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Lipid A and Related Compounds. XVI.[1] Synthesis of Biologically Active Tetraacetyl-3-deoxy-D-*manno*-2-octulosonic Acid (KDO)-(α2→6)-D-Glucosamine-4-phosphates, Novel Analogs of the Nonreducing Sugar Moiety of Lipid A

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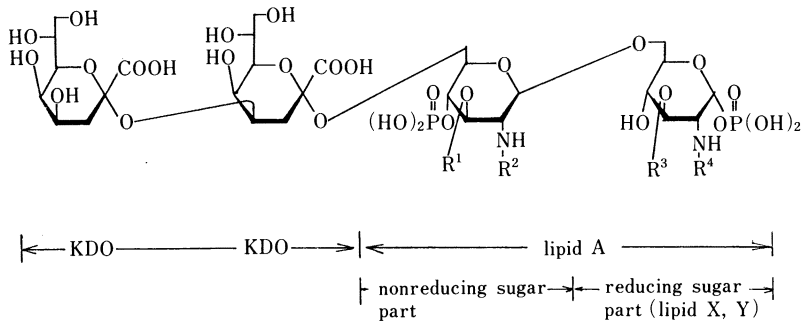
Synthesis of biologically active tetraacetyl-3-deoxy-D-*manno*-2-octulosonic acid-(α2→6)-D-glucosamine-4-phosphate analogs of lipid A is described.

Keywords——lipid A analog; KDO-linked glucosamine; 4-phosphorylated glucosamine; mitogenic activity; antitumor activity; lethal toxicity

Lipopolysaccharide (LPS) isolated from cell walls of various gram-negative bacteria possesses a variety of biological activities, *e.g.*, endotoxicity, adjuvanticity, antitumor activity and so on. These activities has been attributed to the hydrophobic moiety of LPS, termed lipid A.[2] 3-Deoxy-D-*manno*-2-octulosonic acid (KDO) occurs as a ketosidic component of the core region in all LPS and seems to play a biologically important role in being mitogenic and in amplifying the antitumor activity of lipid A.[3] However, the roles of KDO in the various biological activities of LPS are still unclear.

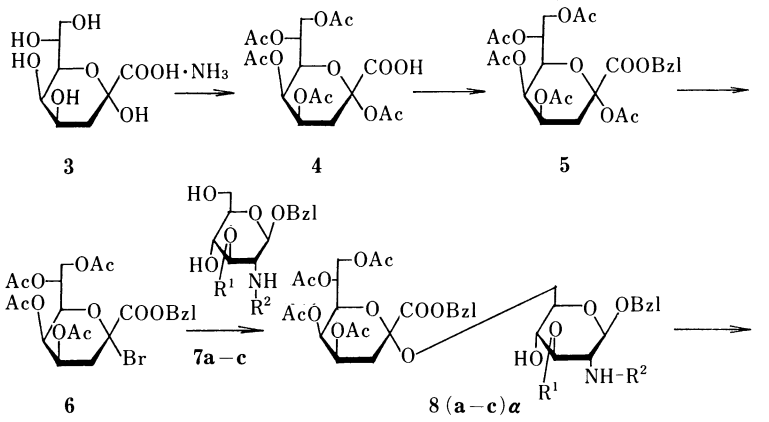
Recently, two research groups revealed that the C-2 position of KDO in the core region of LPS is attached to the C-6 position of the nonreducing moiety of lipid A from the Re mutant of *Salmonella minnesota* (**1a**)[4] and *Escherichia coli* (**1b**)[5] through a α2→6 linkage as shown in Chart 1. In addition, we and the other groups have found that the nonreducing



1a: $R^1=C_{14}$–O–C_{14}, $R^2=C_{14}$–O–C_{12}, $R^3=C_{14}$–OH, $R^4=C_{14}$–O–C_{16}
1b: $R^1=C_{14}$–O–C_{14}, $R^2=C_{14}$–O–C_{12}, $R^3=R^4=C_{14}$–OH

C_{14}–OH: (*R*)-3-hydroxytetradecanoyl C_{14}–O–C_{12}: (*R*)-3-dodecanoyloxytetradecanoyl
C_{14}–O–C_{14}: (*R*)-3-tetradecanoyloxytetradecanoyl C_{14}–O–C_{16}: (*R*)-3-hexadecanoyloxytetradecanoyl

Chart 1



a: $R^1 = C_{14}$–O–C_{14}, $R^2 = C_{14}$–O–C_{12} Bzl: benzyl
b: $R^1 = R^2 = C_{14}$–O–C_{14}
c: $R^1 = R^2 = C_{14}$–O–C_{12}
C_{14}–O–C_{12-14}: $CH_3(CH_2)_{10}CHCH_2CO$–
$CH_3(CH_2)_{10-12}$–CO

Chart 2

sugar moiety of lipid A is more important than the reducing sugar moiety (*cf.* lipid X and Y) for expressing the biological activities of LPS.[1c,h,6]

In a recent communication[1f] we reported the novel synthesis of tetraacetyl-KDO–(α2→6)-D-glucosamine-4-phosphate analogs (nonreducing sugar part) (**2a**—**c**) to clarify the effects of KDO on the biological activities of the nonreducing sugar part of lipid A. This paper describes these results in detail, as summarized in Charts 2 and 3.

The key disaccharide derivatives were synthesized by glycosidation of two monosaccharide components, **6** and **7a**—**c**, as shown in Chart 2. The component (**6**) as a glycosyl acceptor was prepared from ammonium KDO (**3**) (mp 122—124 °C, $[\alpha]_D^{24}$ +41.4 °), which was synthesized by a modification of the method of Hershberger *et al.*[7]

The hydroxyl groups of **3** were protected by acylation with acetic anhydride in the presence of 4-dimethylaminopyridine to give the pentaacetate (**4**) in 64% yield and further protection of the carboxyl group of **4** was carried out by esterification with phenyldiazomethane at room temperature to afford the ester (**5**) in 78% yield. Subsequent bromination at the C-2 position of **5** was accomplished by using $TiBr_4$ in dichloromethane at room temperature to give the bromide (**6**) as a syrup in almost quantitative yield. The bromide (**6**) was submitted to the next glycosidation without purification because of its instability.

On the other hand, the monosaccharide components (**7a**—**c**)[1a] as glycosyl donors were prepared from benzyl 2-amino-2-deoxy-4,6-*O*-isopropyridene-β-D-glucopyranoside.[1a]

Glycosidation of **7a** with **6** freshly prepared from pentaacetyl-KDO (**4**) was carried out in

the presence of $Hg(CN)_2$–$HgBr_2$ and Molecular Sieves 4A in dichloromethane at room temperature for 24 h to give **8aα** and **8aβ** in 31 and 6% yields, respectively; excess addition of the 2-bromo-KDO derivative (**6**) to the glucosamine derivative (**7a**) (molar ratio; 1 : 0.75) was needed owing to the easy formation of the 2-debrominated derivative from 6. The structures of the glycosidic 2→6 linkages of **8aα** and **8aβ** were confirmed by the evidence presented later (see Chart 3).

Phosphorylation of **8aα** with diphenyl phosphorochloridate, pyridine and 4-dimethylaminopyridine in dichloromethane at room temperature for 4 h gave **9aα** in 92% yield. The infrared (IR) spectrum of **9aα** showed the characteristic absorption band of the diphenyl phosphoryl group at 958 cm^{-1} and multiple signals assigned to the four phenyl groups were observed at 7.12—7.31 ppm in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. The protective benzyl and phenyl groups of **9aα** were then removed stepwise by hydrogenolysis catalyzed with 10% Pd-on-carbon at 35 °C for 5 h and PtO_2 at room temperature for 24 h in methanol to give the phosphate (**2aα**) in 54% yield. The phosphate (**2aα**) showed the characteristic blue color with the phosphate-specific spray reagent[8] and the ^{1}H-NMR spectrum of **2aα** showed no proton signal due to phenyl groups. The related phosphates, **2bα** and **2cα**, were similarly synthesized from **7b** and **7c** as illustrated in Chart 2.

The stereochemistry of the 2→6 linkage in the glycosidation products (**8aα** and **8bβ**) was established by analysis of the ^{1}H-NMR spectra of the modified compounds, **12α** and **12β**, bearing two 2,2,2-trichloroethoxycarbonyl (TCEC) substituents instead of the fatty acids on the 2-amino and 3-hydroxyl groups of **8**. In the KDO part of **8aα** and **8aβ**, the 3-H_{eq} proton signals, which are reliable indicators of the α and β (2→6) linkages, overlapped with the methylene proton signals of fatty acids substituted on the glucosamine part, whereas **12α** and **12β** have no methylene proton signals of the fatty acids.

The latter compounds, **12α** and **12β**, were synthesized as shown in Chart 3. Glycosidation of the two components, **6** and **10**, was carried out by the procedure described above to yield **11α** (45%) and **11β** (4%). Each product was then phosphorylated by the above-mentioned procedure to give **12α** [^{1}H-NMR ($CDCl_3$) δ: 2.27 (dd, J = 13.4, 5.9 Hz, 3-H_{eq} of KDO] in 77% yield and **12β** [^{1}H-NMR ($CDCl_3$) δ: 2.47 (dd, J = 11.7, 4.6 Hz, 3-H_{eq} of KDO] in 68% yield. ^{1}H-NMR spectroscopic analysis of the former indicated a smaller chemical shift value of the 3-H_{eq} proton in the KDO residue, which is characteristic of an α-ketoside.[9] Subsequent deprotection of the TCEC group at the 2- and 3-position of the glucosamine skeleton of **12α**

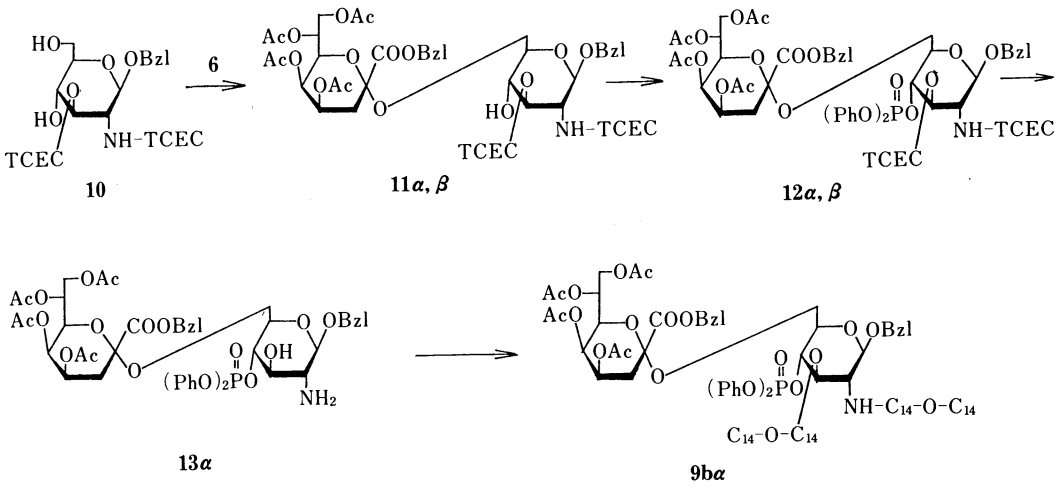


Chart 3

with zinc powder in acetic acid at room temperature for 5 h afforded **13α** in 41% yield. The simultaneous acylation[1o] of the amino and hydroxyl groups of **13α** with (*R*)-3-tetradecanoyloxytetradecanoic acid gave **9bα** in 40% yield. The ^{1}H-NMR and IR spectra and the optical rotation of **9bα** thus obtained were identical with those of **9bα** as indicated in Chart 2.

The antitumor activity, lethal toxicity and mitogenic activity of **2(a—c)α** were determined. Although the antitumor activity of **2(a—c)α** against ascities form of Ehrlich carcinoma in ddY mice was weaker than that of natural lipopolysaccharide, **2bα** was most effective. The lethal toxicity of **2cα** in C_{57}BL/6 mice loaded with galactosamine was most potent among these compounds, whereas **2bα** did not show lethality even at a high dose (50 g/mouse). On the other hand, these compounds (**2(a—c)α**) possess mitogenic activity comparable with that of lipid A.[1g,i]

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. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-FX90Q (90 MHz) FT-NMR spectrometer using tetramethylsilane (TMS) 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 60F_{254} on aluminum sheets, Merck). All compounds were located by spraying the sheets with sulfuric acid and heating on a hot plate. Phosphorus-containing compounds were revealed by spraying with the phosphate-specific spray reagent.[8] Column chromatography was performed on silica gel (Kiesel gel 60, 70—230 mesh, Merck). Evaporations were carried out under reduced pressure at 35 °C. Reaction solvents were dried on Molecular Sieves 4A.

2,4,5,7,8-Penta-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulosonic Acid (4)——A solution of **3** (3.0 g, 11.8 mmol) in pyridine (38.9 g, 0.49 mol) and acetic anhydride (43.5 g, 0.426 mol) was stirred at 5 C, and then 4-dimethylaminopyridine (0.5 g, 4.1 mmol) was added to the mixture. After 24 h, TLC (chloroform : methanol = 10 : 1) showed the reaction to be complete. Chloroform (300 ml) was added to the reaction solution. The mixture was cooled to 5 C and washed twice with 1 N hydrochloric acid (300 ml + 200 ml). The organic layer was washed with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$) and concentrated to dryness. The residual syrup (5.2 g) was purified on a column (50 ml) of silica gel (chloroform : methanol = 20 : 1) to yield the penta-*O*-acetate (**4**) (3.39 g, 64%), amorphous, $[\alpha]_D^{24}$ +93.6 (c = 1.18, $CHCl_3$). IR (KBr): 1755, 1372, 1235, 1160, 1048, 1018 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.92—2.37 (17H, m, $COCH_3$ and H-3 of KDO), 6.77 (1H, brs, COOH).

Benzyl 2,4,5,7,8-Penta-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulosonate (5)——A solution of **4** (2.24 g, 5 mmol) in dichloromethane (20 ml) was stirred at room temperature, and then a solution of phenyldiazomethane in petroleum ether [30 ml, 6.3% (w/v)] was added over 10 min. After 2 h, TLC (chloroform : isopropyl ether = 10 : 3) showed the reaction to be complete. The reaction mixture was adjusted to pH 4 with acetic acid and then washed successively with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$) and concentrated. The residual syrup (4.2 g) was purified on a column (50 ml) of silica gel (chloroform : methanol = 20 : 1) to yield the benzyl ester (**5**) (2.1 g, 78%), mp 96—98 C, $[\alpha]_D^{25}$ +91.9 (c = 0.9, $CHCl_3$). IR (KBr): 1772, 1749, 1371, 1244, 1205, 1088, 1052, 1010 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.95—1.20 (16H, m, $COCH_3$ and H-3 of KDO), 7.32 (5H, s, Ph). ^{13}C-NMR ($CDCl_3$) δ: 20.59 (CH_3CO), 30.83 (C-3), 62.15 (C-8), 63.99 (C-6), 65.94 (C-5), 67.40 (CH_2Ph), 67.83 (C-4), 69.79 (C-7), 97.53 (C-2), 128.14 (arom.). *Anal.* Calcd for $C_{25}H_{30}O_{13}$: C, 55.76; H, 5.62. Found: C, 55.68; H, 5.65.

Benzyl 4,5,7,8-Tetra-*O*-acetyl-2-bromo-3-deoxy-α-D-*manno*-2-octulosonate (6)——A solution of **5** (269 mg, 0.5 mmol) in dichloromethane (5 ml) and ethyl acetate (0.5 ml) was stirred at room temperature. Then, Molecular Sieves 4A (160 mg) was added to the solution and the whole was stirred for 60 min. The reaction mixture was cooled to 5 °C, titanium tetrabromide (350 mg, 0.95 mmol) was added, and stirring was continued at room temperature for 16 h. The reaction was confirmed to be complete by TLC (chloroform : isopropyl ether = 10 : 3), then acetonitrile (2.8 ml) and anhydrous sodium acetate (1.0 g) were added to the reaction mixture and the whole was stirred at room temperature for 30 min to decompose the reagent. Toluene (10 ml) was added to the mixture with cooling, the whole was stirred for 30 min, and then the solids were filtered off and the filtrate was concentrated (vacuum pump) to give **6** (302 mg) (100%) as a syrup. This syrup was used for the following reaction without purification. *Rf* values on TLC (chloroform : acetone = 10 : 2): **5**, 0.22; **6**, 0.31; 2-deoxylate, 0.45.

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α and β-D-*manno*-2-octulopyranosylonate)-2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (8aα and β)——$Hg(CN)_2$ (180 mg, 0.49 mmol), $HgBr_2$ (36 mg, 0.14 mmol) and Molecular Sieves 4A (300 mg) were added to the

solution of **7a** (418 mg, 0.375 mmol) in dichloromethane (5 ml) and the mixture was stirred at room temperature for 2 h. Then, the bromide (**6**) (308 mg) derived from benzyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulosonate (269 mg, 0.50 mmol) in dichloromethane (1 ml) was added to the mixture and the whole was stirred at room temperature for 20 h. The reaction was confirmed to be complete by TLC (chloroform : isopropyl ether = 10 : 3) (the starting bromide was no longer detectable), then the reaction mixture was washed with 10% KI aqueous solution and water, dried ($MgSO_4$), and concentrated. The residual syrup was purified on a column (25 g) of silica gel (chloroform : isopropyl ether = 25 : 1) to give successively the HBr-eliminated product from the bromide (**6**), the α-glycosylate (**8aα**) (187 mg, 31%), the β-glycosylate (**8aβ**) (36 mg, 6%) and recovered **7a** (241 mg, 58%).

Characterizing Data for the α-Glycosylate (**8aα**): Syrup, $[\alpha]_D^{21}$ +9.2 (c = 2.02, $CHCl_3$). IR (KBr): 3370, 2930, 1755, 1661, 1371, 1240, 1161, 1085, 1048, 699 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (12H, t, J = 3.2 Hz, CH_3), 1.27 (80H, s, CH_2), 1.81—2.46 (22H, m, $COCH_2$, $COCH_3$ and H-3 of KDO), 6.15 (1H, d, J = 5.4 Hz, NH), 7.08—7.33 (10H, m, Ph). *Anal.* Calcd for $C_{90}H_{145}NO_{22}\cdot 4H_2O$: C, 64.91; H, 9.26; N, 0.84. Found: C, 65.08; H, 8.97; N, 0.90.

Characterizing Data for the β-Glycosylate (**8aβ**): Syrup, $[\alpha]_D^{22}$ +19.2 (c = 0.24, $CHCl_3$). IR (KBr): 3380, 2935, 1750, 1660, 1371, 1235, 1162, 1089, 1048, 699 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (12H, t, J = 3.4 Hz, CH_3), 1.28 (80H, s, CH_2), 1.70—2.44 (22H, m, $COCH_2$, $COCH_3$ and H-3 of KDO), 5.84 (1H, d, J = 5.4 Hz, NH), 7.22—7.38 (10H, m, Ph). *Anal.* Calcd for $C_{90}H_{145}NO_{22}\cdot 4H_2O$: C, 64.91; H, 9.26; N, 0.84. Found: C, 65.15; H, 9.01; N, 0.87.

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (8bα)——Treatment of **7b** (434 mg, 0.380 mmol) as described for **8aα** gave **8bα** (250 mg, 31%), syrup, $[\alpha]_D^{24}$ +11.5 (c = 1.46, $CHCl_3$).

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-dodecanoyloxytetradecanoyl]-β-D-glucopyranoside (8cα)——Treatment of **7c** (220 mg, 0.202 mmol) as described for **8aα** gave **8cα** (81 mg, 25%), syrup, $[\alpha]_D^{24}$ +10.9° (c = 3.25, $CHCl_3$).

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (9aα)——Pyridine (30 mg, 0.38 mmol), 4-dimethylaminopyridine (43 mg, 0.35 mmol) and Molecular Sieves 4A (13 mg) were added to the solution of **8aα** (100 mg, 0.062 mmol) in dichloromethane (0.5 ml) with stirring at 5 C and after 30 min, diphenyl phosphorochloridate (92 mg, 0.34 mmol) was added to the mixture. The reaction mixture was stirred at room temperature for 4 h, after which time TLC (chloroform : isopropyl ether = 10 : 3) showed the reaction to be complete. The mixture was cooled to 5 C and the excess phosphorylating agent was destroyed by addition of a small amount of cold aqueous sodium hydrogen carbonate. After a short time, the organic layer was separated, washed with water and dried ($MgSO_4$). Solvents were removed under reduced pressure and the residue (208 mg) was purified on a column (10 g) of silica gel (chloroform : isopropyl ether = 20 : 1) to yield the diphenylphosphorylate (**9aα**) (106 mg, 93%), syrup, $[\alpha]_D^{22}$ +17.6 (c = 1.42, $CHCl_3$). IR (KBr): 3400, 2930, 1755, 1235, 1221, 1192, 1162, 1043, 958 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.86 (12H, t, J = 3.5 Hz, CH_3), 1.25 (80H, s, CH_2), 1.77—2.43 (22H, m, $COCH_2$, $COCH_3$ and H-3 of KDO), 6.10 (1H, d, J = 5.7 Hz, NH), 7.12—7.31 (20H, m, Ph). *Anal.* Calcd for $C_{102}H_{154}NO_{25}P$: C, 67.12; H, 8.50; N, 0.77. Found: C, 66.88; H, 8.52; N, 0.72.

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (9bα)——Treatment of **8bα** (175 mg, 0.11 mmol) as described for **9aα** gave **9bα** (160 mg, 80%), syrup, $[\alpha]_D^{21}$ +17.2° (c = 1.50, $CHCl_3$). *Anal.* Calcd for $C_{104}H_{158}NO_{25}P$: C, 67.40; H, 8.59; N, 0.76. Found: C, 67.23; H, 8.72; N, 0.77.

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-dodecanoyloxytetradecanoyl]-β-D-glucopyranosie (9cα)——Treatment of **8cα** (146 mg, 0.093 mmol) as described for **9aα** gave **9cα** (133 mg, 79%), syrup, $[\alpha]_D^{20}$ +15.6 (c = 6.65, $CHCl_3$). *Anal.* Calcd for $C_{100}H_{150}NO_{25}P$: C, 66.83; H, 8.41; N, 0.98. Found: C, 66.90; H, 8.55; N, 1.01.

6-*O*-(4,5,7,8-Tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosonic acid)-2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-4-*O*-phosphono-D-glucopyranose (2aα)——A solution of **9aα** (63 mg, 0.035 mmol) in methanol (4 ml) was hydrogenated at 25 °C under atmospheric pressure in the presence of 10% Pd-on-C catalyst (70 mg). The reaction was monitored by TLC (chloroform : methanol = 10 : 1, Rf = 0.6). The catalyst was filtered off and PtO_2 (30 mg) was added to the filtrate. Hydrogenolysis was continued at room temperature for 18 h, when TLC (chloroform : methanol = 10 : 3, Rf = 0.7) showed the reaction to be complete (there was no UV-absorbing material). The catalyst was then filtered off and the filtrate was concentrated to dryness. The residual solid (47 mg) was purified on a column (10 g) of silica gel (chloroform : methanol = 20 : 1) followed by lyophilization from dioxane to obtain the desired compound (**2aα**) (28 mg, 54%), mp 123—125 C, $[\alpha]_D^{22}$ +19.4 (c = 0.32, $CHCl_3$). IR (KBr): 3450, 2935, 1751, 1630, 1372, 1250, 1108, 1051 cm^{-1}. *Anal.* Calcd for $C_{76}H_{134}NO_{25}P\cdot H_2O$: C, 60.41; H, 9.07; N, 0.93. Found: C, 60.11; H, 8.79; N, 0.79.

6-*O*-(4,5,7,8-Tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosonic acid)-2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-4-*O*-phosphono-D-glucopyranose (2bα)——Treatment of **9bα** (85 mg, 0.046 mmol) as described for **2aα** gave **2bα** (26 mg, 37%), mp 116—120 C, $[\alpha]_D^{24}$ +14.2 (c = 0.5, $CHCl_3$). *Anal.* Calcd for $C_{78}H_{138}NO_{25}P\cdot 2H_2O$: C, 60.21; H, 9.07; N, 0.90. Found: C, 60.05; H, 8.84; N, 1.00.

6-*O*-(4,5,7,8-Tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosonic acid)-2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-dodecanoyloxytetradecanoyl]-4-*O*-phosphono-D-glucopyranose (2cα)——Treatment of **9cα** (128 mg, 0.071 mmol) as described for **2aα** gave **2cα** (36 mg, 35%), mp 122—124 °C, $[\alpha]_D^{25}$ +24.6° (*c* = 1.05, $CHCl_3$). *Anal.* Calcd for $C_{74}H_{130}NO_{25}P \cdot 3H_2O$: C, 58.51; H, 8.76; N, 0.92. Found: C, 58.71; H, 8.53; N, 0.76.

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α and β-D-*manno*-2-octulopyranosylonate)-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl)amino-3-*O*-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranoside (11α and β)——$Hg(CN)_2$ (72 mg, 0.2 mmol), $HgBr_2$ (10 mg, 0.04 mmol) and Molecular Sieves 4A (110 mg) were added to a solution of **10** (124 mg, 0.2 mmol) in dichloromethane (2 ml) and the mixture was stirred at room temperature for 1 h. Then the bromide (**6**, 114 mg) derived from benzyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulosonate (108 mg, 0.20 mmol) in dichloromethane (1 ml) was added to the mixture and the whole was stirred at room temperature for 20 h. It was confirmed by chloroform : isopropyl ether = 10 : 3) that the starting material (the bromide) had disappeared, then the reaction mixture was washed with 10% KI aqueous solution and water, dried ($MgSO_4$), and concentrated. The residual syrup (281 mg) was purified on a column (10 g) of silica gel (chloroform : isopropyl ether = 20 : 1) to yield the α-glycosylate (**11β**) (67 mg, 31%) and the β-glycosylate (**11α**) (8 mg, 4%), respectively.

Characterizing Data of the α-Glycoside (**11α**): Amorphous, $[\alpha]_D^{22}$ + 10.0° (*c* = 0.22, $CHCl_3$). IR (KBr): 3430, 1758, 1370, 1250, 1160, 1085, 1038, 820, 737, 700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.73—2.32 (14H, m, $COCH_3$ and H-3 of KDO), 7.17—7.38 (10H, m, Ph). *Anal.* Calcd for $C_{42}H_{47}Cl_6NO_{20}$: C, 45.92; H, 4.31; N, 1.28. Found: C, 45.85; H, 4.45; N, 1.12.

Characterizing Data of the β-Glycoside (**11β**): Amorphous, $[\alpha]_D^{23}$ + 18.4° (*c* = 1.1, $CHCl_3$). IR (KBr): 3430, 1758, 1370, 1250, 1160, 1085, 1052, 820, 739, 700 cm^{-1}. *Anal.* Calcd for $C_{42}H_{47}Cl_6NO_{20}$: C, 45.92; H, 4.31; N, 1.28. Found: C, 45.88; H, 4.52; N, 1.22.

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-(2,2,2-trichloroethoxycarbonyl)amino-3-*O*-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranoside (12α)——Pyridine (67 mg, 0.84 mmol), 4-dimethylaminopyridine (21 mg, 0.168 mmol) and Molecular Sieves 4A (30 mg) were added to a solution of **11α** (185 mg, 0.168 mmol) in benzene (1 ml) with stirring at 5 °C, and after 20 min diphenyl phosphorochloridate (226 mg, 0.84 mmol) was added. The reaction mixture was stirred at room temperature for 4 h, and monitored by TLC (dichloromethane : isopropyl ether = 10 : 3). After complete disappearance of the starting material, the reaction mixture was cooled, washed with aqueous sodium hydrogen carbonate and water, and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified on a column (25 ml) of silica gel (chloroform : isopropyl ether = 20 : 1) to give the diphenylphosphate (**12α**) (171 mg, 77%), amorphous, $[\alpha]_D^{23}$ + 22.7° (*c* = 0.8, $CHCl_3$). IR (KBr): 3450, 1754, 1495, 1370, 1247, 1163, 1041, 961 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.71—2.46 (14H, m, $COCH_3$ and H-3 of KDO), 2.27 (dd, *J* = 13.4, 5.9 Hz, 3-H_{eq}), 7.11—7.31 (20H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 98.6 (αC-2). *Anal.* Calcd for $C_{54}H_{56}Cl_6NO_{23}P$: C, 48.74; H, 4.24; N, 1.05. Found: C, 49.03; H, 4.33; N, 0.91.

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-(2,2,2-trichloroethoxycarbonyl)amino-3-*O*-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranoside (12β)——Treatment of **11β** (73 mg, 0.0664 mmol) as described for **12α** gave **12β** (60 mg, 68%), amorphous, $[\alpha]_D^{20}$ + 31.4° (*c* = 0.68, $CHCl_3$). ^{13}C-NMR ($CDCl_3$) δ: 99.9 (βC-2). *Anal.* Calcd for $C_{54}H_{56}NO_{23}P$: C, 48.74; H, 4.24; N, 1.05. Found: C, 48.98; H, 4.31; N, 0.99.

Benzyl 2-Amino-6-*O*-(benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-diphenylphosphono-β-D-glucopyranoside (13α)——A solution of **12α** (186 mg, 0.140 mmol) in acetic acid (1.9 ml) was stirred at room temperature. Zinc powder (156 mg, 2.39 mmol) was added to the solution in small portions over 30 min and then the mixture was stirred for 6 h, when TLC (chloroform : methanol = 10 : 1) showed the reaction to be complete. The solid was filtered off and the filtrate was concentrated to dryness. The residual syrup (274 mg) was purified on a column (10 g) of silica gel (chloroform : methanol = 25 : 1) to give **13α** (108 mg, 79%), amorphous, $[\alpha]_D^{24}$ + 29.0° (*c* = 2.26, $CHCl_3$). IR (KBr): 3440, 1753, 1591, 1492, 1372, 1222, 1190, 1162, 1092, 955, 698 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.64—2.24 (14H, m, $COCH_3$ and H-3 of KDO), 7.19—7.38 (20H, m, Ph). *Anal.* Calcd for $C_{48}H_{54}NO_{19}P \cdot H_2O$: C, 56.75; H, 5.36; N, 1.38. Found: C, 56.55; H, 5.46; N, 1.35.

Benzyl 6-*O*-(Benzyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-tetradecanoyloxytetradecanoyl]amino-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (9bα)——A solution of **13α** (70 mg, 0.071 mmol) in dichloromethane (0.7 ml) was stirred at −5 °C and (*R*)-3-tetradecanoyloxytetradecanoic acid (97 mg, 0.213 mmol), 4-dimethylaminopyridine (4 mg, 0.033 mmol) and *N*,*N*-dicyclohexylcarbodiimide (44 mg, 0.213 mmol) was added. The mixture was stirred at −5—5 °C for 1 h, at 5—20 °C for 3 h and at room temperature for 5 h. It was confirmed by TLC (chloroform : isopropyl ether = 10 : 3) that the reaction was complete, then the solid was filtered off and the filtrate was washed with water, dried ($MgSO_4$) and concentrated. The residual syrup (155 mg) was purified on a column (10 g) of silica gel (chloroform : isopropyl ether = 20 : 1) to give the diacylate (**9bα**) (52 mg, 40%), syrup, $[\alpha]_D^{24}$ + 16.9° (*c* = 1.35, $CHCl_3$). IR (KBr): 3420, 2930, 1758, 1236, 1220, 1191, 1164, 1045, 958, 698 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (12H, t, *J* = 3.0 Hz, CH_3), 1.25 (84H, s, CH_2), 1.80—2.60 (22H, m, $COCH_2$, $COCH_3$ and H-3 of KDO), 6.11 (1H, d, *J* = 5.6 Hz, NH), 7.05—7.40 (20H, m, Ph). *Anal.* Calcd for $C_{104}H_{158}NO_{25}P$: C, 67.40; H, 8.59; N, 0.76. Found: C, 67.27; H, 8.68; N, 0.88.

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