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Lipid A and Related Compounds. XVII.[1] Synthesis of 3-Deoxy-D-*manno*-2-octulosonic Acid (KDO)-(α2→6)-D-Glucosamine-4-phosphates, Analogs of the Biologically Active Moiety of Lipopolysaccharide from *Escherichia coli* Re Mutant

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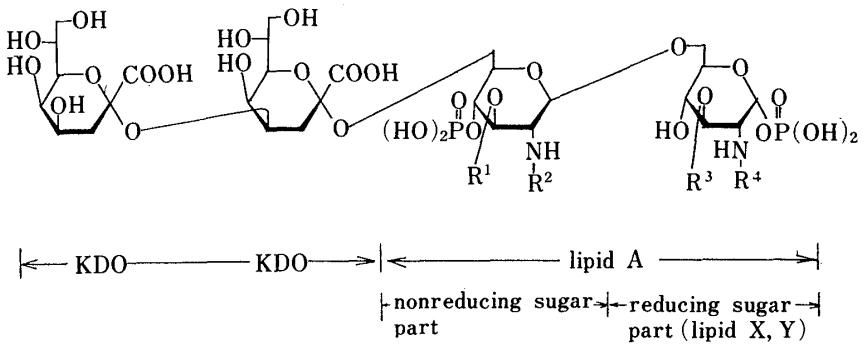
Synthesis of biologically active 3-deoxy-D-*manno*-2-octulosonic acid (KDO)-(α2→6)-D-glucosamine-4-phosphate analogs of lipid A is described.

Keywords——lipid A analog; 3-deoxy-D-*manno*-2-octulosonic acid (KDO); KDO-linked glucosamine; 4-phosphorylated glucosamine; mitogenic activity

Lipopolysaccharide (LPS) located on the cell surface of gram-negative bacteria is composed of three structural regions; *O*-polysaccharide, a common core region, and a lipid component called lipid A. The inner core region of LPS consists of 3-deoxy-D-*manno*-2-octulosonic acid (KDO) and heptose. The outer core region is linked to lipid A through the KDO molecules.[2]

The KDO as a ketosidic component in LPS seems to play a biologically important role in being mitogenic and in amplifying the antitumor activity of lipid A.[3] The KDO region of LPS from *Salmonella minnesota* (**1a**)[4] and *Escherichia coli* (**1b**)[5] Re mutant is bound to lipid A through an (α2→6) linkage, as shown in Chart 1.

Recently, we indicated that the nonreducing sugar moiety has more potent biological activity than the reducing sugar moiety of lipid A, suggesting the importance of the 4-*O*-

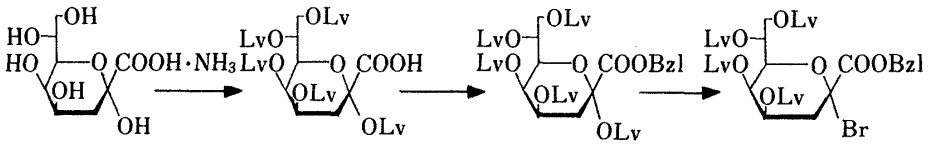


1a: $R^1=C_{14}$–O–C_{14}, $R^2=C_{14}$–O–C_{12}, $R^3=C_{14}$–OH, $R^4=C_{14}$–O–C_{16}
1b: $R^1=C_{14}$–O–C_{14}, $R^2=C_{14}$–O–C_{12}, $R^3=R^4=C_{14}$–OH
C_{14}–OH: (*R*)-3-hydroxytetradecanoyl
C_{14}–O–C_{12}: (*R*)-3-dodecanoyloxytetradecanoyl
C_{14}–O–C_{14}: (*R*)-3-tetradecanoyloxytetradecanoyl
C_{14}–O–C_{16}: (*R*)-3-hexadecanoyloxytetradecanoyl

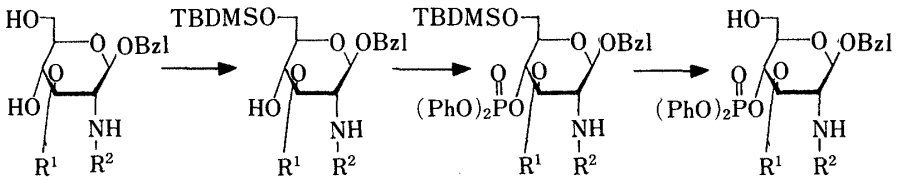
Chart 1

phosphate group for the expression of the biological effects, and we also reported a novel synthesis of tetraacetyl-KDO-(α2→6)-D-glucosamine-4-phosphate analogs (nonreducing sugar part), revealing the α-stereochemistry in the (2→6) glycosidation of the KDO derivatives with the D-glucosamine donor and the amplifying effects of tetraacetyl-KDO on the biological activities of the nonreducing sugar part of lipid A.[1f,g,p]

Here we report the novel synthesis of KDO-(α2→6)-D-glucosamine 4-phosphate (nonreducing sugar part) (**2a**—**c**) to clarify the net effects of KDO instead of the tetraacetyl derivative on the expression of the biological activities of the 4-*O*-phosphate, the nonreducing sugar part of lipid A. The synthetic scheme is shown in Chart 2.



3 **4** **5** **6**



7a—c **8a—c** **9a—c** **10a—c**

a: $R^1 = C_{14}\text{-O-}C_{14}$, $R^2 = C_{14}\text{-O-}C_{12}$ **b**: $R^1 = R^2 = C_{14}\text{-O-}C_{14}$
c: $R^1 = R^2 = C_{14}\text{-O-}C_{16}$
$C_{14}\text{-O-}C_{12-16} = -COCH_2CH(CH_2)_{10}CH_3$
$OCO(CH_2)_{10-14}CH_3$

Chart 2

Our synthetic methodology includes the novel use of the levulinoyl group for protection of the hydroxyl groups of KDO and the assignment of the α-glycosidic structure of **2a**—**c** by ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectroscopic analysis based on the previously reported data on the tetraacetyl derivatives.[1p,6]

The key disaccharide intermediates were synthesized by glycosidation of two monosaccharide components, **6** and **10a**—**c**, as shown in Chart 2. The component (**6**) as a glycosyl acceptor was prepared from KDO (**3**) [mp 122—124 °C, $[\alpha]_D^{24}$ +41.4° (c=1.85, H_2O), lit.[7] mp 121—123 °C, $[\alpha]_D^{27}$ +42.3° (c=1.7, H_2O)], which was prepared according to the modified method of Hershberger *et al.*[7]

Pentalevulinoylation of the hydroxyl groups of KDO was first carried out with levulinoyl anhydride and pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine without solvent by the use of the method reported for the pentaacetate synthesis, but the reaction mixture changed to reddish black and the desired product was not isolated. When acetonitrile was then employed as the solvent, the pentalevulinoylate (**4**) was obtained in 30% yield.

Esterification of the carboxyl group of **4** with phenyldiazomethane at room temperature gave the benzylate (**5**, 56%). The ^1H-NMR spectrum of **5** showed the presence of the phenyl proton signals of the benzyl ester group and methyl proton signals of the levulinoyl groups at 2.18 ppm (15H), and the ^{13}C-NMR spectrum of **5** showed a signal at 97.4 ppm (singlet) suggestive of the α-anomeric configuration.[5]

Subsequent bromination at the C-2 position of **5** was accomplished with $TiBr_4$ in dichloromethane and ethyl acetate (10 : 1) at room temperature to give the bromide (**6**) as a syrup in quantitative yield. Although small amounts of the 2-deoxylated derivative of **5** and the unreacted starting material (**5**) were observed by thin layer chromatography (TLC), the bromide (**6**) was used for the next glycosidation without purification because of its instability.

Other monosaccharide components (**10a—c**) as glycosyl donors were prepared from benzyl 2,3-diacyloxyacylated 2-deoxy-β-D-glucopyranoside (**7a—c**).[1q] As shown in Chart 2, selective protection of the 6-hydroxyl group of the diol (**7a**) with *tert*-butyldimethylsilyl chloride and triethylamine in the presence of a catalytic amount of 4-dimethylaminopyridine in dichloromethane at room temperature gave the monosilylate (**8a**) in 87% yield. Subsequent phosphorylation of **8a** was performed with diphenylphosphoryl chloride, pyridine and 4-dimethylaminopyridine in benzene for 2 h at room temperature to give the phosphate (**9a**) in 98% yield. The infrared (IR) spectrum of **9a** showed a characteristic band of the diphenylphosphoryl group at 953 cm^{-1}. The protective silyl group of **9a** was then removed by treatment with 45% HF in acetonitrile and chloroform (7 : 1) at room temperature for 30 min to afford **10a** in 80% yield. Treatment of **9a** with 90% acetic acid at 80 °C for 4 h also gave **10a** in 46% yield, but removal of the *tert*-butyldimethylsilyl group of **9a** by reaction with *n*-tetrabutylammonium fluoride in tetrahydrofuran failed to give the desired product (**10a**). The related compounds, **10b** and **10c**, were obtained by similar treatment with 45% HF in acetonitrile and chloroform.

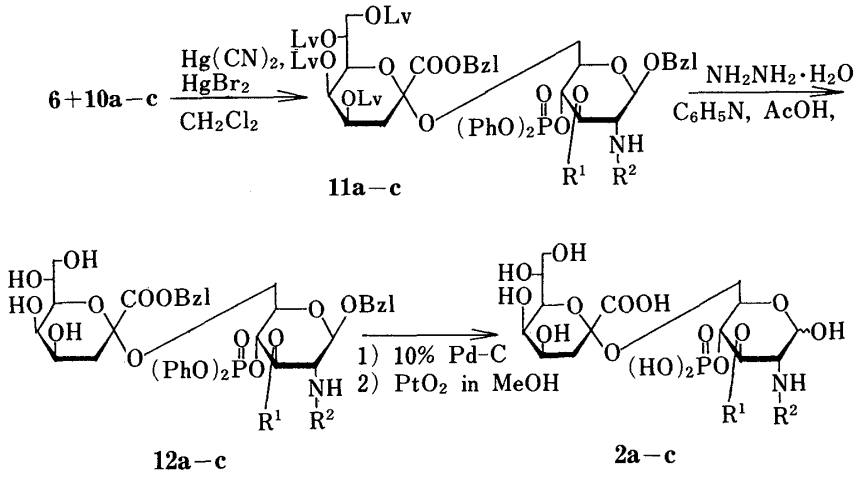


Chart 3

The glycosidation of **10a** and **6**, freshly prepared from pentalevulinoyl KDO with $Hg(CN)_2$, $HgBr_2$ and Molecular sieves 4A in dichloromethane at room temperature for 16 h, gave the single disaccharide (**11**) in 60% yield. The stereochemistry of **11** was assigned as α on the basis of the ^{13}C-NMR chemical shifts (98.5, 98.8, 98.4 ppm) of the C-2 atoms of **11a—c** that were assigned to the α-anomeric carbon atom of the KDO residues,[5,6] because the analysis of the overlapped 3-H_{eq} proton signals of **11a—c** by ^1H-NMR spectroscopy was impossible. Excess 2-bromo-KDO derivative (**6**) was used to avoid the side reaction forming the 2-deoxylated derivative from **6**. We also examined the glycosidation of **6** and **7a** followed by phosphorylation to synthesize **11a**, but the phosphorylation did not proceed. It seems that the intermediary *O*-anion of the 4-hydroxyl group is masked from the attack of the reagent by the electrophilic effect of levulinoyl groups of the KDO part.

Selective removal of the levulinoyl groups of the KDO region of **11a** was achieved by hydrazinoylsis with 0.3 M hydrazine hydrate in chloroform, pyridine and acetic acid (4 : 7 : 1) at room temperature for 30 min to give **12a** in good yield (89%). These results, including the

selective removal of the levulinoyl groups without side reaction with the other ester groups and the glycosyl linkage, indicate the usefulness of a levulinoyl group as a selective protecting group for the hydroxyl function in the field of glycolipids synthesis. The protecting benzyl and phenyl groups of **12a** were then removed stepwise by hydrogenolysis with 10% Pd-on-carbon at 35 °C for 5 h, and then PtO_2 at room temperature for 24 h in methanol to give **2a** in 48% yield. Compounds **2b** and **2c** were similarly synthesized.

Preliminary examination of the biological activity revealed that these compounds (**2a—c**) possess almost the same mitogenic activity as their tetraacetyl derivatives.[1m)]

Further application of this methodology to the synthesis of KDO–KDO–lipid As is under way.[4,5)]

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-NMR 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. TLC was performed on silica gel (Kiesel gel 60F_{254} on aluminum sheet, Merck). All compounds were located by spraying with sulfuric acid and heating on a hot plate. Phosphorus-containing compounds were detected by spraying with the phosphate-specific spray reagent.[7)] 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.

3-Deoxy-2,4,5,7,8-penta-*O*-levulinoyl-α-D-*manno*-2-octulosonic Acid (4)——A solution of levulinic anhydride (34.0 g, 0.15 mmol) in acetonitrile (139 ml) was stirred at room temperature and then pyridine (13.5 g, 0.17 mmol), **3** (3.96 g, 15.5 mmol) and 4-dimethylaminopyridine (248 mg, 2.03 mmol) were added, and the mixture was stirred at room temperature for 48 h when TLC (chloroform : methanol = 10 : 3) showed the reaction to be complete. Solvents were evaporated off and chloroform (300 ml) was added to the residue. The mixture was cooled and extracted with 2 N hydrochloric acid (150 ml). The organic layer was washed with aqueous $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated to dryness. The residual liquid (18 g) was purified on a column (200 g) of silica gel (chloroform : methanol = 40 : 1) to give the pentalevulinate (**4**) (3.32 g, 29%), syrup, $[\alpha]_D^{24}$ +22.2° (c = 1.2, $CHCl_3$). IR (KBr): 3450, 1747, 1720, 1407, 1365, 1208, 1159, 1070 cm^{-1}. *Anal.* Calcd for $C_{33}H_{44}O_{18}$: C, 54.39; H, 6.09. Found: C, 54.66; H, 6.03.

Benzyl 3-Deoxy-2,4,5,7,8-penta-*O*-levulinoyl-α-D-*manno*-2-octulosonate (5)——A solution of **4** (3.32 g, 4.56 mmol) in dichloromethane (30 ml) was stirred at 5 °C, and then a solution of phenyldiazomethane in petroleum ether (15 ml, 6.3% (w/v %), 7.9 mmol) was added over 10 min. After 2 h, TLC (chloroform : acetone = 10 : 2) showed the reaction to be complete. The reaction mixture was adjusted to pH 4 with acetic acid, then washed successively with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$) and concentrated to dryness. The residual syrup (4.83 g) was purified on a column (200 g) of silica gel (chloroform : acetone = 20 : 1) to yield the benzyl ester (**5**) (2.1 g, 56%), syrup, $[\alpha]_D^{25}$ +53.7° (c = 2.0, $CHCl_3$). IR (KBr): 3450, 1748, 1719, 1409, 1363, 1270, 1203, 1158, 1072, 758, 700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.18 (15H, s, $COCH_3$), 2.47—2.88 (20H, m, $-COCH_2CH_2CO-$), 5.32 (2H, s, CH_2Ph), 7.35 (5H, s, Ph). ^{13}H-NMR ($CDCl_3$) δ: 97.42 (αC-2), 128.57 (arom), 206.04 ($\underline{C}OCH_3$). *Anal.* Calcd for $C_{40}H_{50}O_{18}$: C, 58.67; H, 6.16; N, 1.71. Found: C, 58.39; H, 6.44; N, 1.68.

Benyl 2-Bromo-3-deoxy-4,5,7,8-tetra-*O*-levulinoyl-α-D-*manno*-2-octulosonate (6)——A solution of **5** (328 mg, 0.4 mmol) in dichloromethane (4 ml) and ethyl acetate (0.4 ml) was stirred at room temperature. Molecular sieves 4A (80 mg) was added to the solution and the whole was stirred for 30 min. The reaction mixture was cooled at 5 °C, titanium tetrabromide (296 mg, 0.8 mmol) was added, and stirring was continued at room temperature for 5 h. At that time, TLC (chloroform : acetone = 10 : 2) showed the reaction to be complete. Then acetonitrile (2 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 (8 ml) was added with cooling and the mixture was stirred for 15 min. Solids were filtered off and the filtrate was concentrated to dryness *in vacuo*. The residual syrup **6** (340 mg, 100% up) was used for the following reaction without purification because of its easy decomposition to the 2-deoxylate. Rf = 0.45 (chloroform : acetone = 10 : 2).

Benzyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (8a)——A solution of **7a** (557 mg, 0.5 mmol) in dichloromethane (5 ml) was cooled to 5 °C. Triethylamine (56 mg, 0.55 mmol), 4-dimethylaminopyridine (6.1 mg, 50 mmol) and *tert*-butyldimethylsilyl chloride (128 mg, 0.85 mmol) was added to the solution and the mixture was stirred at room temperature for 16 h, when TLC (chloroform : isopropyl ether = 10 : 1) showed the reaction to be complete. The

reaction mixture was washed with water, dried ($MgSO_4$) and concentrated to dryness. The residual syrup (716 mg) was purified on a column (10 g) of silica gel (chloroform : isopropyl ether = 20 : 1) to give the *tert*-butyldimethylsilylate (**8a**) (519 mg, 87%), amorphous, $[\alpha]_D^{24}$ −21.0° (c = 1.16, $CHCl_3$). IR (KBr): 3300, 2930, 1739, 1650, 1551, 1461, 1250, 1168, 1064, 837, 779, 700 cm^{-1}. *Anal.* Calcd for $C_{73}H_{133}NO_{11}Si$: C, 71.34; H, 10.91; N, 1.14. Found: C, 71.22; H, 10.80; N, 1.13.

Benzyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (8b)——*tert*-Butyldimethylsilylation of **7b** (594 mg, 0.52 mmol), as described above for **8a**, give **8b** (565 mg, 86%), amorphous, $[\alpha]_D^{25}$ −20.3° (c = 2.7, $CHCl_3$). *Anal.* Calcd for $C_{75}H_{137}NO_{11}Si$: C, 71.66; H, 10.90; N, 1.11. Found: C, 71.52; H, 11.12; N, 1.01.

Benzyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-hexadecanoyloxytetradecanoyl]-β-D-glucopyranoside (8c)——*tert*-Butyldimethylsilylation of **7c** (623 mg, 0.52 mmol), as described for **8a**, give **8c** (660 mg, 97%), amorphous, $[\alpha]_D^{23}$ −20.5° (c = 1.8, $CHCl_3$). *Anal.* Calcd for $C_{79}H_{145}NO_{11}Si$: C, 72.26; H, 11.13; N, 1.07. Found: C, 72.49; H, 10.98; N, 1.09.

Benzyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (9a)——A solution of **8a** (476 mg, 0.387 mmol) in benzene (2 ml) was stirred at 10 °C. Pyridine (77 mg, 0.967 mmol), 4-dimethylaminopyridine (118 mg, 0.967 mmol) and diphenylphosphoryl chloride (260 mg, 0.967 mmol) were added to the solution and the mixture was stirred at room temperature for 2 h. At that time, TLC (chloroform : isopropyl ether = 10 : 1) showed the reaction to be complete, so the reaction mixture was cooled and washed with aqueous sodium hydrogen carbonate and water, then dried ($MgSO_4$). Solvents were evaporated off and the residual syrup (786 mg) was purified on a column (10 g) of silica gel (chloroform : isopropyl ether = 20 : 1) to give the diphenylphosphate (**9a**) (553 mg, 98%), amorphous, $[\alpha]_D^{25}$ −8.90° (c = 1.9, $CHCl_3$). IR (KBr): 2930, 1739, 1664, 1494, 1465, 1189, 1162, 1022, 953, 835, 775, 685 cm^{-1}. *Anal.* Calcd for $C_{85}H_{142}NO_{14}PSi$: C, 69.87; H, 9.80; N, 0.96. Found: C, 69.77; H, 10.02; N, 0.95.

Benzyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (9b)——Diphenylphosphorylation of **8b** (565 mg, 0.45 mmol), as described above for **9a**, give **9b** (585 mg, 87%), syrup, $[\alpha]_D^{24}$ −8.25° (c = 2.4, $CHCl_3$). *Anal.* Calcd for $C_{87}H_{146}NO_{14}PSi$: C, 70.17; H, 9.88; N, 0.94. Found: C, 70.01; H, 10.04; N, 1.12.

Benzyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-hexadecanoyloxytetradecanoyl]-β-D-glucopyranoside (9c)——Diphenylphosphorylation of **8c** (660 mg, 0.503 mmol), as described for **9a**, give **9c** (672 mg, 87%), syrup, $[\alpha]_D^{25}$ −8.45° (c = 2.1, $CHCl_3$). *Anal.* Calcd for $C_{91}H_{154}NO_{14}PSi$: C, 70.73; H, 10.05; N, 0.91. Found: C, 71.02; H, 9.88; N, 1.12.

Benzyl 2-Deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-gulucopyranoside (10a)——A solution of **9a** (347 mg, 0.237 mmol) in chloroform (0.2 ml) and acetonitrile (1.4 ml) was stirred at room temperature and then 45% HF (0.12 ml, 2.75 mmol) was added. The reaction mixture was stirred for 30 min. At that time, TLC (chloroform : isopropyl ether = 10 : 1) showed the reaction to be complete. Chloroform (15 ml) was added to the mixture and the whole was washed with aqueous sodium hydrogen carbonate (5 ml) and water (5 ml), then dried ($MgSO_4$) and evaporated to dryness. The residual amorphous material (299 mg) was purified on a column (15 g) of silica gel (chloroform : isopropyl ether = 20 : 1) to give the 6-monohydroxylate (**10g**) (255 mg, 80%), amorphous, $[\alpha]_D^{23}$ −19.1° (c = 2.90, $CHCl_3$). IR (KBr): 3270, 2930, 1740, 1653, 1498, 1468, 1193, 1047, 963, 760, 692 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (12H, t, J = 3.0, CH_3), 1.02—1.71 (80H, CH_2), 2.05—2.42 (8H, m, $COCH_2$), 5.56 (1H, t, J = 6.6), 6.20 (1H, d, J = 4.8, NH), 7.0—7.4 (15H, m, Ph). *Anal.* Calcd for $C_{79}H_{128}NO_{14}P \cdot H_2O$: C, 69.52; H, 9.60; N, 1.03. Found: C, 69.35; H, 9.60; N, 1.02.

Benzyl 2-Deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (10b)——Treatment of **9b** (585 mg, 0.393 mmol) as described above for **10a** give **10b** (409 mg, 76%), syrup, $[\alpha]_D^{24}$ −18.3° (c = 2.65, $CHCl_3$). *Anal.* Calcd for $C_{81}H_{132}NO_{14}P \cdot H_2O$: C, 69.84; H, 9.55; N, 1.01. Found: C, 69.58; H, 9.60; N, 1.10.

Benzyl 2-Deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-hexadecanoyloxytetradecanoyl]-β-D-glucopyranoside (10c)——Treatment of **9c** (672 mg, 0.435 mmol) as described for **10a** give **10c** (571 mg, 92%), syrup, $[\alpha]_D^{25}$ −18.5° (c = 2.45, $CHCl_3$). *Anal.* Calcd for $C_{85}H_{140}NO_{14}P \cdot H_2O$: C, 70.46; H, 9.74; N, 0.97. Found: C, 70.21; H, 9.72; N, 0.99.

Benzyl 6-*O*-(Benzyl 3-deoxy-4,5,7,8-tetra-*O*-levulinoyl-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (11a)——$Hg(CN)_2$ (108 mg, 0.30 mmol), $HgBr_2$ (10 mg, 0.04 mmol) and Molecular sieves 4A (140 mg) were added to a solution of **10a** (270 mg, 0.20 mmol) in dichloromethane (2 ml), and the mixture was stirred at room temperature for 2 h. Then the bromide (**6**) (340 mg) derived from benzyl 3-deoxy-2,4,5,7,8-penta-*O*-levulinoyl-α-D-*manno*-2-octulosonate (328 mg, 0.40 mmol) in dichloromethane (0.5 ml) was added, and whole was stirred at room temperature for 20 h. At that time, TLC (chloroform : acetone = 10 : 2) showed the starting material (bromide) to have disappeared, so the reaction mixture was washed with 10% KI aqueous solution and water, then dried ($MgSO_4$), and concentrated to dryness. The residual syrup (501 mg) was purified on a column (25 mg) of silica gel (chloroform : acetone = 50 : 1) to elute successively the starting material (**10a**) (68 mg, 25%) and the glycoside (**11a**) (247 mg, 60%),

amorphous, $[\alpha]_D^{25}$ +13.03° (c=5.0, $CHCl_3$). IR (KBr): 3425, 2930, 1747, 1721, 1491, 1361, 1161, 1025, 960, 755, 695 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (12H, t, J=3.9, CH_3), 1.25 (80H, s, CH_2), 2.04—2.69 (38H, m, $-COCH_2CH_2COCH_3 \times 4$, $COCH_2 \times 4$, H-3 of KDO × 2), 6.10 (1H, d, J=4.9, NH), 7.2—7.4 (20H, m, Ph). ^{13}C(NMR ($CDCl_3$) δ: 98.5 (αC-2). *Anal.* Calcd for $C_{114}H_{170}NO_{29}P$: C, 66.81; H, 8.36; N, 0.68. Found: C, 66.73; H, 8.36; N, 0.82.

Benzyl 6-*O*-(Benzyl 3-deoxy-4,5,7,8-tetra-*O*-levulinoyl-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (11b)——Treatment of **10b** (276 mg, 0.20 mmol) as described for **11a** give **11b** (186 mg, 45%), amorphous, $[\alpha]_D^{25}$ +11.9° (c=3.72, $CHCl_3$). ^{13}C-NMR ($CDCl_3$) δ: 98.8 (αC-2). *Anal.* Calcd for $C_{116}H_{176}NO_{29}P$: C, 67.06; H, 8.54; N, 0.67. Found: C, 67.28; H, 8.51; N, 0.59.

Benzyl 6-*O*-(Benzyl 3-deoxy-4,5,7,8-tetra-*O*-levulinoyl-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-*O*-hexadecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-hexadecanoyloxytetradecanoyl]-β-D-glucopyranoside (11c)——Treatment of **10c** (286 mg, 0.20 mmol) as described for **11a** give **11c** (313 mg, 73%), amorphous, $[\alpha]_D^{25}$ +12.0° (c=4.65, $CHCl_3$). ^{13}C-NMR ($CDCl_3$) δ: 98.4 (αC-2). *Anal.* Calcd for $C_{120}H_{182}NO_{29}P$: C, 67.55; H, 8.60; N, 0.66. Found: C, 67.70; H, 8.56; N, 0.66.

Benzyl 6-*O*-(Benzyl 3-deoxy-α-D-*manno*-2-octuropyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (12a)——Pyridine (1.02 ml) and acetic acid (0.15 ml) were added to a solution of **11a** (103 mg, 0.05 mmol) in chloroform (0.75 ml) and the mixture was stirred at room temperature. Hydrazine hydrate (21.7 mg, 0.43 mmol) was added, and stirring was continued for 20 min. The reaction mixture was evaporated to dryness and the residual syrup was dissolved in benzene. The solution was washed with water, dried ($MgSO_4$) and evaporated to dryness. The residual amorphous material (80 mg) was purified on a column (10 g) of silica gel (chloroform : methanol = 25 : 1) to give the delevulinoylate (**12a**) (76 mg, 92%), mp 50—51 °C, $[\alpha]_D^{26}$ +7.18° (c=3.8, $CHCl_3$). IR (KBr): 3440, 2930, 1740, 1620, 1459, 1190, 1041, 962, 752, 692 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.73—1.03 (12H, m, CH_3), 1.03—1.80 (80H, CH_2), 1.85—2.55 (10H, m, $COCH_2$, H-3 of KDO), 6.13 (1H, d, J=5.4, NH), 7.1—7.4 (20H, m, Ph). *Anal.* Calcd for $C_{94}H_{146}NO_{21}P$: C, 68.13; H, 8.88; N, 0.85. Found: C, 67.88; H, 8.69; N, 1.03.

Benzyl 6-*O*-(Benzyl 3-deoxy-α-D-*manno*-2-octuropyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyloxy]-β-D-glucopyranoside (12b)——Delevulinoylation of **11b** (42 mg, 0.021 mmol) as described for **12a**, give **12b** (28 mg, 84%), mp 63—65 °C, $[\alpha]_D^{25}$ +8.33° (c=3.35, $CHCl_3$). *Anal.* Calcd for $C_{96}H_{150}NO_{21}P$: C, 68.42; H, 8.97; N, 0.83. Found: C, 68.67; H, 8.83, N, 0.85.

Benzyl 6-*O*-(Benzyl 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-hexadecanoyloxytetradecanoyl]-β-D-glucopyranoside (12c)——Delevulinoylation of **11c** (213 mg, 0.10 mmol) as described for **12a**, give **12c** (150 mg, 86%), mp 55—58 °C, $[\alpha]_D^{25}$ +7.38 (c=3.20, $CHCl_3$). *Anal.* Calcd for $C_{100}H_{158}NO_{21}P$: C, 68.98; H, 9.15; N, 0.80. Found: C, 69.23; H, 9.11; N, 0.98.

2-Deoxy-6-*O*-(3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-[(*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-4-*O*-phosphono-D-glucopyranose (2a)——A solution of **12a** (55 mg, 0.033 mmol) in methanol (2.8 ml) was hydrogenated at 30 °C under slight pressure in the presence of 10% Pd-on-carbon catalyst (55 mg), the reaction being monitored by TLC (chloroform : methanol : water = 10 : 10 : 1, Rf=0.7). The catalyst was filtered off and PtO_2 (26 mg) was added to the filtrate. Hydrogenolysis was continued at room temperature for 16 h and TLC (chloroform : methanol : water = 10 : 10 : 1, Rf=0.3) showed the reaction to be complete (no UV absorption). Then the catalyst was filtered off and the filtrate was concentrated to dryness. The residual solid (41 mg) was purified by preparative TLC (1 mm, 20 × 20 cm) on silica gel (chloroform : methanol = 2 : 1) followed by lyophilization from dioxane to obtain the desired compound (**2a**) (21 mg, 48%), mp 176—177 °C, $[\alpha]_D^{24}$ +11.43° (c=1.05, $CHCl_3$). IR (KBr): 3440, 2930, 1738, 1642, 1540, 1468, 1162, 1042 cm^{-1}. *Anal.* Calcd for $C_{68}H_{126}NO_{21}P \cdot 5H_2O$: C, 57.73; H, 9.69; N, 0.99. Found: C, 57.72; H, 9.30; N, 1.28.

2-Deoxy-6-*O*-(3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-[(*R*)-3-tetradecanoyloxytetradecanamido)-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-4-*O*-phosphono-D-glucopyranose (2b)——Treatment of **12b** (26 mg, 0.015 mmol) as described for **2a** give **2b** (12 mg, 58%), mp 175—176 °C, $[\alpha]_D^{24}$ +9.83° (c=1.75, $CHCl_3$). *Anal.* Calcd for $C_{70}H_{130}NO_{21}P \cdot 5H_2O$: C, 58.27; H, 9.08; N, 0.97. Found: C, 58.37; H, 8.78; N, 1.09.

2-Deoxy-6-*O*-(3-deoxy-α-D-*manno*-2-octulopyranosylonate)-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-hexadecanoyloxytetradecanoyl]-4-*O*-phosphono-D-glucopyranose (2c)——Treatment of **12c** (85 mg, 0.049 mmol) as described for **2a** give **2c** (37 mg, 54%), mp 177—178 °C, $[\alpha]_D^{23}$ +11.1° (c=1.35, $CHCl_3$). *Anal.* Calcd for $C_{74}H_{138}NO_{21}P \cdot 4H_2O$: C, 60.02; H, 9.39; N, 0.95. Found: C, 60.03; H, 9.10; N, 1.01.

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