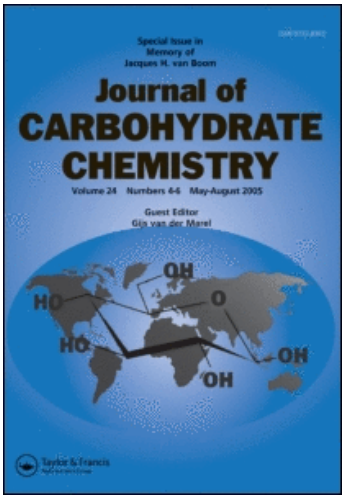

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Synthetic Studies on Sialoglycoconjugates. 5: A Facile, Regio and Stereoselective Synthesis of Ganglioside GM_4 and Its Position Isomer[1]

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SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 5: A FACILE, REGIO AND STEREOSELECTIVE SYNTHESIS OF GANGLIOSIDE GM_4 AND ITS POSITION ISOMER[1]

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ABSTRACT

Ganglioside GM_4 and its positional isomer have been synthesized. 2-(Trimethylsilyl)ethyl 6-O-benzoyl-ß-D-galactopyranoside (4) was prepared from 2-(trimethylsilyl)ethyl ß-D-galactopyranoside (1) by selective 3-O-benzylation, 6-O-benzoylation and subsequent removal of the benzyl group, or by 3,4-O-isopropylidenation, 6-O-benzoylation and O-deisopropylidenation. 2-(Trimethylsilyl)ethyl 3-O-benzoyl-ß-D-galactopyranoside (5) was obtained by selective benzoylation of 1. The glycosidation of 4 or 5 with the methyl α-thioglycoside derivative (9), derived from methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-S-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosonate (8) via selective S-deacetylation and subsequent S-methylation, using dimethyl(methylthio)sulfonium triflate (DMTST) gave the corresponding α-glycosides (10, 12) of Neu5Ac, respectively. Coupling of the trichloroacetimidates (15, 17), obtained from 10 and 12 by O-acetylation, selective removal of the 2-(trimethylsilyl)ethyl group, and imidate formation, with (2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (18) gave the corresponding ß-glycosides (19, 25), which were converted via selective reduction of the azide group, condensation with fatty acids, O-deacylation, and hydrolysis of the methyl ester group into the title compounds.

INTRODUCTION

Sialic acids[2,3] are well known as constituents of glycoproteins and glycolipids of cell membranes and play important roles in biological systems. Naturally occuring sialo-compounds contain sialic acids in α-glycosidic linkages except for CMP-N-acetylneuraminic acid. Recently, among the sialo-glycoconjugates, various types of biological functions[3-5] of

gangliosides have been revealed. In view of these facts, a facile, regio and α-selective glycoside synthesis of sialic acid is critically important for the synthesis of a variety of gangliosides and their various types of analogs, in order to investigate the structure-function relationship of sialoglycoconjugates. Recently, new efforts[6-8] have been made at obtaining α-glycosides, using sialic acid derivatives containing substituents at the C-3 position.

We have previously demonstrated[9,10] that the methyl α-thioglycoside of N-acetylneuraminic acid (Neu5Ac) is a useful glycosyl donor affording directly α-glycosides of Neu5Ac by use of dimethyl(methylthio)sulfonium triflate[9,11] (DMTST) in acetonitrile. We report here a facile, stereoselective synthesis of ganglioside GM_4[12] and its positional isomer.

RESULTS AND DISCUSSION

We have chosen 2-(trimethylsilyl)ethyl 6-O-benzoyl-ß-D-galactopyranoside (4) and 2-(trimethylsilyl)ethyl 3-O-benzoyl-ß-D-galactopyranoside (5) as the suitably protected glycosyl acceptors for the synthesis of ganglioside GM_4 and its positional isomer. Methyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid)onate[9] (9) was used as the glycosyl donor. Dibutyltin oxide mediated, selective etherification[13] of 2-(trimethylsilyl)ethyl ß-D-galactopyranoside[14,15] (1) to give the 3-O-benzyl derivative (2) could be achieved in 76.6% yield after column chromatography. Selective 6-O-benzoylation of 2 with benzoyl chloride in pyridine-dichloromethane at -50 °C gave 2-(trimethylsilyl)ethyl 6-O-benzoyl-3-O-benzyl-ß-D-galactopyranoside (3) in 71% yield. Removal of the benzyl group in compound 3 by hydrogen-transfer reduction with 10% Pd-C catalyst in methanol, in the presence of formic acid as the hydrogen donor, gave one of the desired glycosyl acceptors, 2-(trimethylsilyl)ethyl 6-O-benzoyl-ß-D-galactopyranoside (4) in good yield. Compound 4 was also prepared by another route. Treatment of 1 with 2,2-dimethoxypropane (1.5 equiv to 1) in N,N-dimethylformamide (DMF) containing p-toluenesulfonic acid for 3 h at 80 °C, afforded the 3,4-O-isopropylidene derivative (6) in 86% yield as crystals, which, on selective 6-O-benzoylation according to the methode described for 3, gave 2-(trimethylsilyl)ethyl 6-O-benzoyl-3,4-O-isopropylidene-ß-D-galactopyranoside (7) in good yield. Hydrolytic removal of the isopropylidene group in 7 under mild acidic conditions gave 4 in 84.6% yield. Alternatively, the other glycosyl acceptor, the 3-O-benzoyl derivative (5) of 1, required for the synthesis of the positional isomer of ganglioside GM_4, was prepared by direct,

selective benzoylation of 1 with benzoyl chloride (1.2 equiv) in pyridine-dichloromethane at -50 °C in 67% yield; significant signals in the ^{1}H NMR spectrum were a one-proton doublet of doublets at δ 5.04 ($J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.2 Hz, H-3) and a five-proton multiplet at δ 7.38-8.04 (Ph), indicating the structure assigned.

The glycosyl donor, methyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid)onate (9) was prepared in high yield via selective S-deacetylation of 8[16] with sodium metal (0.95 equiv) in methanol at -40 °C and subsequent S-methylation with methyl iodide in DMF.

The glycosidation of 4 with the methyl α-thioglycoside 9 (2.0 equiv to the acceptor) of Neu5Ac in acetonitrile at -15 °C for 15 h in the presence of DMTST (4.0 equiv to the glycosyl donor), afforded the desired α-glycoside of Neu5Ac, 2-(trimethylsilyl)ethyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-6-O-benzoyl-ß-D-galactopyranoside (10) in 43% yield; no ß-glycoside of Neu5Ac was isolated in this reaction. In the same way, reaction of 9 with the 3-O-benzoyl derivative (5) as the acceptor yielded the α-glycoside (12) at C-6 in compound 5 in 68% yield; again no ß-glycoside was isolated. Acetylation of the glycosides (10,12) thus obtained with acetic anhydride-pyridine gave the acetates (11,13) in quantitative yields, respectively. The structures of the glycosides (10-13) were unambiguously proved by 270 MHz ^{1}H NMR spectroscopy. The observed chemical shifts and coupling constants of Neu5Ac unit in the glycosides for H-3e (δ 2.50-2.72, $J_{3a,3e}$ = 12.6-12.8 Hz, $J_{3e,4}$ = 4.4-4.8 Hz), H-4 (δ 4.76-4.95), and H-7 (δ 5.33-5.39, $J_{7,8}$ = 8.3-9.2 Hz) are characteristic of α-glycosidic linkages[7-9,16,17] of Neu5Ac. Other ^{1}H NMR data are given in the Experimental Section and are consistent with the structures assigned.

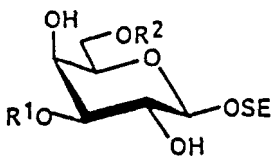
Treatment[15] of compound 11 with boron trifluoride etherate in dichloromethane for 7 h at 0 °C, gave O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2,4-di-O-acetyl-6-O-benzoyl-D-galactopyranose (14) in 84% yield after column chromatography. By essentially the same procedure described for 14, compound 13 afforded the O-(α-N-acetylneuraminyl)-(2→6)-D-galactopyranose derivative (16) in 92% yield. Treatment[12,18] of compound 14 or 16 with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 2 h at 0 °C, gave the corresponding trichloroacetimidates (15,17) in nearly quantitative yields, as a 1:8 mixture and 11:1 mixture of α and ß anomers, respectively. The glycosidation of (2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol[19] (18) with 15 thus obtained in the presence of boron

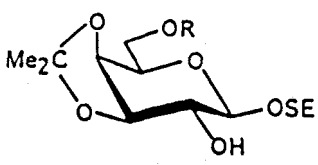
trifluoride etherate[20] for 4 h at 0 °C afforded only the expected ß-glycoside 19 in 82.3% yield. Significant signals in the ^{1}H NMR spectrum of 19 were a one-proton doublet at δ 4.71 ($J_{1,2}$ = 8.1 Hz, H-1, Gal unit) and a one-proton doublet of doublets at δ 5.01 ($J_{2,3}$ = 10.1 Hz, H-2, Gal unit), showing the newly formed ß-glycosidic linkage. Other ^{1}H NMR data are consistent with structure 19. In the same way, when coupled with the acceptor 18, compound 17 gave only the desired ß-glycoside, 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-ß-D-galactopyranosyl)-(1→1)-(2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (25) in high yield.

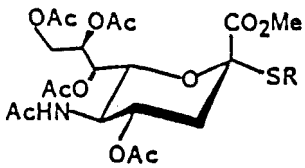
Selective reduction[21] of the azide group in compound 19 with hydrogen sulfide in 5:1 pyridine-water gave the amine 20, which, on condensation with tetradecanoic and octadecanoic acids using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) in dichloromethane, respectively gave the corresponding ganglioside GM_4 derivatives (21,22) in 80 and 81% yields. Finally, O-deacylation of compounds (21,22) with sodium methoxide in methanol, and subsequent saponification of the methyl ester group yielded the two kinds (23,24) of ganglioside GM_4 containing the different fatty acyl groups at the ceramide moiety, in almost quantitative yields. Similarly, selective reduction of the azide group in 25 and subsequent condensation with octadecanoic acid, gave 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-ß-D-galactopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (27) in 75% yield, which was converted, by removal of the protecting groups as described for 23, into a positional isomer of ganglioside GM_4.

In conclusion, regio- and α-stereo-selective glycosidation of Neu5Ac was achieved by using the methyl α-thioglycoside 9 of Neu5Ac as the glycosyl donor and the suitably protected acceptors (4,5) with DMTST in acetonitrile under kinetically controlled conditions. The glycosides (11,12) obtained here were easily converted to ganglioside GM_4 and its positional isomer, indicating that this procedure might be useful for the synthesis of sialoglycoconjugates. It was also shown that the 2-(trimethylsilyl)ethyl group employed here was an efficient protecting group for the anomeric hydroxy group because of the easy and selective deprotection with boron trifluoride etherate, and of the sufficient stability towards many of the reagents used in this paper.

The α-glycosides of Neu5Ac described here could be used as suitable intermediates for other gangliosides synthesis, and may also be important as building units for glycoconjugates syntheses.





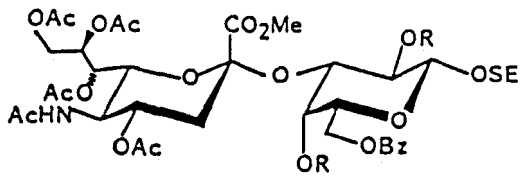


1 $R^1 = R^2 = H$
2 $R^1 = Bn$, $R^2 = H$
3 $R^1 = Bn$, $R^2 = Bz$
4 $R^1 = H$, $R^2 = Bz$
5 $R^1 = Bz$, $R^2 = H$

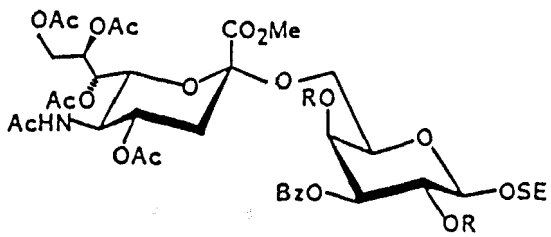
SE = 2-(trimethylsilyl)ethyl
Bn = benzyl
Bz = benzoyl

6 R = H
7 R = Bz

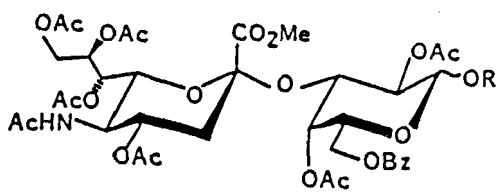
8 R = Ac
9 R = Me



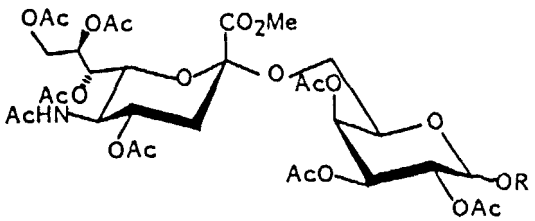
10 R = H
11 R = Ac



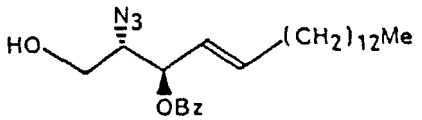
12 R = H
13 R = Ac



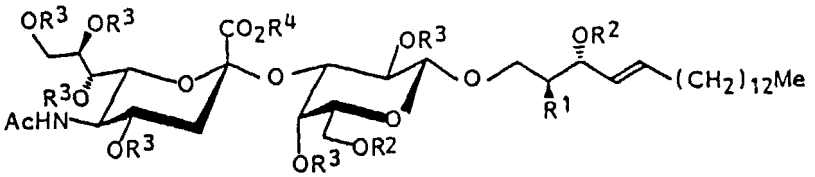
14 R = H
15 R = C(=NH)CCl$_3$



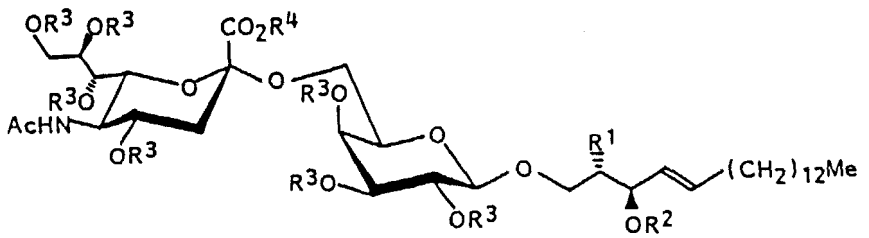
16 R = H
17 R = C(=NH)CCl$_3$



18



19 R^1 = N_3 , R^2 = Bz , R^3 = Ac , R^4 = Me
20 R^1 = NH_2 , R^2 = Bz , R^3 = Ac , R^4 = Me
21,22 R^1 = $NHCO(CH_2)nMe$, R^2 = Bz , R^3 = Ac , R^4 = Me , n = 12,16
23,24 R^1 = $NHCO(CH_2)nMe$, R^2 = R^3 = R^4 = H , n = 12,16



25 R^1 = N_3 , R^2 = Bz , R^3 = Ac , R^4 = Me
26 R^1 = NH_2 , R^2 = Bz , R^3 = Ac , R^4 = Me
27 R^1 = $NHCO(CH_2)_{16}Me$, R^2 = Bz , R^3 = Ac , R^4 = Me
28 R^1 = $NHCO(CH_2)_{16}Me$, R^2 = R^3 = R^4 = H

EXPERIMENTAL

General Procedures. Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union MP-201 polarimeter at 25 °C, and IR spectra were recorded with a Jasco IRA-1 spectrophotometer. ^{1}H-NMR spectra were recorded with a Jeol JNM-GX270 (270 MHz) spectrometer, and the NMR data were confirmed by use of decoupling techniques. Preparative chromatography was performed on silica gel (Waco Co., 200 mesh) with the solvent systems specified. Concentrations and evaporations were conducted in vacuo.

2-(Trimethylsilyl)ethyl 3-O-Benzyl-ß-D-galactopyranoside (2). To a stirred suspension of 2-(trimethylsilyl)ethyl ß-D-galactopyranoside[14] (1, 1.9 g, 6.8 mmol) in dry benzene (50 mL) was added dibutyltin oxide (2.54 g) and the mixture was heated, with stirring, for 5 h at 80 °C. Tetrabutylammonium bromide (1.1 g) and benzyl bromide (10 mL) were added to the mixture, and this was heated, with stirring, for 3 h at 60 °C; the course of the reaction being monitored by TLC. The mixture was concentrated to a syrup and chromatographed over a column of silica gel (100 g) with dichloromethane and then 125:1 dichloromethane-methanol. The latter eluant gave 2 (1.93 g, 76.6%) as a syrup: $[\alpha]_D$ +5.6° (c 0.5, chloroform); IR (film) 3700-3100 (OH), 860 and 840 (TMS), and 750-700 cm^{-1} (Ph); ^{1}H NMR (1:1 CD_3OD-$CDCl_3$) δ 3.39 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 3.60, 4.01 (2m, 2H, $Me_3SiCH_2\underline{CH_2}O$), 3.70 (dd, 1H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.80 (m, 2H, H-6,6'), 4.02 (m, 1H, H-4), 4.24 (d, 1H, H-1), and 7.26-7.38 (m, 5H, Ph).

Anal. Calcd for $C_{18}H_{30}O_6Si$: C, 58.35; H, 8.16. Found: C, 58.39; H, 8.34.

2-(Trimethylsilyl)ethyl 6-O-Benzoyl-3-O-benzyl-ß-D-galactopyranoside (3). To a solution of 2 (3.5 g, 9.1 mmol) in pyridine (10 mL) and dry dichloromethane (40 mL) was added, with stirring, a solution of benzoyl chloride (1.82 g, 12.9 mmol) in dry dichloromethane (20 mL) over 1 h at -50 °C. After completion of the reaction, methanol (1 mL) was added, and the mixture was stirred for 30 min at -20 °C, concentrated, and extracted with dichloromethane. The extract was washed with water, dried (sodium sulfate), and evaporated to a syrup, which was chromatographed on a column of silica gel (150 g) using dichloromethane and then 150:1 dichloromethane-methanol as the eluents. The latter eluant gave compound 3 (3.2 g, 71%) as a syrup: $[\alpha]_D$ -1.55° (c 0.9, chloroform); IR (film) 3700-3200 (OH), 1720 and 1250 (ester), 860 and 840 (TMS), and 710 cm^{-1} (Ph); ^{1}H NMR (1:1 CD_3OD-$CDCl_3$) δ 1.03 (m, 2H, $Me_3Si\underline{CH_2}CH_2O$), 3.47 (dd, 1H, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ =

3.3 Hz, H-3), 3.62 (m, 1H, $Me_3SiCH_2\underline{CH_2}O$), 3.78 (m, 2H, H-2,5), 4.00 (m, 2H, H-4, $Me_3SiCH_2\underline{CH_2}O$), 4.29 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.59 (m, 2H, H-6,6'), 4.77 (s, 2H, benzyl methylene), and 7.29-8.08 (m, 10H, 2Ph).

Anal. Calcd for $C_{25}H_{34}O_7Si$: C, 63.27; H, 7.22. Found: C, 63.32; H, 7.41.

2-(Trimethylsilyl)ethyl 3,4-O-Isopropylidene-ß-D-galactopyranoside (6). To a solution of 1 (2.0 g, 7.13 mmol) in N,N-dimethylformamide (25 mL) were added 2,2-dimethoxypropane (1.3 mL, 10.70 mmol) and p-toluenesulfonic acid monohydrate (30 mg); the pH of the solution reached to about 3. The mixture was heated, with stirring, at 80 °C while the progress of reaction was monitored by TLC; after 3 h, the starting material was no longer detectable, and the acid present was removed by stirring for 15 min with Amberlite IR-410 (OH^-) resin. After removal of the resin, the solution was concentrated to a syrup which was chromatographed on a column of silica gel (150 g) with 200:1 dichloromethane-methanol, to give compound 6 (1.96 g, 86%). Recrystallization from ethyl acetate-hexane gave needles: mp 88-89.5 °C; $[\alpha]_D$ +9.6° (c 1.0, chloroform); IR (KBr) 3450 (OH), 860 and 840 (TMS), and 850 cm^{-1} (Me_2C).

Anal. Calcd for $C_{14}H_{28}O_6Si$: C, 53.14; H, 7.64. Found: C, 53.29; H, 7.63.

A sample (20 mg) of 6 was benzoylated with benzoyl chloride in pyridine to give the di-O-benzoyl derivative. The ^1H NMR data is given here and is consistent with the 2,6-di-O-benzoyl derivative of 6: ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3Si\underline{CH_2}CH_2O$), 1.47, 1.75 (2s, 6H, Me_2C), 3.66, 4.07 (2m, 2H, $Me_3SiCH_2\underline{CH_2}O$), 4.31 (m, 1H, H-5), 4.41 (dd, 1H, $J_{3,4}$ = 5.5 Hz, $J_{4,5}$ = 2.2 Hz, H-4), 4.88 (dd, 1H, $J_{2,3}$ = 7.2 Hz, H-3), 4.64 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.77 (m, 2H, H-6,6'), 5.36 (dd, 1H, H-2), and 7.55-8.16 (m, 10H, 2Ph).

2-(Trimethylsilyl)ethyl 6-O-Benzoyl-3,4-O-isopropylidene-ß-D-galactopyranoside (7). To a stirred solution of 6 (3.0 g, 9.5 mmol) in dry pyridine (50 mL), cooled to -30 °C, was added benzoyl chloride (1.73 g, 12.35 mmol). The mixture was stirred for 4 h at -30 °C, and methanol (1.0 mL) was added. After 30 min, the mixture was concentrated to a syrup, which was extracted with dichloromethane. The extract was successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried (sodium sulfate) and concentrated to a syrup which was purified by chromatography on a column of silica gel (150 g) with 4:1 hexane-ethyl acetate to give compound 7 (2.97 g, 74.7%). Recrystallization from ether-hexane gave needles: mp 93-94 °C, $[\alpha]_D$ +16.8° (c 1.0, chloroform); IR (KBr) 3450 (OH), 1720 and 1250 (ester), 860 and 840 (TMS), 850 (Me_2C), and 710 cm^{-1} (Ph);

^{1}H NMR data ($CDCl_3$) δ 1.02 (m, 2H, $Me_3Si\underline{CH_2}CH_2O$), 1.37, 1.55 (2s, 6H, Me_2C), 3.60 (m, 2H, H-2, $Me_3SiCH_2\underline{CH_2}O$), 3.99 (m, 1H, $Me_3SiCH_2\underline{CH_2}O$), 4.22 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.15 (m, 2H, H-3,5), 4.24 (dd, 1H, $J_{3,4}$ = 5.5 Hz, $J_{4,5}$ = 2.3 Hz, H-4), 4.60 (dd, 1H, J_{gem} = 11.5 Hz, $J_{5,6}$ = 5.0 Hz, H-6), 4.68 (dd, 1H, $J_{5,6'}$ = 7.5 Hz, H-6'), and 7.45 -8.05 (m, 5H, Ph).

Anal. Calcd for $C_{21}H_{28}O_7Si$: C, 59.98; H, 6.71. Found: C, 59.99; H, 6.68.

2-(Trimethylsilyl)ethyl 6-O-Benzoyl-ß-D-galactopyranoside (4). (a) From 3. To a solution of 3 (3.5 g, 7.37 mmol) in methanol (150 mL) were added 10% Pd-C catalyst (2.5 g) and formic acid (2.5 mL). The mixture was heated, with stirring, for 30 min at 60 °C. After completion of the reaction, the catalyst was filtered off, and washed with methanol. The filtrate and washings were combined, and concentrated to a syrup, which was chromatographed on a column of silica gel (150 g) with 40:1 dichloromethane-methanol to give 4 (1.9 g, 67%) as crystals: mp 99-100 °C, $[\alpha]_D$ -6.7° (c 0.92, chloroform); IR (KBr) 3700-3200 (OH), 1720 and 1250 (ester), 860 and 840 (TMS), and 710 cm^{-1} (Ph); ^{1}H NMR (1:1 CD_3OD-$CDCl_3$) δ 1.03 (m, 2H, $Me_3Si\underline{CH_2}CH_2O$), 3.84 (near t, $J_{5,6}$ = 6.6 Hz, H-5), 4.29 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 4.59 (m, 2H, H-6,6'), and 7.43-8.07 (m, 5H, Ph).

Anal. Calcd for $C_{18}H_{28}O_7Si$: C, 56.23; H, 7.34. Found: C, 56.08; H, 7.49.

(b) From 7. To a solution of trifluoroacetic acid (20 mL) and methanol (2 mL), cooled to -3 °C, was gradually added compound 7 (5.4 g, 12.8 mmol); after 15 min, the starting material was no longer detectable by TLC. The mixture was concentrated and toluene was added to the residue, and then concentrated to give crystalline 4. Recrystallization from ether-hexane gave needles (4.14 g, 84.6%): mp 100 °C, $[\alpha]_D$ -8.0° (c 1.0, chloroform); its IR and ^{1}H NMR spectra were identical with those of 4 obtained from 3.

2-(Trimethylsilyl)ethyl 3-O-Benzoyl-ß-D-galactopyranoside (5). To a stirred solution of 1 (1.0 g, 3.6 mmol) in dry pyridine (10 mL), cooled to -50 °C, was added a solution of benzoyl chloride (603 mg, 4.3 mmol) in dry dichloromethane (5 mL) over 10 min. The mixture was stirred for 3 h at -50 °C, and methanol (1 mL) was added. After 30 min, the mixture was concentrated to a syrup, which was extracted with dichloromethane. The extract was washed with water, dried (sodium sulfate), and concentrated. The residue was chromatographed on a column of silica gel (30 g) with 40:1 dichloromethane-methanol to give 5 (920 mg, 67%) as a syrup: $[\alpha]_D$ +30.5° (c 1.2, chloroform); ^{1}H NMR (1:1 CD_3OD-$CDCl_3$) δ 1.00 (m, 2H, $Me_3Si\underline{CH_2}CH_2O$), 3.61, 4.02 (m, 2H, $Me_3SiCH_2\underline{CH_2}O$), 4.01 (dd, 1H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 10.3

Hz, H-2), 4.25 (broad d, 1H, $J_{3,4}$ = 3.2 Hz, H-4), 4.39 (d, 1H, H-1), 5.04 (dd, 1H, H-3), and 7.38-8.08 (m, 5H, Ph).

Anal. Calcd for $C_{18}H_{28}O_7Si$: C, 56.23; H, 7.34. Found: C, 56.05; H; 7.41.

Methyl (Methyl 3-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid)onate (9). To a stirred solution of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-S-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosonate[16] (8, 549 mg, 1.0 mmol) in dry methanol (15 mL), cooled to -40 °C, was added a solution of sodium metal (22 mg, 0.95 mmol) in dry methanol (5 mL). The mixture was stirred for 5 min at -40 °C, and concentrated at 0 °C to an amorphous mass, which was dissolved in dry N,N-dimethylformamide (5 mL). To the stirred solution was added methyl iodide (420 mg, 3.0 mmol), and the mixture was stirred for 6 h at room temperature. Concentration of the mixture gave a syrup which was chromatographed on a column of silica gel (50 g) using (a) dichloromethane, (b) 200:1, (c) 100:1 dichloromethane-methanol. Eluant (c) gave compound 9 (460 mg, 88%) as crystals: mp 80-82 °C, $[\alpha]_D$ +17.8° (c 0.5, chloroform): IR (KBr) 3280 (NH), 1740 and 1230 (ester), and 1660 and 1540 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ 1.88 (s, 3H, AcN), 2.03, 2.04, 2.11, 2.14, 2.17 (5s, 15H, 4AcO, MeS), 2.73 (dd, 1H, $J_{3a,3e}$ = 12.6 Hz, $J_{3e,4}$ = 4.8 Hz, H-3e), 3.81 (s, 3H, MeO), 3.84 (dd, 1H, $J_{5,6}$ = 10.2 Hz, $J_{6,7}$ = 1.8 Hz, H-6), 4.08 (q, 1H, $J_{4,5} = J_{5,6} = J_{5,NH}$ = 10.2 Hz, H-5), 4.11 (dd, 1H, $J_{8,9}$ = 5.1 Hz, $J_{9,9'}$ = 11.7 Hz, H-9), 4.33 (dd, 1H, $J_{8,9'}$ = 2.4 Hz, H-9'), 4.88 (ddd, 1H, H-4), 5.18 (d, 1H, NH), 5.33 (dd, 1H, $J_{7,8}$ = 8.4 Hz, H-7), and 5.38 (m, 1H, H-8).

Anal. Calcd for $C_{21}H_{31}NO_{12}S$: C, 48.36; H, 5.99; N, 2.69. Found: C, 48.33; H, 5.84; N, 2.63.

2-(Trimethylsilyl)ethyl O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-6-O-benzoyl-ß-D-galactopyranoside (10). To a solution of 4 (160 mg, 0.416 mmol) and 9 (430 mg, 0.824 mmol) in dry acetonitrile (2.5 mL) was added Molecular Sieves 3A (MS-3A; 400 mg), and the mixture was stirred for 5 h at room temperature, and cooled to -40 °C. A mixture of dimethyl(methylthio)sulfonium triflate[11b] and MS-3A (1.7g; 50% of DMTST by weight) was added to the reaction mixture at -40 °C and vigorously stirred for 15 h at -15 °C; the course of the reaction being monitored by TLC. The precipitates were filtered off, and washed with dichloromethane. The filtrate and washings were combined, and the solution was successively washed with M sodium carbonate and water, dried (sodium sulfate), and then concentrated to leave a syrup, which was chromatographed on a column of silica gel (50 g) with 1:1

ethyl acetate-hexane to give compound 10 (154 mg, 43%) as an amorphous mass: $[\alpha]_D$ -6.0° (c 2.0, chloroform); IR (KBr) 3700-3150 (OH, NH), 1750 and 1230 (ester), 1670 and 1550 (amide), 860 and 840 (TMS), and 720 cm^{-1} (Ph); ^1H NMR (1:1 CD_3OD-$CDCl_3$) Gal unit δ 4.46 (d, 1H, $J_{1,2}$ = 4.9 Hz, H-1), 4.54 (dd, 1H, $J_{5,6}$ = 6.1 Hz, $J_{6,6'}$ = 11.4 Hz, H-6), 4.62 (dd, 1H, $J_{5,6'}$ = 5.3 Hz, H-6'), and 7.40-8.13 (m, 5H, Ph); Neu5Ac unit δ 1.90 (s, 3H, AcN), 2.05 (2), 2.12 (2) (4s, 12H, 4AcO), 2.72 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.8 Hz, H-3e), 3.78 (s, 3H, MeO), 4.27 (dd, 1H, $J_{8,9'}$ = 2.2 Hz, $J_{9,9'}$ = 12.5 Hz, H-9'), 4.95 (ddd, 1H, $J_{3a,4}$ = 11.4 Hz, $J_{4,5}$ = 9.7 Hz, H-4), 5.32 (d, 1H, $J_{NH,5}$ = 9.3 Hz, NH), 5.33 (dd, 1H, $J_{6,7}$ = 3.65 Hz, $J_{7,8}$ = 8.3 Hz, H-7), and 5.45 (ddd, 1H, $J_{8,9}$ = 4.8 Hz, $J_{8,9'}$ = 2.2 Hz, H-8).

Anal. Calcd for $C_{38}H_{55}NO_{19}Si$: C, 53.20; H, 6.46; N, 1.63. Found: C, 53.29; H, 6.45; N, 1.58.

2-(Trimethylsilyl)ethyl O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2,4-di-O-acetyl-6-O-benzoyl-ß-D-galactopyranoside (11). Compound 10 (100 mg, 0.117 mmol) was acetylated at room temperature with acetic anhydride (2 mL)-pyridine (4 mL), and the product was purified by chromatography on a column of silica gel (20 g) with 80:1 dichloromethane-methanol as the eluent. The acetate 11 was obtained as an amorphous mass (106 mg, quantitative): $[\alpha]_D$ -24.5° (c 2.6, chloroform); ^1H NMR (1:1 CD_3OD-$CDCl_3$) Gal unit δ 0.98 (m, 2H, $Me_3SiCH_2CH_2O$), 4.43 (dd, 1H, $J_{5,6}$ = 7.0 Hz, $J_{6,6'}$ = 12.1 Hz, H-6), 4.59 (dd, 1H, $J_{5,6'}$ = 3.5 Hz, H-6'), 4.63 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 5.05 (dd, 1H, $J_{2,3}$ = 10.3 Hz, H-2), 5.06 (broad s, 1H, H-4), and 7.43-8.04 (m, 5H, Ph); Neu5Ac unit δ 1.85 (s, 3H, AcN), 2.59 (dd, 1H, $J_{3a,3e}$ = 12.6 Hz, $J_{3e,4}$ = 4.4 Hz, H-3e), 3.85 (s, 3H, MeO), 4.22 (dd, 1H, $J_{8,9}$ = 7.0 Hz, $J_{9,9'}$ = 11.4 Hz, H-9), 4.35 (dd, 1H, $J_{8,9'}$ = 2.6 Hz, H-9'), 4.88 (ddd, 1H, $J_{3a,4}$ = 12.4 Hz, $J_{4,5}$ = 11.1 Hz, H-4), 5.19 (d, 1H, $J_{NH,5}$ = 10.4 Hz, NH), 5.37 (dd, 1H, $J_{6,7}$ = 2.6 Hz, $J_{7,8}$ = 9.2 Hz, H-7), 5.56 (ddd, 1H, H-8); O-acyl groups δ 2.00, 2.04, 2.08, 2.11, 2.13, and 2.21 (6s, 18H, 6AcO).

Anal. Calcd for $C_{42}H_{59}NO_{21}Si$: C, 53.55; H, 6.31; N, 1.49. Found: C, 53.44; H, 6.51; N, 1.60.

2-(Trimethylsilyl)ethyl O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-3-O-benzoyl-ß-D-galactopyranoside (12). To a solution of 5 (160 mg, 0.416 mmol) and 9 (430 mg, 0.824 mmol) in dry acetonitrile (2.5 mL), was added MS-3A (400 mg), and the mixture was stirred for 5 h at room temperature, and then cooled to -40 °C. A mixture (1.7 g; 50% of DMTST by weight) of DMTST and MS-3A was added to the reaction mixture at -40 °C, and this was stirred for 15 h at -15 °C. The precipitates were filtered off, and washed with dichlo-

romethane. The filtrate and washings were combined, and the solution was successively washed with M sodium carbonate and water, dried (sodium sulfate), and concentrated to a syrup, which was chromatographed on a column of silica gel (60 g) with 1:1 ethyl acetate-hexane, to give 12 (243 mg, 68%) as an amorphous mass: $[\alpha]_D$ −6.4° (c 0.4, chloroform); ^{1}H NMR (1:1 CD_3OD-$CDCl_3$) Gal unit δ 1.04 (m, 2H, $Me_3SiCH_2CH_2O$), 4.15 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 4.38 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.99 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.3 Hz, H-3), and 7.39-8.10 (m, 5H, Ph); Neu5Ac unit δ 1.82 (s, 3H, AcN), 1.93, 1.98, 2.08, 2.10 (4s, 12H, 4AcO), 2.56 (dd, 1H, $J_{3a,3e}$ = 12.7 Hz, $J_{3e,4}$ = 4.7 Hz, H-3e), 4.36 (dd, 1H, $J_{8,9}$ = 2.6 Hz, $J_{9,9'}$ = 12.1 Hz, H-9), 4.76 (ddd, 1H, H-4), and 5.27-5.33 (m, 2H, H-7,8).

Anal. Calcd for $C_{38}H_{55}NO_{19}Si$: C, 53.20; H, 6.46; N, 1.63. Found: C, 53.32; H, 6.49; N, 1.58.

2-(Trimethylsilyl)ethyl O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2,4-di-O-acetyl-3-O-benzoyl-ß-D-galactopyranoside (13). Compound 12 (200 mg, 0.23 mmol) was acetylated with acetic anhydride (3 mL)-pyridine (5 mL) overnight at room temperature; the product was purified by silica gel column chromatography using 80:1 dichloromethane-methanol as the eluent, to give 13 (quantitative) as an amorphous mass: $[\alpha]_D$ -7.2° (c 1.7, chloroform); ^{1}H NMR ($CDCl_3$) Gal unit δ 1.04 (m, 2H, $Me_3SiCH_2CH_2O$), 3.41 (dd, 1H, $J_{5,6'}$ = 7.8 Hz, $J_{6,6'}$ = 12.3 Hz, H-6'), 3.81 (dd, 1H, $J_{5,6}$ = 5.9 Hz, H-6), 4.62 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.22 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 5.35 (dd, 1H, H-2), 5.62 (broad d, 1H, H-4), and 7.36-7.92 (m, 5H, Ph); Neu5Ac unit δ 1.86 (s, 3H, AcN), 2.50 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.75 (s, 3H, MeO), 4.33 (dd, 1H, $J_{8,9}$ = 2.7 Hz, $J_{9,9'}$ = 12.6 Hz, H-9), 4.83 (ddd, 1H, H-4), and 5.20-5.39 (m, 3H, H-7,8, NH); O-acetyl groups δ 1.96, 1.99, 2.01, 2.06, 2.10, and 2.17 (6s, 18H, 6AcO).

Anal. Calcd for $C_{42}H_{59}NO_{21}Si$: C, 53.55; H, 6.31; N, 1.49. Found: C, 53.49; H, 6.31; N, 1.45.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2,4-di-O-acetyl-6-O-benzoyl-D-galactopyranose (14). To a stirred solution of 11 (860 mg, 1.0 mmol) in dichloromethane (15 mL), cooled to -5 °C, was added boron trifluoride etherate (0.55 mL). The mixture was stirred at 0 °C while the reaction was monitored by TLC; after 7 h, the reaction was complete, and dichloromethane (30 mL) was added to the mixture. The solution was successively washed with M sodium hydrogen carbonate and water, dried (sodium sulfate), and concentrated. The residue was purified by chromatography on a column of

silica gel (70 g) with 40:1 dichloromethane-methanol to give compound 14 (646 mg, 84.1%) as an amorphous mass: $[\alpha]_D$ -16.6° (c 0.78, chloroform); IR (KBr) 3500-3150 (OH, NH), 1740 and 1220 (ester), 1660 and 1540 (amide), and 710 cm^{-1} (Ph).

Anal. Calcd for $C_{37}H_{47}NO_{21}$: C, 52.79; H, 5.63; N, 1.66. Found: C, 52.83; H, 5.66; N, 1.60.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2,4-di-O-acetyl-6-O-benzoyl-D-galactopyranosyl trichloroacetimidate (15). To a stirred solution of 14 (645 mg, 0.77 mmol) in dichloromethane (6 mL), cooled to -5 °C, were added trichloroacetonitrile (2.4 mL) and DBU (66 mg). The mixture was stirred at 0 °C; after 2 h, the starting material was no longer detectable by TLC. The mixture was concentrated to a syrup which was chromatographed on a column of silica gel (50 g) with 70:1 dichloromethane-methanol to give 15 (705 mg, 93.3%) as an amorphous mass. The anomeric ratio (α:ß) was estimated at 1:8 from integration ratio of H-1α and H-1ß: $[\alpha]_D$ -6.2° (c 0.9, chloroform); ^1H NMR ($CDCl_3$) Gal unit δ 4.80 (dd, 1H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 5.14 (dd, 1H, $J_{4,5}$ = 3.1 Hz, H-4), 5.34 (dd, 1H, $J_{1,2}$ = 8.2 Hz, H-2), 5.99 (d, 1H, 8.2 Hz, H-1ß), 7.40-8.04 (m, 5H, Ph), and 8.69 (s, 1H, C=NH); Neu5Ac unit δ 1.85 (s, 3H, AcN), 2.61 (dd, 1H, $J_{3a,3e}$ = 12.7 Hz, $J_{3e,4}$ = 4.8 Hz, H-3e), 3.65 (dd, 1H, $J_{5,6}$ = 10.7 Hz, $J_{6,7}$ = 2.7 Hz, H-6), 3.78 (s, 3H, MeO), 3.98 (dd, 1H, $J_{8,9'}$ = 6.1 Hz, $J_{9,9'}$ = 12.5 Hz, H-9'), 4.06 (q, 1H, $J_{4,5} = J_{5,6} = J_{5,NH}$ = 10.4 Hz, H-5), 4.41 (dd, 1H, $J_{8,9}$ = 2.6 Hz, H-9), 4.90 (ddd, 1H, H-4), 5.19 (d, 1H, NH), and 5.56 (ddd, 1H, $J_{7,8}$ = 8.2 Hz, H-8); O-acetyl group δ 2.01, 2.05, 2.07, 2.14, 2.16, and 2.19 (6s, 18H, 6AcO).

Anal. Calcd for $C_{39}H_{47}N_2O_{21}Cl_3$: C, 47.50; H, 4.80; N, 2.89. Found: C, 47.49; H, 4.92; N, 2.76.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→6)-2,3,4-tri-O-acetyl-D-galactopyranose (16). To an ice-cooled solution of 13 (600 mg, 0.64 mmol) in methanol (50 mL) was added sodium methoxide (50 mg), and the mixture was kept for 1 h at 0 °C, and then treated with Amberlite IR-120 (H^+) resin to remove the base. The solution was evaporated to a syrup which was acetylated with acetic anhydride (2 mL)-pyridine (4 mL) overnight at room temperature. The product was dissolved in dichloromethane (10 mL). To the stirred solution was added boron trifluoride etherate (340 mg) at 0 °C, and the mixture was stirred for 3 h at 0 °C; the course of the reaction being monitored by TLC. Dichloromethane (50 mL) was added to the mixture, and the solution was successively washed with M sodium carbonate and water, dried

(sodium sulfate), and concentrated. The residue was chromatographed on a column of silica gel (70 g) with 60:1 dichloromethane-methanol to give 16 (457 mg, 92%) as an amorphous mass: $[\alpha]_D$ +0.25° (c 0.79, chloroform); ^{1}H NMR ($CDCl_3$) Gal unit δ 3.41 (dd, 1H, $J_{5,6'}$ = 9.0 Hz, $J_{6,6'}$ = 10.6 Hz, H-6'), 3.69 (dd, 1H, $J_{5,6}$ = 5.5 Hz, H-6), 5.47 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.3 Hz, H-3), and 5.54 (broad d, 1H, $J_{4,5}$ 2.4 Hz, H-4); Neu5Ac unit δ 1.85 (s, 3H, AcN), 2.54 (dd, 1H, $J_{3a,3e}$ = 13.1 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.79 (s, 3H, MeO), 4.02 (dd, 1H, $J_{8,9'}$ = 7.7 Hz, $J_{9,9'}$ = 12.3 Hz, H-9'), 4.37 (dd, 1H, $J_{8,9}$ = 2.6 Hz, H-9), 4.88 (ddd, 1H, H-4), and 5.38 (ddd, 1H, $J_{7,8}$ = 7.5 Hz, $J_{8,9}$ = 2.6 Hz, H-8).

Anal. Calcd for $C_{32}H_{45}NO_{21}$: C, 49.30; H, 5.82; N, 1.80. Found: C, 49.21; H, 5.94; N, 1.78.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-O-acetyl-D-galactopyranosyl trichloroacetimidate (17). To a stirred solution of 16 (315 mg, 0.4 mmol) in dichloromethane (3 mL), cooled to -5 °C, were added DBU (32 mg) and trichloroacetonitrile (0.83 mL), and the mixture was stirred for 2 h at 0 °C, and then concentrated to leave a syrup. The product was purified on a column of silica gel (60 g) with 50:1 dichloromethane-methanol to give 17 (344 mg, 92.2%) as an amorphous mass; anomeric ratio (α:ß) was about 11:1: $[\alpha]_D$ +38.4° (c 0.5, chloroform); ^{1}H NMR ($CDCl_3$) Gal unit δ 3.33 (dd, 1H, $J_{5,6'}$ = 6.5 Hz, $J_{6,6'}$ = 10.1 Hz, H-6'), 3.91 (dd, 1H, $J_{5,6}$ = 6.1 Hz, H-6), 4.34 (m, 1H, H-5), 4.86 (broad d, 1H, H-4), 6.61 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1α), and 8.66 (s, 1H, C=NH)); Neu5Ac unit δ 1.87 (s, 3H, AcN), 2.52 (dd, 1H, $J_{3a,3e}$ = 12.9 Hz, $J_{3e,4}$ = 4.7 Hz, H-3e), 3.78 (s, 3H, MeO), 4.09 (dd, 1H, $J_{8,9'}$ = 4.7 Hz, $J_{9,9'}$ = 12.5 Hz, H-9'), 4.25 (dd, 1H, $J_{8,9}$ = 2.4 Hz, H-9), and 4.86 (m, 1H, H-4); O-acetyl groups δ 2.01, 2.02 (2), 2.04, 2.10, 2.15, and 2.18 (7s, 21H, 7AcO).

Anal. Calcd for $C_{34}H_{45}N_2O_{21}Cl_3$: C, 44.19; H, 4.91; N, 3.03. Found: C, 44.25; H, 5.13; N, 3.09.

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-ß-D-galactopyranosyl)-(1→1)-(2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (19). To a solution of 15 (690 mg, 0.7 mmol) and (2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol[19] (600 mg, 1.4 mmol) was added MS-4A (7 g), and the mixture was stirred for 30 min at room temperature, and cooled to 0 °C. Boron trifluoride etherate (200 mg) was added to the cooled mixture, and this was stirred for 4 h at 0 °C. The precipitates were filtered off and washed with dichloromethane. The filtrate and washings were combined, and the solution was successively washed with M sodium hydrogen car-

bonate and water, dried (sodium sulfate), and concentrated. The residue was chromatographed on a column of silica gel (80 g) with 3:2 ethyl acetate-hexane to give compound 19 (722 mg, 82.3%) as an amorphous mass: $[\alpha]_D$ -26.5° (c 0.6, chloroform); IR (KBr) 3300 (NH), 2100 (N_3), 1750 and 1220 (ester), 1660 and 1540 (amide), and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) Gal unit δ 4.62 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 4.71 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 5.06 (broad d, 1H, $J_{4,5}$ = 3.7 Hz, H-4), and 5.10 (dd, 1H, H-2); Neu5Ac unit δ 1.72 (t, 1H, $J_{3a,3e} = J_{3a,4}$ = 12.5 Hz, H-3a), 1.86 (s, 3H, AcN), 2.60 (dd, 1H, $J_{3e,4}$ = 4.7 Hz, H-3e), 3.64 (dd, 1H, $J_{5,6}$ = 10.7 Hz, $J_{6,7}$ = 2.6 Hz, H-6), 3.78 (s, 3H, MeO), 4.90 (ddd, 1H, H-4), 5.19 (d, 1H, $J_{NH,5}$ = 10.3 Hz, NH), and 5.39 (dd, 1H, $J_{6,7}$ = 2.6 Hz, $J_{7,8}$ = 9.1 Hz, H-7); Sphyngosine unit δ 1.23 (m, 22H, $11CH_2$), 5.94 (dt, $J_{4,5}$ = 14.5 Hz, $J_{5,6} = J_{5,6'}$ = 7.0 Hz, H-5); O-acyl groups δ 2.01, 2.03, 2.09, 2.12, 2.13, and 2.25 (6s, 18H, 6AcO), 7.39-8.07 (m, 10H, 2Ph).

Anal. Calcd for $C_{62}H_{84}N_4O_{23}$: C, 59.41; H, 6.70; N, 4.47. Found: C, 59.36; H, 6.75; N, 4.52.

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-ß-D-galactopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-tetradecanamido-4-octadecene-1,3-diol (21). Hydrogen sulfide was bubbled through a solution of 19 (130 mg, 0.1 mmol) in pyridine (10 mL) and water (2 mL) for 48 h while the solution was stirred at room temperature; the course of the reaction being monitored by TLC. The mixture was concentrated to a syrup which was dissolved in dry dichloromethane (6 mL). Tetradecanoic acid (47 mg) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSC; 60 mg) were added to the solution, and the mixture was stirred overnight at room temperature. Dichloromethane (50 mL) was added, and the solution was washed with water, dried (sodium sulfate), and evaporated. The residue was chromatographed on a column of silica gel (30 g) with 80:1 dichloromethane-methanol, to give 21 (119 mg, 80%) as an amorphous mass: $[\alpha]_D$ -13.6° (c 0.2, chloroform); ^1H NMR ($CDCl_3$) Gal unit δ 4.60 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 4.63 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 5.03 (d, 1H, H-4), and 5.04 (dd, 1H, H-2); Neu5Ac unit δ 1.75 (t, 1H, $J_{3a,3e} = J_{3a,4}$ = 11.9 Hz, H-3a), 1.85 (s, 3H, AcN), 2.59 (dd, 1H, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.62 (dd, 1H, $J_{5,6}$ = 10.7 Hz, $J_{6,7}$ = 2.6 Hz, H-6), 3.75 (s, 3H, MeO), 4.35 (dd, 1H, $J_{8,9}$ = 2.6 Hz, $J_{9,9'}$ = 12.6 Hz, H-9), 4.89 (ddd, 1H, H-4), 5.16 (d, 1H, $J_{NH,5}$ = 10.3 Hz, NH), and 5.37 (dd, 1H, $J_{7,8}$ = 9.2 Hz, H-7); Cer unit δ 4.50 (m, 1H, H-2), 5.87 (dt, 1H, $J_{4,5}$ = 14.0 Hz, $J_{5,6} = J_{5,6'}$ = 7.0 Hz, H-5), and 5.88 (d, 1H, $J_{NH,2}$ 9.5 Hz, NH); O-acyl groups δ 2.01, 2.03, 2.07, 2.11, 2.12, 2.19 (6s, 18H, 6AcO), 7.36-8.07 (m, 10H, 2Ph).

Anal. Calcd for $C_{76}H_{112}N_2O_{24}$: C, 63.49; H, 7.85; N, 1.95. Found: C, 63.38; H, 8.19; N, 1.90.

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-ß-D-galactopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (22). The azide group of compound 19 (130 mg, 0.1 mmol) was converted to the amine 20 which was coupled with octadecanoic acid (59 mg), according to the method described for 21, to give 22 (125 mg, 81%) as an amorphous mass: $[\alpha]_D$ -12.5° (c 0.25, chloroform); ^{1}H NMR ($CDCl_3$) Gal unit δ 4.61 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 4.63 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 5.03 (d, 1H, H-4), and 5.04 (dd, 1H, H-2); Neu5Ac unit δ 1.85 (s, 3H, AcN), 2.59 (dd, 1H, $J_{3a,3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.63 (dd, 1H, $J_{5,6}$ = 10.1 Hz, $J_{6,7}$ = 2.6 Hz, H-6), 3.75 (s, 3H, MeO), 4.36 (dd, 1H, $J_{8,9}$ = 2.6 Hz, $J_{9,9'}$ = 12.5 Hz, H-9), 4.89 (ddd, 1H, H-4), 5.20 (d, 1H, $J_{NH,5}$ = 9.9 Hz, NH), and 5.37 (dd, 1H, $J_{7,8}$ = 9.1 Hz, H-7); Cer unit δ 4.49 (m, 1H, H-2), 5.89 (dt, 1H, $J_{4,5}$ = 13.9 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.0 Hz, H-5), and 5.90 (d, $J_{NH,2}$ = 9.3 Hz, NH); O-acyl groups δ 2.00, 2.02, 2.07, 2.11, 2.12, and 2.19 (6s, 18H, 6AcO), 7.36-8.04 (m, 10H, 2Ph).

Anal. Calcd for $C_{80}H_{120}N_2O_{24}$: C, 64.32; H, 8.10; N, 1.88. Found: C, 64.35; H, 8.18; N, 1.92.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-(ß-D-galactopyranosyl)-(1→1)-(2S,3R,4E)-2-tetradecanamido-4-octadecene-1,3-diol (23). To a solution of 21 (116 mg, 0.081 mmol) in methanol (5 mL) was added sodium methoxide (20 mg), and the mixture was stirred for 8 h at room temperature; the course of the reaction being monitored by TLC. Water (0.5 mL) was added to the mixture at 0 °C, and the solution was stirred for 3 h , and then treated with Amberlite-120 (H^+) resin to remove the base. The solution was evaporated to leave a syrup which was chromatographed on a column of Sephadex LH-20 (20 g) with methanol to give 23 (quantitative) as an amorphous mass: $[\alpha]_D$ +2.5°(c 0.35, methanol); IR (KBr) 3700-3000 (OH, NH), 2940 and 2840 (Me, methylene), 1700 (C=O), and 1640 and 1560 cm^{-1} (amide); ^{1}H NMR (CD_3OD) Gal unit δ 4.28 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1); Neu5Ac unit δ 2.01 (s, 3H, AcN), 2.88 (dd, 1H, $J_{3a,3e}$ = 11.9 Hz, $J_{3e,4}$ = 3.9 Hz, H-3e); Cer unit δ 0.90 (t, 6H, J_{Me,CH_2} = 6.7 Hz, 2$\underline{Me}CH_2$), 3.68 (t, 2H, J_{CH_2,CH_2} = 7.4 Hz, CO$\underline{CH_2}CH_2$), 4.10 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.2 Hz, H-3), 4.21 (dd, 1H, $J_{1,2}$ = 4.0 Hz, $J_{1,1'}$ = 10.1 Hz, H-1), 5.44 (dd, 1H, $J_{3,4}$ = 7.3 Hz, $J_{4,5}$ = 14.1 Hz, H-4), and 5.68 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 7.1 Hz, H-5).

Anal. Calcd for $C_{49}H_{90}N_2O_{16}$: C, 61.10; H, 9.42; N, 2.91. Found: C, 61.03; N, 9.45; N, 2.88.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl-onic acid)-(2→3)-O-(ß-D-galactopyranosyl)-(1→1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-1,3-diol (24). The O-acyl and methyl ester groups in compound 22 (123 mg, 0.082 mmol) were removed as described for the preparation of 23, to give 24 (75 mg, 89%) as an amorphous mass: $[\alpha]_D$ +2.2°(c 0.3, methanol); ^{1}H NMR (CD_3OD) Gal unit δ 4.29 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1); Neu5Ac unit δ 2.01 (s, 3H, AcN) and 2.87 (dd, 1H, $J_{3a,3e}$ = 11.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e); Cer unit δ 0.89 (t, 6H, J_{Me,CH_2} = 5.6 Hz, 2$\underline{Me}CH_2$), 2.18 (t, 2H, J_{CH_2,CH_2} = 7.2 Hz, CO$\underline{CH_2}CH_2$), 4.10 (t, 1H, $J_{2,3} = J_{3,4}$ = 8.1 Hz, H-3), 4.23 (dd, 1H, $J_{1,1'}$ = 10.4 Hz, $J_{1,2}$ = 4.2 Hz, H-1), 4.29 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 5.44 (dd, 1H, $J_{4,5}$ = 14.4 Hz, H-4), and 5.66 (dt, 1H, $J_{5,6} = J_{5,6'}$ = 7.4 Hz, H-5).

Anal. Calcd for $C_{53}H_{98}N_2O_{16}$: C, 62.45; H, 9.69; N, 2.75. Found: C, 62.51; H, 9.68; N, 2.70.

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-ß-D-galacto-pyranosyl)-(1→1)-(2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (25). Condensation of 17 (341 mg, 0.37 mmol) with 18 (317 mg, 0.74 mmol), as described for 19, gave compound 25 (340 mg, 77.5%) as an amorphous mass: $[\alpha]_D$ -19.5° (c 2.4, chloroform); IR (KBr) 3300 (NH), 2940 and 2840 (Me, methylene), 2100 (N_3), 1750 and 1220 (ester), and 710 cm^{-1} (Ph); ^{1}H NMR ($CDCl_3$) Gal unit δ 4.61 (1H, $J_{1,2}$ = 7.8 Hz, H-1), 5.07 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 5.22 (dd, 1H, H-2), 5.46 (broad d, 1H, H-4); Neu5Ac unit δ 1.88 (s, 3H, AcN), 2.52 (dd, 1H, $J_{3a,3e}$ = 12.7 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e), 3.75 (s, 3H, MeO), 4.33 (dd, 1H, $J_{9,9'}$ = 12.3 Hz, H-9), 4.85 (ddd, 1H, H-4); Cer unit δ 1.25 (s, 22H, 11CH_2), 5.93 (dt, 1H, $J_{4,5}$ = 14.0 Hz, $J_{5,6} = J_{5,6'}$ = 7.0 Hz, H-5), and 7.43-8.08 (m, 5H, Ph); O-acetyl groups δ 1.99, 2.02, 2.03, 2.11, 2.12, 2.15, and 2.19 (7s, 21H, 7AcO).

Anal. Calcd for $C_{57}H_{82}N_4O_{23}$: C, 57.47; H, 6.94; N, 4.70. Found: C, 57.29; H, 7.05; N, 4.71.

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-ß-D-galacto-pyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (27). Selective reduction of the azide group in 25 (102 mg, 0.086 mmol) with hydrogen sulfide, and subsequent condensation with octadecanoic acid (58 mg) using WSC (58 mg), as described for 21, yielded compound 27 (92 mg, 75%) as an amorphous mass: $[\alpha]_D$ -13.1° (c 1.1, chloroform); ^{1}H NMR ($CDCl_3$) Gal unit δ 3.36 (dd, 1H, $J_{6,6'}$= 10.6 Hz, H-6), 4.56 (d, 1H, $J_{1,2}$= 7.7 Hz, H-1), 5.06 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.1 Hz, H-3),

5.15 (dd, 1H, H-2), and 5.45 (broad d, 1H, H-4); Neu5Ac unit δ 1.83 (s, 3H, AcN), 2.50 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.77 (s, 3H, MeO), 4.30 (dd, 1H, $J_{8,9}$ = 2.4 Hz, $J_{9,9'}$ = 12.4 Hz, H-9), 4.84 (ddd, 1H, H-4), and 5.30 (ddd, 1H, H-8); Cer unit δ 1.25 (s, 52H, 26CH_2), 4.49 (m, 1H, H-2), 5.87 (dt, 1H, $J_{4,5}$ = 14.1 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.1 Hz, H-5), 5.94 (d, 1H, $J_{NH,2}$ = 9.2 Hz, NH), and 7.41-8.05 (m, 5H, ph); O-acetyl groups δ 2.01, 2.04, 2.06, 2.09, 2.10, 2.12, and 2.14 (7s, 21H, 7AcO).

Anal. Calcd for $C_{75}H_{118}N_2O_{24}$: C, 62.92; H, 8.31; N, 1.96. Found: C, 62.86; H, 8.45; N, 1.95.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl-onic acid)-(2→6)-(β-D-galactopyranosyl)-(1→1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-1,3-diol (28). O-Deacylation and subsequent saponification of the methyl ester group of 27 (158 mg, 0.11 mmol), as described for 23, gave 28 (110 mg, quantitative) as an amorphous mass: $[\alpha]_D$ +1.0° (c 0.8, methanol); IR (KBr) 3700-2800 (OH, NH), 2940 and 2840 (Me, methylene), 1700 (C=O), and 1630 and 1560 cm^{-1} (amide); ^1H NMR (CD_3OD) Gal unit δ 4.21 (d, 1H, $J_{1,2}$ = 6.8 Hz, H-1); Neu5Ac unit δ 1.91 (s, 3H, AcN), 2.80 (dd, 1H, $J_{3a,3e}$ = 12.4 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e); Cer unit δ 0.90 (t, 6H, J_{Me,CH_2} = 6.3 Hz, MeCH_2), 2.16 (t, 2H, J_{CH_2,CH_2} = 7.5 Hz, COCH_2CH_2), 4.15 (dd, 1H, $J_{1,1'}$ = 10.3 Hz, $J_{1,2}$ = 4.4 Hz, H-1), 5.43 (dd, 1H, $J_{4,5}$ = 15.5 Hz, H-4), 5.68 (dt, $J_{5,6'}$ = 7.3 Hz, H-5).

Anal. Calcd for $C_{53}H_{98}N_2O_{16}$: C, 62.45; H, 9.69; N, 2.75. Found: C, 62.38; H, 9.84; N, 2.70.

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