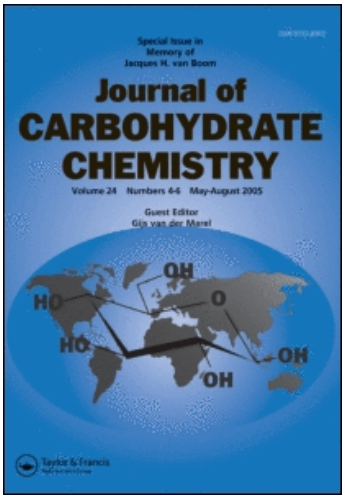

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthetic Studies on Sialoglycoconjugates 53: Synthesis of Novel *N*-Methyl-1-Deoxynojirimycincontaining Sialo-Oligosaccharides Related to Ganglioside GM3 Active as a Biosignal Mediator

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To cite this Article Kiso, Makoto , Katagiri, Hideki , Furui, Hiroyasu , Ando, Keiko , Ishida, Hideharu and Hasegawa, Akira(1994) 'Synthetic Studies on Sialoglycoconjugates 53: Synthesis of Novel *N*-Methyl-1-Deoxynojirimycincontaining Sialo-Oligosaccharides Related to Ganglioside GM3 Active as a Biosignal Mediator', Journal of Carbohydrate Chemistry, 13: 2, 163 — 174

To link to this Article: DOI: 10.1080/07328309408009186

URL: http://dx.doi.org/10.1080/07328309408009186

J. CARBOHYDRATE CHEMISTRY, 13(2), 163-174 (1994)

SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 53: SYNTHESIS OF NOVEL *N*-METHYL-1-DEOXYNOJIRIMYCIN-CONTAINING SIALO-OLIGOSACCHARIDES RELATED TO GANGLIOSIDE GM3 ACTIVE AS A BIOSIGNAL MEDIATOR

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Received June 9, 1993 - Final Form September 1, 1993

ABSTRACT

O-(6-*O*-Benzoyl-β-D-galactopyranosyl)-(1→4)- and *O*-(2, 3, 4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1, 5-dideoxy-1, 5-imino-D-glucitols (**4** and **12**) were each coupled with methyl (methyl 5-acetamido-4,7,8, 9-tetra-*O*-acetyl-3, 5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**5**) in acetonitrile medium in the presence of dimethyl(methylthio)sulfonium triflate (DMTST) or *N*-iodosuccinimide/trifluoromethanesulfonic acid to give the corresponding α-sialyl-(2→3)- and α-sialyl-(2→6)-glycosides (**6** and **13**α), which were converted to novel ganglioside GM3-related trisaccharides (**9** and **15**) containing *N*-methyl-1-deoxynojirimycin.

INTRODUCTION

Sialic acid[1] (*N*-acetylneuraminic acid; Neu5Ac) is known as a unique acidic component of glycolipids and glycoproteins, and plays important roles in a variety of biological processes. 1-Deoxynojirimycin (1,5-dideoxy-1,5-imino-D-glucitol; DNJ) and related compounds have been shown[2] not only to be potent inhibitors of glycosidases and glycoprotein-processing enzymes, but also to be of potential clinical value as antidiabetic, anticancer and anti-HIV agents.

In the course of a synthetic approach to elucidate the biological functions of sialoglycoconjugates, we have systematically synthesized[3] a variety of gangliosides and their analogs. In this connection, some DNJ-containing sialo-oligosaccharides have been designed[4] to evaluate their biomedical usefulness based on a new concept. We describe here the synthesis of novel trisaccharides structurally related to ganglioside GM3[5] that serves not only as the carbohydrate epitopes recognized by influenza A virus[6] and *tripanosoma cruzi*[7] but also as mediators[8] in cell growth and differentiation.

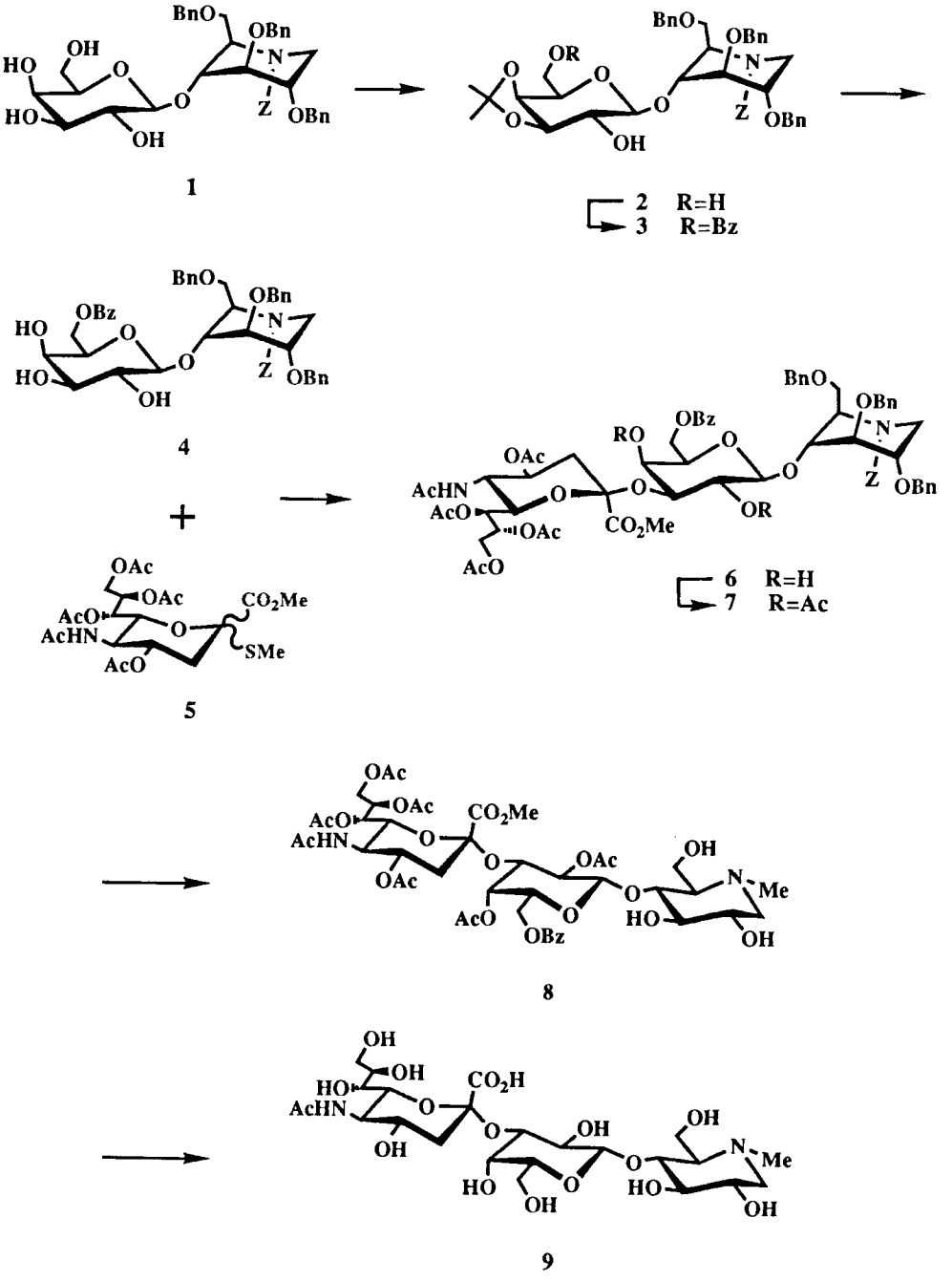
RESULTS AND DISCUSSION

Treatment of *O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol[9] (**1**) with 1.5 mol equivalent of 2,2-dimethoxypropane at 80 °C in *N,N*-dimethylformamide (DMF) gave **2** in a 90% yield. Selective 6'-*O*-benzoylation of **2** and successive deisopropylidenation afforded a partially protected glycosyl acceptor **4** in high yield.

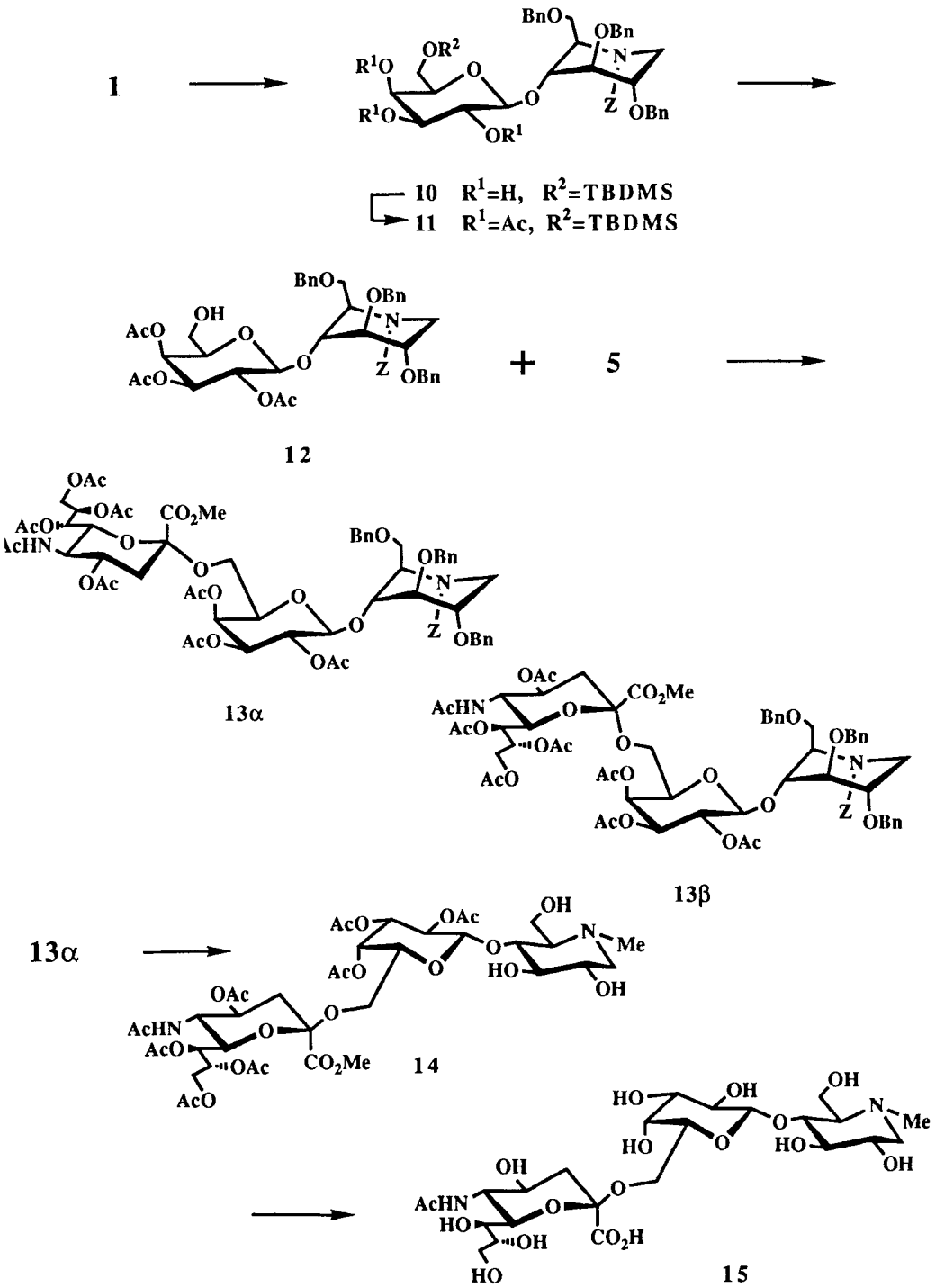
A regio- and α-stereoselective glycosylation of **4** with methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate[10] (**5**) was performed[5,10,11] using DMTST as a thiophilic promoter to give **6** (48% based on the acceptor **4**). The remaining hydroxyls in **6** were acetylated to afford **7**, that was then hydrogenolyzed in the presence of formic acid and palladium black catalyst. The resulting **8** was successively treated with methanolic sodium methoxide and aq 0.2M KOH to give **9**, quantitatively (SCHEME 1).

The 6'-OH in compound **1** was regioselectively protected with *tert*-butyldimethylsilyl (TBDMS) chloride in 2:1 dichloromethane-pyridine at 0 °C to give **10** (90%), and the remaining hydroxyls at C-2'~4' were all acetylated. Significant signals in the ^{1}H NMR spectrum of **11** were a nine-proton singlet at δ 0.87 (*t*-butyl), three three-proton singlets at δ 1.90, 1.99, 2.12 (3AcO), two one-proton doublets of doublets at δ 4.97 ($J_{2,3}$ = 10.3, $J_{3,4}$ = 3.3 Hz, H-3 of Gal), 5.18 ($J_{1,2}$ = 8.1, $J_{2,3}$ = 10.3 Hz, H-2 of Gal), and a one-proton doublet at δ 5.45 ($J_{3,4}$ = 3.3 Hz, H-4 of Gal), indicating the structure assigned. Removal of the TBDMS group in compound **11** with 80% acetic acid gave another glycosyl acceptor **12**.

Glycosylation[3,11] of **12** with **5** (1.7 equiv to the acceptor **12**) in acetonitrile for 5 h at -40 °C in the presence of *N*-iodosuccinimide, trifluoromethanesulfonic acid and 3Å molecular sieves afforded the desired α-glycoside (**13**, 60%) and the β-isomer (30%). These structures were assigned by a ^{1}H NMR empirical rule based on the chemical shifts of H-3*eq*, H-4 and H-8 in the Neu5Ac moiety. The observed chemical shifts for these protons H-3*eq* (δ 2.48 for α, δ 2.44 for β), H-4 (δ 4.82 for α, δ 5.1-5.25 for β), and



SCHEME 1



SCHEME 2

H-8 (δ 5.35 for α, δ 5.1 for β) are characteristic[12] of the respective anomeric configuration. Compound **13α** was hydrogenolyzed in the presence of formic acid and palladium black catalyst to give **14**. *O*-Deacetylation of **14** and saponification of the methyl ester group afforded the desired trisaccharide **15** in almost quantitative yield (SCHEME 2). In the ion-spray mass spectrum (positive ion mode) of **15**, two significant ion peaks that correspond to $[M+H]^+$ (m/z 631.8) and $[M+Na]^+$ (m/z 653.3) were observed in 100% and 50% relative intensities, respectively, unambiguously showing the *N*-methylated structure ($C_{24}H_{42}N_2O_{17}$) assigned. It has been found[9] that the *N*-Boc or *N*-Z protected DNJ derivatives have a unique $^1C_4$ type comformation[13] that easily changes to the normal $^4C_1$ type conformation by deprotection. The similar dramatic conformational change was observed in the hydrogenolysis of **7** and **13α** to give the *N*-methylated DNJ-containing trisaccharides **8** and **14**, respectively. In their ^1H NMR (CD_3OD) spectra, H-1*eq* of the DNJ part appeared at δ 2.96 (dd, J_{gem} = 11.3, $J_{1eq,2}$ = 4.9 Hz), for **8** and at δ 2.91 (dd, J_{gem} = 11.5, $J_{1eq,2}$ = 4.5 Hz) for **14**, respectively, showing the $^4C_1$ type conformation[14, 15] similar to that of the authentic *N*-methyl-1-deoxynojirimycin.[16]

EXPERIMENTAL

General Procedures. Specific rotations were determined with a Union PM-201 polarimeter, and ^1H NMR spectra were recorded at 270 MHz with a JEOL JNM-GX270 spectrometer. Ion-spray MS spectra were recorded with PERKIN ELMER SCIEX API-III. Preparative TLC was performed on silica gel 60 (Merck Co.), and column chromatography on silica gel (Wako Co., 200 mesh) was accomplished with the solvent systems (v/v) specified. Concentrations and evaporations were conducted *in vacuo*.

***O*-(3, 4-*O*-Isopropylidene-β-D-galactopyranosyl)-(1 → 4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1, 5-dideoxy-1, 5-imino-D-glucitol (2) and *O*-(6-*O*-Benzoyl-3, 4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (3).** To a solution of *O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol[9] (**1**, 0.89 g) in *N*,*N*-dimethylformamide (DMF, 5 mL) were added 2,2-dimethoxypropane (0.32 mL) and a catalytic amount of *p*-toluenesulfonic acid monohydrate. The mixture was heated for 4 h at 80 °C and then neutralized with Amberlite IR-410 (OH^-) resin in DMF/methanol. After work-up, the product was purified by chromatography on a column of silica gel with 200:1 CH_2Cl_2-MeOH to give **2** (90%): ^1H NMR ($CDCl_3$-CD_3OD) δ 1.36, 1.52 (2s, 6H, isopropylidene), 3.23 (dd, 1H, J = 14.7, 2.2 Hz, H-1*ax* of DNJ), 3.47 (t, 1H, J = 7.7

Hz), 3.55 (narrow m, 1H), 3.92 (narrow m, 1H), 4.03 (~t, 1H, J = 7.0, 5.5 Hz), 5.0-5.15 (broad t, 2H, CH_2 of Z), and 7.15-7.4 (m, 20H, Ph-*H*).

A solution of **2** (90 mg) in 1:1 dichloromethane-pyridine (10 mL) was cooled to -50 °C, and then benzoyl chloride (1.1 equiv.) was added. The mixture was stirred for 3.5 h at -50 °C and methanol was added. Work-up and chromatography on a column of silica gel with 300:1 CH_2Cl_2-MeOH afforded **3** (84%): $[\alpha]_D$ +22.4° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$-CD_3OD) δ 1.36, 1.51 (2s, 6H, isopropylidene), 1.90 (broad s, 1H, OH), 3.18 (dd, 1H, J = 14.7, 1.8 Hz, H-1*ax* of DNJ), 3.49 (narrow m, 1H), 3.52 (t, 1H, J = 7.7 Hz), 3.67, 3.69 (2broad s, 2H), 3.91 (~s, 1H), 5.0-5.15 (broad t, 2H, CH_2 of Z), 7.05-7.35 (m, 22H, Ph-*H* of OBn and *m*-Ph-*H* of OBz), 7.45 (~t, 1H, J = 7.3 Hz, *p*-Ph-*H* of OBz), and 8.01 (~d, 2H, J = 7.3, 1.1 Hz, *o*-Ph-*H* of OBz).

Anal. Calcd for $C_{51}H_{55}NO_{12}$ (874.00): C, 70.09; H, 6.34; N, 1.60. Found: C, 69.85; H, 6.31; N, 1.46.

***O*-(6-*O*-Benzoyl-β-D-galactopyranosyl)-(1→4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (4).** A mixture of **3** (0.7 g) and 80% aq acetic acid was stirred at 45 °C until the deisopropylidenation was complete. The mixture was concentrated and the syrupy residue was chromatographed on a column of silica gel with 125:1 CH_2Cl_2-MeOH to give **4** (quant): $[\alpha]_D$ +9° (*c* 0.9, $CHCl_3$): ^1H NMR ($CDCl_3$-CD_3OD) δ 3.18 (dd, 1H, J = 14.7, 2.2 Hz, H-1*ax*), 3.43, 3.91, 3.96, 4.13 (4broad s, 4H), 4.57 (d, 1H, J = 6.6 Hz, H-1'), 5.06 (broad t, 2H, CH_2 of Z), 7.05-7.35 (m, 22H, Ph-*H* of OBn and Z, *m*-Ph-*H* of OBz), 7.48 (~t, 1H, J = 7.3-7.7 Hz, *p*-Ph-*H* of OBz), and 8.01 (~d, 2H, J = 7.3, 1.1 Hz, *o*-Ph-*H* of OBz).

Anal. Calcd for $C_{48}H_{51}NO_{12}$ (833.93): C, 69.13; H, 6.16; N, 1.68. Found: C, 68.96; H, 6.09; N, 1.57.

***O*-(Methyl 5-Acetamido-4, 7, 8, 9-tetra-*O*-acetyl-3, 5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-*O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (6).** A mixture of **4** (0.62 g), sialic acid donor **5** (0.74 g, 2 equiv to the acceptor **4**), powdered molecular sieves 3Å (1.5 g) and acetonitrile (8 mL) was stirred overnight at room temperature, and then cooled to -20 °C. To this mixture, dimethyl-(methylthio)sulfonium triflate (DMTST, 3 equiv to the glycosyl donor **5**) was added, and the reaction mixture was stirred for 48 h at -15 °C. The mixture was filtered through Celite and washed with dichloromethane, and the filtrate and washings were combined and concentrated. The residual syrup was chromatographed on a column of silica gel with 2:1 ethyl acetate-hexane to afford **6** (48% yield based on the acceptor **4**): $[\alpha]_D$ -12°(*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.88, 2.00, 2.02, 2.08, 2.10 (5s, 15H, AcN and 4AcO), 2.22 (t, 1H, J = 13 Hz, H-3*ax* of

Neu5Ac), 2.63 (dd, 1H, J_{gem} = 13.2, $J_{3eq,4}$ = 4.8 Hz, H-3*eq* of Neu5Ac), 2.73 (~s, 1H), 3.24 (dd, 1H, J_{gem} = 14.7, $J_{1ax,2}$ = 2 Hz, H-1*ax* of DNJ), 3.48 (narrow m, 1H), 3.68 (t, 1H, J = 8.1 Hz), 3.74 (dd, 1H, J = 11, 4.4 Hz), 3.79 (s, 3H, CO_2CH_3 of Neu5Ac), 4.20 (~s, 1H), 4.32 (dd, 1H, J = 12.5, 2.2 Hz), 4.9-5.15 (m, 3H, CH_2 of Z and H-4 of Neu5Ac), 5.3-5.45 (m, 2H, H-7 and H-8 of Neu5Ac), 7.1-7.4 (m, 22H, Ph-*H* of OBn and Z, *m*-Ph-*H* of OBz), 7.47 (~t, 1H, J = 7.3 Hz, *p*-Ph-*H* of OBz), and 8.00 (~d, 2H, J = 7.3 Hz, *o*-Ph-*H* of OBz).

Anal. Calcd for $C_{68}H_{78}N_2O_{24}$ (1307.36): C, 62.47; H, 6.01; N, 2.14. Found: C, 62.38; H, 5.96; N, 2.11.

***O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-*O*-(2, 4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1, 5-dideoxy-1,5-imino-D-glucitol (7).** Acetylation of **6** with acetic anhydride in pyridine gave **7** (quant): $[\alpha]_D$ -13°(*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.72 (t, 1H, J = 12.5 Hz, H-3*ax* of Neu5Ac), 1.84 (s, 3H, AcN), 1.99-2.13 (6s, 18H, AcO), 2.58 (dd, 1H, J_{gem} = 12.5, $J_{3eq,4}$ = 4.8 Hz, H-3*eq* of Neu5Ac), 3.43 (dd, 1H, J_{gem} = 13.2, $J_{1ax,2}$ = 3.3 Hz, H-1*ax* of DNJ), 3.77 (s, 3H, CO_2Me), 3.92 (narrow t, 1H, J = 4 Hz, H-3 or H-4 of DNJ), 4.00 (t, 1H, J = 10~11 Hz, H-6 of DNJ), 4.16 (dd, 1H, J_{gem} = 11, $J_{5,6'}$ = 7 Hz, H-6' of DNJ), 4.27 (narrow t, 1H, J = 3.3 Hz, H-3 or H-4 of DNJ), 4.38 (~d, 1H, J = 13-14 Hz, H-1*eq* of DNJ), 4.89 (d, 1H, J = 7.7 Hz, H-1 of Gal), 4.90 (m, 1H, H-4 of Neu5Ac), 5.0-5.2 (m, 4H, CH_2 of Z, H-2 and H-4 of Gal), 5.39 (dd, 1H, J = 8.8, 2.6 Hz, H-7 of Neu5Ac), 5.56 (m, 1H, H-8 of Neu5Ac), 7.1-7.4 (m, 22H, Ph-*H* of OBn and Z, *m*-Ph-*H* of OBz), 7.48 (~t, 1H, J = 7.3 Hz, *p*-Ph-*H* of OBz), and 7.96 (~d, 2H, J = 7.3 Hz, *o*-Ph-*H* of OBz).

Anal. Calcd for $C_{72}H_{82}N_2O_{26}$ (1391.44): C, 62.15; H, 5.94; N, 2.01. Found: C, 62.08; H, 5.87; N, 1.92.

***O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-*O*-(2, 4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-1,5-dideoxy-1,5-imino-*N*-methyl-D-glucitol (8).** Compound **7** (110 mg) was hydrogenolyzed for 3 days in methanol (10 mL) in the presence of formic acid (1 mL) and palladium black catalyst (100 mg). The catalyst was filtered off and the filtrate was concentrated. The residue was chromatographed on a column of silica gel with 50:1 CH_2Cl_2-MeOH to give **8** (86%): $[\alpha]_D$ -2°(*c* 1.3, MeOH); ^1H NMR ($CDCl_3$-CD_3OD) δ 1.63 (t, 1H, J = 12.5 Hz, H-3*ax* of Neu5Ac), 1.84 (s, 3H, AcN), 2.00, 2.06, 2.12, 2.14, 2.17, 2.33 (6s, 18H, 6AcO), 2.40 (s, 3H, *N*-Me), 2.63 (dd, 1H, J_{gem} = 12.5, $J_{3eq,4}$ = 4.8 Hz, H-3*eq* of Neu5Ac), 2.96 (dd, 1H, J_{gem} = 11.3, $J_{1eq,2}$ = 4.9 Hz, H-1*eq* of DNJ), 3.38 (dd, 1H, J = 8.8, 6.2

Hz), 3.81 (s, 3H, CO_2Me), 4.02 (t, 1H, J = 10.4 Hz), 4.15 (~t, 1H, J = 6.6, 6.2 Hz), 4.36 (dd, J = 6.6, 2.4 Hz), 4.70 (dd, 1H, $J_{2,3}$ = 10.3, $J_{3,4}$ = 3.5 Hz, H-3 of Gal), 4.86 (d, 1H, J = 7.5 Hz, H-1 of Gal), 4.75-4.95 (m, 1H, H-4 of Neu5Ac), 5.0-5.1 (m, 2H, H-2 and H-4 of Gal), 5.27 (dd, 1H, J = 9.5, 2.6 Hz, H-7 of Neu5Ac), 5.72 (m, 1H, H-8 of Neu5Ac), and 7.37 (~q, 2H, J = 7.3 Hz, *m*-Ph-*H* of OBz), 7.50 (~t, 1H, J = 7.3 Hz, *p*-Ph-*H* of OBz), and 7.97 (~d, 2H, J = 7.3, 1.5 Hz, *o*-Ph-*H* of OBz).

Anal. Calcd for $C_{44}H_{60}N_2O_{24}$ (1000.95): C, 52.80; H, 6.04; N, 2.80. Found: C, 52.52; H, 5.76; N, 2.68.

***O*-(5-Acetamido-3, 5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2 → 3)-*O*-(β-D-galactopyranosyl)-(1 → 4)-1, 5-dideoxy-1, 5-imino-*N*-methyl-D-glucitol (9).** A mixture of **8** (50 mg) and sodium methoxide (10 mg) in methanol (5 mL) was stirred overnight at room temperature, and then 0.2 M aq potassium hydroxide (0.5 mL) was added. The mixture was stirred for 8 h at room temperature, neutralized with Amberlite IR-120(H^+) resin, and filtered. The resin was washed with 1:1 methanol-water, and combined filtrate and washings were concentrated. The product was purified by Sephadex LH-20 column (3:2 EtOH-H_2O) to afford **9** (quant): $[\alpha]_D$ +4°(*c* 0.3, 1:1 EtOH-H_2O); ^1H NMR (D_2O-CD_3OD) δ 1.86 (t, 1H, H-3*ax* of Neu5Ac), 2.02 (s, 3H, AcN), 2.82 (dd, 1H, H-3*eq* of Neu5Ac), 2.99 (s, 3H, N-CH_3), 4.47 (d, 1H, J = 7.9 Hz, H-1 of Gal), and complete disappearance of the methyl protons of CO_2Me.

Anal. Calcd for $C_{24}H_{42}N_2O_{17}$ (630.60): C, 45.71; H, 6.71; N, 4.44. Found: C, 45.70; H, 6.56; N, 4.39.

***O*-(6-*O*-*tert*-Butyldimethylsilyl-β-D-galactopyranosyl)-(1 → 4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (10) and *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranosyl)-(1 → 4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1, 5-dideoxy-1,5-imino-D-glucitol (11).** To a solution of **1** (0.5 g) in 2:1 dichloromethane-pyridine (30 mL) was added *tert*-butyldimethylsilyl chloride (0.3 g, ~3mol equiv of **9**) at 0 °C, and the mixture was stirred overnight at 20 °C. The mixture was diluted with dichloromethane, washed with ice-cold 2M HCl and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a column of silica gel with a) 200:1 and b) 150:1 CH_2Cl_2-MeOH. The title compound **10** (0.517 g, 90%) was obtained from eluent b): $[\alpha]_D$ -3.4°(*c* 0.8, MeOH).

A mixture of **10** (1.27 g), acetic anhydride (10 mL) and pyridine (20 mL) was stirred overnight at room temperature. Methanol (20 mL) was added and the mixture was concentrated to a syrup, which was taken-up in dichloromethane, washed with ice-cold 2M HCl and water, dried, and concentrated to give **11** (1.52 g, quant): $[\alpha]_D$ -12.2°(*c*

1.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.02 (2s, 6H, $SiMe_2$), 0.87 (s, 9H, *t*-Bu), 1.90, 1.99, 2.12 (3s, 9H, 3AcO), 3.42 (dd, 1H, J_{gem} = 13.56, $J_{1ax,2}$ = 3.66 Hz, H-1*ax* of DNJ), 3.84 (t, 1H, J = 4 Hz, H-4 of DNJ), 3.85 (dd, 1H, J = 13.6, 4.4 Hz, H-1*eq* of DNJ), 4.24 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 4 Hz, H-3 of DNJ), 4.52 (d, 1H, J = 8.1 Hz, H-1 of Gal), 4.97 (dd, 1H, $J_{2,3}$ = 10.3, $J_{3,4}$ = 3.3 Hz, H-3 of Gal), 5.18 (dd, 1H, $J_{1,2}$ = 8.1, $J_{2,3}$ = 10.3 Hz, H-2 of Gal), 5.45 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4 of Gal), and 7.2-7.4 (m, 20H, Ph-*H*).

Anal. Calcd for $C_{53}H_{67}NO_{14}Si$ (970.20): C, 65.74; H, 6.96; N, 1.44. Found: C, 65.58; H, 6.66; N, 1.36.

***O*-(2, 3, 4-Tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (12).** A mixture of **11** (1.49 g) and 80% acetic acid (25 mL) was stirred overnight at 40-45 °C, and concentrated in *vacuo*. The syrupy residue was chromatographed on a column of silica gel with 250:1 CH_2Cl_2-MeOH to give **12** (1.1 g, 80%): $[\alpha]_D$ +2.7°(*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.89, 1.98, 2.14 (3s, 9H, 3AcO), 3.37 (dd, 1H, J_{gem} = 14, $J_{1ax,2}$ = 3.3 Hz, H-1*ax* of DNJ), 3.79, 4.20 (2t, 2H, J = 4 Hz, H-3 and H-4 of DNJ), 3.91 (dd, 1H, J_{gem} = 14, $J_{1eq,2}$ = 4 Hz, H-1*eq* of DNJ), 4.91 (dd, 1H, $J_{2,3}$ = 10.3, $J_{3,4}$ = 3.3 Hz, H-3 of Gal), 5.08, 5.13 (2d, 2H, CH_2 of Z), 5.19 (dd, 1H, $J_{1,2}$ = 7.9 Hz, H-2 of Gal), 5.29 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4 of Gal), and 7.2-7.4 (m, 20H, Ph-*H*).

Anal. Calcd for $C_{47}H_{53}NO_{14}$ (855.93): C, 65.95; H, 6.24; N, 1.64. Found: C, 65.70; H, 6.20; N, 1.93.

***O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 6)-*O*-(2, 3, 4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2, 3, 6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (13α) and the β-isomer (13β).** A mixture of **12** (0.1 g), sialic acid donor **5** (0.1 g, 1.7 equiv to the acceptor **12**), powdered molecular sieves 3Å (0.3 g) and acetonitrile (5 mL) was stirred overnight at room temperature, and then cooled to -40 °C. To this mixture, *N*-iodosuccinimide (86 mg) and trifluoromethanesulfonic acid (4 μL) were added, and the reaction mixture was stirred for 5 h at -40 °C. The mixture was filtered through Celite and washed with dichloromethane. The filtrate and washings were combined, successively washed with aq sodium bicarbonate, aq sodium thiosulfate and water, dried, and concentrated. The residual syrup was chromatographed on a column of silica gel with a) 3:2 ethyl acetate-*n*-hexane and b) 50:1 toluene-methanol. Eluant b) gave the desired α-glycoside **13α** (0.18 g, 60%) and the β-isomer **13β** (90 mg, 30%), Compound **13α** had $[\alpha]_D$ -13°(*c* 1.0, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 1.86, 1.95, 1.96, 1.99, 2.03, 2.11, 2.12, 2.35 (8s, 24H, AcN, 7AcO), 2.48 (dd, 1H, J_{gem} = 13, $J_{3eq,4}$ = 4.4 Hz, H-3*eq* of Neu5Ac), 3.46 (dd,

1H, J_{gem} = 13, $J_{1ax,2}$ = 3.3 Hz, H-1*ax* of DNJ), 3.74 (s, 3H, CO_2Me), 4.20 (dd, 1H, J_{gem} = 13, $J_{1eq,2}$ = 2.6 Hz, H-1*eq* of DNJ), 4.63 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1 of Gal), 4.82 (m, 1H, H-4 of Neu5Ac), 4.99 (dd, 1H, $J_{2,3}$ = 10.3, $J_{3,4}$ = 3.3 Hz, H-3 of Gal), 5.16 (dd, 1H, H-2 of DNJ), 5.28 (dd, 1H, J = 9.3, 1.7 Hz, H-7 of Neu5Ac), 5.35 (m, 1H, H-8 of Neu5Ac), 5.39 (~d, 1H, $J_{3,4}$ = 3.3 Hz, H-4 of DNJ), and 7.18-7.33 (m, 20H, Ph-*H*).

Anal. Calcd for $C_{67}H_{80}N_2O_{26}$ (1329.37): C, 60.54; H, 6.07; N, 2.11. Found: C, 60.34; H, 5.79; N, 2.13.

^1H NMR ($CDCl_3$) data for compound **13β**: δ 2.44 (dd, 1H, J_{gem} = 13, $J_{3eq,4}$ = 4.8 Hz, H-3*eq* of Neu5Ac), 3.73 (s, 3H, CO_2Me), 5.01 (dd, 1H, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3 Hz, H-3 of DNJ), 5.19 (dd, 1H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.4 Hz, H-2 of DNJ), 5.05-5.25 (m, 4H, H-4 and H-8 of Neu5Ac, CH_2 of Z), 5.35 (dd, 1H, J = 4.9, 2.5 Hz, H-7 of Neu5Ac), 5.54 (d, 1H, J = 3 Hz, H-4 of DNJ), 5.78 (broad d, 1H, J = 10.6 Hz, NH), and 7.2-7.35 (m, 20H, Ph-*H*).

***O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→ 6)-*O*-(2, 3, 4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-1,5-dideoxy-1,5-imino-D-glucitol (14).** A mixture of **13α** (24 mg), palladium black (24 mg), formic acid (3 mL) and methanol (10 mL) was stirred for 5 days in a hydrogen atmosphere. The mixture was filtered through Celite and washed with methanol. The filtrate and washings were combined and concentrated to a syrup, which was chromatographed on a column of silica gel with a) 25:1, b) 10:1 CH_2Cl_2-MeOH. Eluant b) gave **14** (17 mg) quantitatively: $[\alpha]_D$ -4°(*c* 0.3, MeOH); ^1H NMR (CD_3OD) δ 1.84, 1.95, 1.98, 2.02, 2.09, 2.12, 2.15, 2.18 (8s, 24H, AcN, 7AcO), 2.36 (s, 3H, *N*-Me of DNJ), 2.57 (dd, 1H, J_{gem} = 13, $J_{3eq,4}$ = 4.5 Hz, H-3*eq* of Neu5Ac), 2.91 (dd, 1H, J_{gem} = 11-12, $J_{1eq,2}$ = 4-5 Hz, H-1*eq* of DNJ), and 3.82 (s, 3H, CO_2Me).

Anal. Calcd for $C_{39}H_{58}N_2O_{24}$ (938.88): C, 49.89; H, 6.23; N, 2.98. Found: C, 49.66; H, 6.04; N, 2.78.

***O*-(5-Acetamido-3, 5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2 → 6)-*O*-(β-D-galactopyranosyl)-(1 → 4)-1, 5-dideoxy-1, 5-imino-D-glucitol (15).** Compound **14** (38 mg) was treated with methanolic sodium methoxide and 0.1M aq potassium hydroxide as described for **11**. After work-up the product was purified by Sephadex LH-20 column (3:2 EtOH-H_2O) to afford **15** (quant): $[\alpha]_D$ -11°(*c* 0.9, 1:1 EtOH-H_2O); ^1H NMR (D_2O) δ 1.78 (t, 1H, J = 12 Hz, H-3*ax* of Neu5Ac), 2.07 (s, 3H, AcN), 2.53 (s, 3H, *N*-Me of DNJ), 2.4-2.55 (m, 2H, H-1*ax* and H-5 of DNJ), 2.75 (dd, 1H, J_{gem} = 12, $J_{3eq,4}$ = 4.3 Hz, H-3*eq* of Neu5Ac), 3.12 (dd, 1H, J_{gem} = 11.5, $J_{1eq,2}$ = 4.7 Hz, H-1*eq* of DNJ), 3.5-4.1 (m, 18H), and

4.55 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1 of Gal); ion-spray MS (positive ion mode) m/z (relative intensity) 653.3 $[M+Na]^+$ (50) and 631.8 $[M+H]^+$ (100); MS/MS (daughter ions derived from m/z = 631.6) m/z (relative intensity) 339.8 ($[M+H]^+$-Neu5Ac) (100), 291.7 (7.8), 273.9 (17), 177.9 ($[M+H]^+$-Neu5Ac-Gal) (27) and 159.9 (7.8).

Anal. Calcd for $C_{24}H_{42}N_2O_{17}$ (630.60): C, 45.71; H, 6.71, N, 4.44. Found: C, 45.56; H, 6.57; N, 4.27.

ACKNOWLEDGMENT

This work was supported in part by grants-in-aid for Scientific Research (C) and for Developmental Scientific Research from the Japanese Ministry of Education, Science and Culture (No. 05660386 and 05556019).

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