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SYNTHESES OF (α2-9) and (α2-8) LINKED DINEURAMINYL SACCHARIDES BY USE OF 2β-BROMO-3β-HYDROXY-4,7,8,9-TETRA-O-ACETYL-N-ACETYLNEURAMINIC ACID METHYL ESTER[1]

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Abstract - NeuAc(α2-9)NeuAc and NeuAc(α2-8)NeuAc disacchrides, which are involved in the group C meningococcal polysaccharides and gangliosides, were synthesized by condensation of the acetyl protected 2-deoxy-2,3-dehydroneuraminic acid methyl ester having a hydroxyl group at 9 or 8 position with the acetyl protected 2β-bromo-3β-hydroxy-N-acetylneuraminic ester in preference to the corresponding β-glycosides. The hydroxyl groups of the obtained glycosides were removed by phenoxythiocarbonylation followed by reduction with tributylstannane and the resultant disaccharides were deprotected to afford the free glycosides in good yields.

We have already reported[2,3] that the methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-bromo-2,5-dideoxy-β-D-*erythro*-L-*gluco*-2-nonulopyranosonate (**4**)[4] is a prominent glycosyl donor for the synthesis of 2α-glycosides of N-acetylneuraminic acid (NeuAc), which is located at the non-reducing ends of carbohydrate chains on glycoproteins and glycolipids and plays an important role in biological recognition.[5,6]

In 1983, Nagai et al reported that a tetrasialoganglioside, GQ_{1b}, in a few nanomolar concentrations showed a remarkable enhancement of cell growth and neurite outgrowth in two human neuroblastoma cell lines, GOTO and NB-1.[8] The action of GQ_{1b} was quite specific in regard to sialyl structure of the carbohydrate moiety because deletion of only one terminal NeuAc from its two disialyl residues led to a total loss of the activity. Therefore, synthesis of NeuAc(α2-8)NeuAc linkage involved in the biologically active gangliosides such as GQ_{1b} has become increasingly important. But there has been no report of succeeding in the synthesis of the NeuAc(α2-8)NeuAc linkage. We report here a successful application of our approach to the syntheses of NeuAc(α2-9)NeuAc and NeuAc(α2-8)NeuAc linkages involved in the group C meningococcal polysaccharides[7] and gangliosides.

Prior to the synthesis of a NeuAc(α2-8)NeuAc linkage, we tried to synthesize a NeuAc(α2-9)NeuAc saccharide. The glycosyl acceptor **3** having a free hydroxyl group at 9 position was prepared from the 2-deoxy-2,3-dehydro-NeuAc methyl ester **1**[4,8] in the following two steps (Scheme 1). Compound **1** was treated with chlorotriphenylmethane in pyridine at 60 °C to afford the 9-O-triphenylmethyl-NeuAc derivative. Without purification, the hydroxyl groups at 4, 7, and 8 positions were acetylated by addition of acetic anhydride into the reaction mixture. The overall yield was 84%. Detriphenylmethylation of **2** was achieved by treatment with hot 90% acetic acid to give the acceptor

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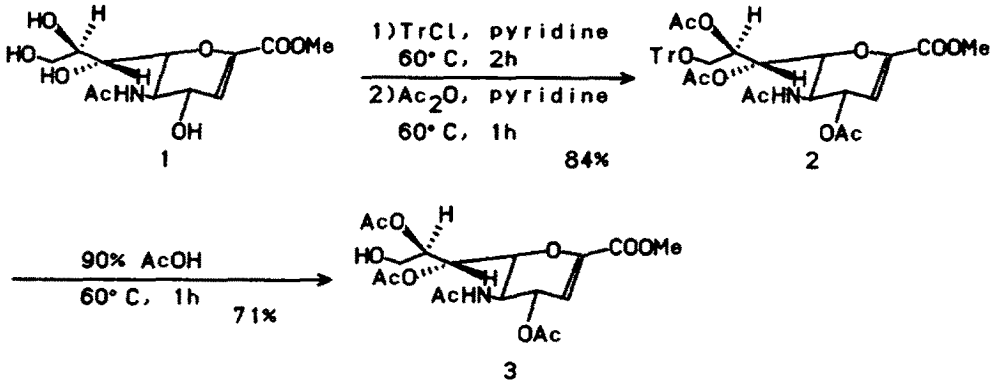
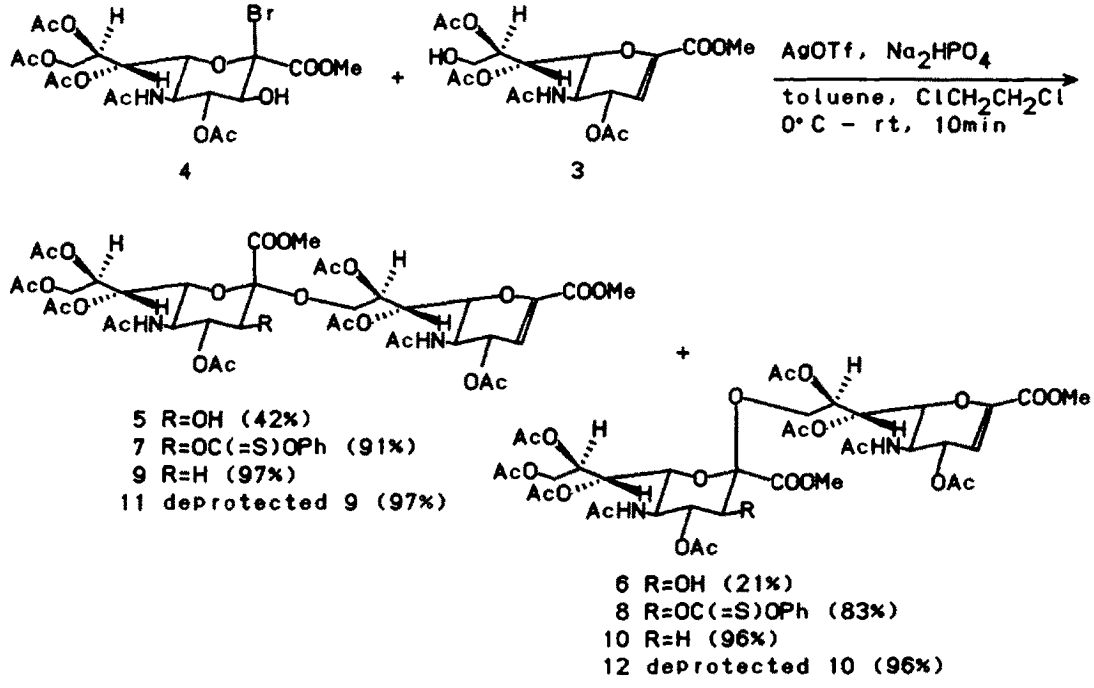
Scheme 1



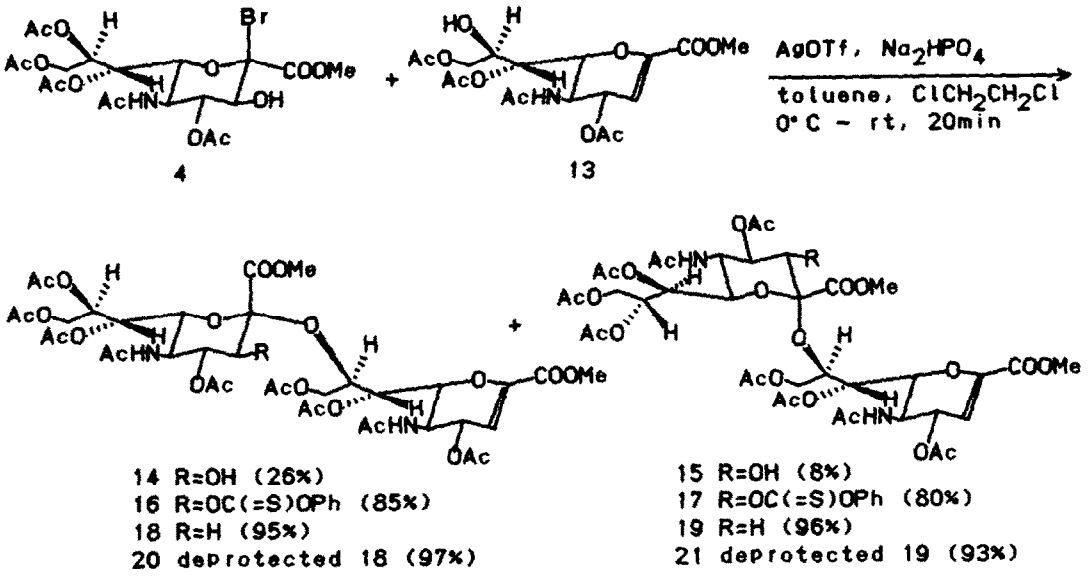
Scheme 2



3 in 71% yield and structure 3 was assigned according to the ^{1}H-NMR spectrum, which showed a deshielded signal at 5.11 ppm for H-8 and a ddd signal at 3.61 ppm for H-9 with a coupling constant between H-9 and OH-9 (7.9 Hz).

Glycosylation of 3 (1.1 equiv) with the acetyl protected 2β-bromo-3β-hydroxy-NeuAc methyl ester 4 in toluene-1,2-dichloromethane (1:1) in the presence of silver trifluoromethanesulfonate (AgOTf) at 0 °C gave an epimeric mixture of 2-9 linked dineuraminyl glycosides (Scheme 2). The mixture could be separated by the repeated silica gel column chromatography (benzene-acetone-methanol, gradient elution from 30:30:0 to 30:30:2) or by preparative ODS HPLC (methanol-water, 46:54) into NeuAc(α2-9)NeuAc glycoside 5 (42% yield) and the corresponding β-glycoside 6 (21% yield). The anomeric configuration was determined by the empirical rule in ^{1}H-NMR as demonstrated earlier.[2,3,9] The coupling constant $J_{7,8}$ value (8.4 Hz) of the NeuAc unit of the α-anomer 5 was larger than that of the β-anomer 6 (2.7 Hz), whereas the $\Delta\delta|$H-9'-H-9$|$ value (0.17 ppm) of 5 was smaller than the value of 6 (0.59 ppm). And the proton at 8 position of the second NeuAc unit of each glycoside remained in a lower field. From these data, we could confirm the structures as shown in Scheme 2. It is noteworthy that this condensation yield is much superior to the yield reported elsewhere

Scheme 3



26.3%; α:β=17.1:9.2).[10]

Reduction of the 3β-hydroxyl group of the glycosides, **5** and **6**, was done by the Robins' method[11] s described earlier;[9] that is carbonothioation followed by reduction with tributylstannane. The -glycoside **5** was easily carbonothioated with phenyl chlorocarbonothioate and 4-dimethylaminopyri- ine (DMAP) for 2.5 h at 50 °C in acetonitrile to afford **7** in 91% yield ($J_{7,8}$=8.9 Hz, $\Delta\delta|$H-9'-H-9$|$= .06 ppm). On the other hand, the β-glycoside **6** was less reactive to the carbonothioation so that he higher temperature (60 °C) and the longer reaction time (12 h) were required to obtain the car- onothioate **8** in 83% yield ($J_{7,8}$=4.0 Hz, $\Delta\delta|$H-9'-H-9$|$=0.68 ppm). Reduction of the both carbonothi- ates with tributylstannane in the presence of azobisisobutyronitrile (AIBN) gave the corresponding -glycoside **9** ($J_{7,8}$=8.9 Hz, $\Delta\delta|$H-9'-H-9$|$=0.18 ppm) and the β-glycoside **10** (3.4 Hz, 0.67 ppm) in 97% nd 96% yield, respectively. Subsequent deprotection of the dineuraminyl saccharides **9** and **10** was chieved as follows: each saccharide was treated with potassium t-butoxide in methanol to give the eacetylated glycoside. Without isolation, 1N NaOH was added to the reaction mixture to hydrolyze he methyl esters. After acidification of the alkaline solution with Dowex 50W-X8 [H^+] resin, the esultant free glycosides, **11** and **12**, were obtained as white powder in quantitative yield.

The next subject was the formation of the NeuAc(α2-8)NeuAc linkage which has never been achieved efore. The above glycosylation condition was applied to the 8-unprotected 2,3-dehydro-NeuAc de- ivative **13**.[3,12] Condensation of the bromohydrin **4** with the acceptor **13** (1.0 equiv) gave a mixture f dineuraminyl saccharides with 2-8 linkage. The mixture could not be separated by silica gel olumn chromatography but preparative ODS HPLC (methanol-water, 45:55) to the NeuAc(α2-8)NeuAc gly- oside **14** (26% yield) and the NeuAc(β2-8)NeuAc glycoside **15** (8% yield). The stereochemistry of the nomeric position was easily determined by the ^{1}H-NMR spectra, in which the coupling constant $J_{7,8}$ alues of **14** and **15** were 7.5 Hz and 1.8 Hz, respectively, and the $\Delta\delta|$H-9'-H-9$|$ values were 0.41 ppm nd 0.91 ppm, respectively. Moreover, the fact that the proton at 8 position remained in a higher ield (**14**: δ 4.63, **15**: δ 4.30) indicated the linkage mode as 2-8. By this condensation method, the euAc(α2-8)NeuAc linkage was constructed for the first time.

The 3β-hydroxyl groups of **14** and **15** were unreactive to the carbonothioation reaction applied to he NeuAc(2-9)NeuAc derivatives. Therefore, the more polar solvents such as N,N-dimethylformamide DMF) for the α-glycoside **14** and dimethylsulfoxide (DMSO) for the β-glycoside **15** were required to onvert to the carbonothioates **16** ($J_{7,8}$=7.5 Hz, $\Delta\delta|$H-9'-H-9$|$=0.42 ppm) and the β-glycoside **17** (2.7 z, 0.85 ppm) in 85% and 80% yield, respectively. The carbonothioates **16** and **17** were easily re- uced with tributylstannane to the α-glycoside **18** ($J_{7,8}$=7.8 Hz, $\Delta\delta|$H-9'-H-9$|$=0.53 ppm) and the β-

glycoside **19** (2.7 Hz, 0.91 ppm) in 95% and 96% yield, respectively, in which **19** was identical with the authentic sample.[3,12] Deacetylation (t-BuOK in methanol) and saponification (1N NaOH in methanol) of **18** and **19** afforded the free dineuraminyl saccharides **20** and **21**, quantitatively.

The coupling constant $J_{7,8}$ and $\Delta\delta|H\text{-}9'\text{-}H\text{-}9|$ values remained unaltered in each series of the acetyl protected α- and β-glycosides, even if the C-3 was substituted with hydroxyl or phenoxythiocarbonate group. The values of each parameter were by no means reversed between α- and β-glycosides.

In conclusion, by use of the 2β-bromo-3β-hydroxy-NeuAc derivative **4** as a donor and one equivalent of the acceptor, the NeuAc(α2-9)NeuAc and NeuAc(α2-8)NeuAc linkages were obtained in good yield in preference to the corresponding β-glycosides.

EXPERIMENTAL

General. Melting points were taken on a Mitamura Riken flat-bulb thermometer with a heating metal block and uncorrected. Elemental analyses were done on a Perkin-Elmer 240C elemental analyzer. Nuclear magnetic resonance spectra (NMR) were obtained with a JEOL GX-500 instrument in the FT mode. Chemical shifts (δ) were expressed in parts per milion from internal tetramethylsilane unless otherwise noted. Coupling constants are in hertz (Hz) and splitting pattern abbreviations are: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of double doublets; m, mutiplet; br, broad. Mass spectra (MS) were obtained on a JEOL DX-300 spectrometer. Infrared spectra (IR) were recorded on a JASCO A-3 spectrophotometer. Optical rotations $[\alpha]_D$ were recorded on a JASCO DIP-181 digital polarimeter.

Preparative high performance liquid chromatography (HPLC) was carried out on a JASCO Trirotor III, IV, or BIP liquid chromatography system and UVIDEC-III, IV, or V as a UV (254 nm) detector by use of a reversed-phase silica-gel (ODS, 10-20 μ Develosil, NOMURA Chemical Co. Ltd.) in a stainless column (10 φ X 250 mm).

Analytical thin layer chromatography (TLC) was conducted on precoated TLC glass sheets (silica gel 60F-254, layer thickness 0.25 mm) manufactured by E. Merck. Detection was effected by dipping into 2% concentrated sulfuric acid ethanol solution followed by heating on a hot plate (ca 120 °C).

Column chromatography was performed with Merck silica gel 60 (70-230 mesh).

^{1}H-NMR data were summarized in Table 1 and 2, and MS, elemental analyses, mp, Rf, $[\alpha]_D$, and IR data were in Table 3.

Methyl 5-acetamido-4,7,8-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-9-O-triphenylmethyl-D-glycero-D-galacto-non-2-eno-pyranosonate (**2**). A mixture of **1**[6] (1.5 g, 5.4 mmol), triphenylmethyl chloride (1.8 g, 6.5 mmol) and pyridine (15 ml) was stirred for 2 h at 60 °C under argon. Acetic anhydride (7 ml) was added to the mixture and the resultant solution was stirred at 60 °C for 1 h. The mixture was condensed and the residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with 5% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give a crude material, which was chromatographed on a silica gel column (ehtyl acetate-acetone, 20:1) to give white crystals. Recrystallization from hexane-ethyl acetate gave **2** (3.1 g, 84%).

Methyl 5-acetamido-4,7,8,-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-eno-pyranosonate (**3**). A solution of **2** (95 mg, 1.4 mmol) in 90% acetic acid (10 ml) was stirred at 60 °C for 1 h and condensed to a syrup, which was chromatographed on a silica gel column (ethyl acetate-acetone, 6:1) to give **3** (430 mg, 71%) as a syrup.

Condensation of **4** with **3**. To a stirred mixture of **4**[4] (400 mg, 0.70 mmol), **3** (330 mg, 0.77 mmol), anhydrous Na_2HPO_4 (360 mg), dry 1,2-dichloroethane (8 ml), and toluene (5 ml) was added a solution of AgOTf (180 mg, 0.70 mmol) in toluene (3 ml) at 0 °C under argon. The mixture was stirred for 15 min and filtered, and the solid was washed with chloroform. The combined filtrates and washings were condensed to a syrup, which was purified by a silica gel column (benzene-acetone-methanol, gradient elution from 30:30:0 to 30:30:2). The fast migrating substance was methyl 5-acetamido-4,7,8-tri-O-acetyl-2,6-anhydro-2,3,5-tri-deoxy-9-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-5-deoxy-β-D-erythro-L-gluco-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**6**) (135 mg, 21%), which was obtained as a syrup, and the slow one was the corresponding α-isomer **5**, which was crystallized from ether-methanol to give white crystals (274 mg, 42%).

Condensation of **4** with **13**. A solution of AgOTf (300 mg, 1.2 mmol) in toluene (4 ml) was added to a stirred mixture of **4** (660 mg, 1.2 mmol), **13** (500 mg, 1.2 mmol), anhydrous Na_2HPO_4 (600 mg), 1,2-dichloroethane (10 ml), and toluene (6 ml) at 0 °C under argon. The mixture was stirred for 10 min at 0 °C and for additional 10 min at room temp, and worked up in the same manner as described above to give a syrup, which was chromatographed on a silica gel column (benzene-acetone-methanol, gradient elution from 30:30:0 to 30:30:2) to give a mixture of **14** and **15** (461 mg, 43%). This was separated by ODS HPLC (methanol-water, 50:50 at 40 °C). The fast eluted isomer was methyl 5-acetamido-4,7,9-tri-O-acetyl-2,6-anhydro-2,3,5-trideoxy-8-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-5-deoxy-α-D-erythro-L-gluco-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**14**) (279 mg, 26%) and the slow one the corresponding β-isomer **15** (86 mg, 8%).

Table 1. ^{1}H-NMR Data for Non-reducing (NeuAc) Unit in Chloroform-d

Compound	Chemical shifts, δ (multiplicities)												
	H-3eq (dd)	H-3ax (dd)	H-4 (dd)	H-5 (ddd)	H-6 (dd)	H-7 (dd)	H-8 (ddd)	H-9 (dd)	H-9' (dd)	Me ester (s)	NH (d)	OH-3 (d)	O-Ac, N-Ac (s)	Phenyl (m)
2		6.05[a]	5.50	4.27	4.48	5.58	5.37	3.24	3.40	3.75	5.66		1.89,1.95,2.05 2.06	7.20-7.45
3		5.98[a]	5.52	4.49	4.39	5.46	5.11	3.61[b]	4.05	3.81	5.91	3.20[c]	1.93,2.07,2.08 2.16	
5		3.77	5.22	4.16	4.62	5.27	5.36	4.08	4.25	3.81[d]	5.73	4.07	1.89,2.04,2.07 2.10,2.14[d]	
6		3.81	5.24	4.16	4.29	5.37	5.05	4.08	4.67	3.82[d]	6.28	3.37	1.90,2.02,2.07 2.13,2.16[d]	
7		5.68[a]	5.73	4.19	4.88	5.30	5.48	4.17	4.23	3.80[d]	5.72		1.93,2.03,2.06 2.10,2.15[d]	7.00-7.45
8		5.82[a]	5.54	4.38	4.44	5.42	5.19	4.09	4.71	3.80[d]	6.52		1.91,2.02,2.06 2.11,2.20[d]	7.00-7.45
9	2.53	1.89	4.94[b]	3.97	4.13	5.33	5.40	4.12	4.30	3.80[d]	5.37		1.89,2.03,2.07 2.13,2.15[d]	
10	2.44	1.84	5.28[b]	4.05	4.30	5.41	5.17	4.13	4.80	3.81[d]	6.26		1.91,2.01,2.06 2.10,2.16[d]	
11[e]	2.70	1.80	3.77[b]	3.88[f]	3.81	3.54	3.87	3.62	3.85				2.02[d]	
12[e]	2.45	1.76	4.10[b]	3.93[f]	3.93	3.56	3.84	3.64	3.83				2.04[d]	
14		4.00	5.24	4.19	4.38	5.24	5.28	3.96	4.37	3.83[d]	5.67	4.05	1.88,2.06,2.09 2.09,2.11[d]	
15		3.85	4.99	4.21	4.66	5.35	5.32	3.95	4.86	3.82[d]	6.03	3.12	1.85,2.04,2.05 2.12,2.20[d]	
16		5.89[a]	5.47	4.40	4.38	5.32	5.34	4.02	4.44	3.81[d]	5.48		1.91,2.06,2.08 2.10,2.13[d]	7.00-7.45
17		5.84[a]	5.26	4.38	4.72	5.38	5.33	4.00	4.85	3.79[d]	6.20		1.88,2.02,2.05 2.13,2.20[d]	7.00-7.45
18	2.65	2.19	4.88[b]	4.00	3.81	5.31	5.31	3.95	4.48	3.83[d]	5.14		1.86,2.05,2.08 2.10,2.16[d]	
20[e]	2.71	1.78	3.66[b]	3.84[f]	3.67	3.54	3.78	3.62	3.83				2.01[d]	

Compound	First-order coupling constants, Hz											
	$J_{3eq,3ax}$	$J_{3eq,4}$	$J_{3ax,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,NH}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$	$J_{OH,3ax}$
2			3.7	5.7	7.0	8.6	5.2	5.5	4.9	4.9	-10.4	
3			2.7	7.9	9.6	9.8	3.2	5.6	3.4	4.3	-12.8	6.4, 7.9[g]
5			9.8	10.4	10.7	9.0	2.0	8.4	6.8	2.4	-12.5	5.5
6			9.8	10.1	10.7	9.8	2.1	2.7	7.9	2.1	-12.5	9.8
7			9.5	9.4	10.7	9.8	1.8	8.9	5.2	2.3	-12.5	
8			9.8	10.7	10.4	9.5	2.1	4.0	7.9	2.4	-12.5	
9	-12.8	4.6	12.0	11.7	10.7	10.8	2.1	8.9	6.0	2.8	-12.5	
10	-13.0	5.0	11.9	11.0	10.7	9.8	2.3	3.4	8.2	2.1	-12.2	
11[e]	-12.2	4.4	12.2	11.5	10.4		0.8	8.9	6.4	2.7	-11.9	
12[e]	-12.8	4.9	11.6	10.8	h		0	9.5	5.9	2.7	-11.9	
14			9.2	9.6	11.7	10.1	1.8	7.5	7.6	2.4	-12.2	6.4
15			10.1	10.1	11.4	9.6	2.1	1.8	8.9	2.4	-12.2	0
16			7.9	9.6	10.8	9.8	1.5	7.5	7.0	2.7	-12.2	
17			9.8	10.4	10.5	9.8	1.5	2.7	8.9	2.4	-12.2	
18	-12.2	4.6	11.8	9.6	10.8	10.1	1.8	7.8	7.6	1.8	-12.2	
20[e]	-12.2	4.5	11.8	9.6	10.7		1.5	9.0	6.1	2.4	-11.9	

[a]Multiplicity: d. [b]Multiplicity: ddd. [c]Assigned to OH-9. [d]Assignments may be interchanged with reducing unit. [e]Measured in D_2O and t-BuOH (1.23 ppm) was used as an internal standard. [f]Multiplicity: dd. [g]$J_{OH,9}$ coupling constant. [h]Not assigned owing to the complexity of the spectrum.

Methyl 5-acetamido-4,7,8-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-9-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-5-deoxy-3-[phenoxy(thiocarbonyl)]-α-D-erythro-L-gluco-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**7**). To a stirred solution of **5** (75 mg, 0.081 mmol) and DMAP (40 mg, 0.33 mmol) was added phenyl chlorocarbonothioate (23 µl, 0.17 mmol) in acetonitrile (1.0 ml) under argon. The mixture was warmed to 50 °C, stirred for 2.5 h, and condensed to a residue. The residue was partitioned between ethyl acetate and water. The ethyl acetate layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The resulting residue was chromatographed on a silica gel column (benzene-acetone, 1:1) to give crystals. Recrystallization from ether-acetone gave **7** (78 mg, 91%) as white crystals.

Methyl 5-acetamido-4,7,8-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-9-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-5-deoxy-3-[phenoxy(thiocarbonyl)]-β-D-erythro-L-gluco-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**8**). A mixture of **6** (45 mg, 0.049 mmol), DMAP (25 mg, 0.21 mmol), and phenyl chlorocarbonothioate (14 µl, 0.10 mmol) was stirred at 60 °C for 12 h under argon. The mixture was worked up in the same manner as described above to give **8** (43 mg, 83%) as a syrup.

Table 2. ^{1}H-NMR Data for Reducing (NeuAc) Unit in Chloroform-d

Compound	Chemical shifts, δ (multiplicities)										
	H-3 (d)	H-4 (dd)	H-5 (ddd)	H-6 (dd)	H-7 (dd)	H-8 (ddd)	H-9 (dd)	H-9' (dd)	Me ester[a] (s)	NH (d)	O-Ac, N-Ac[a] (s)
5	6.11	5.33	4.48	4.67	5.54	5.43	3.85	4.03	3.83	6.12	1.95, 2.07, 2.08, 2.13
6	6.00	5.50	4.45	4.57	5.55	5.45	3.91	3.94	3.83	6.53	1.92, 2.05, 2.07, 2.14
7	6.13	5.32	4.45	4.62	5.54	5.54	3.90	4.14	3.81	5.99	1.94, 2.04, 2.08, 2.14
8	6.06	5.46	4.42	4.67	5.54	5.45	3.81	3.99	3.82	6.18	1.91, 2.03, 2.07, 2.12
9	6.07	5.37	4.40	4.51	5.46	5.38	3.81	3.82	3.83	6.10	1.94, 2.06, 2.09, 2.14
10	6.07	5.47	4.51	4.59	5.52	5.35	3.59	3.86	3.82	6.14	1.94, 2.03, 2.09, 2.13
11[b]	6.04	4.50	4.09	4.28	3.72	4.00	3.78	3.96			2.07
12[b]	6.04	4.51	4.09	4.29	3.70	4.06	3.45	3.92			2.06
14	6.00	5.59	4.41	4.69	5.40	4.63	4.27	4.54	3.86	6.45	1.96, 2.07, 2.09, 2.09
15	6.00	5.51	4.55	4.61	5.54	4.30	4.72	4.76	3.83	6.45	1.89, 2.04, 2.08, 2.17
16	5.98	5.66	4.28	4.57	5.49	4.89	4.26	4.63	3.82	6.16	1.93, 2.07, 2.09, 2.12
17	6.02	5.52	4.56	4.55	5.56	4.72	4.21	4.63	3.84	6.19	1.91, 2.04, 2.08, 2.20
18	5.97	5.63	4.41	4.55	5.24	4.77	4.18	4.37	3.87	6.62	1.94, 2.07, 2.09, 2.12
20[b]	5.97	4.47	4.07	4.18	3.78	4.23	3.85	3.97			2.06

Compound	First-order coupling constants, Hz								
	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,NH}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$
5	3.7	8.2	6.1	9.0	6.4	3.4	5.2	6.7	-10.7
6	3.7	7.6	8.9	9.2	3.7	3.7	6.0	3.3	- 9.9
7	3.7	4.8	6.1	8.2	5.8	c	6.1	6.1	-11.0
8	3.4	5.8	7.6	8.9	3.1	3.3	5.0	5.2	-10.7
9	3.7	7.5	6.1	8.5	5.5	4.4	c	c	c
10	3.6	6.4	7.8	9.2	4.6	3.5	6.1	4.9	-10.7
11[b]	2.4	9.2	10.4		1.0	9.5	2.7	4.9	-10.4
12[b]	2.4	8.9	10.7		1.2	9.3	6.1	2.7	-10.4
14	3.1	7.7	9.3	9.8	3.1	5.5	4.6	4.0	-12.2
15	2.5	8.9	11.4	9.0	1.1	3.0	6.9	11.5	-12.2
16	2.7	9.5	10.7	9.5	2.1	5.8	6.0	2.8	-12.2
17	2.4	9.3	c	9.5	0.5	3.7	7.2	2.7	-12.5
18	2.4	9.2	11.1	10.1	1.8	5.6	8.1	2.5	-12.2
20[b]	2.1	8.9	11.0		0	9.2	2.7	3.0	-12.2

[a] Assignments may be interchanged with non-reducing unit. [b] Measured in D_2O and t-BuOH (1.23 ppm) was used as an internal standard. [c] Not assigned owing to the complexity of the spectrum.

Methyl 5-acetamido-4,7,9-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-8-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-5-deoxy-3-[phenoxy(thiocarbonyl)]-α-D-erythro-L-gluco-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**16**). To a solution of **14** (90 mg, 0.098 mmol) and DMAP (55 mg, 0.45 mmol) in dry DMF (1 ml) was added phenyl chlorocarbonothioate (30 µl, 0.22 mmol) under argon. The mixture was stirred at 60 °C for 24 h and condensed to a residue. The residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to give a crude syrup, which was chromatographed on a silica gel column (benzene-acetone, 1:1) to give **16** (86 mg, 83%) as a viscous syrup.

Methyl 5-acetamido-4,7,9-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-8-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-5-deoxy-3-[phenoxy(thiocarbonyl)]-β-D-erythro-L-gluco-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**17**). A mixture of **15** (24 mg, 0.026 mmol), DMAP (12 mg, 0.098 mmol), phenyl chlorocarbonothioate (7 µl, 0.051 mmol), and DMSO (0.4 ml) was stirred at 60 °C for 3 h under argon. It was evaporated and the residue was treated in the same manner as described above to give **17** (22 mg, 80%) as a syrup.

Methyl 5-acetamido-4,7,8-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-9-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**9**). To a solution of **7** (70 mg, 0.066 mmol) in toluene (1.5 ml) and tetrahydrofuran (1 ml) were added tributylstannane (36 µl, 0.13 mmol) and AIBN (cat. amount) under argon. The mixture was refluxed (bath temp: 110 °C) for 10 min and condensed to a syrup, which was chromatographed on a silica gel column (ethyl acetate-acetone, 1:1) to give a syrup. This syrup was triturated with hexane-ethyl acetate and washed with hexane to give **9** (58 mg, 97%) as a white powder.

Methyl 5-acetamido-4,7,8-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-9-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**10**). A mixture of **8** (28 mg, 0.026 mmol), tributylstannane (14 µl, 0.052 mmol), AIBN (cat. amount), toluene (0.6 ml), and tetrahydrofuran (0.4 ml) was treated in the same manner as described above to give **10** (23 mg, 96%) as a white powder.

Table 3 MS, Elemental Analyses, Mp. Rf, $[\alpha]_D$, and IR Data

Compound	Formula	MS [a] (M+H)	Anal.	% C	% H	% N	Mp (°C)	Rf	$[\alpha]_D$(c)[b] (Temp)	ν_{max}^{KBr} NH,OH	ester	amide I	II
2	$C_{37}H_{39}NO_{11}$	674	Calcd.	65.96	5.83	2.08	203-205[c]	0.36[d]	+38.3°(1.1) (20°C)	3410	1740	1660	1560
			Found	65.83	5.82	1.84							
3	$C_{18}H_{25}NO_{11}$	432	Calcd.	50.12	5.84	3.25	—[e]	0.36[f]	+58.7°(1.1) (15°C)	3420	1740	1660	1540
			Found	49.79	5.95	3.63							
5	$C_{38}H_{52}N_2O_{24}$	921	Calcd.	49.57	5.67	3.04	137-139[g]	0.20[f]	+29.9°(1.2) (13°C)	3430	1740	1660	1540
			Found	49.37	5.92	3.24							
6	$C_{38}H_{52}N_2O_{24}$	921	Calcd.	49.57	5.67	3.04	—[e]	0.29[f]	+23.9°(1.2) (13°C)	3420	1745	1660	1540
			Found	49.44	5.50	3.33							
7	$C_{45}H_{56}N_2O_{25}S$	1057	Calcd.	51.13	5.34	2.65	207-209[h]	0.46[f]	+35.3°(1.3) (12°C)	3420	1745	1660	1540
			Found	51.27	5.55	2.43							
8	$C_{45}H_{56}N_2O_{25}S$	1057	Calcd.	51.13	5.34	2.65	—[e]	0.48[f]	+35.4°(1.3) (12°C)	3420	1750	1660	1540
			Found	51.09	5.24	2.47							
9	$C_{38}H_{52}N_2O_{23}$	905	Calcd.	50.44	5.79	3.10	—[e]	0.30[f]	+17.8°(2.8) (12°C)	3430	1744	1660	1540
			Found	50.66	5.85	3.31							
10	$C_{38}H_{52}N_2O_{23}$	905	Calcd.	50.44	5.79	3.10	—[e]	0.33[f]	+35.1°(1.3) (12°C)	3430	1742	1660	1540
			Found	50.71	5.85	3.03							
11	$C_{22}H_{34}N_2O_{16}$	583	Calcd.	45.36	5.88	4.81	144-146[i]	0.43[j]	+12.7°(1.4)[k] (12°C)	3400	1719[l]	1640	1560
			Found	45.36	5.92	4.89							
12	$C_{22}H_{34}N_2O_{16}$	583	Calcd.	45.36	5.88	4.81	168-172[i]	0.43[j]	+ 6.6°(0.6)[k] (12°C)	3390	1720[l]	1640	1558
			Found	45.64	5.92	4.95							
14	$C_{38}H_{52}N_2O_{24}$	921	Calcd.	49.57	5.67	3.04	—[e]	0.22[f]	+27.4°(1.1) (13°C)	3430	1740	1660	1540
			Found	49.62	5.76	3.21							
15	$C_{38}H_{52}N_2O_{24}$	921	Calcd.	49.57	5.67	3.04	—[e]	0.22[f]	+20.3°(1.6) (13°C)	3430	1745	1660	1538
			Found	49.80	5.39	3.30							
16	$C_{45}H_{56}N_2O_{25}S$	1057	Calcd.	51.13	5.34	2.65	—[e]	0.48[f]	+35.4°(1.3) (12°C)	3430	1743	1660	1540
			Found	51.06	5.57	2.63							
17	$C_{45}H_{56}N_2O_{25}S$	1057	Calcd.	51.13	5.34	2.65	—[e]	0.45[f]	+22.9°(0.8) (12°C)	3420	1748	1660	1530
			Found	51.31	5.66	2.29							
18	$C_{38}H_{52}N_2O_{23}$	905	Calcd.	50.44	5.79	3.10	—[e]	0.32[f]	+22.3°(0.4) (12°C)	3420	1742	1660	1538
			Found	50.34	5.98	3.28							
20	$C_{22}H_{34}N_2O_{16}$	583	Calcd.	45.36	5.83	4.81	163-166[i]	0.46[j]	+35.7°(0.1)[k] (12°C)	3400	1718[l]	1640	1560
			Found	45.34	5.89	4.90							

[a]Fast atom bomberdment method. [b]Measured in chloroform. [c]Recrystallized from hexane-ethyl acetate. [d]Solvent system is benzene-acetone (3:1). [e]Viscous syrup. [f]Solvent system is benzene-acetone (1:1). [g]Recrystallized from ether-methanol. [h]Recrystallized from ether-acetone. [i]Triturated with chloroform-methanol. [j]Solvent system is propanol-water (7:3). [k]Measured in water. [l]Absorption of carboxylic acid.

Methyl 5-acetamido-4,7,9-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-8-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**18**). To a solution of **16** (24 mg, 0.023 mmol) in toluene (1.2 ml) and tetrahydrofuran (0.8 ml) were added tributylstannane (12 µl, 0.045 mmol) and AIBN (cat. amount) under argon. The mixture was refluxed for 10 min and condensed to a residue, which was chromatographed on a silica gel column (ethyl acetate-acetone, 1:1) to give a syrup. This syrup was triturated with hexane-ethyl acetate and washed with hexane to give **18** (19.5 mg, 95%) as a white powder.

Methyl 5-acetamido-4,7,9-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-8-O-(mehtyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosylonate)-D-glycero-D-galacto-non-2-enopyranosonate (**19**). A mixture of **17** (3.4 mg, 0.0032 mmol), tributylstannane (2 µl, 0.0074 mmol), AIBN (cat. amount), toluene (0.3 ml), and tetrahydrofuran (0.2 ml) was treated in the same manner as described above to give **19**[3,12] (2.8 mg, 96%) as a white powder.

5-Acetamido-2,6-anhydro-3,5-dideoxy-9-O-(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)-D-glycero-D-galacto-non-2-enopyranose (**11**). To a solution of **9** (45 mg, 0.050 mmol) in absolute methanol (4.5 ml) was added potassium t-butoxide (cat. amount) under argon. The mixture was stirred for 0.5 h at room temp and to this was added 1N NaOH (0.6 ml). After stirring for additional 1 h, the reaction mixture was cooled to -10 °C, acidified with Dowex 50W-X8 [H^+], and filtered, and the resin was washed well with methanol-water (1:1). The combined filtrates and washings were condensed to a syrup, which was triturated with chloroform-methanol to give **11** (28 mg, 97%) as a white powder.

5-Acetamido-2,6-anhydro-3,5-dideoxy-9-O-(5-acetamido-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosyl)-D-glycero-D-galacto-non-2-enopyranose (**12**). To a solution of **10** (20 mg, 0.022 mmol) in absolute methanol(2 ml) was added potassium t-butoxide (cat. amount) under argon. After stirring for 0.5 h, 1N NaOH (0.25 ml) was added to it and the mixture was treated in the same manner as described above to give **12** (12.3 mg, 96%) as a white powder.

5-Acetamido-2,6-anhydro-3,5-dideoxy-8-O-(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)-D-glycero-D-galacto-non-2-enopyranose (**20**). To a solution of **18** (9.1 mg, 0.010 mmol) in absolute methanol (1 ml) was added potassium t-butoxide (cat. amount) under argon. The mixture was stirred for 0.5 h at room temp and to this was added 1N NaOH (0.11 ml). After stirring for 1 h,

the mixture was cooled (-10 °C), acidified with Dowex 50W-X8 [H^+], and filtered. The resin was washed with methanol-water (1:1) and the combined filtrates and washings were condensed to a residue, which was triturated with chloroform-methanol to give **20** (5.7 mg, 97%) as a white powder.

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