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Synthesis of 2,3-Dideoxy-2-[(3*R*)-3-hydroxytetradecanoylamino]-3-*C*-[(3*R*)-(3-hydroxytetradecanoyloxy)methyl]-4-*O*-phosphono-D-mannopyranose

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2,3-Dideoxy-2-[(3*R*)-3-hydroxytetradecanoylamino]-3-*C*-[(3*R*)-(3-hydroxytetradecanoyloxy)methyl]-4-*O*-phosphono-D-mannopyranose was synthesized from (1*R*,2*S*,4*S*,5*S*)-6-(2,4-dimethoxybenzyl)-4-hydroxy-2-[(1*R*)-1,2-isopropyridenedioxyethyl]-3-oxa-6-azabicyclo[3.2.0]heptan-7-one.

Lipopolysaccharides (LPS)[1] covering the outer surface membrane of various Gram-negative bacteria, such as *Salmonella minnesota*, *Salmonella typhirium*, *Escherichia coli*, etc., are highly potent stimulators of the immune system. Besides this beneficial response, fatal endotoxic shock caused by LPS has precluded its clinical use. Most of the biological activities of LPS reside in Lipid A (Fig. 1), which is composed from *β* (1→6)-linked two D-glucosamine units, and is a unique hydrophobic anchor substance holding an essentially linear polysaccharide chain to the cell wall.

Lipid A was chemically synthesized by Shiba et al. and Achiwa et al.[2] Nishijima and Raets[3] found Lipid X (Fig. 2) in certain mutants of *Escherichia coli* that were defective in phosphatidylglycerol synthesis. Lipid X is a reducing sugar part of Lipid A, and one of the biosynthetic precursors of Lipid A.[4]

In a series of investigations by Hasegawa and Kiso[5] on the relationship between the molecular structure and the biological activity of nonreducing sugar subunit analogues of Lipid A, it has been demonstrated that several kinds of biological activities (immunostimulating, adjuvant, antitumor, antivirus, macrophage activating, and interferon production stimulating activities) of LPS can be expressed by some 4-*O*-phosphono-D-glucosamine derivatives, such as GLA-60 (Fig. 3).[6] According to these results, we were interested in the biological activities of the 4-*O*-phosphono-D-glucosamine derivatives. This paper reports a synthesis of 2,3-dideoxy-3-*C*-hydroxymethyl-4-*O*-phosphono-D-mannosamine derivative.

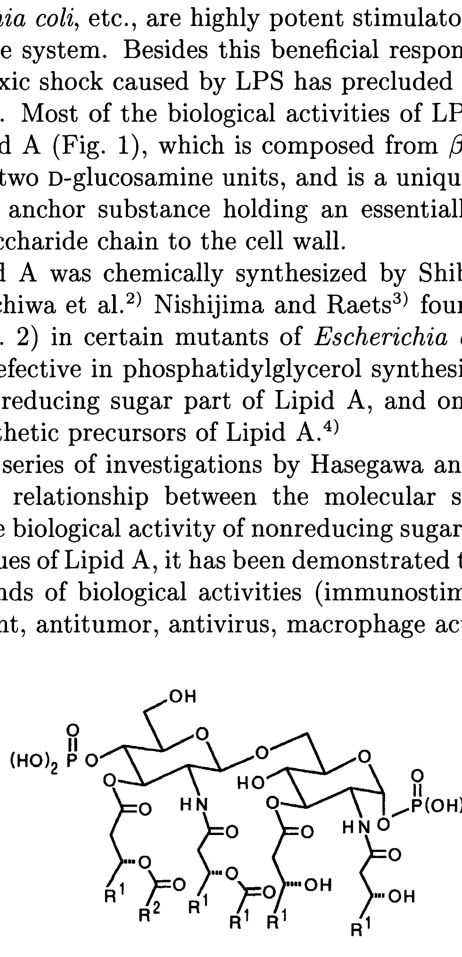


Fig. 1. Structure of Lipid A of *Escherichia coli*. $R^1=n\text{-}C_{11}H_{23}$, $R^2=n\text{-}C_{13}H_{27}$.

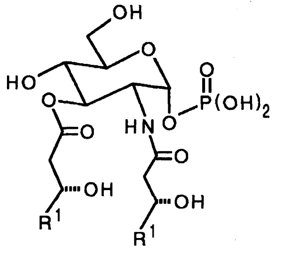


Fig. 2. Structure of Lipid X. $R^1=n\text{-}C_{11}H_{23}$.

Synthesis

(1*R*,2*S*,4*S*,5*S*)-6-(2,4-Dimethoxybenzyl)-4-hydroxy-2-[(1*R*)-1,2-isopropyridenedioxyethyl]-3-oxa-6-azabicyclo[3.2.0]heptan-7-one (**1**)[7] was converted to its allyl furanoside (**2**) by treatment with allyl iodide and silver(I) oxide. Reductive cleavage of *β*-lactam of **2** with lithium aluminum hydride gave the 3-homomannofuranosamine derivative (**3**). The primary alcohol moiety of **3** was acylated to compound **4** with (*R*)-3-benzyloxytetradecanoic acid by using dicyclohexylcarbodiimide (DCC) as a condensing agent. In these reaction conditions, the secondary amine moiety of **3** was not acylated. 2,4-Dimethoxybenzyl protective group was removed from **4** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), giving ester **5**, which gradually converted to amide **6** as a solid (Scheme 1). Compound **6** was further acylated with (*R*)-3-benzyloxytetradecanoic acid, to give **7**, which was treated with (cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate[8] and then iodine-water-pyridine,[9] to yield **8** (mp 78—80 °C).

Treatment of **8** with 90% acetic acid at 60 °C, and then with 2,2-dimethoxypropane, gave an anomeric gummy mixture of pyranose **9**. Treatment of **9** with

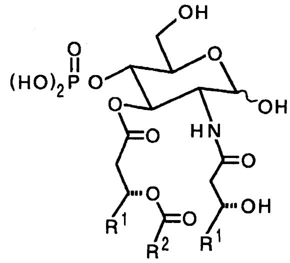
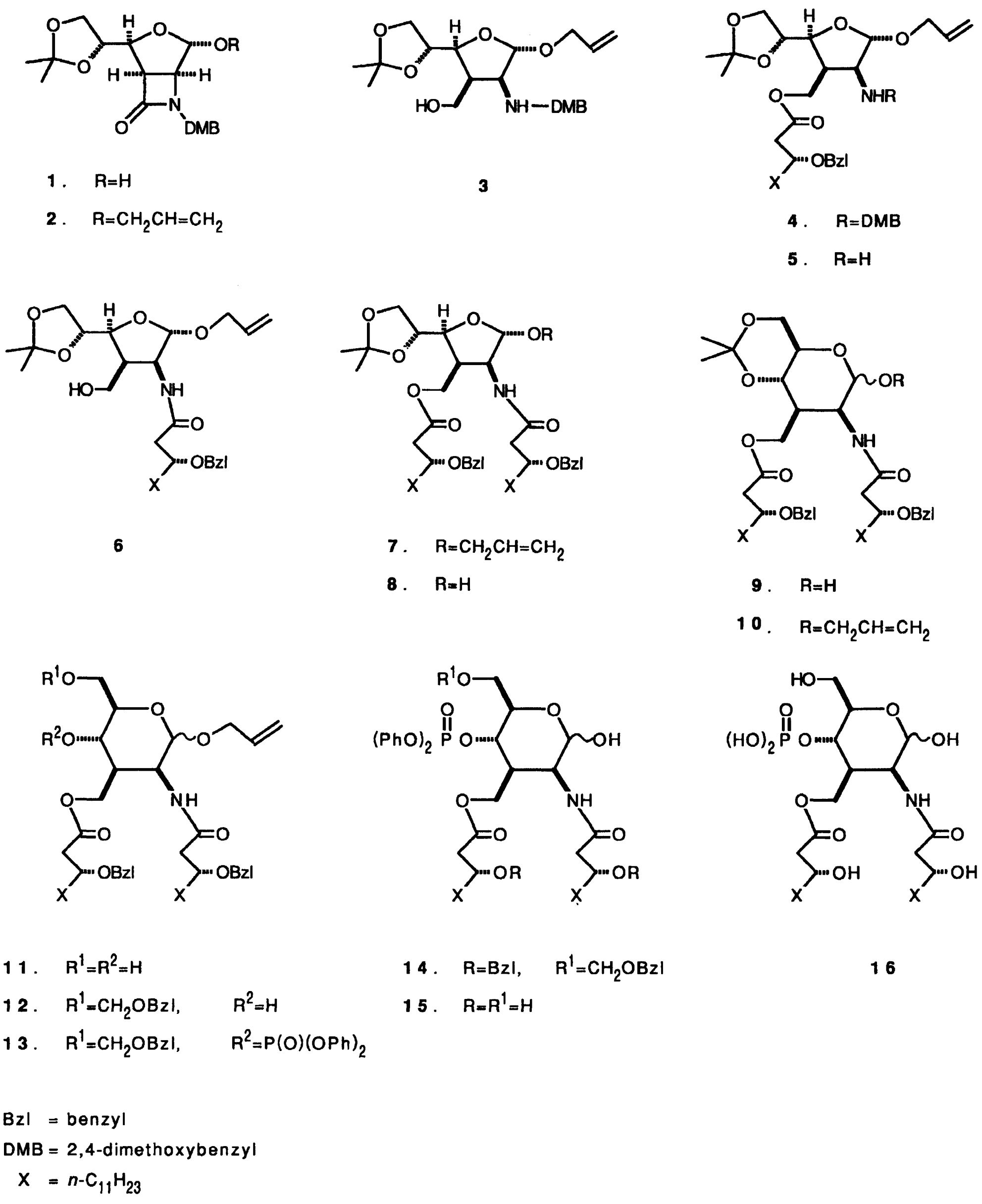


Fig. 3. Structure of GLA-60. $R^1=n\text{-}C_{11}H_{23}$, $R^2=n\text{-}C_{13}H_{27}$.



Scheme 1.

allyl iodide–Ag_2O gave an anomeric mixture of pyranoside **10**. The protective isopropylidene group of **10** was removed to form **11** by treatment with 85% acetic acid, and the primary alcohol moiety of **11** was protected by benzyl chloromethyl ether by using tetramethylurea as a weak base to give **12**. The secondary alcohol moiety of **12** was phosphorylated with diphenyl phosphorochloridate-*N*,*N*-dimethylaminopyridine to give **13**. The anomeric mixtures of **10**, **11**, **12**, and **13** were separable on silica-gel column chromatography, respectively. Treatment of **13** according to the same procedure as described in the formation of **8** from **7** gave **14**. Hydrogenolysis of the benzyl ester moiety of **14** using 10% palladium on carbon as a catalyst gave **15**. By successive hydrogenolysis of phosphoric ester moiety using platinum catalyst, the target compound **16** was obtained.

Compound **16** was inactive with respect to prostaglandin D_2 production in macrophage-like cell line J774.1, an indicator of macrophage activation.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were obtained by the used of a Perkin–Elmer 241 polarimeter. ^1H NMR spectra were recorded at 60 MHz on a Varian T-60, and at 270 MHz on a JEOL JNN-270, using tetramethylsilane as an internal standard. The IR absorption spectra were determined on a JASCO IR A-2 spectrophotometer. Mass spectra were obtained on a JMS-OlSG mass spectrometer. Preparative TLC was performed on silica-gel plates (Merck, Silica Gel 60 F_{245}), and column chromatography was carried out on columns packed with E.

Merck Silica Gel 60 (230—400 mesh) using slightly increased pressure (1.4 atm) for elution.

(1*R*,2*S*,4*S*,5*S*)-4-Allyloxy-6-(2,4-dimethoxybenzyl)-2-[(1*R*)-1,2-*O*-isopropylideneethyl]-3-oxa-6-azabicyclo[3.2.0]heptan-7-one (2). To a solution of the compound **1**[7)] (2.17 g, 5.17 mmol) in DMF (30 mL) and allyl iodide (5 mL, 54.7 mmol) was added Ag_2O (6 g, 25.9 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was filtered, and the filtrate was concentrated in vacuo by a pump, to give an oily mixture that was purified on a silica-gel column. Elution with cyclohexane–EtOAc (1:1) gave 1.70 g (70.8 %) of **2** (R_f=0.558) as a gum. IR ν_{max} (film) 1747, 1608, 1585 cm^{-1}. ^1HNMR (60 MHz, $CDCl_3$) δ=1.37 (3H, s), 1.42 (3H, s), 3.6—4.6 (16H, m, containing 6H singlet of two methoxy groups at δ=3.82), 4.83 (1H, s), 5.0—5.4 (2H, m), 5.5—6.2 (1H, m), 6.35—6.60 (2H, m), 7.15 (1H, d, J=9 Hz). MS m/z 419 (M^+), 404, 362, 193, 151, 121. High Resolution MS of M^+, Found: 419.19462. Calcd for $C_{22}H_{29}NO_7$: 419.19482.

Allyl 2,3-Dideoxy-2-(2,4-dimethoxybenzylamino)-3-*C*-hydroxymethyl-5,6-*O*-isopropylidene-α-D-mannofuranoside (3). To a solution of the crude **2** obtained above (1.7 g, 4.05 mmol) in THF (70 mL) was added $LiAlH_4$ (500 mg, 13.2 mmol) little by little with stirring at an ice-cooling temperature. The mixture was stirred for 15 min at this temperature, and then at room temperature for 30 min. The reaction mixture was quenched with AcOH and diluted with EtOAc that had been washed with aq $NaHCO_3$ and brine. (In the event separation is not good, the mixture should be filtered on celite.) The solution was dried over $MgSO_4$, filtered, concentrated in vacuo, and chromatographed on a silica-gel column. Elution with cyclohexane–EtOAc (1:1) gave **3** (1.53 g, R_f=0.351, 63.2% from **1**) as a gum. $[\alpha]_D^{24}$ +28.2° (c=2.15, $CHCl_3$). IR ν_{max} (film) 3450, 1613, 1588 cm^{-1}. ^1HNMR (60 MHz, $CDCl_3$) δ=1.34 (3H, s), 1.40 (3H, s), 2.66 (1H, quintet, J=6.5 Hz), 2.66 (2H, broad, D_2O exchanged), 3.34 (1H, dd, J=2.5, 6.5 Hz), 3.7—4.4 (16H, m, containing 6H singlet at δ=3.80), 4.89 (1H, d, J=2.5 Hz), 5.0—6.3 (3H, m), 6.3—6.5 (2H, m), 7.12 (1H, d, J=9 Hz). MS m/z 424 (M^++1), 423 (M^+), 408, 382, 365, 306, 236, 167, 151, 121. High Resolution MS of 423, Found: 423.22581. Calcd for $C_{22}H_{33}NO_7$: 423.22561.

Allyl 3-*C*-[[(3*R*)-3-(Benzyloxy)tetradecanoyloxy]methyl]-2,3-dideoxy-2-(2,4-dimethoxybenzylamino)-5,6-*O*-isopropylidene-α-D-mannofuranoside (4). To a solution of **3** (1.50 g, 3.54 mmol) and (3*R*)-3-(benzyloxy)tetradecanoic acid (1.30 g, 3.9 mmol) in CH_2Cl_2 (25 mL) were added DCC (876 mg, 4.25 mmol) and DMAP (476 mg, 3.90 mmol) with stirring at room temperature. After 10 h, the reaction mixture was filtered to remove dicyclohexylurea, which was washed with a small volume of CH_2Cl_2. The combined filtrate was concentrated and chromatographed on a silica-gel column. Elution with cyclohexane–EtOAc (3:1) gave **4** (2.09 g, 79.8% yield) and 0.19 g of a closely related unknown isomer. $[\alpha]_D^{24}$ +20.1° (c=2.11, $CHCl_3$). IR ν_{max} (film) 2930, 2850, 1730, 1610, 1585 cm^{-1}. ^1HNMR (60 MHz, $CDCl_3$) δ=0.8—1.0 (3H, m), 1.1—1.6 (26H, m), 2.45 (2H, dd like, J=3, 6 Hz), 1.64 (1H, bs, NH), 2.78 (1H, broad m, C_3–H), 3.25 (1H, dd, J=2, 6 Hz, C_2–H), 3.70 (2H, s, NCH_2Ar), 3.75 (6H, s), 3.8—4.45 (9H, m), 4.50 (2H, s, OCH_2Ph), 4.87 (1H, d, J=2 Hz, C_1–H), 5.0—6.2 (3H, m, $-CH{=}CH_2$), 6.3—6.5 (2H, m), 7.14 (1H, d, J=9 Hz), 7.30 (5H, s). MS m/z 739 (M^+), 724, 682, 633, 590, 575, 552, 530, 364, 320, 246, 196, 167, 151, 121. Positive High Resolution FAB-MS of M^++H, Found: 740.47595. Calcd for $C_{43}H_{65+1}NO_9$: 740.47375.

Allyl 2-[(3*R*)-3-(Benzyloxy)tetradecanoylamino]-2,3-dideoxy-3-*C*-hydroxymethyl-5,6-*O*-isopropylidene-α-D-mannofuranoside (6). To a solution of **4** (2.03 g, 2.74 mmol) in CH_2Cl_2 (45 mL) were added H_2O (2.25 mL) and DDQ (760 mg, 3.35 mmol), and the mixture was stirred vigorously for 2 h at room temperature. The reaction mixture was filtered to remove precipitate, and the filtrate was concentrated in vacuo to give an oily mixture that consists mainly of Schiff base derived from liberated amine **5** and 2,4-dimethoxybenzaldehyde. The mixture was dissolved in 99.5% EtOH (100 ml), and then to this solution was added Girard-T (920 mg). The solution was stirred for 3 h at room temperature. The reaction mixture was concentrated in vacuo to a one-third its volume, diluted with EtOAc, washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated to give an oily mixture that was chromatographed on a silica-gel column. Elution with cyclohexane–EtOAc (3:1) and then (1:1) gave 964 mg (60%) of a mixture of an amine **5** and an alcohol **6**. Compound (**5**) (IR ν_{max} (film) 1730 cm^{-1}, ester) gradually changed to the acyl-exchanged amide **6** as a crystalline solid. Mp 89—90 °C (from cyclohexane). $[\alpha]_D^{24}$ +45.1° (c=0.20, $CHCl_3$). IR ν_{max} (Nujol), 3220, 2920, 2860, 1630 (amide) cm^{-1}. ^1HNMR (60 MHz, $CDCl_3$) δ=0.7—1.0 (6H, m), 1.1—1.7 (26H, m), 1.9—2.6 (3H, m), 3.6—4.2 (9H, m), 4.50 (2H, s, CH_2Ph), 4.80 (1H, s, C_1–H), 4.9—6.0 (3H, m), 6.71 (1H, d, J=7 Hz, NH), 7.33 (5H, s). MS m/z 590 (M^++1), 589 (M^+), 588, 574. Anal. Found: C, 68.92; H, 9.48; N, 2.40%. Calcd for $C_{34}H_{55}NO_7$: C, 69.23; H, 9.40; N, 2.38%.

Allyl 2-[(3*R*)-3-(Benzyloxy)tetradecanoylamino]-3-*C*-[[(3*R*)-3-(benzyloxy)tetradecanoyloxy]methyl]-2,3-dideoxy-5,6-*O*-isopropylidene-α-D-mannofuranoside (7). To a solution of the alcohol **6** (892 mg, 1.51 mmol) in CH_2Cl_2 (15 mL) were added a solution of (3*R*)-(3-benzyloxy)tetradecanoic acid (595 mg, 1.78 mmol) in CH_2Cl_2 (5 mL), DMAP (200 mg, 1.64 mmol), and then DCC (590 mg, 2.86 mmol) with stirring at room temperature. After 45 min., the reaction mixture was filtered and concentrated to give a mixture, which was chromatographed on a silica-gel short column. Elution with cyclohexane–EtOAc (3:1) gave 1.66 g of a crude mixture. This mixture was employed for the next reaction without further purification. A small amount of this mixture was separated on a silica-gel preparative TLC plate. Development by cyclohexane–EtOAc (3:1) gave **7** (R_f=0.511). $[\alpha]_D^{24}$ +16.7° (c=0.16, $CHCl_3$). IR ν_{max} (film) 3330 (w), 2930, 2860, 1735, 1675, 1650 cm^{-1}. ^1HNMR (60 MHz, $CDCl_3$) δ=0.8—1.0 (6H, m), 1.0—1.7 (46H, m), 2.3—2.6 (5H, m, C_3–H, $CH_2CO\times2$), 2.7—3.3 (1H, m, C_2–H), 3.6—6.3 (18H, m), 6.77 (1H, d, J=9 Hz, NH), 7.32 (10H, s). MS m/z 905 (M^+), 890 (M^+-15).

2-[(3*R*)-3-(Benzyloxy)tetradecanoylamino]-3-*C*-[[(3*R*)-3-(benzyloxy)tetradecanoyloxy]methyl]-2,3-dideoxy-5,6-*O*-isopropylidene-α-D-mannofuranoside (8). To a solution of the crude mixture **7** (1.60 g) in THF (80 mL) was added $[Ir(C_8H_{10})(PMePh_2)_2]PF_6$ (100 mg). The air in the reaction flask was completely replaced with nitrogen, and then with hydrogen, to activate the iridium complex. After 1 or 2 min, when the red-colored iridium complex became colorless and in solution, immediately the hydrogen was completely replaced again with nitrogen. This solution was stirred for 2 h at room temperature. After checking for double bond shift to an enol ether from the 1-allyloxyl group by TLC, H_2O (10 mL), pyridine (1.0 g, 12.4 mmol), and I_2 (700 mg, 2.76 mmol) were added to this solution with stirring at room temperature. After 15 min, the reaction mixture was concentrated in vacuo and diluted with EtOAc. The solution was washed with aqueous 5% Na_2SO_3, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo to give a residue that was chromatographed on a silica-gel column. Elution with cyclohexane–EtOAc (2 : 1) gave 1.06 g (82% from **6**) of **8** as a crystalline solid, mp 78—80 °C (needles from MeOH). $[\alpha]_D^{24}$ +4.5° (c=0.73, $CHCl_3$). IR ν_{max} (Nujol), 3350, 1727, 1650 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ=0.88 (6H, t, J=6.6 Hz), 1.25 (36H, bs), 1.29 (3H, s), 1.38 (3H, s), 1.4—1.7 (4H, m), 2.31—2.53 (4H, m), 2.77—2.86 (1H, m), 3.21 (1H, d, J=7.0 Hz, OH, exchanged with D_2O), 3.77—4.64 (13H, m, containing 4H singlet at δ=4.51), 5.22 (1H, dd, J=5.5, 7.0 Hz, changed to a doublet, J=5.5 Hz, on addition of D_2O), 6.75 (1H, d, J=7.7 Hz, NH), 7.24—7.33 (10H, m). Anal. Found: C, 71.64; H, 9.68; N, 1.63%. Calcd for $C_{52}H_{83}NO_9$ (866.1): C, 72.10; H, 9.66; N, 1.62%.

2-[(3*R*)-3-(Benzyloxy)tetradecanoylamino]-3-*C*-[[(3*R*)-3-(benzyloxy)tetradecanoyloxy]methyl]-2,3-dideoxy-4,6-*O*-isopropylidene-D-mannopyranose (9). A solution of **8** (500 mg, 0.577 mmol) in 90% AcOH (25 mL) was warmed at 60 °C for 4.5 h with stirring, concentrated, and dried with a pump to give an oily residue (500 mg), which was dissolved in DMF (6 mL). To this solution was added 2,2-dimethoxypropane (0.6 mL, 4.88 mmol) and TsOH–H_2O (6 mg, 0.032 mmol). The mixture was allowed to stand at room temperature overnight, and was then concentrated with a pump, to give an oily residue which was diluted with EtOAc, washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. Concentration of the solvent gave a crude mixture, which was chromatographed on a silica-gel column. Elution with cyclohexane–EtOAc (3 : 2) gave **9** (454 mg, 91% yield) as a 1 : 3 gummy anomeric mixture. The R_f value of the starting **8** was 0.407, and that of **9** was 0.475. IR ν_{max} (film), 3340, 2915, 2840, 1734, 1652 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ=0.88 (6H, t, J=6.2—7.0 Hz), 1.18—1.80 (46H, m), 2.30—2.68 (4H, m), 2.74 (1H, d, J=3.7 Hz, D_2O exchanged, OH), 3.15—4.67 (14H, m), 4.77 (1/4H, dd, J=1.0, 8.5 Hz, C_1–H), 5.03 (3/4H, d, J=2.6 Hz, changed to a singlet on addition of D_2O, C_1–H), 6.34 (1/4H, d, J=9.5 Hz, NH), 6.79 (3/4H, d, J=9.5 Hz, NH), 7.2—7.4 (10H, m). Anal. Found: C, 72.27; H, 9.25; N, 1.44%. Calcd for $C_{52}H_{83}NO_9$ (866.2): C, 72.10; H, 9.66; N, 1.62%. MS m/z 848 (M^+-18), 757, 739, 633.

Allyl 2-[(3*R*)-3-(Benzyloxy)tetradecanoylamino]-3-*C*-[[(3*R*)-3-(benzyloxy)tetradecanoyloxy]methyl]-2,3-dideoxy-4,6-*O*-isopropylidene-D-mannopyranoside (10). A mixture of **9** (379 mg, 0.438 mmol), DMF (2 mL), allyl iodide (2 mL, 21.9 mmol), and Ag_2O (1.0 g, 4.32 mmol) was stirred at room temperature for 5 h. The reaction mixture was filtered. The filtrate was concentrated in vacuo with a pump. The residual oil was chromatographed on a silica-gel column. Elution with cyclohexane–EtOAc (3 : 1) gave an anomeric mixture of **10** (357 mg, 90% yield) having the same R_f value (0.456). IR ν_{max} (film) 3350, 2920, 2850, 1733, 1652 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ=0.88 (6H, t, J=6.6 Hz), 1.0—1.8 (47H, m), 3.1—4.3 (11H, m), 4.4—4.7 (5H, m), 5.1—5.4 (2H, m), 5.8—6.0 (1H, m), 6.76 (1H, d, J=9.9 Hz), 7.2—7.5 (10H, m). Anal. Found: C, 73.09; H, 9.61; N, 1.25%. Calcd for $C_{55}H_{87}NO_9$ (906.2): C, 72.89; H, 9.68; N, 1.55%. MS m/z 905 (M^+), 890, 848.

Allyl 2-[(3*R*)-3-(Benzyloxy)tetradecanoylamino]-3-*C*-[[(3*R*)-3-(benzyloxy)tetradecanoyloxy]methyl]-2,3-dideoxy-D-mannopyranoside (11). A solution of **10** (357 mg, 0.394 mmol) in AcOH (85%, 40 mL) was stirred at 60 °C for 30 min. The reaction mixture was concentrated with a pump and chromatographed on a silica-gel column. Elution with cyclohexane–EtOAc (1 : 1) gave 228 mg of β-anomer of **11** (R_f=0.383) and 68 mg of α-anomer of **11** (R_f=0.270), (total 87% yield). β-Anomer: $[\alpha]_D^{24}$ +10.6° (c=0.17, $CHCl_3$). IR ν_{max} (film) 3350, 2920, 2860, 1732, 1675, 1655 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ=0.88 (6H, t, J=6.6—7.0 Hz), 1.20—1.40 (36H, m), 1.43—1.70 (4H, m), 1.74 (1H, t, J=5.9 Hz, OH), 2.31—2.62 (6H, m), 3.13 (1H, m, changed to dd on addition of D_2O, J=5.4, 10.8 Hz, C_4–H), 3.49—3.60 (3H, m, C_6–H_2, C_5–H), 3.77—4.30 (6H, m), 4.35 (1H, dd, J=2.7, 9.9 Hz, C_2–H), 4.49, 4.61 (2H, AB-q, J=11.4 Hz), 4.51, 4.55 (2H, AB-q, J=11.7 Hz), 4.59 (1H, d, $J_{1a,2e}$=1 Hz, C_1–H), 5.16—5.31 (2H, m), 5.80—5.95 (1H, m), 6.55 (1H, d, J=9.9 Hz, NH), 7.15—7.64 (10H, m). Found: C, 72.16; H, 9.63; N, 1.37%. Calcd for $C_{52}H_{83}NO_9$: C, 72.10; H, 9.66; N, 1.62%. α-Anomer: $[\alpha]_D^{24}$ −5.4° (c=0.26, $CHCl_3$). IR ν_{max} (film) 3350, 2920, 2850, 1732, 1673, 1650 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ=0.88 (6H, t, J=6.2—7.0 Hz), 1.19—1.40 (36H, m), 1.43—1.70 (4H, m), 1.73 (1H, t, J=6.8 Hz, OH), 2.31—2.64 (6H, m, containing OH), 3.02—3.18 (1H, m, C_4–H), 3.44—4.65 (15H, m), 5.14—5.27 (2H, m), 5.78—5.92 (1H, m), 6.45(1H, d, J=9.5 Hz, NH), 7.30—7.64 (10H, m).

Allyl 6-*O*-Benzyloxymethyl-2-[(3*R*)-3-(benzyloxy)tetradecanoylamino]-3-*C*-[[(3*R*)-3-(benzyloxy)tetradecanoyloxy]methyl]-2,3-dideoxy-D-mannopyranoside (12). A solution of an anomeric mixture (β: α=10 : 3) of **11** (212 mg, 0.245 mmol) in CH_2Cl_2 (2 mL), tetramethylurea (700 mg, 6.03 mmol), and benzyl chloromethyl ether (700 mg, 4.47 mmol) was stirred overnight at 15—20 °C. The reaction mixture was purified on a silica-gel column. Elution with cyclohexane–EtOAc (3 : 1) gave 310 mg of a mixture of **12** (anomeric mixture, R_f=0.314 (from the higher R_f anomer of **11**) and 0.176 (from the lower R_f anomer of **11**), respectively) and benzyl alcohol (R_f=0.35). It was difficult to separate benzyl alcohol from the higher R_f product. The higher R_f product: IR ν_{max} (film) 3340, 2920, 2850, 1733, 1675, 1652 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ=0.88 (6H, t, J=6.2—7.0 Hz), 1.20—1.40 (36H, m), 1.45—1.67 (5H, m, containing OH), 2.29—2.67 (5H, m), 3.13 (1H, m, changed to triplet-like on addition of D_2O, C_4–H), 3.55—4.78 (19H, m), 5.15—5.32 (2H, m), 5.88 (1H, m), 6.47 (1H, d, J=9.5 Hz, NH), 7.2—7.7 (15H, m). The lower R_f product: ^1H NMR (270 MHz, $CDCl_3$) δ=0.88 (6H, t, J=6.2—7.0 Hz), 1.20—1.40 (36H, m), 1.45—1.80 (45H, m), 1.94 (1H, broad m), 2.40—2.68 (4H, m), 3.10 (1H, dt, J=5.2—7.0, 9.1—11 Hz, changed to triplet on addition of D_2O, C_4–H), 3.27 (1H, m), 3.53—4.26 (9H, m), 4.44—4.85 (9H, m), 4.95 (1H, d, J=7.7 Hz), 5.10—5.27 (2H, m), 5.75—5.88 (1H, m), 6.30 (1H, d, J=9.5 Hz, NH), 7.13—7.36 (15H, m).

Allyl 6-*O*-Benzyloxymethyl-2-[(3*R*)-3-benzyl-

oxytetradecanoylamino]-3-*C*-[[(3*R*)-3-(benzyloxy)tetradecanoyloxy]methyl]-2,3-dideoxy-4-*O*-diphenoxyphosphinyl-D-mannopyranoside (13). To a solution of the mixture **12** (310 mg, 0.314 mmol) obtained above in CH_2Cl_2 (20 mL) were added diphenyl phosphorochloridate (500 mg, 1.86 mmol) and DMAP (500 mg, 4.05 mmol). The mixture was stirred for 30 min at room temperature, and then concentrated in vacuo to give a crude mixture that was chromatographed on a silica-gel column. Elution with cyclohexane–EtOAc (3:1) gave 580 mg of a mixture of **13** (anomeric mixture, $R_f=0.357$ (from the higher R_f anomer of **12**) and 0.304 (from the lower R_f anomer of **12**), respectively) and $PhCH_2OP(O)(OPh)_2$ ($R_f=0.402$). It was difficult to separate $PhCH_2OP(O)(OPh)_2$ from the higher R_f product. A small amount of the mixture was separated on a silica-gel preparative TLC plate. The higher R_f product: $[\alpha]_{\mathrm{D}}^{24}$ +2.7° ($c=0.22$, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) $\delta=0.88$ (6H, t, $J=6.2$—7.0 Hz), 1.1—1.6 (40H, m), 2.35—2.66 (4H, m), 2.80 (1H, m, C_3–H), 3.59—4.68 (20H, m), 5.18—5.34 (2H, m), 5.90 (1H, m), 6.59 (1H, d, $J=9.9$ Hz, NH), 7.10—7.37 (25H, m). The lower R_f product: ^1H NMR (270 MHz, $CDCl_3$) $\delta=0.88$ (6H, t, $J=5.9$—7.3 Hz), 1.20—1.40 (36H, m), 1.45—1.80 (4H, m), 2.34—2.70 (5H, m), 3.58—4.79 (19H, m), 5.11—5.25 (2H, m), 5.74—5.88 (1H, m), 6.48 (1H, d, $J=9.5$ Hz, NH), 7.13—7.38 (25H, m). Found: C, 70.76; H, 8.62; N, 1.10; P, 2.48%. Calcd for $C_{72}H_{100}NO_{13}P$: C, 70.97; H, 8.27; N, 1.15; P, 2.54%.

6-*O*-Benzyloxymethyl-2-[(3*R*)-3-(benzyloxy)tetradecanoylamino]-3-*C*-[[(3*R*)-3-(benzyloxy)tetradecanoyloxy]methyl]-2,3-dideoxy-4-*O*-diphenoxyphosphinyl-D-mannopyranose (14). The mixture **13** obtained above (370 mg, 0.304 mmol) was treated according to the same procedure as described in the formation of **8** from **7** to give 87 mg (28% yield from **11**) of **14** and $PhCH_2OP(O)(OPh)_2$, which were easily separable by silica gel column chromatography. IR $\nu_{\max}$ (film), 3330, 2920, 2860, 1735, 1670 (shoulder), 1653, 1590 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) $\delta=0.88$ (6H, t, $J=6.6$ Hz), 1.16—1.59 (40H, m), 2.30—2.68 (4H, m), 2.84 (1H, m), 2.88 (1H, b, OH), 3.52—4.80 (17H, m), 5.03 (1H, bs, C_1–H), 6.62 (1H, d, $J=9.5$ Hz, NH), 7.10—7.38 (25H, m). Found: C, 69.85; H, 7.94; N, 1.23; P, 3.09%. Calcd for $C_{69}H_{96}NO_{13}P$: C, 70.32; H, 8.21; N, 1.19; P, 2.63%.

2,3-Dideoxy-2-[(3*R*)-3-hydroxytetradecanoylamino]-3-*C*-[[(3*R*)-3-hydroxytetradecanoyloxy]methyl]-4-*O*-diphenoxyphosphinyl-D-mannopyranose (15). An anomeric mixture of **14** (63 mg, 0.053 mmol) in acetic acid (4 mL) containing 10% palladium on carbon (150 mg) was hydrogenolized for 5 h. The solution was filtered, and the filtrate was concentrated in vacuo to give **15** (46 mg, 98% yield). $[\alpha]_{\mathrm{D}}^{24}$ −25.0° ($c=0.14$, $CHCl_3$). IR $\nu_{\max}$ (film) 3330, 2920, 2850, 1730, 1653, 1590 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) $\delta=0.88$ (6H, t, $J=6.6$—7.0 Hz), 1.20—1.62 (40H, m), 2.20—2.48 (4H, m), 2.90 (1H, m, C_3–H), 3.32—4.70 (13H, m, containing 4×OH), 5.08 (1H, b, changed to singlet on addition of D_2O, C_1–H), 6.63 (1H, d, $J=10.3$ Hz, NH), 7.15—7.39 (10H, m). Anal. Found: C, 64.04; H, 8.95; N, 1.41; P, 3.39%. Calcd for $C_{47}H_{76}NO_{12}P$ (878.1): C, 64.29; H, 8.72; N, 1.60; P, 3.53%.

2,3-Dideoxy-2-[(3*R*)-3-hydroxytetradecanoylamino]-3-*C*-[(3*R*)-(3-hydroxytetradecanoyloxy)methyl]-4-*O*-phosphono-D-mannopyranose (16). An anomeric mixture of **15** (29 mg, 0.0330 mmol) in acetic acid (6 mL) was hydrogenolized for 6 h using PtO_2 (10 mg) as a catalyst. The solution was filtered and concentrated in vacuo to give **16** (24 mg, 98% yield) as a mixture of anomers (3:1). $[\alpha]_{\mathrm{D}}^{24}$ −17.8° ($c=0.09$, MeOH). IR $\nu_{\max}$ (Nujol) 3300 (broad), 1720, 1655 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3+D_2O$) $\delta=0.88$ (6H, t, $J=6.0$—7.0 Hz), 1.1—1.7 (40H, m), 2.2—2.8 (5H, m), 3.6—5.0 (10H, m). Found: C, 57.61; H, 8.81; N, 1.86; P, 4.25%. Calcd for $C_{35}H_{68}NO_{12}P$ (725.9): C, 57.91; H, 9.44; N, 1.93; P, 4.27%.

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