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SYNTHESIS OF 9-*O*-ACYL- AND 4-*O*-ACETYL-SIALIC ACIDS*

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ABSTRACT

Various 9-*O*-acyl derivatives of *N*-acetyl- and *N*-glycoloyl-neuraminic acid, and *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl-onic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose were regioselectively synthesized by use of ortho esters. In addition, 5-acetamido-4-*O*-acetyl-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid was prepared starting from the benzyl and methyl esters of *N*-acetylneuraminic acid.

INTRODUCTION

Sialic acids are biologically important compounds widely distributed in Nature in various forms. The occurrence of partially *O*-acetylated derivatives of *N*-acetyl and *N*-glycoloyl-neuraminic acid has been investigated by Schauer and associates[1,2]. These compounds have significant effects on enzyme function, complement activation, and antigenicity[3]. 5-Acetamido-9-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonic acid (**3**) was recognized as an essential determinant of the cell surface receptors of influenza A and B viruses[4].

We have been interested in various *O*-acylated derivatives of neuraminic acid as new blockers of sialidase, and as starting materials for the synthesis of other sialic acids[5,6]. The synthesis of **3** was reported by Schauer[7,8] and by Augé *et al.*[9]. We describe herein a regioselective synthesis giving a high yield of 9-*O*-acylated-*N*-acetyl- and -*N*-glycoloyl-neuraminic acid, as well as the synthesis of 5-acetamido-4-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonic acid.

RESULTS AND DISCUSSION

The highly regioselective acylation at OH-9 of *N*-acetylneuraminic acid (**1**) and *N*-glycoloylneuraminic acid (**2**) was carried out by the treatment of **1** or **2** with

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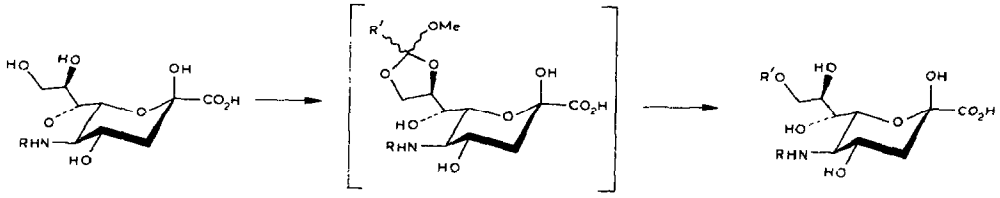


1 R = Ac, R′ = H
2 R = Gc, R′ = H

Gc = Glycoloyl

3 R = Ac, R′ = Ac
4 R = Ac, R′ = formyl
5 R = Ac, R′ = butyroyl
6 R = Ac, R′ = valeryl
7 R = Ac, R′ = Bz
8 R = Gc, R′ = Ac
9 R = Gc, R′ = butyroyl
10 R = Gc, R′ = Bz

such 1,1,1-trimethyl orthoesters as trimethyl orthoformate, trimethyl orthovalerate, and trimethyl orthobenzoate, in dimethyl sulfoxide in the presence of a catalytic amount of *p*-toluenesulfonic acid, to give the corresponding 9-*O*-acylated derivatives **3–10** in 60–92% yield. These structures were ascertained by ^{1}H-n.m.r. spectroscopy, the chemical shifts at δ 4.10–4.30 for H-9a and 4.28–4.48 for H-9b being strongly indicative of an *O*-monoacylated position[1]. The regioselective acylation clearly suggested the formation of an internal ortho ester. In the same

11 R = Lac

12 R = Lac, R′ = Ac
13 R = Lac, R′ = butyroyl

14 R = Lac

15 R = Lac, R′ = Ac
16 R = Lac, R′ = butyroyl

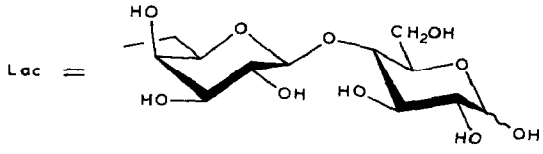


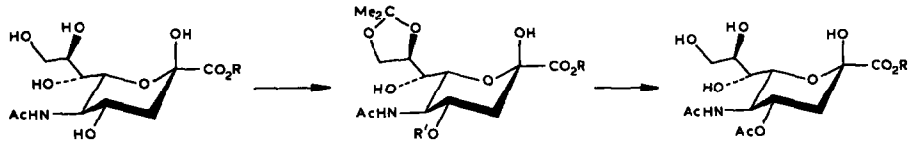
TABLE I

^{1}H-N.M.R. DATA FOR COMPOUNDS **12**, **13**, **15** AND **17**[a]

Compound	*Chemical shifts (δ)*				
	H-1α	*H-1β*	*H-1'*	*H-9'a*	*H-9'b*
12	5.20 (4.0)	4.48 (9.2)	4.34 (9.0)	4.12 (13.0, 3.5)	4.23 (13.0, 2.0)
13	5.20 (4.5)	4.55 (9.0)	4.30 (8.5)	4.12 (12.3, 4.1)	4.28 (12.3, 3.5)
15	5.14 (4.5)	4.67 (9.6)	4.35 (8.6)	4.10 (12.0, 4.5)	4.31 (12.0, 1.5)
16	5.16 (4.5)	4.60 (9.0)	4.38 (8.5)	4.18 (11.0, 4.5)	4.30 (11.0)

[a]For a solution in D_2O at 400 MHz. Coupling constants (Hz) in parentheses.

manner, treatment of *N*-acetyl-α-neuraminyl- (**11**) and *N*-acetyl-β-neuraminyl-(2→6)-lactose[10] (**14**) in dimethyl sulfoxide with 1,1,1-trimethyl orthoacetate or trimethyl orthobutylate and *p*-toluenesulfonic acid gave, in 82–92% yield, the corresponding 9''-*O*-acylated derivatives **12**, **13**, **15**, and **16** without *O*-acylation at the other hydroxy groups. Their structures were ascertained by 400-MHz, ^{1}H-n.m.r. spectroscopy (see Table I).



17 R = Bn
18 R = Me

19 R = Me, R' = H
20 R = R' = H
21 R = H, R' = Ac
22 R = Bn, R' = H
23 R = Bn, R' = Ac

24 R = Bn
25 R = H

Benzyl esterification of **1** gave crystalline benzyl 5-acetamido-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**17**) in 80% yield. Treatment with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid of the methyl (**18**) and benzyl ester (**17**) of **1** gave methyl 5-acetamido-8,9-*O*-isopropylidene-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**19**) and benzyl 5-acetamido-8,9-*O*-isopropylidene-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**22**), respectively, in good yield. Saponification of the methyl ester group of **19** afforded the intermediate **20** which was regioselectively acetylated at O-4 to give **21**, further deblocked by treatment with 90% acetic acid to give, in 30% yield, 5-acetamido-4-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonic acid (**25**).

TABLE II

^{1}H-N.M.R. DATA FOR **17**[a] AND **23**[b]

Proton	*Chemical shifts (δ) for*[c]	
	17[d]	**24**[e]
3a	1.822	2.294
	$J_{3,4}$ 11.5	$J_{3,4}$ 12.8
	$J_{3,3'}$ 13.0	$J_{3,3'}$ 12.8
3e	2.233	2.204
	$J_{3',4}$ 5.0	$J_{3',4}$ 5.4
	$J_{3',3}$ 13.0	$J_{3',3}$ 12.8
4	3.982	5.338
	$J_{4,3'}$ 5.0	$J_{4,3'}$ 5.4
	$J_{4,3}$ 10.5	$J_{4,3}$ 12.8
	$J_{4,5}$ 10.5	$J_{4,5}$ 10.6
5	3.827	3.984
	$J_{5,4}$ 10.2	$J_{5,4}$ 10.6
	$J_{5,6}$ 10.2	$J_{5,6}$ 10.8
6	3.985	3.940
	$J_{6,5}$ 10.5	$J_{6,5}$ 10.8
	$J_{6,7}$ 1.2	$J_{6,7}$ 1.3
7	3.469	3.437
	$J_{7,6}$ 1.2	$J_{7,6}$ 1.3
	$J_{7,8}$ 9.4	$J_{7,8}$ 4.8
		$J_{7,OH}$ 4.8
8	3.634	4.172
	$J_{8,9'}$ 2.8	$J_{8,9'}$ 11.2
	$J_{8,9}$ 6.1	$J_{8,9}$ 10.0
	$J_{8,7}$ 9.4	$J_{8,7}$ 4.8
9a	3.573	4.038
	$J_{8,9}$ 6.1	$J_{8,9}$ 10.0
	$J_{9,9'}$ 12.0	$J_{9,9'}$ 9.0
9b	3.743	4.050
	$J_{9',8}$ 2.8	$J_{9',8}$ 11.2
	$J_{9',9}$ 12.0	$J_{9',9}$ 9.0

[a]Solution in D_2O. Internal water was used as a secondary reference and chemical shifts were converted to the Me_4Si scale by use of the water–Me_4Si shift difference of 4.700 p.p.m. [b]Solution in $CDCl_3$. [c]Coupling constants in Hz. [d]1.961 (NAc), 5.219 (*J* 12.0 Hz, PhC*H*), 5.230 (*J* 12.0 Hz, PhC*H*), and 7.038 (Ph). [e]1.306 and 1.387 (CMe_2), 2.001 (NAc), 2.083 (OAc), 4.598 ($J_{OH,4}$ 4.8, OH-7), 5.170 (*J* 12.0 Hz, PhC*H*), 5.295 (*J* 12.0 Hz, PhC*H*), 6.058 ($J_{NH,5}$ 7.3, NH), and 7.365 (Ph).

Compound **25** was also prepared by acetylation of **22** to give, in 95% yield, benzyl 5-acetamido-4-*O*-acetyl-8,9-*O*-isopropylidene-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**23**), the structure of which was assigned by 400-MHz ^{1}H-n.m.r. spectrometry (see Table II); the OAc-4 group was confirmed by the downfield shift of the signal for H-4 as compared with the spectrum of **17**. Removal of the *O*-isopropylidene group gave the intermediate **24**, the benzyl ester group of which was hydrogenolyzed to afford **25** in 52% yield. The structures of **3** and **25** were confirmed by comparison of their ^{1}H-n.m.r. spectra with those published earlier[1].

EXPERIMENTAL

General methods. — Melting points were determined in a capillary tube. Optical rotations were measured with a JASCO-JIP-4 polarimeter. I.r. spectra were recorded as films and KBr disks with a JASCO-A2 spectrometer. ^{1}H-N.m.r. spectra were recorded with Varian EM-390 and XL-400 spectrometers with tetramethylsilane as an internal standard for solutions in (^{2}H)chloroform and sodium 4,4-dimethyl-4-silapentane-1-sulfonate for solutions in deuterium oxide.

*5-Acetamido-9-*O*-acetyl-3,5-dideoxy*-D-glycero-*β*-D-galacto-*2-nonulopyranosonic acid* (**3**). — To a solution of **1** (155 mg, 0.5 mmol) in dimethyl sulfoxide (1 mL) was added trimethyl orthoacetate (120 mg, 1 mmol) and *p*-toluenesulfonic acid monohydrate (5 mg) at room temperature. After being stirred for 20 min, the mixture was applied to a column of Dowex 1-X8 (HCO_3^-) anion-exchange resin (10 mL; 100–200 mesh). The column was washed with water (100 mL) and eluted with M formic acid (50 mL). The eluent was lyophilized, and the residue dissolved with a little water and precipitated with ethanol to give **3** (162 mg, 92%), amorphous powder, $[\alpha]_D^{28}$ −10° (*c* 1, water); ν_{max}^{film} 1720, 1620, and 1550 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.78 (dd, 1 H, *J* 13.0 and 5.0 Hz, H-3*a*), 2.01 (s, 3 H, NAc), 2.16 (dd, 1 H, *J* 13.0 and 5.0 Hz, H-3*e*), 4.13 (dd, 1 H, *J* 12.0 and 5.5 Hz, H-9a), and 4.32 (dd, 1 H, *J* 12.0 and 2.8 Hz, H-9b).

Anal. Calc. for $C_{13}H_{21}NO_{10}$: C, 44.45; H, 6.02; N, 3.99. Found: C, 44.60; H, 5.95; N, 3.96.

*5-Acetamido-3,5-dideoxy-9-*O*-formyl*-D-glycero-*β*-D-galacto-*2-nonulopyranosonic acid* (**4**). — To a solution of **1** (155 mg, 0.5 mmol) in dimethyl sulfoxide (1 mL) was added trimethyl orthoformate (106 mg, 1 mmol) and *p*-toluenesulfonic acid (5 mg), and the mixture processed as described for **3** to give **4** (155 mg, 92%), amorphous powder, $[\alpha]_D^{28}$ −15° (*c* 1, water); ν_{max}^{film} 1710, 1620, and 1560 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.84 (dd, 1 H, *J* 12.2 and 10.8 Hz, H-3*a*), 2.02 (s, 3 H, NAc), 2.23 (dd, 1 H, *J* 12.2 and 4.2 Hz, H-3*e*), 4.26 (dd, 1 H, *J* 11.1 and 4.7 Hz, H-9a), 4.48 (dd, 1 H, *J* 11.1 and 3.1 Hz, H-9b), and 8.20 (s, 1 H, CHO).

Anal. Calc. for $C_{12}H_{19}NO_{10}$: C, 42.73; H, 5.68; N, 4.15. Found: C, 42.70; H, 5.75; N, 4.08.

*5-Acetamido-9-*O*-butyroyl-3,5-dideoxy*-D-glycero-*β*-D-galacto-*2-nonulopyranosonic acid* (**5**). — To a solution of **1** (155 mg, 0.5 mmol) in dimethyl sulfoxide (1 mL) was added trimethyl orthobutyrate (148 mg, 1 mmol) and *p*-toluenesulfonic acid (5 mg), and the mixture processed as described for **3** to give **5** (167 mg, 88%), amorphous powder, $[\alpha]_D^{28}$ −11° (*c* 1, water); ν_{max}^{film} 1720, 1630, and 1540 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 0.88 (t, 3 H, *J* 6.7 Hz, CH_3-3′), 1.82 (dd, 1 H, *J* 13.2 and 10.8 Hz, H-3*a*), 2.02 (s, 3 H, NAc), 2.24 (dd, 1 H, *J* 13.2 and 4.2 Hz, H-3*e*), 2.37 (t, 2 H, *J* 6.6 Hz, H_2-2′), 4.20 (dd, 1 H, *J* 12.3 and 4.2 Hz, H-9a), and 4.34 (dd, 1 H, *J* 12.3 and 3.3 Hz, H-9b).

Anal. Calc. for $C_{15}H_{25}NO_{10}$: C, 47.49; H, 6.64; N, 3.69. Found: C, 47.22; H, 6.83; N, 3.54.

5-Acetamido-3,5-dideoxy-9-O-valeroyl-D-glycero-*β*-D-galacto-*2-nonulopyranosonic acid* (**6**). — To a solution of **1** (155 mg, 0.5 mmol) in dimethyl sulfoxide (1 mL) was added trimethyl orthovalerate (162 mg, 1 mmol) and *p*-toluenesulfonic acid (5 mg), and the mixture processed as described for **3** to give **6** (167 mg, 85%), amorphous powder, $[\alpha]_D^{28}$ −11° (*c* 1, water); ν_{max}^{film} 1710, 1620, and 1550 cm^{-1}; ^1H-n.m.r. (D_2O): *δ* 0.88 (t, 3 H, *J* 6.2 Hz, CH_3-4'); 1.83 (dd, 1 H, *J* 12.6 and 10.8 Hz, H-3*a*), 2.06 (s, 3 H, NAc), 2.22 (dd, 1 H, *J* 12.6 and 4.2 Hz, H-3*e*), 2.44 (t, 2 H, *J* 6.7 Hz, H-2'), 4.20 (dd, 1 H, *J* 12.0 and 3.9 Hz, H-9a), and 4.36 (dd, 1 H, *J* 12.0 and 3.5 Hz, H-9b).

Anal. Calc. for $C_{16}H_{27}NO_{10}$: C, 48.85; H, 6.92; N, 3.56. Found: C, 48.66; H, 7.05; N, 3.29.

5-Acetamido-9-O-benzyl-3,5-dideoxy-D-glycero-*β*-D-galacto-*2-nonulopyranosonic acid* (**7**). — To a solution of **1** (155 mg, 0.5 mmol) in dimethyl sulfoxide (1 mL) was added trimethyl orthobenzoate (182 mg, 1 mmol) and *p*-toluenesulfonic acid (5 mg), and the mixture processed as described for **3** to give **7** (124 mg, 60%), amorphous powder, $[\alpha]_D^{28}$ +4° (*c* 1, water); ν_{max}^{film} 1720, 1620, and 1550 cm^{-1}; ^1H-n.m.r. (D_2O): *δ* 1.85 (dd, 1 H, *J* 13.0 and 10.8 Hz, H-3*a*), 2.03 (s, 3 H, NAc), 2.22 (dd, 1 H, *J* 13.0 and 4.8 Hz, H-3*e*), 4.30 (dd, 1 H, *J* 12.8 and 5.0 Hz, H-9a), and 4.45 (dd, 1 H, *J* 12.8 and 3.2 Hz, H-9b), and 7.2–8.2 (5 H, Ph).

Anal. Calc. for $C_{18}H_{23}NO_{10}$: C, 52.30; H, 5.61; N, 3.39. Found: C, 52.18; H, 5.82; N, 3.36.

9-O-Acetyl-3,5-dideoxy-5-glycoloylamido-D-glycero-*β*-D-galacto-*2-nonulopyranosonic acid* (**8**). — To a solution of **2** (163 mg, 0.5 mmol) in dimethyl sulfoxide (1 mL) was added trimethyl orthoacetate (120 mg, 1 mmol) and *p*-toluenesulfonic acid (5 mg), and the mixture processed as described for **3** to give **8** (165 mg, 90%), amorphous powder, $[\alpha]_D^{28}$ −12° (*c* 1, water); ν_{max}^{film} 1720, 1620, and 1540 cm^{-1}; ^1H-n.m.r. (D_2O): *δ* 1.78 (dd, 1 H, *J* 11.0 and 12.5 Hz, H-3*a*), 2.03 (s, 3 H, OAc-9), 2.18 (dd, 1 H, *J* 12.5 and 5.0 Hz, H-3*e*), 3.51 (dd, 1 H, *J* 1.4 and 9.5 Hz, H-7), 4.06 (s, 2 H, NGc-5), 4.10 (dd, 1 H, *J* 5.5 and 12.0 Hz, H-9a), and 4.28 (dd, 1 H, *J* 2.5 and 12.0 Hz, H-9b).

Anal. Calc. for $C_{13}H_{21}NO_{11}$: C, 42.51; H, 5.76; N, 3.81. Found: C, 42.48; H, 5.79; N, 3.80.

9-O-Butyroyl-3,5-dideoxy-5-glycoloylamido-D-glycero-*β*-D-galacto-*2-nonulopyranosonic acid* (**9**). — To a solution of **2** (163 mg, 0.5 mmol) in dimethyl sulfoxide (1 mL) was added trimethyl orthobutyrate (148 mg, 1 mmol) and *p*-toluenesulfonic acid (5 mg), and the mixture processed as described for **3** to give **9** (174 mg, 88%), amorphous powder, $[\alpha]_D^{28}$ −8° (*c* 1, water); ν_{max}^{film} 1720, 1620, and 1560 cm^{-1}; ^1H-n.m.r. (D_2O): *δ* 0.88 (t, 3 H, *J* 6.7 Hz, CH_3-3'), 1.80 (dd, 1 H, *J* 13.1 and 10.8 Hz, H-3*a*), 2.24 (dd, 1 H, *J* 13.1 and 6.0 Hz, H-3*e*), 4.05 (s, 2 H, NGc-5), 4.12 (dd, 1 H, *J* 5.5 and 12.2 Hz, H-9a), and 4.30 (dd, 1 H, *J* 3.2 and 12.2 Hz, H-9b).

Anal. Calc. for $C_{15}H_{25}NO_{11}$: C, 45.57; H, 6.37; N, 3.54. Found: C, 43.52; H, 6.41; N, 3.35.

9-O-Benzoyl-3,5-dideoxy-5-glycoloylamido-D-glycero-β-D-galacto-*2-nonulopyranosonic acid* (**10**). — To a solution of **2** (163 mg, 0.5 mmol) in dimethyl sulfoxide (1 mL) was added trimethyl orthobenzoate (182 mg, 0.5 mmol) and *p*-toluenesulfonic acid (5 mg), and the mixture processed as described for **3** to give **10** (150 mg, 70%), amorphous powder, $[\alpha]_D^{28}$ +6° (*c* 1, water); ν_{max}^{film} 1720, 1630, and 1550 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.88 (dd, 1 H, *J* 12.8 and 10.5 Hz, H-3*a*), 2.15 (dd, 1 H, *J* 12.8 and 4.6 Hz, H-3*e*), 4.08 (s, 2 H, NGc-5), 4.26 (dd, 1 H, *J* 12.8 and 5.2 Hz, H-9a), 4.45 (dd, 1 H, *J* 12.8 and 4.1 Hz, H-9b), and 7.2–8.2 (5 H, Ph).

Anal. Calc. for $C_{18}H_{27}NO_{11}$: C, 49.89; H, 6.28; N, 3.23. Found: C, 49.67; H, 6.50; N, 3.02.

O-*(5-Acetamido-9-O-acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→6)*-O-β-D-*galactopyranosyl-(1→4)*-D-*glucopyranose* (**12**). — To a solution of **11** (32 mg, 0.05 mmol) in dimethyl sulfoxide (0.4 mL) was added trimethyl orthoacetate (60 mg, 0.5 mmol) and *p*-toluenesulfonic acid (1 mg), and the mixture processed as described for **3** to give **12** (29 mg, 85%), amorphous powder, $[\alpha]_D^{23}$ +4° (*c* 0.5 water); ν_{max}^{KBr} 1720, 1615, and 1560 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.75 (dd, 1 H, *J* 12.5 and 11.5 Hz, H-3″*a*), 1.95 (s, 3 H, NAc), 2.08 (s, 3 H, OAc-9″), 2.65 (dd, 1 H, *J* 12.5 and 3.6 Hz, H-3″), 4.12 (dd, 1 H, *J* 13.0 and 3.5 Hz, H-9″a), 4.23 (dd, 1 H, *J* 13.0 and 2.0 Hz, H-9″b), 4.34 (d, 1 H, *J* 9.0 Hz, H-1″), 4.48 (d, 2/3 H, *J* 9.2 Hz, H-1), 5.20 (d, 1/3 H, *J* 4.0 Hz, H-1).

Anal. Calc. for $C_{25}H_{41}NO_{20}\cdot H_2O$: C, 42.20; H, 6.37; N, 1.97. Found: C, 41.99; H, 6.45; N, 1.88.

O-*(5-Acetamido-9-O-butyroyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→6)*-O-β-D-*galactopyranosyl-(1→4)*-D-*glucopyranose* (**13**). — To a solution of **11** (32 mg, 0.05 mmol) in dimethyl sulfoxide (0.4 mL) was added trimethyl orthobutyrate (74 mg, 0.5 mmol) and *p*-toluenesulfonic acid (1 mg), and the mixture processed as described for **3** to give **13** (29 mg, 82%), amorphous powder, $[\alpha]_D^{23}$ +1° (*c* 0.5 water); ν_{max}^{KBr} 1720, 1620, and 1560 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 0.88 (t, 3 H, *J* 6.8 Hz, CH_3-3 of butyroyl), 1.78 (dd, 1 H, *J*13.2 and 10.5 Hz, H-3″*a*), 1.97 (s, 3 H, NAc), 2.65 (dd, 1 H, *J* 13.2 and 3.5 Hz, H-3″*e*), 2.37 (t, 2 H, *J* 6.6 Hz, H_2-2 of butyroyl), 4.12 (dd, 1 H, *J* 12.3 and 4.1 Hz, H-9″a), 4.28 (dd, 1 H, *J* 12.3 and 3.5 Hz, H-9″b), 4.30 (d, 1 H, *J* 8.5 Hz, H-1′), 4.55 (d, 2/3 H, *J* 9.0 Hz, H-1), and 5.20 (d, 1/3 H, *J* 4.5 Hz, H-1).

Anal. Calc. for $C_{27}H_{45}NO_{20}\cdot 2\ H_2O$: C, 43.84; H, 6.63; N, 1.90. Found: C, 43.65; H, 6.78; N, 1.77.

O-*(5-Acetamido-9-O-acetyl-3,5-dideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosylonic acid)-(2→6)*-O-β-D-*galactopyranosyl-(1→4)*-D-*glucopyranose* (**15**). — To a solution of **14** (32 mg, 0.05 mmol) in dimethyl sulfoxide (0.4 mL) was added trimethyl orthoacetate (60 mg, 0.05 mmol) and *p*-toluenesulfonic acid (1 mL), and the mixture processed as described for **3** to give **15** (34 mg, 90%), amorphous powder, $[\alpha]_D^{23}$ +2° (*c* 0.5 water); ν_{max}^{KBr} 1720, 1626, and 1550 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.67 (dd, 1 H, *J* 13.0 and 11.5 Hz, H-3*a*), 1.98 (s, 3 H, NAc), 2.03 (s, 3 H, OAc-9″), 2.39 (dd, 1 H, *J* 13.0 and 3.0 Hz, H-3″), 4.10 (dd, 1 H, *J* 12.0 and

4.5 Hz, H-9″a), 4.31 (dd, 1 H, *J* 12.0 and 1.5 Hz, H-9″b), 4.35 (d, 1 H, *J* 8.6 Hz, H-1′), 4.67 (d, 2/3 H, *J* 9.6 Hz, H-1), and 5.14 (d, 1/3 H, *J* 4.5 Hz, H-1).

Anal. Calc. for $C_{25}H_{41}NO_{20} \cdot 4\ H_2O$: C, 40.16; H, 6.61; N, 1.87. Found: C, 40.14; H, 6.83; N, 1.78.

O-*(5-Acetamido-9-O-butyroyl-3,5-dideoxy*-D-glycero-*β*-D-galacto-*2-nonulopyranosylonic acid)-(2→6)*-O-*β*-D-*galactopyranosyl-(1→4)*-D-*glucopyranose* (**16**). — To a solution of **14** (32 mg, 0.05 mmol) in dimethyl sulfoxide (0.4 mL) was added trimethyl orthobutyrate (74 mg, 0.5 mmol) and *p*-toluenesulfonic acid (1 mg), and the mixture processed as described for **3** to give **16** (30 mg, 86%), amorphous powder, $[\alpha]_D^{23}$ +1° (*c* 0.5 water); ν_{max}^{KBr} 1720, 1620, and 1550 cm^{-1}; ^{1}H-n.m.r. (D_2O): *δ* 1.88 (t, 3 H, *J* 6.6 Hz, CH_3-3 of butyroyl), 1.98 (s, 3 H, NAc), 1.35 (t, 2 H, H_2-2 of butyroyl), 4.18 (dd, 1 H, *J* 11.0 and 4.5 Hz, H-9″a), 4.30 (br. d, 1 H, *J* 11.0 Hz, H-9″b), 4.38 (d, 1 H, *J* 8.5 Hz, H-1′), 4.60 (d, 2/3 H, *J* 9.0 Hz, H-1), and 5.16 (d, 1/3 H, *J* 4.5 Hz, H-1).

Anal. Calc. for $C_{27}H_{45}NO_{20}$: C, 46.09; H, 6.45; N, 1.99. Found: C, 45.86; H, 6.62; N, 1.75.

Benzyl 5-acetamido-3,5-dideoxy-D-glycero-*β*-D-galacto-*2-nonulopyranosonate* (**17**). — A solution of **1** (2 g) in water (10 mL) was made neutral with 10% Cs_2CO_3 and evaporated, and the residue dried in the presence of P_2O_5 to give the cesium salt of **1**. This was dissolved in *N,N*-dimethylformamide (20 mL) and to the stirred solution was added benzyl bromide. The mixture was stirred under Ar for 2 h at room temperature, filtered, and the filtrate evaporated to a syrup which was purified by crystallization from 2-propanol to give **17** (2.0 g, 80%), m.p. 183–185°, $[\alpha]_D^{28}$ −43° (*c* 1, water); ν_{max}^{KBr} 1710, 1620, and 1520 cm^{-1}; ^{1}H-n.m.r., see Table I.

Anal. Calc. for $C_{17}H_{25}NO_9$: C, 52.71; H, 6.51; N, 3.28. Found: C, 52.68; H, 6.55; N, 3.24.

Methyl 5-acetamido-3,5-dideoxy-8,9-O-isopropylidene-D-glycero-*β*-D-galacto-*2-nonulopyranosonate* (**19**). — To a solution of methyl 5-acetamido-D-*glycero-β*-D-*galacto*-2-nonulopyranosonate[11] (**18**, 5 g) in acetone (100 mL) were added with stirring 2,2-dimethoxypropane (8 g) and *p*-toluenesulfonic acid (10 mg) for 1 h at room temperature. The mixture was treated with Dowex 1 (OH^-) anion-exchange resin (1 g) to remove the acid, and the resin filtered off and washed with acetone. The combined filtrate and washings were evaporated under reduced pressure, and the residue was purified by crystallization from 2-propanol to give **19** (4.56 g, 81%), m.p. 115–118°, $[\alpha]_D^{28}$ −25° (*c* 1, methanol); ν_{max}^{KBr} 1745, 1640, and 1520 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): *δ* 1.33 (s, 3 H, ≡C–CH_3), 1.39 (s, 3 H, ≡C–CH_3), 2.08 (s, 3 H, NAc), 2.08 (dd, 1 H, *J* 13.0 and 11.0 Hz, H-3*a*), and 2.24 (dd, 1 H, *J* 13.0 and 5.0 Hz, H-3*e*).

Anal. Calc. for $C_{25}H_{25}NO_9$: C, 62.11; H, 5.21; N, 2.90. Found: C, 62.09; H, 5.35; N, 2.68.

Benzyl 5-acetamido-8,9-O-isopropylidene-D-glycero-*β*-D-galacto-*2-nonulopyranosonate* (**22**). — To a solution of **17** (1 g) in acetone (20 mL) was added with stirring 2,2-dimethoxypropane (1 g) and *p*-toluenesulfonic acid (2 mg). After 30

min at room temperature, the mixture was treated with Dowex 1 (OH^-) anion-exchange resin (1 g) to remove the acid, and the resin was filtered off and washed with acetone. The combined filtrate and washings were evaporated under reduced pressure, and the residue was purified by crystallization from ether to give **22** (*c* 1, methanol), m.p. 178–179°, $[\alpha]_D^{28}$ −22° (*c* 1, methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 1.33 (s, 3 H, ≡C–CH_3), 1.39 (s, 3 H, ≡C–CH_3), 2.08 (s, 3 H, NAc), 2.08 (dd, 1 H, *J* 13.0 and 11.0 Hz, H-3*a*), 2.24 (dd, 1 H, *J* 13.0 and 5.0 Hz, H-3*e*), 3.82 (s, 3 H, –CO_2CH_3), 5.15 (d, 1 H, *J* 12.5 Hz, PhC*H*), 5.26 (d, 1 H, *J* 12.5 Hz, PhC*H*), and 7.34 (s, 5 H, Ph).

Anal. Calc. for $C_{21}H_{29}NO_9$: C, 57.40; H, 6.65; N, 3.19. Found: C, 57.53; H, 6.80; N, 3.07.

*Benzyl 5-acetamido-4-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-*D-glycero-β-D-galacto-*nonulopyranosonate* (**23**). — To a solution of **22** (430 mg) in pyridine (1 mL) was added acetic anhydride (1 mL). The mixture was stirred for 1 h at room temperature, and ethanol (10 mL) was added. The solution was evaporated to dryness and the residue purified by crystallization from isopropyl ether to give **22** (297 mg, 75%), m.p. 178–179°, $[\alpha]_D^{28}$ −24° (*c* 1, methanol); ^{1}H-n.m.r., see Table II.

Anal. Calc. for $C_{15}H_{23}NO_{11}$: C, 45.80; H, 5.89; N, 3.56. Found: C, 45.75; H, 5.93; N, 3.54.

*5-Acetamido-4-*O*-acetyl-3,5-dideoxy-*D-glycero-β-D-galacto-*2-nonulopyranosonic acid* (**25**). — (*a*). A solution of **19** (726 mg) in M NaOH (2 mL) was stirred for 4 h at room temperature, and then diluted with water and de-ionized with Dowex 50 (H^+) cation-exchange resin. The filtrate was lyophilized and the residue acetylated with acetic anhydride (1 mL) and pyridine (1 mL) for 1 h at room temperature. The excess of acetic anhydride was decomposed by the addition of ethanol, and the solvents were evaporated off. The residue was treated with 9:1 acetic acid–water for 2 h at 60°. The mixture was evaporated to give a syrup which was chromatographed on Dowex 1-X8 (20 mL; HCO^-) anion-exchange resin (100–200 mesh) with a formic acid gradient (0–1M). The eluate was lyophilized, and the residue dissolved in a little water and precipitated with ethanol to give **25** (220 mg, 31%), amorphous powder, $[\alpha]_D^{20}$ −34° (*c* 1, water); ν_{max}^{KBr} 1745, 1620, and 1550 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.90 (s, 3 H, NAc), 2.00 (s, 3 H, OAc-4), 2.16 (dd, 1 H, *J* 12.8 and 5.4 Hz, H-3*e*), 3.47 (br. d, 1 H, *J* 9.0 Hz, H-7), 3.53 (dd, 1 H, *J* 12.0 and 6.2 Hz, H-9a), 3.76 (dd, 1 H, *J* 12.0 and 3.0 Hz, H-9b), and 5.19 (m, 1 H, H-4).

Anal. Calc. for $C_{13}H_{21}NO_{10}$: C, 44.45; H, 6.03; N, 3.99. Found: C, 44.42; H, 6.11; N, 3.94.

(*b*). A solution of **23** (393 mg) in 90% acetic acid was stirred for 2 h at 60°. The mixture was evaporated to give a syrup which was dissolved in methanol (10 mL) and treated with H_2 in the presence of 10% Pd–C for 2 h at room temperature. The solution was filtered through Celite and evaporated under reduced pressure. The residue was dissolved in a little water and precipitated with ethanol to give **25** (182 mg, 52%), amorphous powder showing an optical rotation, and i.r. and ^{1}H-n.m.r. spectra identical with those of **25** described under (*a*).

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