Lipid A and Related Compounds. XXVI.[1] Syntheses of Biologically Active Penta-*O*-acetyl-*N*-glycoloylneuraminyl- and Penta-*O*-acetyl-3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic Acid-(α 2→6)-D-glucosamine-4-phosphate Analogs of Lipid A

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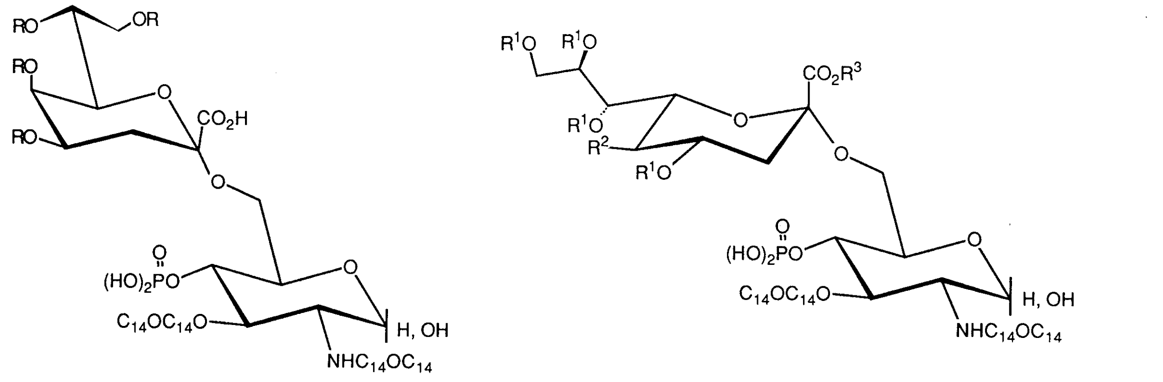
The syntheses of novel penta-*O*-acetyl-*N*-glycoloylneuraminyl- and penta-*O*-acetyl-3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid-(α 2→6)-D-glucosamine-4-phosphate analogues of lipid A containing sialic acid in place of 3-deoxy-D-*manno*-2-octulosonic acid are described. Preliminary examination of the biological activity revealed that two synthetic disaccharides showed mitogenic activities and weak antitumor activities.

Keywords *N*-acetylneuraminic acid; *N*-glycoloylneuraminyl-glucosamine-4-phosphate; KDN-glucosamine-4-phosphate; lipid A analog; mitogenic activity

Lipid A is known to be responsible for the expression of many biological activities of lipopolysaccharide (LPS) of gram-negative bacteria.[2] In previous papers,[3] we and other groups demonstrated that acyloxyacyl glucosamine-4-phosphates as the nonreducing sugar moiety of lipid A is more important than the reducing sugar moiety (lipid X and lipid Y) for expressing the biological activities of lipid A. Furthermore, 2-keto-3-deoxyoctulosonic acid (KDO) linked-acylated 4-*O*-monophosphorylglucosamine derivatives (**1** and **2** in Chart 1) possessed mitogenic activity comparable to that of lipid A.[4] Recently, we prepared *N*-acetylneuraminyl-(α 2→6)-D-glucosamine-4-phosphate analogues[5] (**3** and **4** in Chart 1) of lipid A containing *N*-acetylneuraminic acid, whose structure is similar to KDO and whose function has attracted much interest.[6] Compounds **3** and **4** possessed weak mitogenic activities.[7] *N*-Glycoloylneuraminic acid[8] is of relatively widespread occurrence, more resistant to cleavage by neuraminidase than *N*-acylneuraminic acid. And 3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (KDN)[9] bearing an hydroxyl group instead of the acetylamino group at C-5 of *N*-acetylneuraminic acid is an important terminal unit of the oligosaccharide chain of polysialoglycoproteins of the membranes of the rainbow trout.

As a synthetic approach to investigate the relationship between the chemical structure and the biological activity of the nonreducing subunit of lipid As, we now describe the syntheses of two new penta-*O*-acetyl-*N*-glycoloylneuraminyl- (**5**) and penta-*O*-acetyl-KDN-(α 2→6)-D-glucosamine-4-phosphate analogs (**6**) of lipid As.

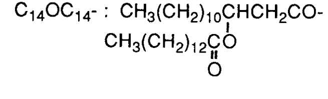
First, we describe a synthetic sequence for the penta-*O*-acetyl *N*-glycoloylneuraminyl glucosamine-4-phosphate derivative (**5**). Treatment of 2-chloro per-*O*-acetylated methyl ester derivative (**7**) with benzyl alcohol in the presence of silver salicylate[10] gave stereospecificially the corresponding 2-*O*-α-benzyl compound (**8**) in a 96% yield. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **8** showed signals of the equatorial proton H-3_{eq} at 2.66 ppm, suggestive of an α-anomeric configuration (ranges for α-linked sialyl derivatives: H-3_{eq} at 2.6—2.8 ppm).[11] The replacement of the *N*-acetyl group of **8** by an *N*-glycoloyl group was carried out first by alkylation with phosphorus pentachloride and methanol,[12] then reacylation by acetyl glycoloyl chloride to give the *N*-glycoloylamino compound (**9**) in a 51% yield. The presence of the acetoxymethyl group of **9** was suggested by the



1: R= Ac **2**: R= H

3: R^1= Ac, R^2= AcNH, R^3= H **4**: R^1= H, R^2= AcNH, R^3= H

5: R^1= Ac, R^2= $AcOCH_2CONH$, R^3= Me **6**: R^1= Ac, R^2= AcO, R^3= H

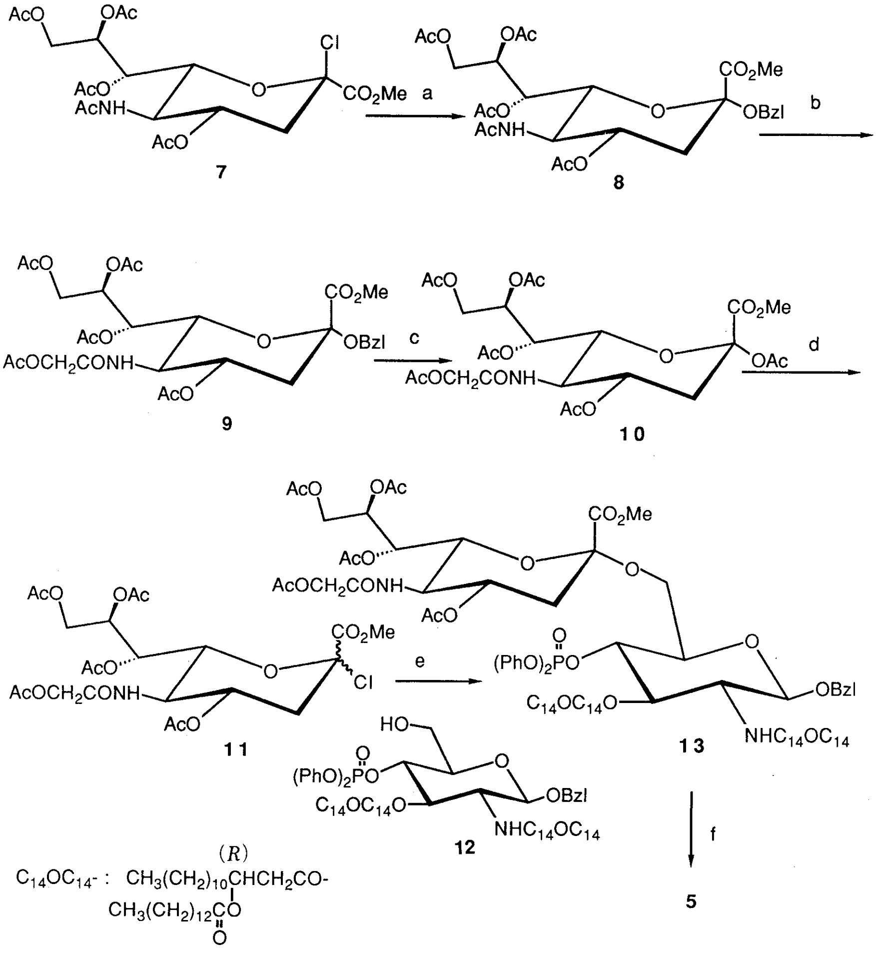


$$C_{14}OC_{14}\text{-}:\ CH_3(CH_2)_{10}\overset{(R)}{\underset{\substack{|\\ CH_3(CH_2)_{12}\underset{\substack{\|\\ O}}{C}O}}{CH}}CH_2CO\text{-}$$

Chart 1

^{1}H-NMR spectrum [δ 2.19 (3H, s), 4.31, 4.60 (each 1H, d, J=15.4 Hz)]. Removal of the benzyl group of **9** was performed by hydrogenation in the presence of palladium–carbon followed by acylation with acetic anhydride and pyridine to give **10**[13] in a 57% overall yield. Treatment of **10** with acetyl chloride saturated with dry HCl at room temperature for 24 h gave the 2-chloro compound (**11**) in quantitative yield. The unstable compound (**11**) was used for subsequent glycosylation without further purification, because of its instability. In our previous investigations,[3e,f] 2-deoxy-4-*O*-phosphono-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucose showed some distinct biological activities, such as antitumor activity. Therefore, we used benzyl 2-deoxy-4-*O*-phosphono-[(*R*)-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glycopyranoside (**12**) carrying the tetradecanoyloxytetradecanoyl group at both N-2 and O-3 of the glucosamine residue, as the reducing part. Glycosylation of the glycosyl acceptor (**12**), easily prepared from benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene β-D-glucopyranoside[3d] in 5 steps, with the glycosyl donor (**11**) in the presence of $Hg(CN)_2$, $HgBr_2$ and Molecular Sieves (MA) 4A in CH_2Cl_2 at room temperature for 2 d gave the desired (α 2→6) linked disaccharide (**13**α) in an 11% yield, and its β-anomer (**13**β) in a 6% yield, respectively, after separation by preparative thin layer chromatography. The signal due to the H-3_{eq} proton of the sialosyl moiety of **13**α was observed at 2.70 ppm (α-anomer). The IR spectrum of **13**α showed characteristic absorptions of P–O–Ph at 955 cm^{-1}. The protective benzyl and phenyl groups of **13**α were cleaved stepwise by hydrogenation by palladium–carbon and then platinum oxide as catalysts in MeOH to give the penta-*O*-acetyl *N*-glycoloylneuraminyl glucosamine-4-phosphate derivative (**5**) in a 17% yield. Compound **5** gave a positive test with the specific spray-reagent for phosphate.[14]

Next, the synthesis of penta-*O*-acetyl KDN-(α 2→6)-glucosamine-4-phosphate derivative (**6**) was carried out as follows. Benzyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl bromid) onate (**17**)[15] was synthesized by the following procedures. The hydroxyl group and carboxyl group of **14** were successfully protected by acetylation with acetic anhydride/pyridine, then phenyldiazomethane, to give **16** in a 50% yield from **14**. The anomerical acetyl group of **16** was quantitatively converted into the 2-bromo derivative (**17**) with titanium(IV) bromide in ethyl acetate at room temperature for 24 h. Glycosylation of **12** with **17** in the presence

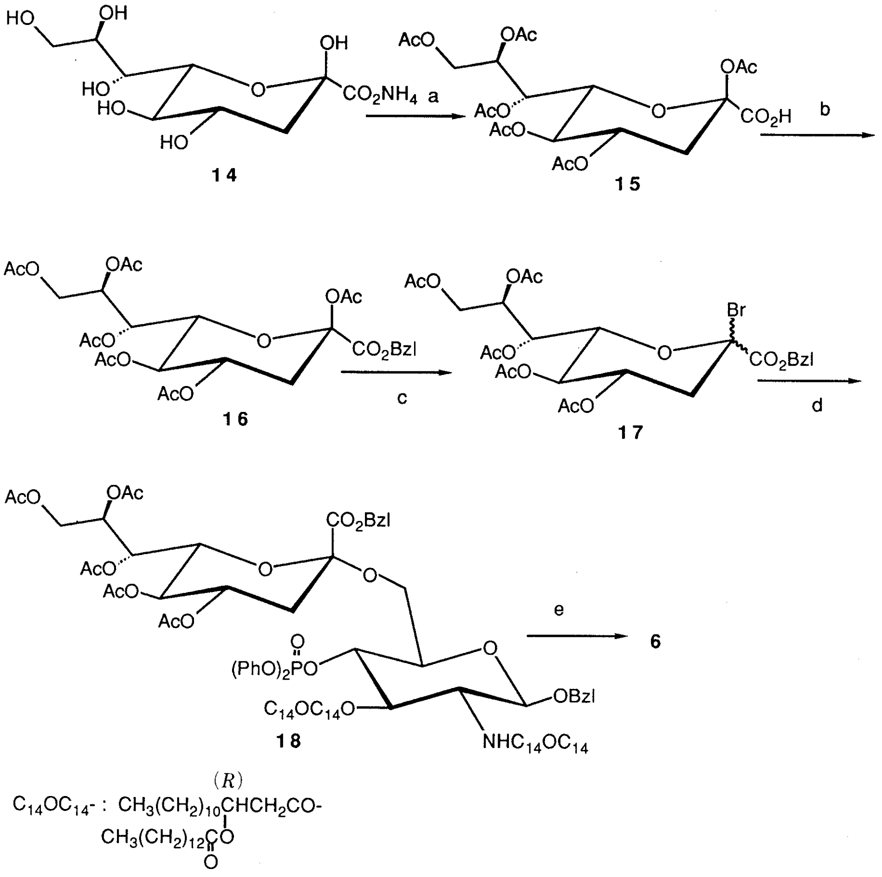


reagents : a) Ag salicylate, BzlOH ; b) i) PCl_5, then MeOH, ii) $AcOCH_2COCl$, DMAP, pyridine ;

c) i) Pd / C, H_2, ii) Ac_2O, DMAP, pyridine; d) AcCl, HCl ; e) **12**, $Hg(CN)_2$, $HgBr_2$, MS4A ;

f) i) Pd / C, H_2, ii) PtO_2, H_2

Chart 2



reagents : a) Ac_2O, pyridine; b) $PhCHN_2$; c) $TiBr_4$; d) **12**, $Hg(CN)_2$, $HgBr_2$, MS4A ; e) i) Pd / C, H_2 , ii) PtO_2, H_2

Chart 3

of $Hg(CN)_2$, $HgBr_2$ and MS 4A in CH_2Cl_2 at room temperature for 2 d gave the α (2→6) linked disaccharide (**18**α) in a 20% yield, and its β-anomer (**18**β) in a 17% yield, respectively. The stereochemistry of **18**α was confirmed by ^{1}H-NMR spectrum of the chemical shift of the H-3_{eq} proton of the sialosyl moiety of **18**α at 2.68 ppm. Finally, deprotection of **18**α as described for the preparation of **5**, gave compound **6** in a 37% yield. The structures of all compounds were characterized by ^{1}H-NMR and infrared (IR) spectroscopy and elemental analyses.

Preliminary examination of the biological activity showed that two chemically synthesizing disaccharides (**5**), (**6**) possessed mitogenic activities and weak antitumor activities.

Experimental

All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. IR spectra were recorded on a JASCO A-202 infrared spectrophotometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-FX 90 (90 MHz) and JEOL JNM-GX 270 (270 MHz) spectrometer with tetramethylsilane (in $CDCl_3$) as an internal standard, and the chemical shifts are given in δ values. The abbreviations of signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Column chromatography was carried out on Silica gel 60 (70—230 mesh, Merck). Thin layer chromatography (TLC) on Silica gel 60-F_{254} (Merck) was used to monitor the reaction and to ascertain the purity of the reaction products. The spots were visualized by spraying with a 5% aqueous sulfuric acid solution and then heating.

Methyl (Benzyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (8) Silver salicylate (5.38 g, 22.1 mmol) was added to a stirred solution of compound **7**, freshly prepared from *N*-acetylneuraminic acid (4.75 g, 14.7 mmol) and benzyl alcohol (10 ml) in CH_2Cl_2 (90 ml) at room temperature in the dark under argon. After stirring for 5 h, the precipitate was filtered off and the filtrate was washed successively with ice-cold 5% aqueous $NaHCO_3$, 5% aqueous $Na_2S_2O_3$, brine and dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was chromatographed on a column of silica gel with $CHCl_3$–acetone (5 : 1) to give **8** (8.23 g, 96.4%), mp 64—65 °C. $[\alpha]_D^{19}$ +2.38° (c=1.79, $CHCl_3$). IR (KBr): 1740 (ester), 1650 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.89 (3H, s, AcN), 1.95 (1H, dd, $J_{3a,3e}$=12.8, $J_{3a,4}$=8.1 Hz, H-3_{ax}), 2.03, 2.04, 2.14, 2.17 (each 3H, s, OAc), 2.66 (1H, dd, $J_{3e,4}$=4.6 Hz, H-3_{eq}), 3.68 (3H, s, OMe), 4.03—4.17 (2H, m, H-5,6), 4.15 (1H, dd, $J_{9,9'}$=12.4, $J_{8,9'}$=2.7 Hz, H-9'), 4.34 (1H, dd, $J_{8,9}$=3.0 Hz, H-9), 4.44 (1H, d, J_{gem}=11.6 Hz, OCH_2Ph), 4.82 (1H, d, OCH_2Ph), 4.89 (1H, ddd, $J_{4,5}$=10.5 Hz, H-4), 5.14 (1H, br d, $J_{NH,5}$=9.2 Hz, NH), 5.35 (1H, dd, $J_{7,8}$=8.4, $J_{6,7}$=2.2 Hz, H-7), 5.46 (1H, ddd, H-8), 7.26—7.35 (5H, m, aromatic H). *Anal.* Calcd for $C_{27}H_{30}NO_{13}\cdot H_2O$: C, 54.55; H, 5.42; N, 2.36. Found; C, 54.18; H, 5.77; N, 2.47.

Methyl (Benzyl 5-Acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (9) Phosphorus pentachloride (2.17 g, 10.4 mmol) was added to a solution of **g** (3.03 g, 5.21 mmol) and *N*,*N*-dimethylaniline (2.52 g, 20.8 mmol) in dry CH_2Cl_2 (40 ml), and cooled at −35 °C with stirring. The mixture was stirred at the same temperature for 7 h, then MeOH (15 ml) was added to the mixture. After stirring for 2.5 h at −35 °C, H_2O (10 ml) was added to the mixture, warmed at room temperature and stirred overnight. The resulting mixture was washed with aqueous saturated $NaHCO_3$ and brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness *in vacuo*. The residual oil was redissolved in dry CH_2Cl_2 (40 m), pyridine (1.24 g, 15.6 mmol) and 4-dimethylaminopyridine (1.27 g, 10.4 mmol), and acetyl glycoloyl chloride (2.13 g, 15.6 mmol) was added to the mixture which was cooled at 0 °C and kept at 0 °C for 0.5 h, then warmed at room temperature. After stirring overnight, the resulting mixture was washed with saturated aqueous $NaHCO_3$, and brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was purified by a column of silica gel ($CHCl_3$–acetone = 10 : 1) to give **9** (1.68 g, 51%), mp 63—64 °C.

$[\alpha]_D^{20}$ −1.29° (c=1.40, $CHCl_3$). IR (KBr): 3354 (NH), 1749 (ester), 1650 (amide), 699 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.01, 2.04, 2.14, 2.18, 2.19 (each 3H, s, Ac), 2.03 (1H, t, $J_{3a,3e}=J_{3a,4}=12.7$ Hz, H-3_{ax}), 2.68 (1H, dd, $J_{3e,4}=4.9$ Hz, H-3_{eq}), 3.80 (3H, s, OMe), 4.08 (1H, ddd, $J_{4,5}=J_{NH,5}=J_{6,5}=10.0$ Hz, H-5), 4.12 (1H, dd, $J_{9,9'}=12.4$, $J_{8,9'}=5.7$ Hz, H-9′), 4.22 (1H, dd, $J_{6,7}=2.2$ Hz, H-6), 4.35 (1H, dd, $J_{8,9}=2.7$ Hz, H-9), 4.31 (1H, d, $J_{gem}=15.4$ Hz, $OC\underline{H}_2OAc$), 4.44 (1H, d, $J_{gem}=11.9$ Hz, OCH_2Ph), 4.60 (1H, d, $OC\underline{H}_2OAc$), 4.83 (1H, d, OCH_2Ph), 4.96 (1H, ddd, H-4), 5.30 (1H, dd, $J_{7,8}=8.1$ Hz, H-7), 5.47 (1H, ddd, H-8), 5.85 (1H, br d, NH), 7.28—7.34 (5H, m, aromatic H). *Anal.* Calcd for $C_{29}H_{32}NO_{15}\cdot 2H_2O$: C, 51.94; H, 5.41; N, 2.09. Found: C, 51.52; H, 5.89; N, 2.04.

Methyl (5-Acetoxyacetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate (10) Compound **9** (0.43 g, 0.67 mmol) in methanol (2 ml) was hydrogenated in the presence of 10% Pd-on-carbon (0.05 g) at room temperature for 5 h. The catalyst was filtrated and the filtrate was concentrated to dryness *in vacuo*. The residue was redissolved in dry CH_2Cl_2 (2 ml), pyridine (0.08 g, 1.0 mmol) and 4-dimethylaminopyridine (DMAP) (0.061 g, 0.5 mmol), and Ac_2O (0.10 g, 1.0 mmol) was added to the mixture cooled at 0 °C. After stirring at room temperature overnight, the resulting mixture was washed successively with ice-cold 1 N HCl, aqueous saturated $NaHCO_3$, and brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was purified by a column of silica gel ($CHCl_3$–acetone = 5 : 1) to give **10** (0.225 g, 57%), as a syrup. $[\alpha]_D^{23}$ +1.83° (c=0.76, $CHCl_3$). IR (film): 3354 (NH), 1742 (ester), 1650 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.02, 2.04, 2.07, 2.14, 2.16, 2.20 (each 3H, s, Ac), 2.10 (1H, dd, $J_{3a,3e}=13.8$, $J_{3a,4}=11.1$ Hz, H-3_{ax}), 2.56 (1H, dd, $J_{3e,4}=5.1$ Hz, H-3_{eq}), 3.80 (3H, s, OMe), 4.09 (1H, ddd, $J_{5,6}=10.8$, $J_{4,5}=9.2$, $J_{NH,5}=9.5$ Hz, H-5), 4.14 (1H, dd, $J_{9,9'}=12.4$, $J_{8,9'}=6.2$ Hz, H-9′), 4.18 (1H, dd, $J_{5,6}=10.8$, $J_{6,7}=2.2$ Hz, H-6), 4.30 (1H, d, $J_{gem}=15.4$ Hz, $OC\underline{H}_2OAc$), 4.49 (1H, dd, $J_{8,9}=2.7$ Hz, H-9), 4.61 (1H, d, $OC\underline{H}_2OAc$), 5.09 (1H, ddd, $J_{4,5}=9.2$ Hz, H-4), 5.32 (1H, dd, $J_{7,8}=5.4$ Hz, H-7), 5.34 (1H, ddd, H-8), 5.95 (1H, d, NH). *Anal.* Calcd for $C_{24}H_{28}NO_{16}\cdot H_2O$: C, 47.69; H, 5.00; N, 2.32. Found: C, 47.99; H, 5.32; N, 2.43.

Methyl (5-Acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chlorid)onate (11) Compound **10** (0.067 g, 0.11 mmol) was dissolved in AcCl (5 ml) and cooled at −5—−10 °C. Dry hydrogen chloride gas was passed through the solution for 10 min. The mixture was kept at room temperature for 24 h and concentrated to syrup. After further coevaporations of the syrup from dry toluene (3 × 5 ml) and then dry ether (5 × 5 ml), the resulting residue was dried under reduced pressure to give a white powder **11** (quantitative). The unstable chloride (**11**) was used for the subsequent glycosylation without further purification.

Benzyl [2-Deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(methyl 5-acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α,β-D-*galacto*-2-nonulopyranosyl)-4-*O*-diphenylphosphono-β-D-glucopyranoside (13) Compound **12** (0.36 g, 0.64 mmol), pulverized MS 4A (0.5 g), $Hg(CN)_2$ (0.11 g, 0.45 mmol), and $HgBr_2$ (0.069 g, 0.19 mmol) were dried by a high vacuum-pump for 3 h. The mixture was dissolved in dry CH_2Cl_2 (3 ml) and stirred at room temperature for 1 h under argon. The chloride **11** in dry CH_2Cl_2 (2 ml) was added to the mixture over a period of 1 h, and then the suspension was stirred at room temperature for 4 h under argon. The resulting mixture was filtered off and the filtrate was washed with 10% aqueous KI solution and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated to dryness *in vacuo*. The residue was applied to a column of silica gel with ether to give **13α** and **13β**. The anomeric mixture (**13α** and **13β**) was further purified by preparative TLC ($CHCl_3$–acetone = 20 : 1) to give (α-linked) (**13α**) (0.135 g, 11%) and (β-linked) (**13β**) (0.072 g, 6%). **13α**: Syrup, $[\alpha]_D^{22}$ −11.0° (c=0.14, $CHCl_3$). IR (film): 3352 (NH), 1749 (ester), 1650 (amide), 955 (PO), 700 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (12H, t, $J=5.9$ Hz, $-CH_3$), 1.25 (80H, br s, $-CH_2-$), 1.99, 2.02, 2.14, 2.15, 2.19 (each 3H, s, Ac), 2.66 (1H, dd, $J=12.8$ Hz, H-3_{eq}), 3.72 (3H, s, OMe), 4.54 (2H, s, OCH_2O), 7.26—7.35 (15H, m, Ph). *Anal.* Calcd for $C_{103}H_{156}N_2O_{28}P\cdot H_2O$: C, 64.46; H, 8.30; N, 1.46. Found: C, 64.60; H, 8.33; N, 1.43. **13β**: Syrup, $[\alpha]_D^{22}$ −5.88° (c=0.10, $CHCl_3$). IR (film): 3352 (NH), 1751 (ester), 1654 (amide), 955 (PO), 700 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (12H, t, $J=5.8$ Hz, $-CH_3$), 1.26 (80H, br s, $-CH_2-$), 1.99, 2.01, 2.02, 2.06, 2.13 (each 3H, Ac), 2.48 (1H, dd, $J=5.1$, 13.5 Hz, H-3_{eq}), 3.72 (3H, s, OMe), 4.56 (2H, s, OCH_2O), 7.29—7.36 (15H, m, Ph). *Anal.* Calcd for $C_{103}H_{156}N_2O_{28}P$: C, 65.07; H, 8.27; N, 1.47. Found: C, 65.33; H, 8.75; N, 1.55.

2-Deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(methyl 5-acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-didoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)-4-*O*-phosphono-D-glucopyranose (5) Compound **13α** (0.135 g, 0.07 mmol) in MeOH (2 ml) was hydrogenated in the presence of 10% Pd-on-carbon (0.07 g) at 30 °C for 4 h. The catalyst was filtered off and Adams' platinum catalyst (0.135 g) was added to the filtrate. Hydrogenolysis was continued at 30 °C for 18 h. The catalyst was filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was purified by preparative TLC ($CHCl_3$–MeOH = 5 : 1) to give **5** (0.02 g, 17%), as a syrup. $[\alpha]_D^{18}$ +9.9° (c=0.38, $CHCl_3$), IR (film): 3405 (NH, OH), 1750 (ester), 1650 (amide), 1260 (HO–P) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (12H, t, $J=5.9$ Hz, $-CH_3$), 1.25 (80H, br s, $-CH_2-$), 1.93—2.20 (16H, m, OAc, H-3_{ax}), 3.72 (3H, s, OMe). *Anal.* Calcd for $C_{77}H_{136}N_2O_{28}P$: C, 58.95; H, 8.74; N, 1.79. Found: C, 58.62; H, 8.42; N, 1.69.

2,4,5,7,8,9-Hexa-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosylonic Acid (15) Ammonium 3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**14**) (0.242 g, 0.85 mmol) was dissolved in pyridine (2.5 ml) and DMAP (0.01 g, 0.82 mmol), and then acetic anhydride (2.5 ml) was added to the mixture cooled first at 0 °C, and then at room temperature for 1 d. The reaction mixture was diluted with CH_2Cl_2 and washed with ice-cold 1 N HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated to dryness *in vacuo*. The residue was purified by a column of silica gel ($CHCl_3$–MeOH = 5 : 1) to give **15** (0.20 g, 50%), mp 128—130 °C. $[\alpha]_D^{20}$ −19.5° (c=2.74, MeOH). IR (film): 1730 (ester), 1650 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 2.00, 2.02, 2.04, 2.07, 2.09, 2.13 (each 3H, s, OAc), 2.58 (1H, dd, $J_{3e,3a}=14.0$, $J_{3e,4}=5.4$ Hz, H-3_{eq}), 4.07 (1H, dd, $J_{5,6}=10.0$, $J_{6,7}=2.7$ Hz, H-6), 4.10 (1H, dd, $J_{9,9'}=12.1$, $J_{8,9'}=6.5$ Hz, H-9′), 4.51 (1H, dd, $J_{8,9}=2.2$ Hz, H-9), 4.93 (1H, t, $J=10.0$ Hz, H-5), 5.14 (1H, ddd, H-8), 5.28 (1H, ddd, H-4), and 5.39 (1H, dd, $J_{7,8}=7.0$ Hz, H-7), *Anal.* Calcd for $C_{21}H_{28}O_{15}$: C, 48.46; H, 5.42. Found: C, 48.13, H, 5.08.

Benzyl 2,4,5,7,8,9-Hexa-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonolopyranosonate (16) Phenyldiazomethane was added to a suspension of **15** (0.22 g, 0.42 mmol) and methanol (2 ml) with stirring at room temperature. The mixture was stirred at room temperature overnight, then the excess phenyldiazomethane was decomposed with acetic acid until the red color disappeared. The resulting solution was evaporated to dryness *in vacuo*. The resulting residue was purified on a column of silica gel ($CHCl_3$–isopropyl ether (PE) = 20 : 1) to give **16** (quantitative), as a syrup. $[\alpha]_D^{20}$ −15.4° (c=1.19, $CHCl_3$). IR (film): 1753 (ester), 700 (Ph) cm^{-1}. EI-MS m/z: 551 (M^+-59). ^{1}H-NMR ($CDCl_3$) δ: 2.00, 2.01, 2.02, 2.09, 2.11 (18H, s, OAc), 2.09 (1H, dd, $J_{3a,3e}=14.3$, $J_{3a,4}=11.8$ Hz, H-3_{ax}), 2.63 (1H, dd, $J_{3e,4}=5.1$ Hz, H-3_{eq}), 4.15 (1H, dd, $J_{9,9'}=12.4$, $J_{8,9'}=4.9$ Hz, H-9′), 4.20 (1H, dd, $J_{5,6}=10.3$ Hz, $J_{6,7}=2.7$ Hz, H-6), 4.42 (1H, dd, $J_{8,9}=2.7$ Hz, H-9), 4.97 (1H, t, $J=10.0$ Hz, H-5), 5.15 (1H, ddd, $J_{8,7}=6.8$ Hz, H-8), 5.16 (1H, d, $J_{gem}=12.4$ Hz, $-OC\underline{H}_2Ph$), 5.23 (1H, d, $-OC\underline{H}_2Ph$), 5.26 (1H, m, H-4), 5.39 (1H, dd, $J_{6,7}=2.7$ Hz, H-7), 7.34—7.36 (5H, m, aromatic H). *Anal.* Calcd for $C_{28}H_{34}O_{15}\cdot H_2O$: C, 53.50; H, 5.77. Found: C, 53.92; H, 5.48.

Benzyl (4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl bromid)onate (17) Compound **16** (0.12 g, 0.20 mmol) was dissolved in CH_2Cl_2–AcOEt (10 : 1) and MS 4A (0.10 g), and stirred at room temperature for 1 h. $TiBr_4$ (0.14 g, 0.37 mmol) was added to the mixture cooled at 0 °C, then stirred at the same temperature overnight. Acetonitrile (3 ml) and anhydrous NaOAc (1.0 g) was added to the reaction mixture, and stirred for 0.5 h, then toluene (5 ml) was added to the mixture and stirred a further 15 min. The insoluble materials were filtered off and the filtrate was evaporated to dryness *in vacuo*. The unstable bromide **17** was used for the subsequent glycosylation without further purification.

Benzyl 2-Deoxy-2-[(*R*)-3-tetradecanoyloxytetradecamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(benzyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α,β-D-*galacto*-2-nonulopyranosyl)-4-*O*-diphenylphosphono-β-D-glucopyranoside (18) Compound **12** (0.107 g, 0.18 mmol), $Hg(CN)_2$ (0.137 g, 0.54 mmol) and $HgBr_2$ (0.032 g, 0.09 mmol) was dried by the use of a high vacuum-pump for 3 h. The mixture was dissolved in dry CH_2Cl_2 (3 ml) and stirred at room temperature for 1 h under argon. The bromide **17** in dry CH_2Cl_2 (2 ml) was added at room temperature over a period of 1 h, and then the suspension was stirred at room temperature for 2 d. The resulting mixture was filtered off and the filtrate was washed with 10% aqueous KI solution and brine. The organic phase was concentrated to dryness *in vacuo* and the residue was purified by column chromatography on silica gel with $CHCl_3$–IPE = 20 : 1 to give **18α** and **18β**. The anomeric mixture (**18α** and **18β**) was further purified by preparative TLC to give (α-linked) **18α** (0.026 g, 20%) and (β-linked) **18β** (0.022 g, 17%), respectively. **18α**: Syrup, $[\alpha]_D^{23}$ −3.60° (c=1.20, $CHCl_3$). IR (film): 3352 (NH), 1749 (ester), 1650 (amide), 955 (PhOP), 700 (Ph) cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 0.89 (12H, t, $J=5.7$ Hz, –CH$_3$), 1.26 (80H, br s, –CH$_2$–), 1.85—2.15 (16H, m, OAc, H-3$_{ax}$), 2.68 (1H, dd, $J=4.4$, 13.4 Hz, H-3$_{eq}$), 5.20 (2H, s, PhCH$_2$), and 7.30—7.35 (20H, m, Ph). *Anal.* Calcd for $C_{107}H_{157}N_2O_{27}P$: C, 66.44; H, 8.18; N, 1.45. Found: C, 66.69; H, 8.63; N, 1.70 **18**β: Syrup, $[\alpha]_D^{22}$ $-2.91°$ ($c=0.10$, CHCl$_3$). IR (film): 3354 (NH), 1751 (ester), 1654 (amide), 955 (PhOP), 700 (Ph) cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 0.87 (12H, t, $J=5.9$ Hz, –CH$_3$), 1.25 (80H, br s, –CH$_2$–), 1.85—2.15 (16H, m, OAc, H-3$_{ax}$), 2.66 (1H, dd, $J=4.4$, 12.3 Hz, H-3$_{eq}$), 5.20 (2H, s, PhCH$_2$).

2-Deoxy-2-[(*R*)-3-tetradecanoyloxytetradecamido]-3-*O*-[(*R*)-3-tetradecanoxyloxytetradecanoyl]-6-*O*-(4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)-4-*O*-phosphono-D-glucopyranose (6) Compound **18**α (0.026 g, 0.013 mmol) in MeOH (1.0 ml) was hydrogenated in the presence of 10% Pd-on-carbon (0.013 g) at 30 °C for 4 h. The catalyst was filtered off and Adams' platinum catalyst (0.026 g) was added to the filtrate. Hydrogenolysis was continued at 30 °C for 18 h. The catalyst was filtered off and the filtrate was evaporated to an oil and applied to preparative TLC (CHCl$_3$–MeOH = 5 : 1) to give **6** (8 mg, 37%), as a syrup. $[\alpha]_D^{28}$ $-7.6°$ ($c=0.08$, CHCl$_3$). IR (film): 3405 (NH, OH), 1750 (ester), 1650 (amide), 1260 (HOP) cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 0.89 (12H, t, $J=7.1$ Hz, –CH$_3$), 1.27 (80H, br s, –CH$_2$–), 1.93—2.20 (16H, m, OAc, H-3$_{ax}$), 3.72 (3H, s, OMe). *Anal.* Calcd for $C_{81}H_{137}N_2O_{27}P$: C, 60.73; H, 8.62; N, 1.75. Found: C, 60.35; H, 8.17; N, 1.55.

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