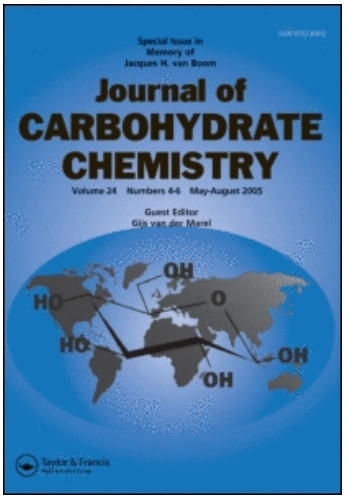

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SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 15: SYNTHESIS OF GANGLIOSIDE GM_3 ANALOGS CONTAINING A VARIETY OF LIPOPHILIC PARTS

Akira Hasegawa,* Takatoshi Murase, Minoru Morita, Hideharu Ishida, and Makoto Kiso

Department of Applied Bioorganic Chemistry, Gifu University, Yanagido, Gifu 501-11 (Japan)

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ABSTRACT

Several ganglioside GM_3 analogs, containing a variety of lipophilic parts in place of the ceramide moiety have been synthesized. Glycosylation of (2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecen-1,3-diol (2) with 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-β-D-galactopyranosyl)-(1→4)-3-O-acetyl-2,6-di-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate (1) gave the β-glycoside (5), which was converted, via selective reduction of the azide group, introduction of acyl groups, O-deacylation, and de-esterification, into the desired compounds (10-12). On the other hand, coupling of 1 with 3-benzyloxycarbonylamino-1-propanol (3) or (2RS)-3-benzyloxycarbonylamino-2-O-benzoyl-1,2-propanediol (4) gave the corresponding β-glycosides 13 and 14, respectively. These were converted by N-debenzyloxycarbonylation, coupling with 2-tetradecylhexadecanoic acid, O-deacylation, and hydrolysis of the methyl ester group, into the end products (17 and 18).

INTRODUCTION

Ganglioside GM_3 was first detected from horse erythrocytes by Yamakawa et al.[1] in 1952, and is the major ganglioside component in erythrocytes of many animal species.[2-5] Recently, various types of important biological functions of this ganglioside, such as influenza A virus receptor,[6] induction[7] of monocytic differentiation of human myeloid, and enhancement or inhibition of protein kinase activity,[8] have been reported.

Ganglioside GM_3, as well as other gangliosides, is a polymorphous molecule at the sialic acid and ceramide moieties. Therefor, in order to investigate the functions of gangliosides at the molecular level, the synthesis of a variety of gangliosides and a variety of analogs is of critical importance. Previously,[9] we report- ed a facile, regio- and stereo-selective synthesis of ganglioside GM_3. As a part of our continuing efforts[10,11] on the synthesis and structure-function relationship of sialoglycoconjugates, we describe here the synthesis of ganglioside GM_3 analogs containing a variety of lipophilic parts in the place of ceramide. Our aim is to elucidate the role of the ceramide pertion in the functions of the gangliosides.

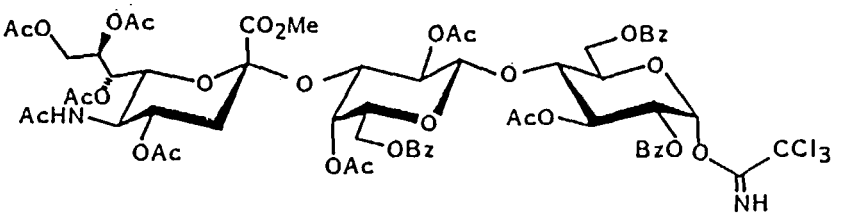
RESULTS AND DISCUSSION

For the synthesis of the desired ganglioside GM_3 analogs, in which the ceramide moiety is replaced by other lipophilic groups, we have chosen 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)-3-O-acetyl-2,4-di-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate[9] (1) as the glycosyl donor, and (2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecen-1,3-diol (2), 3-benzyloxycarbonylamino-1-propanol (3) and (2RS)-3-benzyloxycarbonylamino-2-O-benzoyl-1,2-propanediol (4) as the glycosyl acceptors. Selective reduction of the azide group or reductive cleavage of the benzyloxycarbonyl group in the glycosides obtained, in-

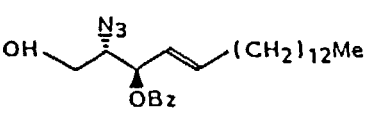
troduction of the fatty acyl groups, and removal of the protecting groups will give the end products.

Selective reduction[9,10,13] of the azide group in 5, obtained by glycosylation of 2 with the glycosyl donor 1 according to the method[9] described by us, with hydrogen sulfide in pyridine-water, gave the amine derivative, which was converted by N-acylation using acetic anhydride, (RS)-2-acetoxytetradecanoic acid or (R)-3-acetoxytetradecanoic acid, into the corresponding derivatives (6-8) in high yields, respectively. Finally, O-deacylation of 5-8 with sodium methoxide in methanol, and subsequent saponification of the methyl ester group yielded the four kinds (9-12) of ganglioside GM_3 analogs.

Glycosylation of 3 with 1 in dry dichloromethane for 4 h at 0 °C in the presence of boron trifluoride etherate[14] and molecular sieves 4A afforded only the desired β-glycoside, 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)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-3-benzyloxycarbonylamino-1-propanol (13) in 88% yield. Significant signals in the ^1H NMR spectrum of 13 were a one-proton doublet at δ 4.63 ($J_{1,2}$ = 7.3 Hz, H-1, lactose unit) and a one-proton doublet of doublets at δ 5.19 ($J_{2,3}$ = 9.5 Hz, H-2, lactose unit), showing the newly formed β-glycosidic linkage. Other ^1H NMR data are consistent with structure 13. In the same way, when coupled with the acceptor 4, compound 1 gave the expected β-glycoside 14 in 85% yield. Hydrogenolysis of the benzyloxycarbonyl group in compounds 13 and 14 with hydrogen in the presence of 10% Pd-C catalyst in ethanol and subsequent condensation with 2-tetradecylhexadecanoic acid using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSC) in dichloromethane-1,4-dioxane, respectively, gave the corresponding derivatives of ganglioside GM_3 analogs (15 and 16) in 72 and 71% yields. Finally, O-deacylation of 15 and 16 with sodium methoxide in methanol, and subsequent saponification of the methyl ester group yielded the additional, two kinds (17 and 18) of ganglioside GM_3 analogs containing the different,



1



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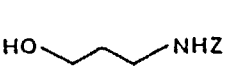

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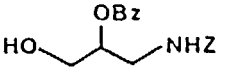
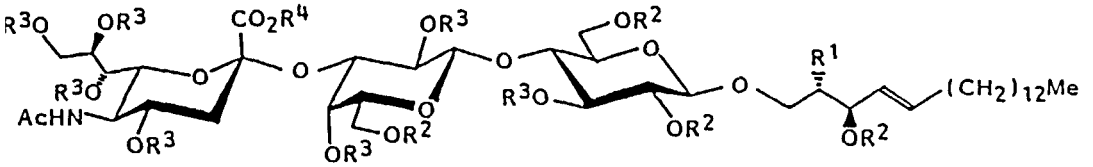
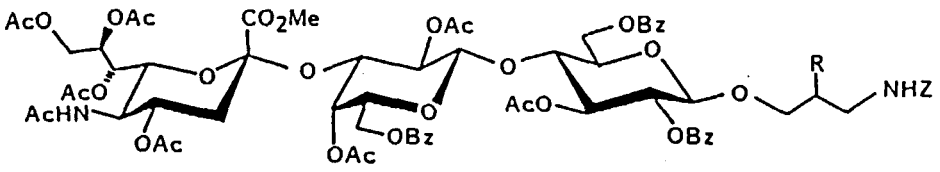


4

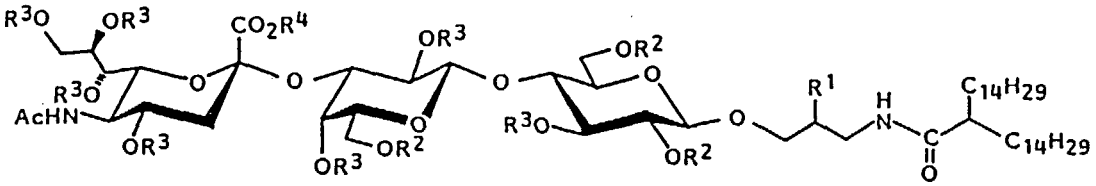
Bz = benzoyl
Z = benzyloxycarbonyl



5 $R^1 = N_3$, R^2 = Bz, R^3= Ac, R^4 = Me
6 R^1 = NHAc, R^2 = Bz, R^3 = Ac, R^4 = Me
7 R^1 = NHCOCH(OAc)$(CH_2)_{11}$Me, R^2 = Bz, R^3 = Ac, R^4 = Me
8 R^1 = NHCOCH$_2$CH(OAc)$(CH_2)_{10}$Me, R^2 = Bz, R^3 = Ac, R^4 = Me
9 $R^1 = N_3$, $R^2 = R^3 = R^4$ = H
10 R^1 = NHAc, $R^2 = R^3 = R^4$ = H
11 R^1 = NHCOCH(OH)$(CH_2)_{11}$Me, $R^2 = R^3 = R^4$ = H
12 R^1 = NHCOCH$_2$CH(OH)$(CH_2)_{10}$Me, $R^2 = R^3 = R^4$ = H



13 R = H
14 R = OBz



15 R^1 = H, R^2 = Bz, R^3 = Ac, R^4 = Me
16 R^1 = OBz, R^2 = Bz, R^3 = Ac, R^4 = Me
17 R^1 = R^2 = R^3 = R^4 = H
18 R^1 = OH, R^2 = R^3 = R^4 = H

lipophilic parts in the place of ceramide in almost quantitative yields.

EXPERIMENTAL

General Procedures. Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union PM-201 polarimeter at 25 °C, and IR spectra were recorded with a Jasco IRA-100 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 (Wako Co., 200 mesh) with the solvent systems specified. Concentrations and evaporations were conducted in vacuo.

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)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-acetamido-3-O-benzoyl-4-octadecen-1,3-diol (6). Hydrogen sulfide was bubbled through a solution of 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)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecen-1,3-diol[9] (5, 100 mg, 0.06 mmol) in pyridine (10 mL) and water (2 mL) for 36 h while the solution was stirred at room temperature; the course of the reaction was monitored by TLC. The mixture was concentrated to a syrup which was acetylated with acetic anhydride (2 mL) in pyridine overnight at room temperature, and the product was purified by chromatography on a column of silica gel (30 g) with 4:1 ethyl acetate-hexane, to give 6 (93 mg, 92%) as an amorphous mass: $[\alpha]_D$ +13.5° (c, 0.56, chloroform); ^{1}H NMR ($CDCl_3$) lactose unit δ 4.63 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.84 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.99 (m, 2H, H-2',4'), and 5.18 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2); Neu5Ac unit δ 2.56 (dd, 1H, $J_{3a,3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.8 Hz, H-3e), 3.72 (s, 3H, MeO), and 4.90 (m, 1H, H-4); cer unit δ 0.88 (t, 3H, $MeCH_2$), and 5.76 (dt, 1H, $J_{4,5}$ = 14.4 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.2 Hz, H-5); O-and N-acyl groups δ 1.82, 1.90 (2), 2.00 (2), 2.02 (2), 2.11, and 2.18 (9s, 27H, 7AcO, 2AcN).

Anal. Calcd for $C_{86}H_{105}N_2O_{32}$ (1681.1): C, 61.42; H, 6.47; N, 1.67. Found: C, 61.23; H, 6.55; N, 1.53.

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)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-[(2RS)-2-acetoxytetradecanamido]-3-O-benzoyl-4-octadecen-1,3-diol (7). To a stirred solution of the amine, prepared from 5 (100 mg, 0.06 mmol) as described for 6, in dry dichloromethane (5 mL), were added (RS)-2-acetoxytetradecanoic acid (34 mg, 0.12 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC, 35 mg, 0.18

mmol). The mixture was stirred overnight at room temperature and then extracted with dichloromethane. The extract was washed with water, dried (sodium sulfate), and concentrated to a syrup, which was chromatographed on a column of silica gel (30 g) with 3:1 ethyl acetate-hexane as the eluent, to give 7 (103 mg, 90%) as an amorphous mass: $[\alpha]_D$ +10.7° (c, 0.66, chloroform); ^{1}H NMR (1:1 $CDCl_3$ -CD_3OD) lactose unit δ 4.84 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 5.01 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1'), and 5.21 (t, 1H, $J_{2,3}$ = 8.5 Hz, H-2); Neu5Ac unit δ 1.63 (t, 1H, $J_{3a,3e} = J_{3a,4}$ = 12.3 Hz, H-3e), 1.83 (s, 3H, AcN), and 2.56 (dd, 1H, $J_{3e,4}$ = 4.8 Hz, H-3e), and 3.71 (s, 3H, MeO); cer unit δ 0.88 (t, 6H, 2MeCH$_2$); O-acyl groups δ 1.89, 1.98, 1.99, 2.00, 2.02, 2.11, 2.14, and 2.18 (8s, 24H, 8AcO), and 7.26-8.06 (m, 20H, 4Ph).

Anal. Calcd for $C_{100}H_{134}N_2O_{34}$ (1908.2): C, 62.95; H, 7.08; N, 1.47. Found: C, 62.74; H, 7.35; N, 1.52.

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-β-D-galactopyranosyl)-(1→4)-O-(3-O-acetyl-2,6-di-O-benzoy-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-[(R)-3-acetoxytetradecanamido]-3-O-benzoyl-4-octadecen-1,3-diol (8). Condensation of the amine, derived from 5 (90 mg, 54 μmol), with (R)-3-acetoxytetradecanoic acid (28 mg) in the presence of WSC (31 mg) as described for 7, gave compound 8 (90 mg, 89%) as an amorphous mass: $[\alpha]_D$ +9.1° (c, 0.44, chloroform); ^{1}H NMR (1:1 $CDCl_3$-CD_3OD) lactose unit δ 4.66 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.85 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.99 (m, 2H, H-2',4'), and 5.12 (dd, 1H, $J_{2,3}$ =9.9 Hz, H-2); Neu5Ac unit δ 1.64 (t, 1H, $J_{3a,3e} = J_{3a,4}$ = 12.5 Hz, H-3a), 1.83 (s, 3H, AcN), and 2.56 (dd, 1H, $J_{3e,4}$ = 4.8 Hz, H-3e), and 3.72 (s, 3H, MeO); cer unit δ 0.88 (t, 6H, 2MeCH$_2$), and 5.75 (dt, 1H, $J_{4,5}$ = 15.4 Hz, $J_{5,6} = J_{5,6'}$ = 7.5 Hz, H-5); O-acyl groups δ 2.00 (2), 2.01 (2), 2.02 (2), 2.12, and 2.18 (8s, 24H, 8AcO), and 7.26-8.06 (m, 20H, 4Ph).

Anal. Calcd for $C_{100}H_{134}N_2O_{34}$ (1908.2): C, 62.95; H, 7.08; N, 1.47. Found: C, 62.69; H, 7.08; N, 1.53.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-O-(β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-azido-4-octadecen-1,3-diol (9). To a solution of 5 (148 mg, 0.09 mmol) in methanol (7 mL) was added sodium methoxide (30 mg) and the mixture was stirred for 5 h at room temperature; the course of the reaction was monitored by TLC. Water (0.5 mL) was added to the mixture at 0 °C, and this was stirred for 5 h, and then treated with Amberlite IR-120 (H^+) resin to remove the base. The solution was concentrated to a syrup which was chromatographed on a column of Sephadex LH-20 (40 g) with methanol, to give 9 (70 mg, 84%) as an amorphous mass: $[\alpha]^D$ +0.9° (c 0.48, methanol); ^1H NMR (CD_3OD) lactose unit δ 4.32 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1) and 4.44 (d, 1H, $J_{1',2'}$ = 7.0 Hz, H-1'); Neu5Ac unit δ 1.75 (t, 1H, $J_{3a,3e} = J_{3a,4}$ = 12.5 Hz, H-3a), 2.01 (s, 3H, AcN), and 2.86 (dd, 1H, $J_{3e,4}$ = 4.6Hz, H-3e); cer unit δ 0.93 (t, 3H, $MeCH_2$), 5.51 (dd, 1H, $J_{3,4}$ = 7.4 Hz, $J_{4,5}$ = 15.5 Hz, H-4), and 5.77 (dt, 1H, $J_{5,6} = J_{5,6'}$ = 7.1 Hz, H-5).

Anal. Calcd for $C_{41}H_{72}N_4O_{20}$ (941.0): C, 52.33; H, 7.71; N, 5.95. Found: C, 52.30; H, 7.91; N, 5.84.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-O-(β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-acetamido-4-octadecen-1,3-diol (10). The O-acyl and methyl ester groups in 6 (85 mg, 0.05 mmol) were removed, as described for 9, to give compound 10 (47 mg, 97%) as an amorphous mass: $[\alpha]_D$ +0.6° (c 1.0, 1:1 chloroform-methanol); ^1H NMR (1:1 $CDCl_3$-CD_3OD) lactose unit δ 4.31 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1) and 4.44 (d, 1H, $J_{1',2'}$ = 7.2 Hz, H-1'); Neu5Ac Unit δ 2.86 (dd, 1H, $J_{3a,3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.7 Hz, H-3e); cer unit δ 0.89 (t, 3H, $MeCH_2$), 5.46 (dd, 1H, $J_{3,4}$ = 7.1 Hz, $J_{4,5}$ = 15.4 Hz, H-4), and 5.70 (dt, 1H, $J_{5,6} = J_{5,6'}$ = 7.1 Hz, H-5); N-acyl groups δ 1.96 and 2.04 (2s, 6H, 2AcN).

Anal. Calcd for $C_{43}H_{76}N_2O_{21}$ (957.1): C, 53.96; H, 8.00; N, 2.93. Found: C, 53.75; H, 8.23; N, 2.81.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-O-(β-D-

glucopyranosyl)-(1→1)-(2S,3R,4E)-2-[(RS)-2-hydroxytetradecanamido]-4-octadecen-1,3-diol (11). Saponification of 7 (90 mg, 47 μmol), as described for the preparation of 9, gave 11 (53 mg, 98%) as an amorphous mass: $[\alpha]_D$ +1.5° (c 1, 1:1 chloroform-methanol); IR (KBr) 3400 (OH, NH), 2940 and 2860 (Me, methylene), 1700 (C=O), and 1630 and 1550 cm^{-1} (amide); ^1H NMR (1:1 $CDCl_3$-CD_3OD) lactose unit δ 4.31 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1) and 4.33 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1'); Neu5Ac unit δ 2.03 (s, 3H, AcN) and 2.86 (dd, 1H, $J_{3a,3e}$ = 12.2 Hz, $J_{3e,4}$ = 4.7 Hz, H-3e); cer unit δ 0.89 (t, 6H, 2MeCH$_2$), 4.96 (dd, 1H, H-4), and 5.73 (dt, 1H, $J_{4,5}$ = 15.0 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.5 Hz, H-5).

Anal. Calcd for $C_{55}H_{100}N_2O_{22}$ (1141.4): C, 57.88; H, 8.83; N, 2.45. Found: C, 57.63; H, 8.79; N, 2.40.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-O-(β-D-glucopyranosyl-(1→1)-(2S,3R,4E)-2-[(R)-3-hydroxytetradecanamido]-4-octadecen-1,3-diol (12). Saponification of 8 (80 mg, 43 μmol), as described for 9, gave 12 (50 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -0.6° (1:1 chloroform-methanol); IR (KBr) 3500-3300 (OH, NH), 2940 and 2850 (Me, methylene), 1700 (C=O), and 1650 and 1550 cm^{-1} (amide); ^1H NMR (1:1 $CDCl_3$-CD_3OD) lactose unit δ 4.32 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1) and 4.44 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'); Neu5Ac unit δ 2.04 (s, 3H, AcN) and 2.86 (dd, 1H, $J_{3a,3e}$ = 10.6 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e); cer unit δ 0.89 (t, 6H, 2MeCH$_2$), 5.46 (dd, 1H, $J_{3,4}$ = 7.1 Hz, $J_{4,5}$ =15.6 Hz, H-4), and 5.70 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 7.5 Hz, H-5).

Anal. Calcd for $C_{55}H_{100}N_2O_{22}$ (1141.4): C, 57.88; H, 8.83; N, 2.45. Found: C, 57.69; H, 8.93; N, 2.46.

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)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-3-benzyloxycarbonylamino-1-propanol (13). To a solution of 1 (200 mg, 0.143 mmol) and 3-benzyloxycarbonylamino-1-propanol (60 mg, 0.3 mmol) in dry dichloromethane (4 mL) was added molecular sieves 4A type AW-300 (MS-4A-AW-300, 1.8 g),

and the mixture was stirred for 30 min at room temperature and then cooled to 0 °C. Boron trifluoride etherate (40 mg) was added to the mixture, and this was stirred for 4 h at 0 °C; the progress of the reaction being monitored by TLC. The precipitates were filtered off and washed throughly with dichloromethane. The solution was successively washed with M sodium carbonate and water, dried (sodium sulfate), and concentrated to a syrup that was chromatographed on a column of silica gel (50 g) with 3:1 ethyl acetate-hexane, to give 13 (182 mg, 88%) as an amorphous mass: $[\alpha]_D$ +5.2° (c 0.58, chloroform); ^{1}H NMR ($CDCl_3$) lactose unit δ 4.61 (dd, 1H, $J_{2',3'}$ = 13.6 Hz, $J_{3',4'}$ = 3.6 Hz, H-3'), 4.63 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 4.89 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 5.19 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), and 5.49 (t, 1H, $J_{3,4}$ = 9.5 Hz, H-3); Neu5Ac unit δ 1.83 (s, 3H, AcN), 2.57 (dd, 1H, $J_{3a,3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.70 (s, 3H, MeO), 4.87 (m, 1H, H-4), 5.19 (d, 1H, $J_{5,NH}$ = 10.3 Hz, NH), 5.35 (dd, 1H, $J_{6,7}$ = 2.6 Hz, $J_{7,8}$ = 8.9 Hz, H-7), and 5.55 (m, 1H, H-8); O-acyl groups δ 1.97, 2.00, 2.01 (2), 2.02, 2.12, and 2.22 (7s, 21H, 7AcO), and 7.27-8.06 (m, 20H, 4Ph).

Anal. Calcd for $C_{70}H_{80}N_2O_{31}$ (1445.4): C, 58.17; H, 5.58; N, 1.94. Found; C, 58.25; H, 5.72; N, 1.94.

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)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-(2RS)-2-O-benzoyl-3-benzyloxycarbonylamino-1,2-propanediol (14). Condensation of 1 (150 mg, 0.11 mmol) with (2RS)-2-O-benzoyl-3-benzyloxycarbonylamino-1,2-propanediol (4, 74 mg, 0.22 mmol), as described for 13, gave compound 14 (142 mg, 85%) as an amorphous mass: $[\alpha]_D$ +7.0° (c 0.9, chloroform); ^{1}H NMR ($CDCl_3$) lactose unit δ 4.60 (d, 1H, $J_{1,2}$ = 9.0 Hz, H-1), 4.64 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1'), 5.20 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.0 Hz, H-2), and 5.45 (near t, H-3); Neu5Ac unit δ 1.63 (t, 1H, $J_{3a,3e}$ = $J_{3a,4}$ = 12.5 Hz, H-3a), 1.82 (s, 3H, AcN), 2.56 (dd, 1H, $J_{3e,4}$ = 4.7 Hz, H-3e), 3.59 (broad d, 1H, $J_{5,6}$ = 10.6 Hz, H-6), 3.71 (s, 3H, MeO), 4.85 (m, 1H, H-4), 5.33 (dd, 1H, $J_{6,7}$ = 2.0 Hz, $J_{7,8}$ = 8.8 Hz, H-7), and 5.48 (m, 1H, H-8); O-acyl groups δ 1.98, 1.99, 2.00, 2.02, 2.07, 2.12 and 2.21 (7s, 21H, 7AcO), and 7.22-8.05 (m, 25H, 5Ph).

Anal. Calcd for $C_{77}H_{84}N_2O_{33}$ (1565.5): C, 59.08; H, 5.41; N, 1.79. Found; C, 59.05; H, 5.66; N, 1.63.

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)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-3-(2-tetradecylhexadecanamido)-1-propanol (15). Compound 13 (80 mg, 55 μmol) was dissolved in ethanol (5 mL), 10% Pd-C catalyst (60 mg) and acetic acid (0.2 mL) were added, and hydrogen was bubbled through while the mixture was stirred for 2 h at room temperature. The catalyst was removed by filtration, and the solution was concentrated. To a solution of the residue in dichloromethane (3 mL)-1,4-dioxane (1 mL) were added 2-tetradecylhexadecanoic acid (50 mg) and WSC (32 mg), and the mixture was stirred overnight at room temperature, and then extracted with dichloromethane. The extract was washed with water, dried (sodium sulfate), and concentrated to a syrup which was chromatographed on a column of silica gel (30 g) with 50:1 chloroform-methanol, to give 15 (72 mg, 73%) as an amorphous mass: $[\alpha]_D$ +1.0° (c 0.6, chloroform); ^1H NMR (1:1 $CDCl_3$-CD_3OD) lactose unit δ 4.63 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3'), 4.64 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.98 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1'), 5.17 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 5.50 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), and 7.31-8.06 (m, 15H, 3Ph); Neu5Ac unit δ 1.82 (s, 3H, AcN), 2.57 (dd, 1H, $J_{3a,3e}$ = 13.1 Hz, $J_{3e,4}$ = 4.3 Hz, H-3e), 3.71 (s, 3H, MeO), 4.89 (m, 1H, H-4), 5.33 (dd, 1H, $J_{6,7}$ = 2.4 Hz, $J_{7,8}$ = 9.5 Hz, H-7), and 5.50 (m, 1H, H-8); lipophilic part δ 0.88 (m, 6H, 2MeCH_2), and 1.25 (s, 52H, 26CH_2); O-acetyl groups δ 1.99, 2.00, 2.01 (2), 2.03, 2.13, and 2.22 (7s, 21H, 7AcO).

Anal. Calcd for $C_{92}H_{132}N_2O_{30}$ (1746.1): C, 63.29; H, 7.62; N, 1.60. Found: C, 63.12; H, 7.65; N, 1.49.

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)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-(2RS)-2-O-benzoyl-3-(2-tetradecylhexadecanamido)-1,2-propanediol (16). Hydrogenation of 14 (100 mg,

64 μmol) with hydrogen in the presence of 10% Pd-C catalyst (60 mg) in ethanol (5 mL), and subsequent N-acylation with 2-tetradecylhexadecanoic acid (57 mg) using WSC (36 mg), as described for 15, afforded 16 (84 mg, 71%) as an amorphous mass: $[\alpha]_D$ + 6.2° (c 0.58, chloroform); ^{1}H NMR (1:1 $CDCl_3$-CD_3OD) lactose unit δ 4.73 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.91 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 5.20 (t, $J_{2,3}$ = 8.2 Hz, H-2), and 5.49 (t, 1H, H-3); Neu5Ac unit δ 1.63 (t, 1H, $J_{3a,3e} = J_{3a,4}$ = 12.2 Hz, H-3a), 1.82 (s, 3H, AcN), 2.57 (dd, 1H, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.72 (s, 3H, MeO), 4.81 (m, 1H, H-4), 5.34 (dd, 1H, $J_{6,7}$ = 2.7 Hz, $J_{7,8}$ = 8.9 Hz, H-7), and 5.48 (m, 1H, H-8); lipophilic part δ 0.88 (t, 6H, 2MeCH$_2$) and 1.26 (s, 52H, 26CH$_2$; O-acyl groups δ 1.97, 1.99, 2.01, 2.12 (2), 2.20, and 2.22 (7s, 21H, 7AcO), and 7.20-8.06 (m, 20H, 4Ph).

Anal. Calcd for $C_{99}H_{136}N_2O_{32}$ (1866.2): C, 63.72; H, 7.35; N, 1.50. Found: C, 63.79; H, 7.33; N, 1.52.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-O-(β-D-glucopyranosyl)-(1→1)-3-(2-tetradecylhexadecanamido)-1-propanol (17). The O-acyl and methyl ester groups in 15 (70 mg, 40 μmol) were removed, as described for the preparation of 9, to give compound 17 (45 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -5.8° (c 0.9, 1:1 chloroform-methanol); IR (KBr) 3700-3400 (OH, NH), 2940 and 2850 (Me, methylene), 1700 (C=O), and 1620 and 1560 cm^{-1} (amide); ^{1}H NMR (98:2 $(CD_3)_2SO$-D_2O) lactose unit δ 4.17 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1) and 4.21 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1'); Neu5Ac unit δ 1.90 (s, 3H, AcN) and 2.75 (dd, 1H, $J_{3a,3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e); lipophilic part δ 0.85 (t, 6H, 2MeCH$_2$) and 1.23 (m, 52H, 26CH$_2$)

Anal. Calcd for $C_{56}H_{104}N_2O_{20}$ (1125.4): C, 59.76; H, 9.31; N, 2.49. Found: C, 59.71; H, 9.60; N, 2.33.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-β-D-galactopyranosyl)-(1→4)-O-β-D-glucopyranosyl)-(1→1)-(2RS)-3-(2-tetradecylhexadecanamido)-1,2-propanediol (18). Saponification of 16 (83 mg, 44 umol), as described for 9, gave 18 (50 mg, quantitative) as an amorphous mass:

$[\alpha]_D$ -5.0° (c 1.0, 1:1 chloroform-methanol); IR (KBr) 3700-3350 (OH, NH), 2940 and 2850 (Me, methylene), 1700 (C=O), and 1630 and 1540 cm^{-1} (amide); ^1H NMR (98:2 $(CD_3)_2SO$-D_2O) lactose unit δ 4.16 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.20 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'); Neu5Ac unit δ 1.89 (s, 3H, AcN) and 2.75 (dd, 1H, $J_{3a,3e}$ = 12.2 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e); lipophilic part δ 0.85 (t, 6H, 2$\underline{Me}CH_2$) and 1.23 (m, 52H, 26CH_2).

Anal. Calcd for $C_{56}H_{104}N_2O_{21}$ (1141.4): C, 58.93; H, 9.18; N, 2.45. Found: C, 58.69; H, 9.35; N, 2.39.

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