Synthesis of 6-*O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α- and -β-D-*galacto*-nonuropyranosonic Acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose[1)]

Kazuo SUZUKI, Rika KOBAYASHI, Kimio FURUHATA, and Haruo OGURA*

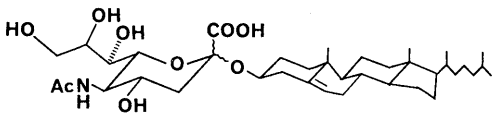
School of Pharmaceutical Science, Kitasato University, Shirokane, Minato-ku, Tokyo 108, Japan. *Received November 24, 1989*

6-*O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α- and -β-D-*galacto*-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galacto-pyranosyl-(1→4)-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose was synthesized under various conditions through a Koenigs–Knorr-like reaction. The stereochemistry of the products was confirmed by analysis of the nuclear magnetic resonance and circular dichroism spectra.

Keywords *N*-acetyl-D-neuraminic acid; *O*-glycoside; lactose; cholesterol; glycolipid; NMR; CD; stereochemistry

N-Acetyl-D-neuraminic acid is widely distributed in membrane glycoproteins and glycolipids. Recently, we reported on the synthesis of *N*-acetyl-D-neuraminic acid derivatives[2)] and 5-acetamido-2-(5-cholesten-3β-yloxy)-3,5-dideoxy-D-*glycero*-α- and -β-D-*galacto*-nonulopyranosonic acid (**1** and **2**)[3)] (Chart 1). These compounds (**1** and **2**) are known to have the biological activity of neuritogenesis that was recognized as [Neuro 2a] by Nagai *et al.*,[4)] and reported to induce the morphological conversion of normal rat glioblasts by Kato *et al.*[5)]

In this paper, we would like to report the synthesis of D-lactose containing derivatives of **1** and **2** as part of a series of studies on the structure–activity relationship of *N*-acetyl-D-neuraminic acid derivatives. The structures of the products were confirmed by analyses of the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra and the circular dichroism (CD) spectra.



1: α-anomer
2: β-anomer

Chart 1

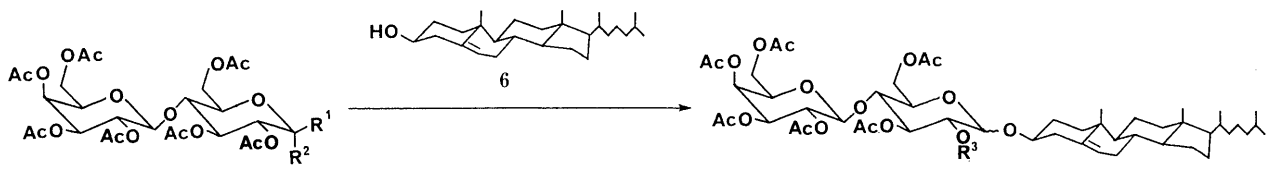
Koenigs–Knorr-like reaction of hepta-*O*-acetyl-D-lactosyl halides (**3**, **4** and **5**) and cholesterol (**6**) under various conditions (Table I) gave 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**7**) as an α-anomer and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,6-di- and 2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**8** and **9**) as β-anomers (Chart 2). As can be seen from Table I, when silver trifluoromethanesulfonate (AgOTf) and anhydrate tin (II) chloride ($SnCl_2$) (1.2 eq) were used as promoters for 20 min at room temperature (entry 5), the yield was 38% after chromaseparation. When the same promoters (2 eq) were used for 18 h at 0 °C (entry 1), the yield was low (25%) after chromatographic separation, but the α-anomer (**7**) and β-anomer (**9**) were obtained in 3:2 ratio. The structures were elucidated by analyses of the fast atom bombardment mass spectra (FAB-MS) ahd ^{1}H-NMR spectra (Table II).

The α-anomer (**7**) has been deacetylated at the 2-position of the glucose moiety. In the ^{1}H-NMR spectrum, the signal due to a proton at the 2-position on the glucose moiety of

TABLE I. Koenigs–Knorr-like Reactions of **3**, **4** and **5** with **6** in Dry CH_2Cl_2

Entry	Donor	Promoter	Temp. (°C)	Reaction time (h)	Total yield (%)	Ratio of product	
						7+**8**	**9**
1	**5**	AgOTf/$SnCl_2$(2 eq)[6,7)]	0	18	25	3[a)]	2
2	**5**	AgOTf/$SnCl_2$(1.2 eq)	0	1	14	0	1
3	**5**	$BF_3 \cdot Et_2O$(4 eq)[8)] (Et_3N(1 eq))	R.t.	2.25	13	1	2
4	**3**	AgOTf/$SnCl_2$(1.2 eq)	R.t.	24	36	1	1
5	**3**	AgOTf/$SnCl_2$(1.2 eq)	R.t.	20 (min)	38	2	3
6	**4**	$BF_3 \cdot Et_2O$ (4 eq) (Et_3N(1 eq))	R.t.	1	16	2[b)]	3

a) The only product **7**. *b*) The products were **7**, **8** and another compound.



3: R^1=H, R^2=Br
4: R^1=H, R^2=F
5: R^1=F, R^2=H

7: α-anomer (R^3=H)
8: β-anomer (R^3=H)
9: β-anomer (R^3=Ac)

Chart 2

the α-anomer (**7**) was observed at 3.50 ppm (1H, ddd), being shifted to higher field than that of the corresponding β-anomer (**9**) (4.86 ppm). In the FAB-MS, molecular ion peaks of **7** and **9** were observed at (*m/z*) 985 ($M^+ + Na$) and (*m/z*) 1027 ($M^+ + Na$), respectively.

By applying conventional methods (Chart 3),[9] **9** was deacetylated with sodium methoxide (MeONa) in methanol (MeOH), the hydroxy groups at the 4- and 6-positions on the galactose moiety of **10** were protected by benzylidenation with benzaldehyde, the residual hydroxy groups of **11** were acetylated with acetic anhydride in pyridine to form **12**, and finally **12** was converted to 2,3-di-*O*-acetyl-β-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**13**) by debenzylidenation with 80% acetic acid in 56% yield from **9** (Chart 3).

TABLE II. ^{1}H-NMR Chemical Shifts (δ ppm) of **7** and **9** in Chloroform-*d*

Compound	Chemical shifts ppm (multiolicities, *J* Hz)	
	7	**9**
Glucose moiety		
H-1	4.97 (1H, d, *J*=4.5)	4.54 (1H, d, *J*=7)
H-2	3.50 (1H, ddd, *J*=12, 10, 4.5)	4.86 (1H, dd, *J*=9, 7)
H-3	5.21 (1H, dd, *J*=10, 10)	5.18 (1H, dd, *J*=9, 9)
H-4	3.63 (1H, dd, *J*=10, 10)	3.77 (1H, dd, *J*=9, 9)
H-5	3.99 (1H, ddd, *J*=10, 5, 2)	3.58 (1H, ddd, *J*=9, 5, 2)
H-6	4.40 (1H, dd, *J*=12, 2)	4.44 (1H, dd, *J*=11, 2)
H-6′	4.11 (1H, dd, *J*=12, 5)	4.09 (1H, dd, *J*=11, 5)
Galactose moiety		
H-1	4.49 (1H, d, *J*=8)	4.47 (1H, d, *J*=8)
H-2	5.12 (1H, dd, *J*=10, 8)	5.10 (1H, dd, *J*=10, 8)
H-3	4.95 (1H, dd, *J*=10, 4)	4.94 (1H, dd, *J*=10, 3)
H-4	5.34 (1H, dd, *J*=3, 1)	5.34 (1H, dd, *J*=3, 1.5)
H-5	3.86 (1H, ddd, *J*=7.5, 6, 1)	3.86 (1H, ddd, *J*=7, 7, 1.5)
H-6	4.17 (1H, dd, *J*=10, 6)	4.13 (1H, dd, *J*=11, 7)
H-6′	4.06 (1H, dd, *J*=10, 7.5)	4.07 (1H, dd, *J*=11, 7)
Cholesterol moiety		
H-3	3.44 (1H, m)	3.44 (1H, m)
H-6	5.34 (1H, br)	5.34 (1H, br)
18-Me	0.65 (3H, s)	0.66 (3H, s)
19-Me	1.00 (3H, s)	0.97 (3H, s)
21-Me	0.90 (3H, d, *J*=6.5)	0.90 (3H, d, *J*=6)
26-Me	0.86 (3H×2, d, *J*=1.5)	0.86 (3H×2, d, *J*=2)
27-Me	0.84 (3H, s)	0.84 (3H, s)

Compound **13** and methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-D-*glycero*-β-D-*galacto*-2-nonuropyranosyl chlorid)-onate (**14**) were subjected to Koenigs–Knorr-like reaction under various conditions (Table III), and gave α- and β-anomers of 6-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-

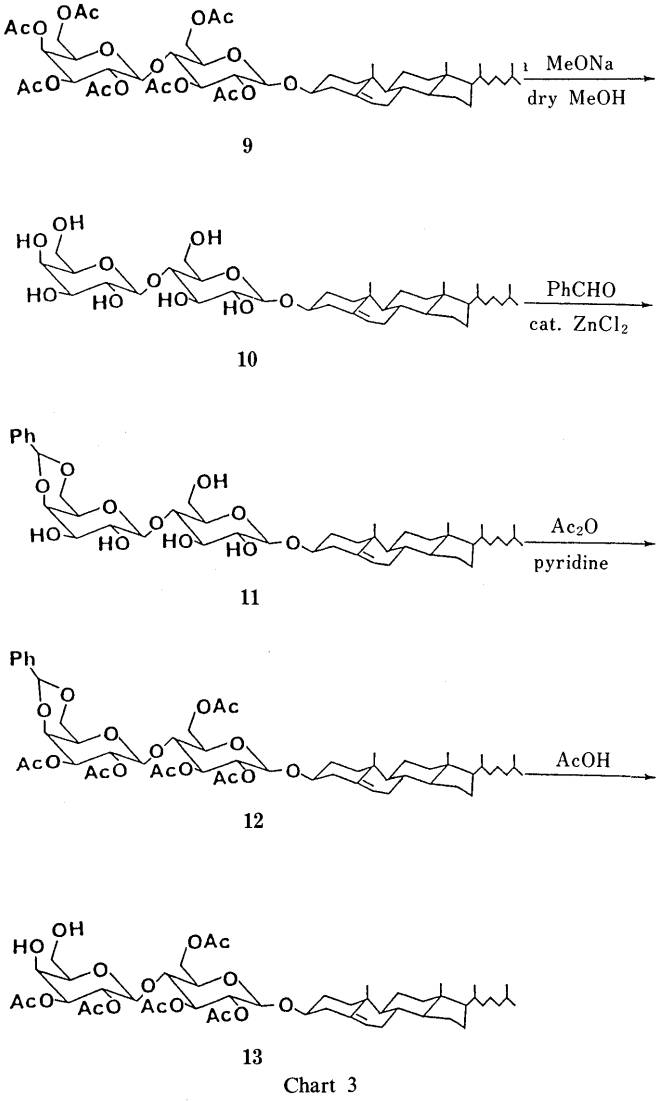
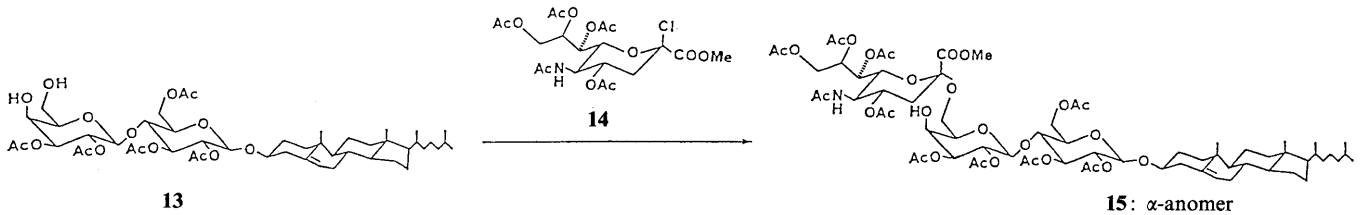


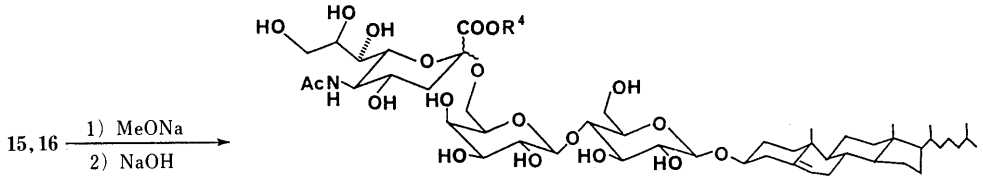
Chart 3

TABLE III. Koenigs–Knorr-like Reaction of **13** and **14**



Entry	**14** (eq)	Promoter	Reaction time (d)	Total yield (%)	Ratio of products **15**	**16**
1	1.3	$HgBr_2/Hg(CN)_2$	4.5 (h)		—— No reaction ——	
2	1.5	$BF_3 \cdot Et_2O/Et_3N$	7		—— No reaction ——	
3	2.0	$AgOTf/Na_2HPO_3$	5	28	1	0[a]
4	2.0	$AgOTf/Na_2HPO_3$	10	10	7	3
5[b]	1.0	$Cp_2ZrCl_2/AgClO_4$[9]	10		—— No reaction ——[a]	

a) A very small amount of product was detected by TLC. *b*) The soluvent was dry benzene.



17: α-anomer (R^4=Me)
18: β-anomer (R^4=Me)
19: α-anomer (R^4=H)
20: β-anomer (R^4=H)

Chart 4

TABLE IV. ^{1}H-NMR Chemical Shifts (δ ppm) of **15** and **16** in Chloroform-*d*

Compound	Chemical shifts ppm (multiplicities, *J* Hz)	
	15	**16**
Sialic acid moiety		
H-3ax	1.97 (1H, dd, *J*=12, 8)	1.79 (1H, dd, *J*=13, 11)
H-3eq	2.55 (1H, dd, *J*=12, 5)	2.46 (1H, dd, *J*=13, 5)
H-4	5.28 (1H, ddd, *J*=10, 8, 5)	5.31 (1H, ddd, *J*=11, 11, 5)
H-5	4.05 (1H, ddd, *J*=12, 10, 9.5)	3.76 (1H, ddd, *J*=11, 11, 9)
H-6	4.34 (1H, dd, *J*=12, 2.5)	4.26 (1H, dd, *J*=11, 2)
H-7	5.25 (1H, dd, *J*=10, 2.5)	5.34 (1H, dd, *J*=3, 2)
H-8	5.27 (1H, ddd, *J*=10, 6, 5)	5.26 (1H, ddd, *J*=8, 3, 2)
H-9	4.88 (1H, dd, *J*=12, 5)	4.70 (1H, dd, *J*=12, 2)
H-9′	4.03 (1H, dd, *J*=12, 6)	4.17 (1H, dd, *J*=12.5, 8)
NHCOOCH$_3$	5.31 (1H, d, *J*=9.5)	6.10 (1H, d, *J*=9)
NHCOOCH$_3$	4.81 (3H, s)	3.81 (1H, s)
Galactose moiety		
H-1	4.47 (1H, d, *J*=8)	4.44 (1H, d, *J*=8)
H-2	5.17 (1H, dd, *J*=11, 8)	5.17 (1H, d, *J*=10.5, 8)
H-3	4.85 (1H, dd, *J*=11, 3)	4.88 (1H, dd, *J*=10.5, 3)
H-4	4.06 (1H, d, *J*=3)	4.19 (1H, *J*=3)
H-5	3.63 (1H, dd, *J*=6.5, 6.5)	3.40 (1H, dd, *J*=8, 4.5)
H-6	3.79 (1H, dd, *J*=10, 6.5)	3.80 (1H, dd, *J*=9.5, 8)
H-6′	3.75 (1H, dd, *J*=10, 6.5)	3.70 (1H, dd, *J*=9.5, 4.5)
4-OH	4.06 (1H, s)	3.81 (1H, s)
Glucose moiety		
H-1	4.53 (1H, d, *J*=8)	4.53 (1H, d, *J*=8)
H-2	4.85 (1H, dd, *J*=9.5, 8)	4.82 (1H, dd, *J*=10, 8)
H-3	5.16 (1H, dd, *J*=9.5, 9.5)	5.14 (1H, dd, *J*=10, 8)
H-4	3.76 (1H, dd, *J*=10, 9.5)	3.75 (1H, dd, *J*=10, 8)
H-5	5.58 (1H, ddd, *J*=10, 5.5, 2)	3.55 (1H, ddd, *J*=10, 5, 2)
H-6	4.43 (1H, dd, *J*=12, 2)	4.43 (1H, dd, *J*=12, 2)
H-6′	4.12 (1H, dd, *J*=12, 5.5)	4.10 (1H, dd, *J*=12, 5)
Cholesterol moiety		
H-3	3.43 (1H, m)	3.43 (1H, m)
H-6	5.33 (1H, dd, *J*=4, 1.5)	5.34 (1H, d, *J*=8.5)
18-Me	0.66 (3H, s)	0.65 (3H, s)
19-Me	0.96 (3H, s)	0.96 (3H, s)
21-Me	0.89 (3H, d, *J*=6.5)	0.90 (3H, d, *J*=6.5)
26-Me	0.86 (3H×2, d, *J*=1.5)	0.86 (3H×2, d, *J*=2)
27-Me	0.84 (3H, s)	0.84 (3H, s)

acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosyl)-onate]-(2→6)-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**15** and **16**). As can be seen from Table III, when AgOTf and sodium phosphite (Na_2HPO_3) (2 eq) were used as a promoter for 5 d at room temperature (entry 3), the α-anomer (**15**) was obtained selectively in 28% yield after chromatographic separation. When the same promoters (2 eq) were used for 10 d at room temperature (entry 4), the yield was low (10%), but the α-anomer (**15**) and β-anomer (**16**) were obtained in 7:3 ratio. The ^{1}H-NMR spectra of **15** and **16** are summarized in Table IV. The signal due to

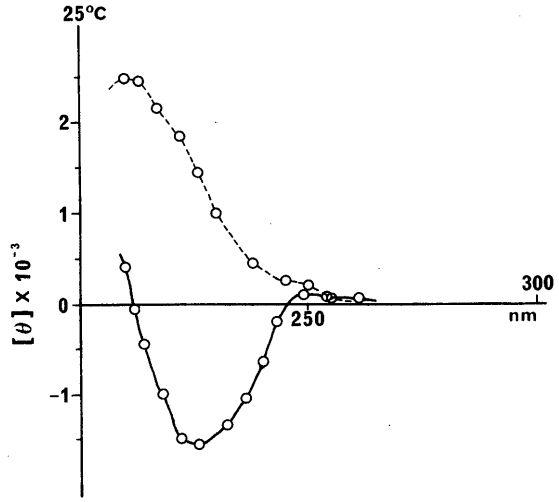


Fig. 1. CD Curves of **17** (——) and **18** (------) in MeOH

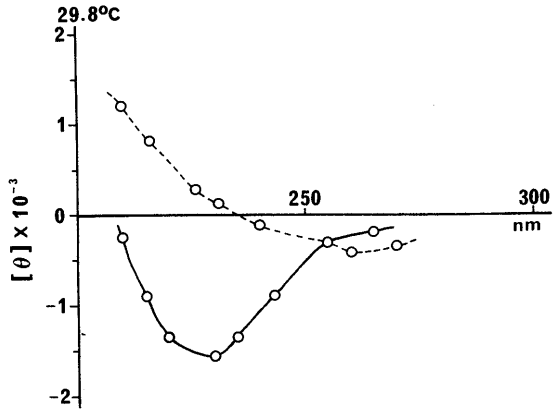


Fig. 2. CD Curves of **19** (——) and **20** (------) in MeOH

the H-3eq proton of the sialosyl moiety of **15** and **16** was observed at 2.55 ppm (α-anomer) or 2.46 ppm (β-anomer), respectively, as shown in Table IV.

Furthermore, the H-4 protons of the sialosyl moiety of **15** and **16** was observed at 5.28 ppm (α-anomer) or 5.31 ppm (β-anomer), and the coupling constants between H-7 and H-8 of the sialosyl moiety were 10 Hz for **15** and 3 Hz for **16**. These results are consistent with the report by Paulsen and Tiets.[11]

These compounds (**15** and **16**) were deacetylated using sodium methoxide in methanol, and the CD spectra of the resulting products (**17** and **18**) (Chart 4) were measured (Fig. 1). The CD spectra of the demethylated compounds (**19** and **20**) were also measured (Fig. 2).

The peak around 220 nm in the CD spectra is assigned

to the n–π^* Cotton effect of the carboxyl group, as shown in Figs. 1 and 2. The negative sign of the Cotton effect was assigned to the α-anomer and the positive sign to the β-anomer. These results are consistent with previous reports[1,11]

The biological activities of these compounds (**15**, **16**, **19** and **20**) are under investigation.

Experimental

Melting points (mp) were measured with a Yamato melting point apparatus and the results are uncorrected. Optical rotations $[\alpha]_D$ were measured with JASCO DIP-4 digital polarimeter. Thin layer chromatography (TLC) was performed on Silica gel $60F_{254}$ plates (Merck Art. 5719) and spots were detected under ultraviolet (UV) irradiation and by heating on a hot plate after spraying the plate with 5% sulfuric acid in aqueous methanol. FAB-MS and infrared (IR) spectra were measured with JEOL JMS-3100 and JASCO IR-A2 instruments, respectively. NMR spectra were recorded by using tetramethylsilane as an internal standard on Varian 300 and 400 spectrometers. Chemical shifts are quoted in parts per million (δ), and signals are expressed as s (singlet), d (doublet), m (multiplet) and br (broad). CD spectra were measured in a 0.1 cm cell with JASCO J-20 spectrometer. Column chromatography was conducted on silica gel; Wakogel C-200 (100—200 mesh) or C-300 (200—300 mesh).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-acetyl-1-(5-cholesten-3β-yloxy)-α-and-β-D-glucopyranose (7 and 8) and 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (9) Cholesterol (16.5 mmol) and a promoter (Table I) were added to a solution of compounds **3**, **4** or **5** (16.5 mmol) and powdered molecular sieves 4A (6.5 g) in dry CH_2Cl_2 (50 ml). The mixture was stirred in the dark under an argon atmosphere. This mixture was filtered and washed with CH_2Cl_2 (50 ml). The resulting solution was washed with a saturated NaCl solution. The CH_2Cl_2 extract was dried with anhydrous Na_2SO_4 and evaporated *in vacuo*.

The residue was separated and purified by silica gel column chromatography (AcOEt–hexane, 2 : 3) to give the α-anomer (**7**), β-anomer (**8**) and β-anomer (**9**). These compounds were recrystallized from hexane–AcOEt (1 : 200) as colorless needles. The yields and anomeric ratios are summarized in Table I.

α-Anomer (**7**): Colorless needles, mp 182—183 °C (dec.). The NMR ($CDCl_3$) data are summarized in Table II. FAB-MS (*m/z*): 985 ($M^+ + Na$).

α-Anomer (**7**) + β-Anomer (**8**): Colorless needles, mp 83—86 °C (dec.).

β-Anomer (**9**): Colorless needles, mp 160–188 °C (dec.). The NMR ($CDCl_3$) data are summarized in Table II. FAB-MS (*m/z*): 1027 ($M^+ + Na$).

2,3-Di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glycopyranose (13) A solution of **9** (800 mg, 0.8 mmol) in dry MeOH was added to 28% MeONa (in MeOH, 0.5 ml), and the mixture was stirred at room temperature for 4 h. The white powdered precipitate was collected by filtration to give *O*-β-D-galactopyranosyl-(1→4)-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**10**) (84%), mp 256 °C (dec.). Further, the filtrate was neutralized with Dowex 50W-X8 [H^+] and then evaporated to give **10** (9%), mp 217—220 °C (dec.). ^{1}H-NMR (MeOH-d_4) δ: glucose moiety; 4.41 (1H, d, J=7.5 Hz, H-1), galactose moiety; 4.35 (1H, d, J=7.5 Hz, H-1), cholesterol moiety; 3.39 (1H, br, H-6), 0.71 (3H, s, Me-18), 0.99 (3H, s, Me-19), 0.95 (3H, d, J=6.5 Hz, Me-21), 0.90, 0.88 (3H×2, d, J=1.5 Hz, Me-26, 27). *Anal.* Calcd for $C_{39}H_{66}O_{11}$: C, 65.89; H, 9.36. Found: C, 65.34; H, 9.30.

Compound **10** (400 mg, 0.56 mmol) and $ZnCl_2$ (384 mg, 2.8 mmol) were added to benzaldehyde (8 ml), and the mixture was stirred at room temperature for 4 h. The mixture was washed with ice water and suspended in AcOEt. The precipitate was filtered to give 4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**11**) as a white powder (70%). mp 210—213 °C. FAB-MS (*m/z*: 822 ($M^+ + Na + 1$).

11 (300 mg, 0.376 mmol) was added to anhydrous acetic acid (1 ml) in pyridine (1 ml). The solution was stirred at room temperature for 3 h. The mixture was poured into ice water, and the precipitate was filtered off to give 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**12**) as a white powder (92%). mp 210—220 °C. FAB-MS (*m/z*): 1047 ($M^+ + Na$).

A solution of **12** (300 mg, 0.3 mmol) was added to 80% acetic acid (15 ml), and the mixture was refluxed for 3 h, added to toluene and then evaporated *in vacuo*. The residue was suspended in hexane. The precipitate was filtered off to give **13** as a white powder (94%). mp 170—176 °C (dec.). FAB-MS (*m/z*): 947 ($M^+ + Na$). ^{1}H-NMR ($CDCl_3$) δ: glucose moiety; 4.55 (1H, d, J=8 Hz, H-1), galactose moiety; 4.47 (1H, d, J=8 Hz, H-1), 3.80 (1H, br, OH-4), 3.46 (1H, br, OH-6), cholesterol moiety; 3.43 (1H, m, H-3), 5.33 (1H, br, H-6), 0.65 (3H, s, Me-18), 0.96 (3H, s, Me-19), 0.89 (3H, d, J=6.5 Hz), Me-21), 0.85, 0.84 (3H×2, J=2.0 Hz, Me-26, 27).

This compound (**13**) was used in following reaction.

6-*O*-[Methyl (5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-di-deoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate]-(2→6)-2,3-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (15) and 6-*O*-[Methyl (5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonuropyranosyl)-onate]-(2→6)-2,3-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (16) A solution of **13** (500 mg, 0.543 mmol) and **14** (Table III) in CH_2Cl_2 (20 ml) was added to powdered molecular sieves 4A (1.25 g) and stirred at room temperature. The solution was added to a catalyst (Table III) (1.09 mmol) and then stirred at room temperature in the dark under an argon atmosphere. The mixture was filtered, and the filtrate was washed with saturated $NaHCO_3$ solution and saturated NaCl solution. The CH_2Cl_2 extract was dried with anhydrous Na_2SO_4 and then evaporated *in vacuo*. The residue was separated and purified by silica gel column chromatography ($CHCl_3$–MeOH, 25 : 1, followed by AcOEt–hexane, 5 : 1) and Lober column chromatography (Merck) ($CHCl_3$–MeOH, 50 : 1) to give the α-anomer (**15**) and β-anomer (**16**) (Table III).

α-Anomer (**15**): Colorless powder, mp 127—130 °C (dec.). $[\alpha]_D^{29}$ −16.4° (c=1, $CHCl_3$). FAB-MS (*m/z*): 1417 ($M^+ + Na$). IR ν_{max}^{KBr} cm^{-1}: 2960, 1760. The NMR ($CDCl_3$) data are summarized in Table IV. *Anal.* Calcd for $C_{69}H_{103}NO_{28}$: C, 59.43; H, 7.45; N, 1.00. Found: C, 59.01; H, 7.31; N, 1.30.

β-Anomer (**16**): Colorless powder, mp 124—126 °C (dec.). $[\alpha]_D^{29}$ −13.2° (c=1, $CHCl_3$). FAB-MS (*m/z*): 1417 ($M^+ + Na$). IR ν_{max}^{KBr} cm^{-1}: 2950, 1730. The NMR ($CDCl_3$) data are summarized in Table IV. *Anal.* Calcd for $C_{69}H_{103}NO_{28}$: C, 59.43; H, 7.45; N, 1.00. Found: C, 59.08; H, 7.41; N, 1.10.

6-*O*-[Methyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-*galacto*-nonulopyranosyl)onate]-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (17) A solution of **15** (100 mg, 0.072 mmol) in dry MeOH (10 ml) was added to 28% MeONa (in MeOH, 0.5 ml), and the mixture was stirred at room temperature for 3 h, then treated with Dowex 50W-X8 (H^+) resin in an ice bath to remove sodium ion. The whole was filtered and washed with MeOH. The resulting solution was evaporated *in vacuo* to yield **17** (93%) as a white powder. mp 205—217 °C (dec.). $[\alpha]_D^{29}$ −28.6° (c=0.37, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 3950. ^{1}H-NMR (MeOD-d_4) δ: glucose moiety; 4.42 (1H, d, J=8 Hz, H-1), galactose moiety; 4.32 (1H, d, J=7Hz, H-1), sialic acid moiety; 2.64 (1H, dd, J=12.5, 4.5 Hz, H-3eq), 1.80 (1H, dd, J=12.5, 12Hz, H-3ax), 3.80 (3H, s, $COOCH_3$), cholesterol moiety; 3.40 (1H, br, H-3), 5.36 (1H, br, H-6), 0.71 (3H, s, Me-18), 1.02 (3H, s, Me-19), 0.94 (3H, d, J=6.5 Hz, Me-21), 0.88, 0.86 (3H×2, d, J=1.5 Hz, Me-26, 27). The CD spectra were summarized in Fig. 1. *Anal.* Calcd for $C_{51}H_{85}NO_{19}$: C, 60.28; H, 8.43; N, 1.38. Found: C, 59.97; H, 8.42; N, 1.62.

6-*O*-[Methyl (5-Acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonuropyranosyl)onate]-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (18) A solution of **16** (50 ml, 0.0359 mmol) in dry MeOH (5 ml) was added to 28% MeONa (in MeOH, 0.2 ml). The mixture was stirred at room temperature for 2 h, and processed as described for **17** to give **18** as a white powder (98%). mp 210—220 °C (dec.). $[\alpha]_D^{29}$ −15.1° (c=0.37, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 3930. ^{1}H-NMR (MeOD-d_4) δ: glucose moiety; 4.43 (1H, d, J=8 Hz, H-1), galactose moiety; 4.33 (1H, d, J=7 Hz, H-1), sialic acid moiety; 2.49 (1H, dd, J=13, 5 Hz, H-3eq, 1.64 (1H, dd, J=13, 11 Hz, H-3ax), 3.80 (3H, s, $COOCH_3$), 2.01 (3H, s, $NHCOCH_3$), cholesterol moiety; 3.43 (1H, m, H-3), 5.37 (1H, br, H-6), 0.71 (3H, s, Me-18), 1.03 (3H, s, Me-19), 0.94 (3H, d, J=6.5 Hz, Me-21), 0.88, 0.87 (3H×2, d, J=1.5 Hz, Me-26, 27). The CD spectra are shown in Fig. 1. *Anal.* Calcd for $C_{51}H_{85}NO_{19}$: C, 60.28; H, 8.43; N, 1.38. Found: C, 60.29; H, 8.59; N, 1.46.

6-*O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosylonic Acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (19) A solution of **17** (34 mg, 0.0335 mmol) in MeOH was added to 1 N NaOH (0.2 ml). The reaction mixture was stirred at room temperature for 1.5 h, and deionized on Dowex 50W-X8 (H^+) resin in an ice bath. The whole was filtered and washed with MeOH. The resulting solution was evaporated *in vacuo* to yield **18** (93%) as a colorless powder. mp 185—195 °C (dec.). $[\alpha]_D^{29}$ −22.7° (c=0.03, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2930. ^{1}H-NMR (MeOH-d_4) δ: glucose moiety; 4.43 (1H, d, J=8.0 Hz, H-1), galactose moiety; 4.33 (1H, d, J=7.5 Hz, H-1), sialic acid moiety; 2.67 (1H, dd, J=12.5, 4.5 Hz, H-3eq), 1.80 (1H, dd J=12.5,

12.0 Hz, H-3ax), 1.99 (3H, s, NHCOCH$_3$), cholesterol moiety; 3.41 (1H, br, H-3), 5.36 (1H, br, H-6), 0.71 (3H, s, Me-18), 1.02 (3H, s, Me-19), 0.94 (3H, d, J=7 Hz, Me-21), 0.88, 0.87 (3H, d, J=1.5 Hz, Me-26, 27). The CD spectra are shown in Fig. 2. *Anal.* Calcd for $C_{50}H_{83}NO_{19}$: C, 59.92; H, 8.35; N, 1.40. Found: C, 59.79; H, 8.46; N, 1.71.

6-*O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonuropyranosylonic Acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (20) A solution of **18** (20 mg, 0.0197 mmol) in MeOH was added to 1 N NaOH (0.2 ml), and the same procedures as above gave **20** as a colorless powder (94%). mp 155—162 °C (dec.). $[\alpha]_D^{29}$ −17.9° (c=0.29, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3370, 3930. ^{1}H-NMR (MeOH-d_4) δ: glucose moiety; 4.42 (1H, d, J=8.0 Hz, H-1), galactose moiety; 4.33 (1H, d, J=7.5 Hz, H-1), sialic acid moiety; 2.47 (1H, dd, J=13.0, 5.0 Hz, H-3eq), 1.63 (1H, dd, J=13.0, 11.5 Hz, H-3ax), 2.02 (3H, s, NHCOCH$_3$), cholesterol moiety; 3.43 (1H, br, H-3), 5.37 (1H, br, H-6), 0.72 (3H, s, Me-18), 1.03 (3H, s, Me-19), 0.94 (3H, d, J=6.5 Hz, Me-21), 0.88, 0.86 (3H×2, d, J=1.5 Hz, Me-26, 27). The CD spectra are shown in Fig. 2. *Anal.* Calcd for $C_{50}H_{83}NO_{19}$: C, 59.92; H, 8.35; N, 1.40. Found: C, 59.90; H, 8.43; N, 1.58.

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