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Neuraminic Acid and Related Compounds. I. Syntheses of Biologically Active 4',7',8',9'-Tetra-*O*-acetyl-sialyl- and Sialyl-(α 2-6)-D-glucosamine-4-phosphate Analogues of Lipid A

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The syntheses of novel tetraacetylsialyl- and sialyl-(α 2-6)-D-glucosamine-4-phosphate analogues of lipid A containing sialic acid in place of 3-deoxy-D-manno-2-octulosonic acid (KDO) are described. Preliminary examination of the biological activity revealed that two synthetic disaccharides possessed weak mitogenic activities.

Keywords—sialic acid; sialyl-glucosamine-4-phosphate; lipid A analog; mitogenic activity

Lipopolysaccharide (LPS) of gram-negative bacteria possesses a variety of biological activities and is composed of three structural regions; an inner part, a polysaccharide region and a lipid part called lipid A.¹⁾ 3-Deoxy-D-manno-2-octulosonic acid (KDO) as a ketosidic component of the polysaccharide part is attached to lipid A, a biologically active fragment of LPS. Recently, two research groups reported the structure of the KDO region of LPS from *Salmonella minnesota*²⁾ and *Escherichia coli*,³⁾ in which the KDO group was attached to lipid A with a (α 2-6) linkage.

It was also found by us and the other groups that the nonreducing sugar moiety of lipid A is more important than the other parts (lipid X and lipid Y) for expressing the biological activities of LPS.⁴⁾ Furthermore, tetraacetyl-KDO-⁵⁾ and KDO⁶⁾ (α 2-6)-D-glucosamine-4-phosphate analogues (**1** and **2** in Chart 1) possess mitogenic activity comparable to that of lipid A.

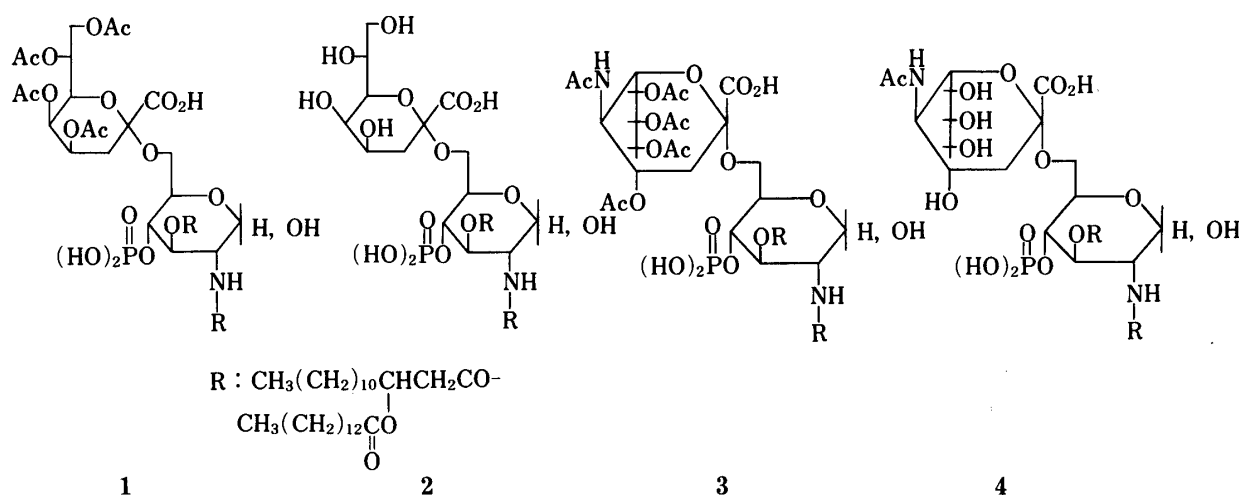


Chart 1

We report herein the syntheses of two new compounds, tetraacetylsialyl- and sialyl-(α 2—6)-D-glucosamine-4-phosphate analogues (**3** and **4** in Chart 1) of lipid A containing sialic acid, whose structure is similar to KDO and whose function has recently attracted interest.⁷⁾

First, we describe a synthetic sequence for the tetraacetyl-sialyl glucosamine-4-phosphate derivative (**3**). (Chart 2) Esterification of *N*-acetylneuraminic acid (**5**) with phenyldiazomethane in methanol at room temperature afforded the benzyl ester (**6**) in 76.9% yield. The hydroxyl groups of **6** were acetylated with acetic acid, pyridine, and 4-dimethylaminopyridine (DMAP) at room temperature for 20 h to give **7** in 73.8% yield. Treatment of **7** with acetyl chloride saturated with dry hydrogen chloride at room temperature for 24 h gave the 2-chloro compound (**8**) in nearly quantitative yield. The unstable chloride (**8**) was used for the subsequent glycosylation without further purification. In our previous investigations,^{4e,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-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]- β -D-glucopyranoside (**9**) carrying the tetradecanoyloxytetradecanoyl group at both N-2 and O-3 of the glucosamine residue, as the reducing part. The 6-OH group of **9** should be much more reactive than the 4-OH group and is therefore suitable as a substrate for the reaction with the halide (**8**). Glycosylation of the glycosyl acceptor (**9**), easily prepared from benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene- β -D-glucopyranoside in 2 steps,^{4c)} with the glycosyl donor (**8**) in the presence of Hg(CN)₂, HgBr₂, and Molecular Sieves 4A in CH₂Cl₂ at room temperature for 4 d gave the desired (2—6)-linked disaccharide containing sialic acid (**10**) in 34.0% yield as a mixture of anomers. Separation by preparative thin layer chromatography (TLC (CHCl₃—MeOH = 20 : 1)) afforded **10 α** and **10 β** in yields of 23.4 and 10.6%, respectively. The by-product of the reaction was mainly the 2,3-dehydrosialic acid derivative. The presence of an α -glycosidic linkage in **10 α** was indicated by the proton nuclear magnetic resonance (¹H-NMR) spectrum, which showed signals for the equatorial proton H-3_{eq} of sialic acid at 2.69 ppm (ranges for α -linked sialyl derivatives: H-3_{eq} at 2.6—2.8 ppm).⁸⁾ In the case of **10 β** , the H-3_{eq} signals overlapped with the methylene proton signals (2.22—2.67 ppm) of fatty acids in the ¹H-NMR spectrum.⁹⁾ Therefore, the confirmation of the β linkage of **10 β** was based on the ¹H-NMR spectral data (the H-3_{eq} signals of **10 β** were not observed downfield from 2.67 ppm). In addition, signals at 99.7 ppm (singlet) of **10 α** and 98.5 ppm (singlet) of **10 β** in the carbon-13 nuclear magnetic resonance (¹³C-NMR) spectrum were assigned to the α - and β -ketosidic carbon atom, respectively.¹⁰⁾ The structure of **10 α** was confirmed by the ¹H-NMR spectrum; the signals at 1.25 ppm were assigned to the methylene protons of fatty acids, and the signals at 1.84—2.22 ppm to the acetyl group protons. The 4-hydroxyl group of **10 α** was phosphorylated with diphenylphosphorochloridate, pyridine, and DMAP in CH₂Cl₂ to give the phosphate (**11**) in 57% yield. The infrared (IR) spectrum of **11** showed a characteristic band of >P—O—Ph at 955 cm⁻¹. The protective benzyl and phenyl groups were cleaved stepwise by hydrogenation in the presence of palladium-carbon and then of platinum oxide as catalysts in methanol solution to yield the tetraacetylsialyl glucosamine-4-phosphate derivative (**3**) in 23% yield as the free acid form. Compound **3** gave a positive test with the specific spray-reagent for phosphate.¹¹⁾

Next, for the synthesis of the sialyl glucosamine-4-phosphate derivative (**4**), we used the monochloroacetyl group in place of the acetyl group as a protecting group for the hydroxyl groups of sialic acid. The monochloroacetyl group can be removed selectively in the presence of other ester functions. The synthesis of **4** was carried out by a method similar to that used for **3**, as shown in Chart 2.

The hydroxyl groups of the benzyl ester (**6**) were acylated with monochloroacetic anhydride, pyridine, and DMAP to give the monochloroacetyl compound (**12**) in 77.7% yield.



The anomeric monochloroacetyl group of **12** was quantitatively converted into the 2-chloro derivative (**13**) as described for the preparation of **8**. Condensation of the two components, **9** and **13** in the presence of $\text{Hg}(\text{CN})_2$, HgBr_2 , and Molecular Sieves 4A in CH_2Cl_2 at room temperature for 2 d gave the expected ($\alpha 2 \rightarrow 6$) sialyl disaccharide (**14**) in poor yield (4.3%), as a single anomer. The ^1H -NMR spectrum of **14** showed signals of $\text{H}-3_{\text{eq}}$ at 2.65 ppm, suggestive of the α -anomeric configuration. The low yield can be accounted for by the lack of reactivity of **13**, probably due to the steric hindrance of monochloroacetyl groups. The stereoselective formation of the thermodynamically more stable α -anomer (**14a**) in glycosylation may be attributable to the low reactivity of **13**. Phosphorylation of the 4-hydroxyl group of **14** was effected with diphenylphosphorochloridate, pyridine, and DMAP to give the 4-phosphate (**15**). The monochloroacetyl groups of **15** were selectively removed by diisopropylethylamine and thiourea to afford **16** in 43% yield. The ^1H -NMR spectrum of **16** showed that **16** has no chloroacetyl groups. Finally, deprotection of **16** as described for **11** gave the ($\alpha 2 \rightarrow 6$) sialyl glucosamine-4-phosphate derivative (**4**) in 56% yield. The structure of **4** was assigned on the basis of the positive fast atom bombardment mass spectrometry (FAB-MS), which showed an $(\text{M} + \text{K})^+$ ion at m/z 1462. The structures of all compounds were characterized by ^1H -NMR and ^{13}C -NMR spectroscopies, as well as IR spectroscopy and elemental analyses.

Preliminary examination of the biological activity revealed that two synthetic disaccharides, **3** and **4**, possessed weak mitogenic activities.

Experimental

All melting points were determined with a micro-melting point apparatus (Yanagimoto) and are uncorrected. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. IR spectra were measured on a JASCO A-202 infrared spectrophotometer. ^1H -NMR spectra were recorded on a JEOL JNM-FX90Q (90 MHz) FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard. ^{13}C -NMR spectra were recorded on a JEOL JNM-FX90Q (22.5 MHz) FT-NMR spectrometer using tetramethylsilane (in CDCl_3) as an internal standard. Chemical shifts were recorded in values (δ) downfield from TMS, and the abbreviations of signal patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Thin layer chromatography (TLC) was performed on silica gel (Kiesel 60F₂₅₄ on aluminium sheet, Merck). All compounds were located by spraying with sulfuric acid and heating on a hot plate. Phosphorus-containing compounds were revealed by spraying with the phosphate-specific spray-reagent.¹¹⁾ Preparative TLC was performed on preparative layer chromatography plates (Kieselgel 60F₂₅₄, Merck). Column chromatography was performed on silica gel (Kiesel 60, 70–230 mesh, Merck). Evaporations were carried out under reduced pressure at 35 °C.

5-Acetamido-3,5-dideoxy-D-glycero- β -D-galacto-2-nonulosonic Acid Benzyl Ester (6)—Phenyldiazomethane (about 9 mmol) was added dropwise to a suspension of *N*-acetylneuraminic acid (**5**) (1.50 g, 4.85 mmol) and methanol (80 ml) with stirring. The mixture was stirred at room temperature for 3 h, then the excess of phenyldiazomethane was decomposed with acetic acid until the red color disappeared. The resulting solution was evaporated to dryness. The residue was purified on a column of silica gel (chloroform : methanol = 3 : 1) to give **6** (1.24 g, 76.9%), mp 163–165 °C. $[\alpha]_{\text{D}}^{20} -21.9^\circ$ ($c = 1.0$, methanol). IR (KBr): 3360, 1755, 1661, 687 cm^{-1} . ^1H -NMR (CD_3OD) δ : 2.01 (3H, s, $-\text{COCH}_3$), 2.25 (1H, dd, $J = 4.5, 12.8 \text{ Hz}$, 3-H_{eq}), 5.22 (2H, s, $-\text{COOCH}_2\text{Ph}$), 7.31–7.36 (5H, m, $-\text{CH}_2\text{Ph}$). Anal. Calcd for $\text{C}_{18}\text{H}_{23}\text{NO}_9$: C, 54.13; H, 6.13; N, 3.51. Found: C, 54.23; H, 6.23; N, 3.73.

5-Acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-D-glycero- β -D-galacto-2-nonulosonic Acid Benzyl Ester (7)—Acetic anhydride was added to a solution of **6** (1.24 g, 4 mmol), pyridine (3.16 g, 40 mmol), and 4-dimethylaminopyridine (1.22 g, 10 mmol) in dichloromethane (20 ml) cooled at 0 °C with stirring. The mixture was warmed at room temperature and stirred for 15 h. The resulting mixture was washed with 1 N HCl, saturated aqueous NaCl, saturated aqueous NaHCO_3 , and saturated aqueous NaCl. The organic phase was dried (MgSO_4) and concentrated to dryness. The residue was purified on a column of silica gel (chloroform : ether : acetone = 1 : 2 : 1) to afford **7** (1.80 g, 73.8%), mp 113–115 °C. $[\alpha]_{\text{D}}^{20} -29.1^\circ$ ($c = 1.0$, CHCl_3). IR (KBr): 3400, 1756, 1671, 700 cm^{-1} . ^1H -NMR (CDCl_3) δ : 1.83–2.28 (19H, m, $-\text{COCH}_3 \times 6$ and 3-H_{ax}), 2.56 (1H, dd, $J = 5.1, 13.2 \text{ Hz}$, 3-H_{eq}), 5.20 (2H, s, $-\text{COOCH}_2\text{Ph}$), 7.35 (5H, s, $-\text{CH}_2\text{Ph}$). Anal. Calcd for $\text{C}_{28}\text{H}_{35}\text{NO}_{14}$: C, 55.17; H, 5.79; N, 2.30. Found: C, 55.13; H, 6.16; N, 2.18.

5-Acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-tri-deoxy-D-glycero- β -D-galacto-2-nonulosonic Acid Benzyl Ester (8)—Compound **7** (200 mg, 0.33 mmol) was dissolved in acetyl chloride (7 ml) and cooled in -5°C – -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 a syrup. After further coevaporations of the syrup from toluene ($3 \times 5 \text{ ml}$)

and then ether (5 × 5 ml), the residue was dried under reduced pressure to give a white powder (**8**) (quantitative). TLC (ethyl acetate, $R_f=0.62$) showed the reaction to be complete.

Benzyl 2-Deoxy-2-[(R)-3-tetradecanoyloxytetradecanamido]-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]-6-O-(benzyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α , β -D-galacto-2-nonulopyranosyl)- β -D-glucopyranoside (10 α** and **10 β**)**—Benzyl 2-deoxy-2-[(R)-3-tetradecanoyloxytetradecanamido]-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]- β -D-glucopyranoside (**9**) (366 mg, 3.28×10^{-4} mol), pulverized Molecular Sieves 4A (230 mg), Hg(CN)₂ (58 mg, 2.30×10^{-4} mol), and HgBr₂ (28 mg, 7.65×10^{-5} mol) were dried by the use of a high vacuum-pump for 2 h. The mixture was dissolved in dichloromethane (5 ml) and stirred at room temperature for 1 h under argon. The chloride (**8**) (192 mg, 3.28×10^{-4} mol) in CH₂Cl₂ (5 ml) was added to the mixture over a period of 1 h, and then the suspension was stirred at room temperature for 4 d. The resulting mixture was filtered and the filtrate was washed with 10% aqueous KI solution and saturated aqueous NaCl. The organic phase was concentrated to dryness and the residue was purified on a column of silica gel with ether to yield **10 α** and **10 β** . The anomeric mixture (**10 α** and **10 β**) was further purified by preparative TLC to afford (α -linked) **10 α** (108 mg, 23.4%) and (β -linked) **10 β** (51 mg, 10.6%). **10 α** : mp 73–76 °C. $[\alpha]_D^{20} -6.91^\circ$ ($c=0.9$, CHCl₃). IR (KBr): 3352, 1740, 1658, 697 cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.88 (12H, t, $J=5.9$ Hz, $-\text{CH}-(\text{CH}_2)_{10}\text{CH}_3 \times 2$ and $-\text{CO}(\text{CH}_2)_{12}\text{CH}_3 \times 2$), 1.25 (80H, s, $-\text{CH}-\text{CH}_2(\text{CH}_2)_9\text{CH}_3 \times 2$ and $-\text{COCH}_2(\text{CH}_2)_{11}\text{CH}_3 \times 2$), 1.84–2.22 (16H, m, $\text{CH}_3\text{CO} \times 5$ and H-3_{ax}), 2.22–2.64 (8H, m, $\text{COCH}_2 \times 4$), 2.69 (1H, dd, $J=4.4$, 14.4 Hz, 3-H_{eq}), 5.21 (2H, s, $-\text{COOCH}_2\text{Ph}$), 5.85 (1H, d, $J=9.0$ Hz, $-\text{NH}-$), 7.29, 7.36 (10H, s, $-\text{CH}_2\text{Ph} \times 2$). ¹³C-NMR (CDCl₃) δ : 99.7 (s, ketosidic carbon atom), 100.3 (d, glycosidic carbon atom). Anal. Calcd for C₉₅H₁₅₄N₂O₂₃: C, 67.42; H, 9.17; N, 1.65. Found: C, 67.75; H, 8.93; N, 1.63. **10 β** : Amorphous. $[\alpha]_D^{20} -13.1^\circ$ ($c=0.94$, CHCl₃). IR (KBr): 3400, 1754, 1668, 702 cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.88 (12H, t, $J=5.9$ Hz, $-\text{CH}-(\text{CH}_2)_{10}\text{CH}_3 \times 2$ and $-\text{CO}(\text{CH}_2)_{12}\text{CH}_3 \times 2$), 1.25 (80H, s, $-\text{CH}-\text{CH}_2(\text{CH}_2)_9\text{CH}_3 \times 2$ and $-\text{COCH}_2(\text{CH}_2)_{11}\text{CH}_3 \times 2$), 1.89–2.18 (16H, m, $\text{CH}_3\text{CO} \times 5$ and H-3_{ax}), 2.22–2.67 (9H, m, $\text{COCH}_2 \times 4$ and H-3_{eq}), 5.23 (2H, s, $-\text{COOCH}_2\text{Ph}$), 5.85 (1H, d, $J=9.3$ Hz, $-\text{NH}-$), 7.32, 7.35 (10H, s, $-\text{CH}_2\text{Ph} \times 2$). ¹³C-NMR (CDCl₃) δ : 98.5 (s, ketosidic carbon atom), 100.2 (s, glycosidic carbon atom). Anal. Calcd for C₉₅H₁₅₄N₂O₂₃·2H₂O: C, 66.02; H, 9.21; N, 1.62. Found: C, 66.20; H, 9.07; N, 1.52.

Benzyl 2-Deoxy-2-[(R)-3-tetradecanoyloxytetradecanamido]-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]-6-O-(benzyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosyl)-4-O-diphenylphosphono- β -D-glucopyranoside (11**)**—Diphenylphosphorochloridate (36 mg, 1.33×10^{-4} mol) was added to a solution of **10 α** (45 mg, 2.66×10^{-5} mol), 4-dimethylaminopyridine (16 mg, 1.33×10^{-4} mol), and pyridine (11 mg, 1.33×10^{-4} mol) in CH₂Cl₂ (5 ml) at 0 °C under argon. The reaction mixture was warmed at room temperature and stirred for 3 h. The solution was washed with saturated aqueous NaHCO₃ and saturated aqueous NaCl. The organic phase was dried with MgSO₄ and evaporated. Purification of the residue was achieved with preparative TLC (chloroform:methanol=20:1) to give **11** (29 mg, 57%), mp 49–52 °C. $[\alpha]_D^{21} -8.89^\circ$ ($c=0.36$, CHCl₃). IR (KBr): 3352, 1740, 1658, 955, 697 cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.88 (12H, t, $J=5.6$ Hz, $-\text{CH}-(\text{CH}_2)_{10}\text{CH}_3 \times 2$ and $-\text{CO}(\text{CH}_2)_{12}\text{CH}_3 \times 2$), 1.25 (80H, s, $-\text{CH}-\text{CH}_2(\text{CH}_2)_9\text{CH}_3 \times 2$ and $-\text{COCH}_2(\text{CH}_2)_{11}\text{CH}_3 \times 2$), 1.84–2.22 (16H, m, $\text{CH}_3\text{CO} \times 5$ and H-3_{ax}), 2.69 (1H, dd, $J=4.6$, 12.9 Hz, 3-H_{eq}), 5.21 (2H, s, $-\text{COOCH}_2\text{Ph}$), 5.81 (1H, d, $J=8.8$ Hz, $-\text{NH}-$), 7.29, 7.36 (20H, s, $-\text{CH}_2\text{Ph} \times 2$ and $(\text{PhO})_2\text{PO}-$). Anal. Calcd for C₁₀₇H₁₆₃N₂O₂₆P: C, 66.78; H, 8.54; N, 1.46. Found: C, 66.39; H, 9.01; N, 1.33.

2-Deoxy-2-[(R)-3-tetradecanoyloxytetradecanamido]-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]-6-O-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosyl)-4-O-phosphono-D-glucopyranose (3**)**—The phosphate (**11**) (16 mg, 8.31×10^{-6} mol) in methanol (0.3 ml) was hydrogenated in the presence of 10% Pd-on-carbon (8 mg) at 40 °C for 4 h. The catalyst was filtered off and Adams platinum catalyst (10 mg) was added to the filtrate. Hydrogenolysis was continued at 35 °C for 15 h and TLC (chloroform:methanol=5:1) showed the reaction to be complete. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was purified by preparative TLC (chloroform:methanol=10:3) to give **3** (3 mg, 23%), mp 41–44 °C. $[\alpha]_{450}^{21} -55.6^\circ$ ($c=0.018$, methanol). IR (KBr): 3352, 1742, 1650, 1262 cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.88 (12H, t, $J=6.1$ Hz, $-\text{CH}-(\text{CH}_2)_{10}\text{CH}_3 \times 2$ and $-\text{CO}(\text{CH}_2)_{12}\text{CH}_3 \times 2$), 1.25 (80H, s, $-\text{CH}-\text{CH}_2(\text{CH}_2)_9\text{CH}_3 \times 2$ and $-\text{COCH}_2(\text{CH}_2)_{11}\text{CH}_3 \times 2$), 1.93–2.20 (16H, m, $\text{CH}_3\text{CO} \times 5$ and H-3_{ax}). Anal. Calcd for C₈₁H₁₄₃N₂O₂₆P: C, 61.11; H, 9.05; N, 1.76. Found: C, 61.49; H, 9.05; N, 1.66.

5-Acetamido-2,4,7,8,9-penta-O-monochloroacetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulosonic Acid Benzyl Ester (12**)**—Monochloroacetic anhydride (4.44 g, 2.60×10^{-2} mol) in CH₂Cl₂ (45 ml) was added to a suspension of pyridine (2.06 g, 2.60×10^{-2} mol), 4-dimethylaminopyridine (790 mg, 6.50×10^{-3} mol), and the benzyl ester (**6**) (1.58 g, 2.60×10^{-3} mol) in CH₂Cl₂ (45 ml) at 0 °C. The resulting mixture was stirred at 0 °C for 3 h, and then at room temperature for 15 h. The solution was worked up in the same manner as described for **7**. The residue was chromatographed on silica gel with CH₂Cl₂:ether (2:1) to afford **12** (1.58 g, 77.7%), mp 58–59 °C. $[\alpha]_D^{21} -20.1^\circ$ ($c=1.6$, CHCl₃). IR (KBr): 3276, 1769, 1689, 698 cm⁻¹. ¹H-NMR (CDCl₃) δ : 1.90 (3H, s, $\text{CH}_3\text{CO}-$), 2.65 (1H, dd, $J=5.0$, 13.2 Hz, 3-H_{eq}), 3.99–4.25 (10H, m, $\text{ClCH}_2\text{CO}- \times 5$), 5.23 (2H, s, $\text{PhCH}_2\text{OCO}-$), 6.14 (1H, d, $J=9.3$ Hz, $-\text{NH}-$), 7.36 (5H, s, $-\text{CH}_2\text{Ph}$). Anal. Calcd for C₂₈H₃₀Cl₅NO₁₄: C, 43.02; H, 3.87; N, 1.79. Found: C, 43.38; H, 3.80; N, 1.90.

5-Acetamido-4,7,8,9-tetra-O-monochloroacetyl-2-chloro-2,3,5-trideoxy-D-glycero- α -D-galacto-2-nonulosonic Acid Benzyl Ester (13**)**—The monochloroacetyl compound (**12**) (250 mg, 3.20×10^{-4} mol) was dissolved in acetyl

chloride (6 ml) saturated with dry hydrogen chloride gas at -5°C — -10°C . The solution was stirred at room temperature for 24 h and concentrated to dryness. Traces of acetyl chloride, monochloroacetic acid, and hydrogen chloride were removed by coevaporation from toluene (5×5 ml) and then ether (5×5 ml) to yield **13** (quantitative). TLC (CH_2Cl_2 : ether = 5:1) showed the reaction to be complete.

Benzyl 2-Deoxy-2-[(R)-3-tetradecanoyloxytetradecanamido]-3-O-[(R)-tetradecanoyloxytetradecanoyl]-6-O-(benzyl-5-acetamido-3,5-di-deoxy-D-glycero- α -D-galacto-2-nonulopyranosyl)- β -D-glucopyranoside (14)—The glucosamine derivative (**9**) (366 mg, 3.23×10^{-4} mol), pulverized Molecular Sieves 4A (400 mg), $\text{Hg}(\text{CN})_2$ (58 mg, 2.30×10^{-4} mol), and HgBr_2 (28 mg, 7.65×10^{-5} mol), dried with a high vacuum-pump for 2 h, were dissolved in CH_2Cl_2 (7 ml) and the mixture was stirred under argon for 1 h. The chloride (**13**) (231 mg, 3.20×10^{-4} mol) in CH_2Cl_2 (7 ml) was then added dropwise, and the whole was stirred at room temperature for 2 d. The resulting mixture was worked up as described for **10**. The crude product (**14**) was purified by a column of silica gel (CH_2Cl_2 : ether = 5:1) to afford **14** (25 mg, 4.3%), mp $32-34^{\circ}\text{C}$. $[\alpha]_D^{21} -13.3^{\circ}$ ($c=0.18$, CHCl_3). IR (KBr): 3296, 1746, 1663, 700 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 0.88 (12H, t, $J=5.9$ Hz, $-\text{CH}-(\text{CH}_2)_{10}\text{CH}_3 \times 2$ and $-\text{CO}(\text{CH}_2)_{12}\text{CH}_3 \times 2$), 1.25 (80H, s, $-\text{CH}-\text{CH}_2(\text{CH}_2)_9\text{CH}_3 \times 2$ and $-\text{COCH}_2(\text{CH}_2)_{11}\text{CH}_3 \times 2$), 1.89 (3H, s, $\text{CH}_3\text{CO}-$), 2.65 (1H, dd, $J=5.4$, 14.4 Hz, $3-\text{H}_{\text{eq}}$), 3.98–4.26 (8H, m, $-\text{COCH}_2\text{Cl} \times 4$), 5.22 (2H, s, $-\text{COOCH}_2\text{Ph}$), 7.42, 8.19 (10H, m, $\text{PhCH}_2- \times 2$). Anal. Calcd for $\text{C}_{95}\text{H}_{150}\text{Cl}_4\text{N}_2\text{O}_{23}$: C, 62.35; H, 8.26; N, 1.63. Found: C, 62.42; H, 8.26; N, 1.42.

Benzyl 2-Deoxy-2-[(R)-3-tetradecanoyloxytetradecanamido]-3-O-[(R)-3-tetradecanoyloxytetradecanol]-6-O-(benzyl-5-acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosyl)-4-O-diphenylphosphono- β -D-glucopyranoside (15)—Diphenylphosphorochloridate (22 mg, 8.32×10^{-6} mol) was added dropwise to a solution of the disaccharide (**14**), pyridine (7 mg, 8.32×10^{-5} mol), and 4-dimethylaminopyridine (10 mg, 8.32×10^{-5} mol) in CH_2Cl_2 under argon at 0°C . The mixture was stirred at room temperature for 3 h and worked up as described for **11**. The crude product (**11**), thiourea (13 mg, 1.66×10^{-4} mol), and diisopropylethylamine (22 mg, 1.66×10^{-4} mol) were dissolved in tetrahydrofuran (THF) (3 ml), and then refluxed for 3 h. The solvent was evaporated off and the residue was purified by preparative TLC (CHCl_3 : methanol = 5:1) to give **15** (6 mg, 43%), mp $64-67^{\circ}\text{C}$. $[\alpha]_D^{21} -7.7^{\circ}$ ($c=0.26$, CHCl_3). IR (KBr): 3400, 1742, 1675, 972, 695 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 0.88 (12H, t, $J=6.1$ Hz, $-\text{CH}-(\text{CH}_2)_{10}\text{CH}_3 \times 2$ and $-\text{CO}(\text{CH}_2)_{12}\text{CH}_3 \times 2$), 1.26 (80H, s, $-\text{CH}-\text{CH}_2(\text{CH}_2)_9\text{CH}_3 \times 2$ and $-\text{COCH}_2(\text{CH}_2)_{11}\text{CH}_3 \times 2$), 1.97 (3H, s, $\text{CH}_3\text{CO}-$), 7.26–7.45 (20H, m, $\text{PhCH}_2- \times 2$ and $(\text{PhO})_2\text{PO}-$). Anal. Calcd for $\text{C}_{99}\text{H}_{155}\text{N}_2\text{O}_{22}\text{P}$: C, 67.70; H, 8.90; N, 1.59. Found: C, 67.98; H, 8.40; N, 1.55.

2-Deoxy-2-[(R)-3-tetradecanoyloxytetradecanamido]-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]-6-O-(5-acetamide-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosyl)-4-O-phosphono-D-glucopyranose (4)—The phosphate (**12**) (11 mg, 6.26×10^{-6} mol) in methanol (0.1 ml) was hydrogenated in the presence of Pd-on-carbon (5 mg) at 40°C for 3 h. The catalyst was filtered off and Adams platinum catalyst (10 mg) was added to the filtrate. Hydrogenolysis was run at 35°C for 18 h and TLC (chloroform: methanol = 2:1) showed the reaction to be complete. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was purified by preparative TLC (chloroform: methanol = 2:1) to give **4** (5 mg, 56%), mp $183-187^{\circ}\text{C}$. $[\alpha]_D^{22} -25^{\circ}$ ($c=0.04$, methanol). IR (KBr): 3448, 1743, 1645, 1262 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 0.88 (12H, t, $J=6.1$ Hz, $-\text{CH}-(\text{CH}_2)_{10}\text{CH}_3 \times 2$ and $-\text{CO}(\text{CH}_2)_{12}\text{CH}_3 \times 2$), 1.26 (80H, s, $-\text{CH}-\text{CH}_2(\text{CH}_2)_9\text{CH}_3 \times 2$ and $-\text{COCH}_2(\text{CH}_2)_{11}\text{CH}_3 \times 2$), 1.93 (3H, s, $\text{CH}_3\text{CO}-$). Positive FAB-MS ($\text{M}+\text{K}$) $^+$ at m/z 1462.

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