through Celite, and evaporated to dryness. The residue was dissolved in a small amount of ethanol and precipitated with ether to give **8** (12 mg, 92%) as a colorless powder. $[\alpha]_D^{16}$ +24.0° (c=0.5, in MeOH). *Anal.* Calcd for $C_{23}H_{27}NO_{11}$: C, 54.22; H, 5.34; N, 2.75. Found: C, 54.18; H, 5.04; N, 2.51. IR ν_{max}^{KBr} cm^{-1}: 17020, 1610, 1560. ^{1}H-NMR (300 MHz) data are shown in Table I.

Benzyl (4-Methylcoumarin-7-yl 5-Acetamido-7-*O*-acetyl-4-*O*-butyldimethylsilyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (10) *tert*-Butyldimethylchlorosilane (100 mg) and imidazole (100 mg) were added to a solution of **5** (179 mg) in acetonitrile (5 ml). The mixture was stirred for 1 h at room temperature, and the progress of the reaction was monitored by TLC. Then pyridine (0.5 ml) and acetic anhydride (0.5 ml) were added. The mixture was stirred for 16 h at room temperature, poured into ice-water (15 ml), and extracted with chloroform. The extract was washed with water, dried over sodium sulfate, filtered, and evaporated to dryness. The residue was purified by silica gel column chromatography with chloroform–methanol (50 : 1) to give **10** (135 mg, 60%) as a colorless powder. $[\alpha]_D^{16}$ −8.6° (c=1, in MeOH). *Anal.* Calcd for $C_{39}H_{51}NO_{12}Si$: C, 62.13; H, 6.81; N, 1.85. Found: C, 62.11; H, 6.95; N, 2.01. IR ν_{max}^{KBr} cm^{-1}: 1745, 1615, 1550. ^{1}H-NMR (400 Hz, $CDCl_3$): −0.04 (6H, s, Si(Me)$_2$), 0.825

(9H, s, $SiC(Me)_3$), 1.32 (3H, s, NAc), 1.97 (1H, dd, J=13.0, 11.5 Hz, 3-H_{ax}), 2.17 (3H, s, OAc), 2.32 (3H, br s, 4′-Me) 2.64 (1H, dd, J=13.0, 4.5 Hz, 3-H_{eq}), 3.88 (1H, dd, J=6.4, 8.5 Hz, 9-H), 3.93 (1H, dd, J=6.4, 8.5 Hz, 9-H′), 4.05 (1H, dt, J=6.4, 5.5 Hz, 8-H), 4.21 (1H, q, J=5.5 Hz, 5-H), 4.55 (1H, dd, J=11.0, 1.9 Hz, 6-H), 5.15 (1H, d, J=12.0 Hz, –CHPh), 5.17 (1H, d, J=12.0 Hz, –CHPh), 5.29 (1H, dd, J=1.9, 5.5 Hz, 7-H), 5.44 (1H, d, J=8.5 Hz, NH), 6.19 (1H, br s, 3′-H), 6.98 (1H, dd, J=9.0, 2.5 Hz, 6′-H), 7.08 (1H, d, J=2.5 Hz, 8′-H), 7.21—7.27 (5H, m, Ph), 7.37 (1H, d, J=9.0 Hz, 5′-H).

Benzyl (4-Methylcoumarin-7-yl 5-Acetamido-7-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (11) and Its 4-*O*-*tert*-Butyldimethylsilyl Ether (12) A solution of **10** (100 mg) in 80% acetic acid was stirred for 72 h at room temperature. The mixture was evaporated to yield an amorphous powder, which was purified by preparative TLC on silica gel (20 × 20 cm, 0.25 mm thick) with chloroform–methanol (10 : 1) to give **11** (45 mg, 48%) and **12** (20 mg, 21%).

11: $[\alpha]_D^{16}$ +39.0° (c=1, in MeOH). *Anal.* Calcd for $C_{30}H_{33}NO_{12}$: C, 60.10; H, 5.55; N, 2.34. Found: C, 60.28; H, 5.48; N, 2.32. IR ν_{max}^{KBr} cm^{-1}: 1735, 1605, 1540. ^{1}H-NMR (300 MHz, CD_3OD): 1.98(3H, s, NAc), 2.12 (1H, dd, J=13.0, 11.5 Hz, 3-H_{ax}), 2.14 (3H, s, OAc), 2.36 (3H, br s, 4′-Me), 2.88 (1H, dd, J=13.0, 4.5 Hz, 3-H_{eq}), 3.55 (1H, dd, J=11.5, 6.5 Hz, 9-H), 3.68 (1H, dd, J=11.5, 4.0 Hz, 9-H′), 4.01 (1H, t, J=10.5 Hz, 5-H), 4.05 (1H, ddd, J=6.5, 4.0, 8.8 Hz, 8-H), 4.59 (1H, dd, J=2.0, 10.5 Hz, 6-H), 5.09 (1H, dd, J=2.0, 8.8 Hz, 7-H), 5.18 (1H, d, J=12.0 Hz, –CHPh), 5.33 (1H, d, J=12.0 Hz, –CHPh), 6.33 (1H, br s, 3′-H), 7.12 (1H, d, J=2.0 Hz, 5′-H), 7.17 (1H, dd, J=2.0, 9.0 Hz, 6′-H), 7.64 (1H, d, J=9.0 Hz, 8′-H).

12: $[\alpha]_D^{16}$ +33.0° (c=1, MeOH). *Anal.* Calcd for $C_{36}H_{47}NO_{12}Si$: C, 60.57; H, 6.63; N, 1.96. Found: C, 60.45; H, 6.66; N, 1.89. IR ν_{max}^{KBr} cm^{-1}: 1732, 1605, 1545. ^{1}H-NMR (300 MHz, CD_3CD): 0.10 (6H, s, $-Si(Me)_2$), 0.95 (9H, s, $-SiC(Me)_3$), 1.97 (3H, s, NAc), 2.15 (1H, dd, J=13.0, 11.0 Hz, 3-H_{ax}), 2.52 (3H, s, 4′-H), 2.79 (1H, dd, J=4.5, 13.0 Hz, 3-H_{eq}), 3.55 (1H, dd, J=11.5, 6.5 Hz, 9-H), 3.65 (1H, dd, J=11.5, 3.5 Hz, 9-H′), 3.76 (1H, ddd, J=11.0, 4.5, 10.5 Hz, 4-H), 4.02 (1H, t, J=10.5 Hz, 5-H), 4.05 (1H, ddd, J=6.5, 3.5, 9.0 Hz, 8-H), 4.54 (1H, dd, J=10.5, 2.0 Hz, 6-H), 5.11 (1H, dd, J=2.0, 9.0 Hz, 7-H), 5.25 (1H, d, J=12.0 Hz, –CHPh), 5.29 (1H, d, J=12.0 Hz, –CHPh), 6.32 (1H, br s, 3′-H), 7.15 (1H, d, J=2.0 Hz, 8′-H), 7.19 (1H, dd, J=2.0, 9.0 Hz, 6′-H), 7.69 (1H, d, J=9.0 Hz, 5′-H).

4-Methylcoumarin-7-yl 5-Acetamido-7-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (13) A solution of **11** (20 mg) in ethanol was treated with hydrogen over 10% palladium-on-charcoal for 2 h at room temperature, and the mixture was processed as described for **4** to give **13** (14 mg, 85%) as a colorless powder. $[\alpha]_D^{16}$ +55.0° (c=0.5, in H_2O). *Anal.* Calcd for $C_{23}H_{27}NO_{12}$: C, 54.22; H, 5.34; N, 2.74. Found: C, 54.18; H, 5.10; N, 2.62. IR ν_{max}^{KBr} cm^{-1}: 1730, 1610, 1560. ^{1}H-NMR (300 MHz) data are shown in Table I.

Benzyl (4-Methylcoumarin-7-yl 5-Acetamido-4,9-di-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (14) *tert*-Butyldimethylchlorosilane (600 mg) and imidazole (400 mg) were added to a solution of **2** (560 mg) in acetonitrile (10 ml). The mixture was stirred for 18 h at room temperature, poured into water (20 ml), and extracted twice with chloroform. The extract was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was purified by silica gel column chromatography with chloroform–methanol (20 : 1) to give **14** (355 mg, 45%) as a colorless powder. $[\alpha]_D^{16}$ +34.4° (c=1, in MeOH). *Anal.* Calcd for $C_{40}H_{59}NO_{11}Si_2$: C, 61.11; H, 7.56; N, 1.78. Found: C, 61.08; H, 7.71; N, 1.76. IR ν_{max}^{KBr} cm^{-1}: 1735, 1625, 1605, 1550. ^{1}H-NMR (300 MHz, $CDCl_3$); −0.03 (3H, s, SiMe), 0.00 (3H, s, SiMe), 0.01 (3H, s, SiMe), 0.015 (3H, s, SiMe), 0.85 (9H, s, $SiC(Me)_3$), 0.92 (9H, s, $SiC(Me)_3$), 1.98 (3H, s, NAc), 2.12 (1H, t, J=12.5 Hz, 3-H_{ax}), 2.36 (3H, br s, 4′-Me), 2.55 (1H, dd, J=4.5, 12.5 Hz, 3-H_{eq}), 3.35 (1H, br d, J=10.5 Hz, 6-H), 5.05 (1H, d, J=12.0 Hz, –CHPh), 5.14 (1H, d, J=12.0 Hz, –CHPh), 5.25 (1H, d, J=8.2 Hz, NH), 6.18 (1H, br s, 3′-H), 6.9—7.3 (8H, aromatic protons).

Benzyl (4-Methylcoumarin-7-yl 5-Acetamido-7,8-di-*O*-acetyl-4,9-di-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (15) Acetic anhydride (5 ml) was added to a solution of **14** (197 mg) in pyridine (5 ml). The mixture was stirred for 16 h at room temperature, poured into water (30 ml), and extracted with chloroform. The extract was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was purified by silica-gel column chromatography with chloroform-methanol (20 : 1) to give **15** (170 mg, 78%) as a colorless powder. $[\alpha]_D^{16}$ +36° (c=1, in MeOH). *Anal.* Calcd for $C_{44}H_{63}NO_{13}Si_2$: C, 60.73; H, 7.29; N, 1.61. Found: C, 60.00; H, 7.39; N, 1.49. ^{1}H-NMR (300 MHz, $CDCl_3$): −0.08 (3H, s, –SiMe), −0.06 (3H, s, –SiMe), −0.01 (3H, s, –SiMe), 0.00 (3H, s, –SiMe), 0.82 (9H, s, $-SiC(Me)_3$), 0.86 (9H, s, $-SiC(Me)_3$), 1.94 (3H, s, NAc), 1.99 (1H, t, J=12.5 Hz, 3-H_{ax}), 2.14 (3H, s, OAc), 2.16 (3H, s, OAc), 2.38 (3H, d, J=0.1 Hz, 4′-Me), 2.65 (1H, dd, J=4.5, 13.0 Hz, 3-H_{eq}), 3.16 (1H, br q, J=9.0 Hz, 5-H), 3.69 (1H, dd, J=11.8, 3.8 Hz, 9-H), 3.85 (1H, dd, J=2.5, 11.8 Hz, 9-H′), 4.21 (1H, ddd, J=12.5, 4.5, 9.0 Hz, 4-H), 4.84 (1H, dd, J=1.0, 11.0 Hz, 6-H), 5.23 (1H, ddd, J=3.8, 2.5, 9.0 Hz, 8-H), 5.44 (1H, dd, J=9.0, 1.0 Hz, 7-H), 5.55 (1H, br d, J=8.0 Hz, NH), 5.07 (2H, s, –CHPh), 6.18 (1H, d, J=1.0 Hz, 3′-H), 6.9—7.4 (8H, aromatic protons).

4-Methylcoumarin-7-yl 5-Acetamido-7,8-di-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (17) A solution of **15** (150 mg) in 80% acetic acid was stirred for 90 min at 60 °C. The mixture was evaporated at 20 °C to yield an amorphous powder, which was purified by silica-gel column chromatography with chloroform–methanol (10 : 1) to give benzyl (4-methylcoumarin-7-yl 5-acetamido-7,8-di-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**16**, 61 mg, 55%).

A solution of **16** (40 mg) in ethanol was treated with hydrogen over 10% palladium-on-charcoal for 2 h at room temperature, and the mixture was processed as described for **4** to give **17** (27 mg, 80%) as a colorless powder.

16: ^{1}H-NMR (300 MHz, $CDCl_3$): 2.03 (3H, s, NAc), 2.17 (1H, dd, J=13.5, 12.0 Hz, 3-H_{ax}), 2.19 (3H, s, OAc), 2.23 (3H, s, OAc), 2.38 (3H, br s, 4′-Me), 2.84 (1H, dd, J=4.5, 13.5 Hz, 3-H_{eq}), 3.54 (1H, dd, J=2.0, 14.0 Hz, 9-H), 3.68 (1H, ddd, J=10.0, 4.5, 12.0 Hz, 4-H), 3.87 (1H, dd, J=14.0, 2.0 Hz, 9-H), 3.94 (1H, q, J=10.0 Hz, 5-H), 4.58 (1H, dd, J=1.0, 10.5 Hz, 6-H), 4.91 (1H, d, J=12.0 Hz, –CHPh), 5.12 (1H, br d, J=9.5 Hz, 8-H), 5.15 (1H, d, J=12.0 Hz, –CHPh), 5.32 (1H, dd, J=1.0, 9.5 Hz, 7-H), 5.74 (1H, d, J=9.0 Hz, NH), 6.18 (1H, br s, 3′-H), 6.70 (8H, aromatic protons).

17: $[\alpha]_D^{16}$ +40.0° (c=0.5, in MeOH). *Anal.* Calcd for $C_{25}H_{29}NO_{13}$: C, 54.45; H, 5.30; N, 2.54. Found: C, 54.42; H, 5.39; N, 2.53. IR ν_{max}^{KBr} cm^{-1}: 1735, 1610, 1570, ^{1}H-NMR (300 MHz) data are shown in Table I.

Acknowledgements We gratefully acknowledge support of this work by a Grant-in-Aid for Scientific Research (No. 63470129) from the Ministry of Education, Science and Culture, and by a grant from the Waksman Foundation of Japan.

References

1) A. P. Corfield, M. Sander-Wewer, R. W. Veh, M. Wember, and R. Schauer, *Biol. Chem. Hoppe-Seyler*, **367**, 433 (1986).
2) A. K. Shukla, C. Schroder, U. Nohle, and R. Schauer, *Carbohydr. Res.*, **168**, 199 (1987).
3) G. N. Rogers, G. Herrler, J. C. Paulson, and H.-D. Klenk, *J. Biol. Chem.*, **261**, 5947 (1986).
4) R. Schauer, *Methods Enzymol.*, **138**, 611 (1987).
5) J. J. Thomas, E. C. Folger, D. L. Nist, B. J. Thomas, and R. H. Jones, *Anal. Biochem.*, **88**, 461 (1978).
6) N. Baggett and B. J. Marsden, *Carbohydr. Res.*, **110**, 11 (1982).
7) H. Ogura, K. Furuhata, S. Sato, K. Anazawa, M. Itoh, and Y. Shitori, *Carbohydr. Res.*, **167**, 77 (1987).
8) K. Anazawa, K. Furuhata, and H. Ogura, *Chem. Pharm. Bull.*, **36**, 4976 (1988).
9) M. Potier, L. Mameli, M. Belisle, L. Dallaire, and S. B. Melancon, *Anal. Biochem.*, **94**, 287 (1979).
10) J. Haverkamp, H. Van Halbeek, L. Dorland, J. F. G. Vleigenthart, R. Pfeil, and R. Schauer, *Eur. J. Biochem.*, **122**, 305 (1982).
11) R. W. Myers, R. T. Lee, Y. C. Lee, G. H. Thomas, L. W. Reynolds, and Y. Uchida, *Anal. Biochem.*, **101**, 166 (1980).