

Enzymatic Preparation of (*S*)-3-Hydroxytetradecanoic Acid and Synthesis of Unnatural Analogues of Lipid A Containing the (*S*)-Acid

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Synthesis of unnatural analogues, that contain (*S*)-3-hydroxytetradecanoyl moieties in place of the corresponding natural (*R*)-isomers, of both lipid A and its biosynthetic precursor, designated precursor Ia or lipid IV_A, has been achieved through our recently developed procedure. (*S*)-3-Hydroxytetradecanoic acid was prepared from its racemate through the optical resolution by the use of a lipase and subsequent fractional recrystallization. The (*S*)-acyl analogue of lipid A exhibited slightly stronger interleukin-6 inducing activity than the corresponding natural lipid A, and the (*S*)-acyl analogue of the biosynthetic precursor was far more active than the natural precursor in inhibiting the induction of interleukin-6 by lipopolysaccharide.

Many structural variations have been reported of lipid A, the bioactive principle of lipopolysaccharide (LPS) in the cell surface of Gram-negative bacteria, including their biosynthetic precursors.¹⁾ Interestingly, their bioactivities can be quite different. The main structural differences among these lipid A analogues are based on the variation of the number and type of acyl groups, and their location on the hydrophilic sugar backbone. As one of the several explanations for the diverse activity, the acyl part is supposed to influence the overall conformation of the hydrophilic moiety which would bind to the receptor on the macrophages. However, the detailed structure-activities relationship and the active conformation of lipid A as well as the nature of the receptor still remain to be studied.

Thus it seemed to be of value to synthesize analogues that possess other types of fatty acyl moieties for better understanding of these issues. So we planned to synthesize the unnatural analogues **3** and **4** (Chart 1) corresponding to lipid A **1**²⁾ from *Escherichia coli* and its biosynthetic precursor **2**,³⁾ respectively, but containing 3-hydroxytetradecanoyl moieties of (*S*)-configuration in place of the natural (*R*)-isomer, based on an improved new synthetic procedure presented in a preceding paper.^{3c)} The biological activity of the synthetic materials were examined by the use of human peripheral whole-blood cells. Irrespective of the configuration of the hydroxyacyl components, **3** induced interleukin-6 as natural lipid A **1** and LPS do, and **4** was antagonistic toward LPS like the natural biosynthetic precursor **2**.⁴⁾

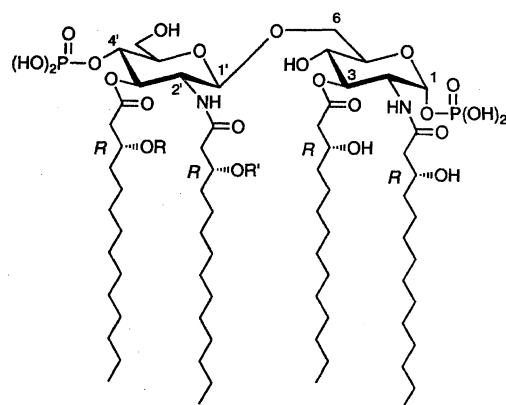
Results and Discussion

Preparation of Optically Pure 3-Hydroxytetradecanoic Acid. Several routes had been reported for the preparation of optically pure 3-hydroxytetradecanoic acid by us⁵⁾ as well as other researchers.⁶⁾ In this study, we prepared (*S*)-

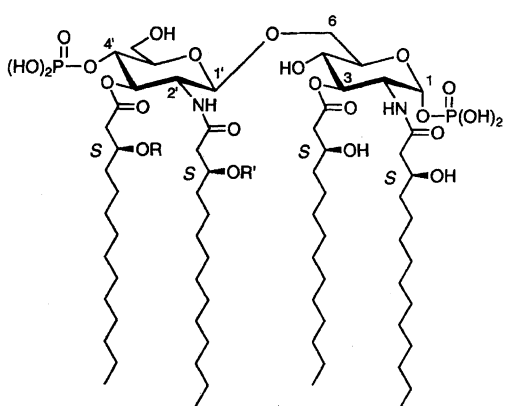
3-hydroxytetradecanoic acid ((*S*)-**7**) by enzymatic resolution of the racemate, i.e., through lipase-catalyzed transesterification of the racemic methyl ester, followed by fractional recrystallization.

Racemic methyl 3-hydroxytetradecanoate ((±)-**5**), which was readily obtained from Meldrum's acid and dodecanoyl chloride in 3 steps,^{5a,7)} was treated with immobilized lipase (Amano PS) and vinyl acetate in THF at 26–28 °C (Scheme 1).^{4,5c)} In this reaction, (*S*)-**5** was predominantly acetylated as in the resolution of racemic 3-hydroxytetradecanoic acid (±)-**7** reported by Wong et al.^{6c)} The reaction was monitored by gas-liquid chromatography and was stopped by filtration when 50% of the substrate was transformed into the corresponding acetate (after 5 d). Thus, the acetate (*S*)-**6** (ca. 70% ee) and the unchanged hydroxy ester (*R*)-**5** (70% ee) were obtained in 46 and 49% yields, respectively, after silica-gel column chromatography.⁸⁾ The enantiomeric purity of (*R*)-**5** was determined by ¹H NMR analysis using a chiral shift reagent, europium tris[3-(heptafluoropropyl)hydroxymethylene-(+)-camphorate] (Eu(hfc)₃), and that of desired (*S*)-**6** was estimated from the purity of (*R*)-**5** (70% ee) thus obtained. When the reaction was carried out in diisopropyl ether for 34 h, the ee of the remaining free (*R*)-**5** fell down to 53%, indicating that the reaction rate was higher but the selectivity was lower in this solvent than those in THF.

Optical resolution of the hydroxy ester (±)-**5** described here is more advantageous from a practical point of view than that of the corresponding hydroxy acid ((±)-**7**) using a *Pseudomonas* lipase reported by Wong et al.^{6c)} The latter reaction provided crystalline (*R*)-3-hydroxytetradecanoic acid ((*R*)-**7**) and oily (*S*)-3-(acetoxyl)tetradecanoic acid each in 80% ee, but the complete separation of these products can be achieved in our hand neither by a simple filtration nor by



1: Lipid A from *Escherichia coli*
(R = tetradecanoyl, R' = dodecanoyl)
2: Biosynthetic precursor (R = R' = H)



3 (R = tetradecanoyl, R' = dodecanoyl)
4 (R = R' = H)

Chart 1.

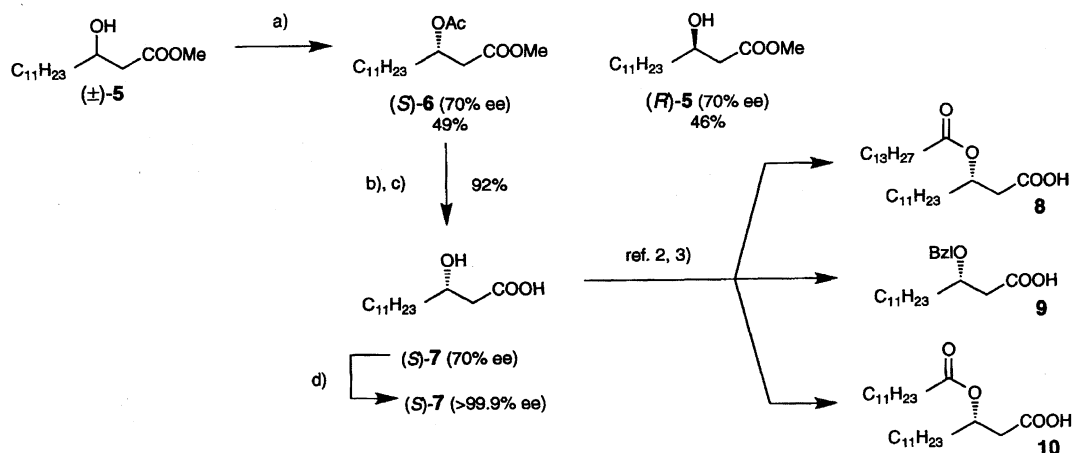
silica-gel chromatography. In the present method, the separation of the acetylated and unacetylated enantiomers by silica-gel chromatography was easier.^{5c)}

The acetate (*S*)-**6** was then deacetylated (6 M hydrochloric acid, MeOH, 40 °C) (1 M = 1 mol dm⁻³), and the subsequent hydrolysis of the methyl ester provided (*S*)-**7** in 92% yield.

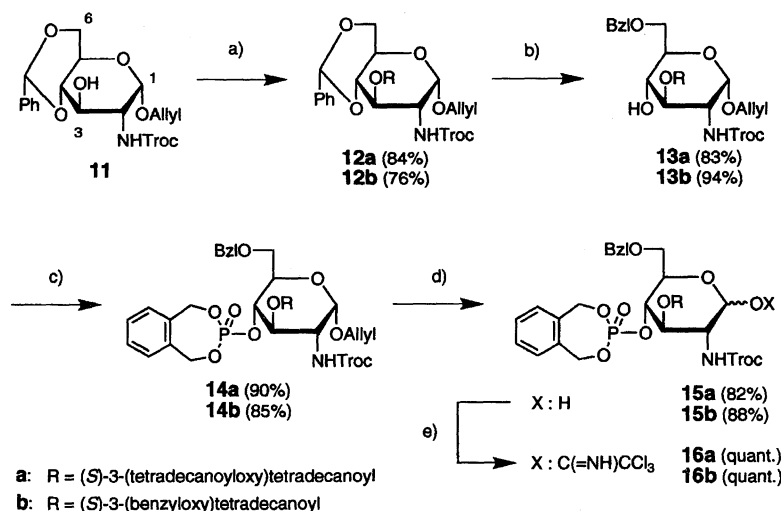
For this transformation, concurrent alkaline hydrolysis of both the acetyl group and the methyl ester induced a substantial amount of the β -elimination product (up to ca. 30%).

Next, fractional recrystallization of the partially resolved 3-hydroxytetradecanoic acid ((*S*)-**7**) was investigated to improve the enantiomeric purity. The use of (*S*)-1-phenylethylamine was found to be more effective for this purpose than that of achiral dibenzylamine so far employed for the same purpose in this laboratory. The (*S*)-amine was added to a solution of the optically impure (*S*)-**7** (70% ee) in CH₃CN at 20 °C. A slight excess (1.18 mol amt.) of the amine was used over the amount required to neutralize the enantiomerically pure (*S*)-acid present in the mixture. Crystals of the salt separated were collected by filtration and treated with aqueous citric acid to yield (*S*)-**7** of > 90% ee. The operation was repeated once more, providing enantiomerically pure (*S*)-**7** (> 99.9% ee). (*R*)-3-Hydroxytetradecanoic acid ((*R*)-**7**) of > 99.9% ee was also obtained from the crude methyl ester (*R*)-**5** by the same transformation (alkaline hydrolysis followed by fractional recrystallization using (*R*)-1-phenylethylamine) in 50% yield. Three (*S*)-3-hydroxytetradecanoic acid derivatives (**8**, **9**, and **10**) were synthesized from enantiomerically pure (*S*)-**7** by means of our published procedures.^{2,3)}

Syntheses of the Glycosyl Donors. The glycosyl donors **16a** and **16b**⁹⁾ corresponding to the distal sugar units of **3** and **4** were both prepared from the known compound **11** as summarized in Scheme 2.^{3c)} The transformation of **11** toward the donors includes five conventional manipulations: i) *O*-acylation with (*S*)-3-(tetradecanoyloxy)tetradecanoic acid (**8**) or (*S*)-3-(benzyloxy)tetradecanoic acid (**9**) using dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) at the 3-hydroxy group, ii) reductive opening of the benzylidene ring using sodium cyanotrihydroborate and HCl, iii) introduction of Watanabe's phosphoryl group¹⁰⁾ to the 4-hydroxy functions, iv) deprotection of the 1-*O*-allyl group by a cationic iridium complex ([Ir(cod)(MePh₂P)₂][PF₆]) and aqueous iodine,¹¹⁾ and v) the final trichloroacetimidate formation (CCl₃CN, Cs₂CO₃). Total yields from **11** were 51%



Scheme 1. a) immobilized lipase (Amano PS), vinyl acetate, THF, 26–28 °C, 5 d. b) hydrochloric acid, MeOH, 40 °C, 2 d. c) NaOH, H₂O, MeOH, 90 °C, 30 min. d) fractional crystallization as the (*S*)-1-phenylethylammonium salt.



Scheme 2. Troc = 2,2,2-trichloroethoxycarbonyl. Bzl = benzyl. a) **8** or **9**, DCC, DMAP, CH_2Cl_2 . b) $\text{Na}[\text{BH}_3(\text{CN})]$, HCl, THF. c) *N,N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine, 1*H*-tetrazole, CH_2Cl_2 . d) $[\text{Ir}(\text{cod})(\text{MePh}_2\text{P})_2]\text{PF}_6$, THF; I_2 , H_2O . e) CCl_3CN , Cs_2CO_3 , CH_2Cl_2 .

for **16a**, and 53% for **16b**.

Syntheses of the Glycosyl Acceptors. The synthetic route to **19** and **20** (Scheme 3) is almost the same as reported previously²⁾ except the reaction sequence. The known protected glucosamine **17**, prepared from *D*-glucosamine hydrochloride in 3 steps,²⁾ was acylated with (S)-3-(benzyloxy)-tetradecanoic acid (**9**) (DCC, DMAP) to give **18** in 81% yield. The isopropylidene group was removed (aqueous AcOH) to give the acceptor **19** for the synthesis of **4** in 92% yield. Because the *N*-acyl groups on the two glucosamine residues are the same in analogue **4**, both were introduced after the disaccharide formation to avoid the repetition of the same synthetic operation. The glycosyl acceptor **20** for the synthesis of **3** was obtained by the deprotection of the Troc group (zinc-copper couple)¹²⁾ of **19**, followed by selective acylation with **9** at the resultant amino group (83% yield).

Disaccharide Formations and Synthesis of the Desired (S)-Acyl Analogues of Lipid A. Trimethylsilyl trifluoromethanesulfonate-mediated glycosidation¹³⁾ of **16a** with **20** and **16b** with **19** proceeded smoothly and selectively at -20°C to afford $\beta(1\rightarrow6)$ disaccharide **21a** and **21b** in 82 and 74% yields, respectively (Scheme 4). The structures of these products were established by extensive ^1H NMR measurements.

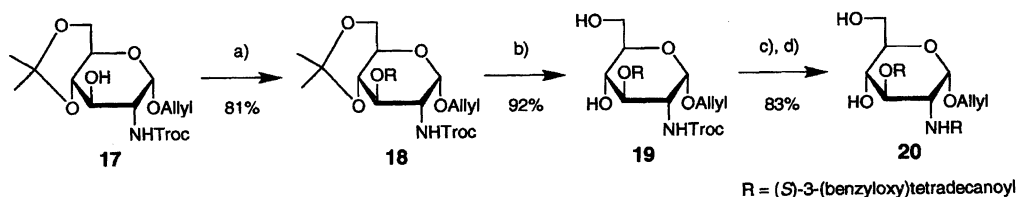
Both disaccharides **21a** and **21b** were treated with zinc-copper couple to remove the Troc protecting group-(s),¹²⁾ the products were mono- or bis-*N*-acylated with (S)-3-(benzyloxy)tetradecanoic acid (**9**) to yield **22a** or **22b**, re-

spectively. The allyl protection at the 1-position was then removed by the usual sequence (the iridium complex, then aqueous iodine), and the resultant hydroxy group was phosphorylated by butyllithium and tetrabenzyl diphosphate to give the 1-phosphates **24a** and **24b** in moderate yields (56 and 46%), owing to the lability of the products. The anomeric selectivity was, however, satisfactory to give the α -phosphates solely. Finally, hydrogenolysis (7 kg cm^{-2} of H_2 , Pd black, THF) of all the benzyl-type protecting groups for three or five hydroxy and two phosphoryl groups furnished desired **3** and **4** in high yields after purification by centrifugal partition chromatography.

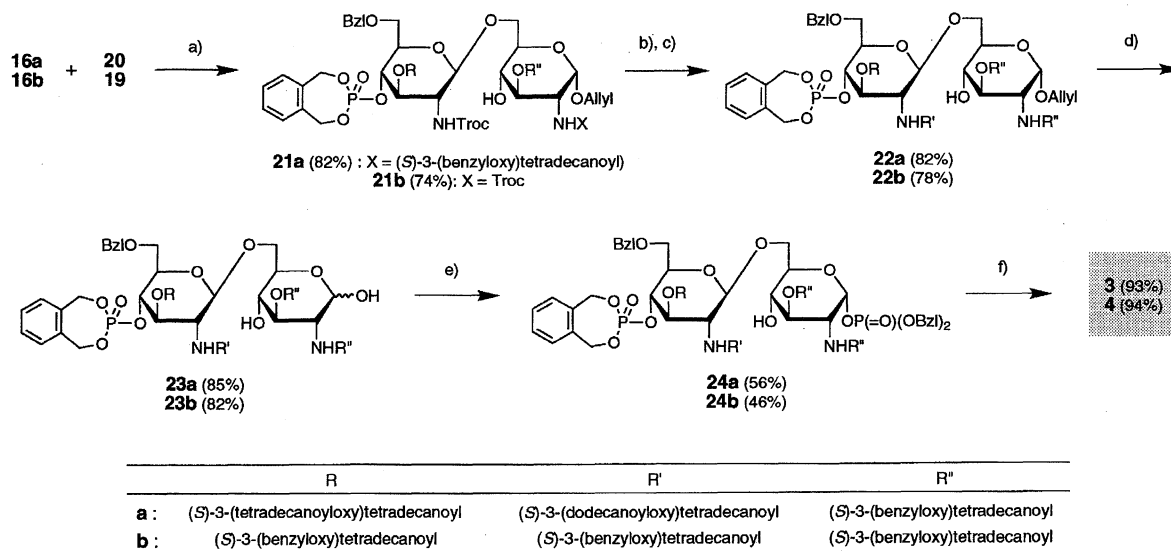
Induction of Interleukin-6. The biological activity of (S)-acyl analogues **3** and **4** prepared in this work, synthetic lipid A **1** of *Escherichia coli*-type,¹⁴⁾ natural-type biosynthetic precursor of lipid A **2**,³⁾ and a natural LPS from *E. coli* 0111:B4¹⁴⁾ was examined by testing the induction of interleukin-6 (IL-6) in heparinized human peripheral whole-blood cells prepared from the blood of M.H. or Y.S.¹⁵⁾

As shown in Fig. 1, the (S)-acyl analogue of lipid A **3** synthesized in this study was found to induce IL-6 as natural lipid A **1** and LPS do. Moreover, at every dose (0.1, 0.3, 1, and 3 ng mL^{-1}) except for the lowest one (0.03 ng mL^{-1}) tested, the IL-6 inducing activity of **3** was apparently stronger than that of natural-type lipid A **1** which contains (*R*)-acyl groups.

The IL-6 inducing activity of LPS (*E. coli* 0111:B4), the natural-type biosynthetic precursor **2**, and its (S)-acyl



Scheme 3. a) **9**, DCC, DMAP, CH_2Cl_2 , 24 h. b) AcOH- H_2O (9:1), 95°C , 10 min. c) Zn-Cu, AcOH, 30 min. d) **9**, DCC, CH_2Cl_2 , 2 d.



Scheme 4. a) TMSOTf, MS4A, (CH₂Cl)₂, -20 °C. b) Zn-Cu, AcOH. c) **10** or **9**, DCC, CH₂Cl₂. d) [Ir(cod)(MePh₂P)₂]PF₆, THF; I₂, H₂O. e) *n*-BuLi, ((BzlO)₂PO)₂O, THF, -78→23 °C. f) H₂ (7 kg cm⁻²), Pd black, THF.

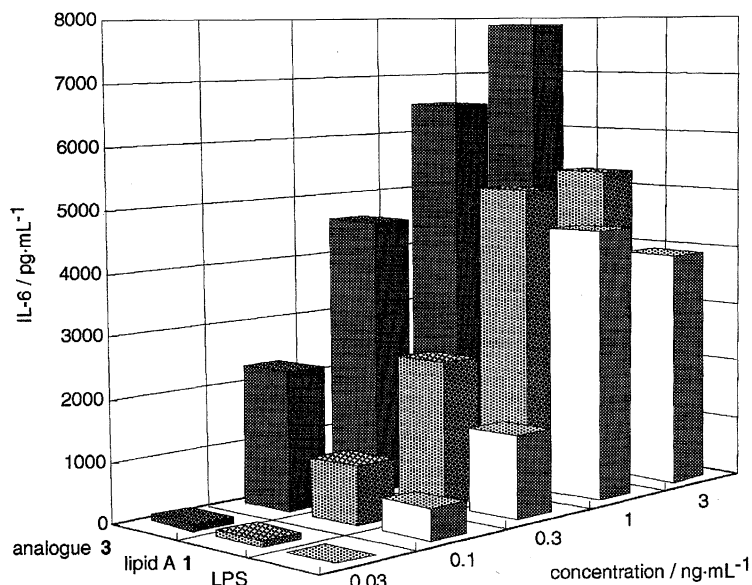


Fig. 1. IL-6 induction of **3**, synthetic lipid A **1** of *E. coli*-type, and LPS (*E. coli* 0111 : B4). The blood donor was M.H.

analogue **4**, was examined (Table 1). In this assay, LPS induced more than 2.3 ng mL⁻¹ of IL-6 at a dose of 0.1 ng mL⁻¹, whereas both **2** and **4** induced no IL-6 even with ten-times higher doses.

Next, the inhibitory activities of the natural-type precursor

2 and the (S)-acyl analogue **4** against IL-6 induction by LPS (0.1 ng mL⁻¹) were examined using the same cells as that for Table 1 (Fig. 2). As described, LPS (0.1 ng mL⁻¹ dose) alone induced 2.3 ng mL⁻¹ of IL-6. When the same amount of **2** was added to LPS, the level of induced IL-6 fell

Table 1. IL-6 Induction by LPS, the Natural-Type Precursor **2**, and (S)-Acyl Analogue **4**

Test compound	Dose (ng mL ⁻¹)	IL-6 induced (ng mL ⁻¹) ^{a)}
LPS	0.1	2.3
	1.0	2.5
Biosynthetic precursor 2	1.0	0.018
Analogue 4	1.0	0.014

a) The blood donor was Y.S.

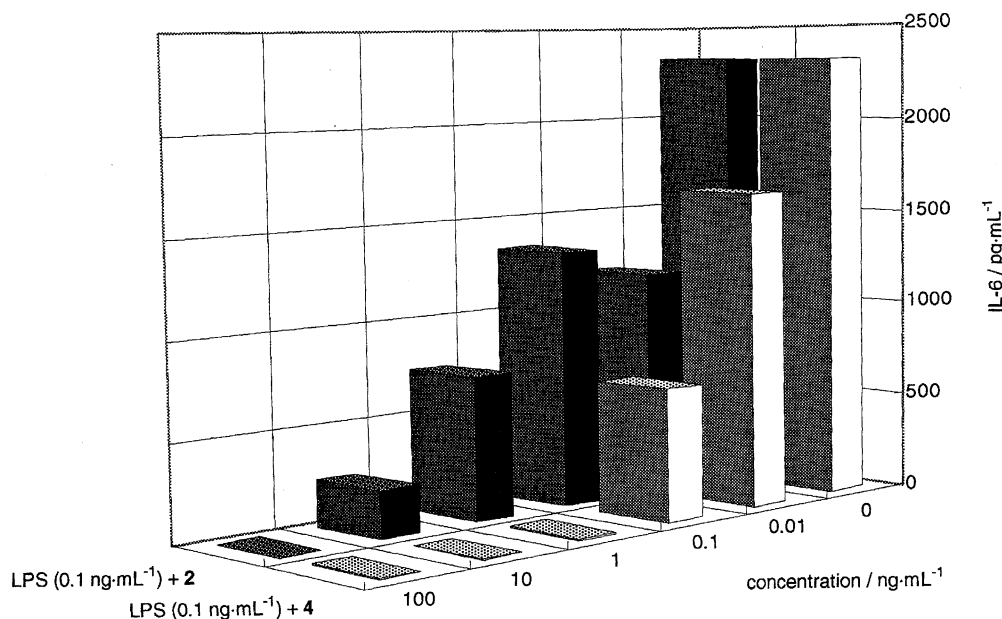


Fig. 2. Inhibitory activity by **2** and **4** against IL-6 induction by LPS. The blood donor was Y.S.

down to 1.34 ng mL^{-1} . A higher dose (100 ng mL^{-1}) of **2** completely inhibited the induction of IL-6 by LPS. The (*S*)-acyl analogue **4** was also found to show even more potent antagonistic activity than the natural-type compound **2**. For the complete inhibition of IL-6 production by LPS (0.1 ng mL^{-1}), 1 ng mL^{-1} of **4** was sufficient, as compared to the amount of natural precursor (10 ng mL^{-1}) required for the same effect. Thus, in a rough estimation, **4** with (*S*)-hydroxyacyl groups is ca. 10 times more potent than the natural-type **2** in inhibiting the IL-6 induction of LPS.

The other detailed bioactivities of **3** and **4** are under further investigation.

Conclusions

The synthesis of (*S*)-acyl analogues of lipid A and its biosynthetic precursor, **3** and **4**, have been accomplished by means of enzymatic preparation of (*S*)-3-hydroxytetradecanoic acid and its use in a recently improved synthetic strategy. Both syntheses require 13 steps from *N*-Troc-D-glucosamine; the total yields were 6.9% for **3** and 4.9% for **4**.

The positive bioactivity of the (*S*)-acyl analogue **3**, and the potent antagonistic activity of the (*S*)-acyl analogue **4** of a biosynthetic precursor showed that the (*R*)-configuration of the all 3-hydroxytetradecanoyl groups in lipid As is not necessarily required for their bioactivity. There might hence be another compounds of stronger bioactivity in the series of lipid A analogue containing different acyl groups.

These results also suggest that the molecular conformations of **1** and **3**, or **2** and **4** are similar to each other. This might be due to a similar arrangement of fatty acid moieties in both pairs of compounds.¹⁶ The conformational study of the lipid A analogues is important to understand the precise structure-activity relationships. Fortunately, salts of **3** and some other lipid A analogues are stable enough in several solvents to apply the NMR technique for their conforma-

tional analysis; this is now under way and the result will be reported in due course.

Experimental

The experimental techniques and the characterizing apparatuses used are summarized in our previous paper.^{5b} Gas-liquid chromatography was performed on a Shimadzu GC-14B gas chromatograph, and centrifugal partition chromatography was done on a model LLB-M apparatus (Sanki Engineering Ltd., Kyoto). IL-6 induction in human peripheral whole-blood cell cultures were measured as reported recently by us.¹⁵

Methyl (*S*)-3-Acetoxytetradecanoate ((*S*)-6**) [Enzymatic Optical Resolution].** To a solution of racemic methyl 3-hydroxytetradecanoate (**5**) (20.0 g, 0.080 mol) in THF (20 mL) were added vinyl acetate (80 mL, 0.86 mol) and immobilized lipase (Amano PS, 5.0 g).¹⁷ The reaction was monitored by gas-liquid chromatography (column, 2% OV-1 HP uniport; column temperature, 190°C ; nitrogen as a carrier gas at 40 mL min^{-1}). The mixture was stirred at $26\text{--}28^\circ\text{C}$ until the reaction was stopped by filtration of the immobilized lipase at 50% of the conversion. After removing the solvent in vacuo, the residue was purified by silica-gel flash chromatography (500 g, toluene/EtOAc=20 : 1) to give (*S*)-**6** (9.89 g, 49%) and (*R*)-**5** (10.8 g, 46%). The ^1H NMR data of (*S*)-**6** were identical with those reported.⁷

The enantiomeric purities were determined on (*R*)-**5** by ^1H NMR (270 MHz) using a chiral shift reagent, $\text{Eu}(\text{hfc})_3$ as follows. A portion of (*R*)-**5** (5 mg) and $\text{Eu}(\text{hfc})_3$ (10 mg) were dissolved in CDCl_3 (0.7 mL). The signal of the methoxy group of the ester (*R*)-**5** appeared at 4.3 ppm, while that of (*S*)-**5** at 4.6 ppm. In this case the enantiomeric purity of (*R*)-**5** was found to be 70% ee, thus the ee of (*S*)-**6** was estimated also to be 70%.

(*S*)-3-Hydroxytetradecanoic Acid ((*S*)-7**).** To a solution of methyl 3-acetoxytetradecanoate ((*S*)-**6**) (33.8 g, 0.11 mol) in MeOH (200 mL) was added aqueous HCl (6 M, 80 mL). After stirring at 40°C for 2 d, the solvent was removed in vacuo. The residue was dissolved in MeOH (400 mL) and to this was added aqueous NaOH (4.6 M, 40 mL). After stirring at 90°C for 30 min, the solvent was removed in vacuo to give (*S*)-**7** (70% ee) as a pale yellow solid (24.6 g, 92%).

This product was then fractionally crystallized as the (*S*)-1-phenylethylamine salt from CH₃CN as follows. The optically impure 3-hydroxytetradecanoic acid ((*S*)-**7**, 70% ee, 26.6 g) obtained above and (*S*)-1-phenylethylamine (12.5 mL, 0.10 mol) were dissolved in MeOH (60 mL). CH₃CN (300 mL) was added to the solution, and the precipitate was collected by filtration. The crystalline mass obtained was treated with 10% aqueous citric acid (200 mL) and EtOAc (250 mL). The organic layer was separated, washed with saturated aqueous NaHCO₃ (250 mL) and water (200 mL) successively, then dried over Na₂SO₄. Evaporation of the solvent in vacuo gave a white solid. The above operation was repeated until ee of (*S*)-**7** exceeded 99% (two times). The ee was determined after converting to (*S*)-**5** by CH₂N₂ treatment, using the ¹H NMR technique as described above. The yield of (*S*)-**7** was 11.6 g (52%). [α]_D²⁵ = +15.2 (*c* 1.00, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ = 4.03 (m, 1H), 2.58 (dd, *J* = 16.5, 3.0 Hz, 1H), 2.47 (dd, *J* = 16.5, 8.9 Hz, 1H), 1.62–1.26 (m, 20H), 0.88 (t, *J* = 6.9 Hz, 3H).

Enantiomerically pure (*R*)-**7** was also obtained from (*R*)-**5** by alkaline hydrolysis followed by fractional crystallization using (*R*)-1-phenylethylamine.

Allyl 4,6-*O*-Benzylidene-2-deoxy-3-*O*-[(*S*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (12a**).** (*S*)-3-(Tetradecanoyloxy)tetradecanoic acid (**8**) (1.47 g, 3.2 mmol), DCC (596 mg, 3.2 mmol), and DMAP (38 mg, 0.31 mmol) were added to a solution of allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (**11**) (1.50 g, 3.1 mmol) in CH₂Cl₂ (20 mL). The mixture was stirred at room temperature for 24 h. Then MeOH (1.0 mL) and AcOH (0.5 mL) were added, and the mixture was stirred for 30 min. The insoluble materials were filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in EtOAc (100 mL), and washed successively with saturated aqueous NaHCO₃ (50 mL $\times$ 2) and brine (40 mL). The EtOAc solution was dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (180 g, toluene/EtOAc = 50 : 1) to give **12a** as a colorless powder (2.39 g, 84%). [α]_D²⁴ = +26.0 (*c* 0.93, CHCl₃). FAB-MS (positive) *m/z* 918 [(*M*+H)⁺]. Found: C, 61.28; H, 8.08; N, 1.52%. Calcd for C₄₇H₇₄Cl₃NO₁₀: C, 61.40; H, 8.11; N, 1.52%. ¹H NMR (500 MHz, CDCl₃) δ = 7.45–7.32 (m, 5H), 5.88 (m, 1H), 5.50 (s, 1H), 5.39 (dd, *J* = 10.1, 9.6 Hz, 1H), 5.33 (d, *J* = 9.8 Hz, 1H), 5.29 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.24 (dd, *J* = 10.3, 1.6 Hz, 1H), 5.19–5.14 (m, 1H), 4.93 (d, *J* = 3.6 Hz, 1H), 4.78 (d, *J* = 12.1 Hz, 1H), 4.68 (d, *J* = 12.1 Hz, 1H), 4.28 (dd, *J* = 10.0, 4.8 Hz, 1H), 4.21 (dd, *J* = 12.9, 5.3 Hz, 1H), 4.06 (ddd, *J* = 10.1, 9.8, 3.6 Hz, 1H), 4.02 (dd, *J* = 12.9, 6.7 Hz, 1H), 3.93 (ddd, *J* = 10.0, 9.6, 4.8 Hz, 1H), 3.77 (dd, *J* = 10.0, 10.0 Hz, 1H), 3.69 (dd, *J* = 9.6, 9.6 Hz, 1H), 2.59 (dd, *J* = 15.6, 6.7 Hz, 1H), 2.52 (dd, *J* = 15.6, 5.9 Hz, 1H), 2.15–2.03 (m, 2H), 1.56–1.49 (m, 4H), 1.31–1.25 (m, 38H), 0.88 (t, *J* = 6.9 Hz, 6H).

Allyl 4,6-*O*-Benzylidene-3-*O*-[(*S*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (12b**).** In a manner similar to that for the synthesis of **12a**, **11** (0.63 g, 1.3 mmol) was acylated with **9** to yield **12b** as a colorless solid (0.790 g, 76%). [α]_D²⁹ = +24.4 (*c* 0.91, CHCl₃). FAB-MS (positive) *m/z* 799 [(*M*+H)⁺]. Found: C, 60.12; H, 6.80; N, 1.52%. Calcd for C₄₀H₅₄Cl₃NO₉: C, 60.11; H, 6.81; N, 1.75%. ¹H NMR (500 MHz, CDCl₃) δ = 7.42–7.22 (m, 10H), 5.89 (m, 1H), 5.48 (s, 1H), 5.41 (dd, *J* = 10.3, 9.6 Hz, 1H), 5.37 (d, *J* = 9.8 Hz, 1H), 5.31 (dd, *J* = 17.1, 1.1 Hz, 1H), 5.24 (dd, *J* = 10.3, 1.1 Hz, 1H), 4.93 (d, *J* = 3.6 Hz, 1H), 4.76 (d, *J* = 12.1 Hz, 1H), 4.49 (d, *J* = 11.4 Hz, 1H), 4.45 (d, *J* = 12.1 Hz, 1H), 4.36 (d, *J* = 11.4 Hz, 1H), 4.28 (dd, *J* = 10.3, 4.8 Hz, 1H), 4.22 (dd, *J* = 12.8, 5.3 Hz,

1H), 4.05 (ddd, *J* = 10.3, 9.8, 3.6 Hz, 1H), 4.02 (dd, *J* = 12.8, 6.6 Hz, 1H), 3.95 (ddd, *J* = 10.3, 9.6, 4.8 Hz, 1H), 3.81–3.79 (m, 1H), 3.76 (dd, *J* = 10.3, 10.3 Hz, 1H), 3.70 (dd, *J* = 9.6, 9.6 Hz, 1H), 2.60 (dd, *J* = 15.1, 6.2 Hz, 1H), 2.49 (dd, *J* = 15.1, 5.9 Hz, 1H), 1.60–1.46 (m, 2H), 1.40–1.20 (m, 18H), 0.88 (t, *J* = 6.9 Hz, 3H).

Allyl 6-*O*-Benzyl-2-deoxy-3-*O*-[(*S*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (13a**).** To a solution of **12a** (1.32 g, 1.4 mmol) in anhydrous THF (40 mL) were added Na[BH₃CN] (0.95 g, 0.014 mol) and dry hydrogen chloride in THF (20% (w/v), 5 mL). After stirring for 10 min, saturated aqueous NaHCO₃ (50 mL) and acetone (30 mL) were added to the mixture, and stirring was continued for 30 min. The insoluble materials were filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in ethyl acetate (50 mL), and washed successively with saturated aqueous NaHCO₃ (30 mL) and brine (40 mL). The EtOAc solution was dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (180 g, toluene/EtOAc = 10 : 1) to give **13a** as a colorless syrup (1.09 g, 83%). [α]_D²¹ = +36.2 (*c* 1.36, CHCl₃). FAB-MS (positive) *m/z* 920 (*M*⁺). Found: C, 61.11; H, 8.35; N, 1.52%. Calcd for C₄₇H₇₄Cl₃NO₁₀: C, 61.26; H, 8.31; N, 1.52%. ¹H NMR (500 MHz, CDCl₃) δ = 7.38–7.24 (m, 5H), 5.88 (m, 1H), 5.34–5.31 (m, 1H), 5.27 (d, *J* = 10.0 Hz, 1H), 5.26 (dd, *J* = 15.6, 1.3 Hz, 1H), 5.20 (dd, *J* = 10.3, 1.3 Hz, 1H), 5.13 (m, 1H), 4.91 (d, *J* = 3.6 Hz, 1H), 4.76 (d, *J* = 11.9 Hz, 1H), 4.63 (d, *J* = 11.9 Hz, 1H), 4.61 (d, *J* = 3.0 Hz, 2H), 4.20 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.00 (dd, *J* = 12.8, 5.4 Hz, 1H), 3.95 (ddd, *J* = 10.2, 10.0, 3.6 Hz, 1H), 3.87–3.65 (m, 4H), 3.65 (s, 1H), 2.53 (dd, *J* = 14.1, 3.0 Hz, 1H), 2.41 (dd, *J* = 14.1, 9.7 Hz, 1H), 2.27 (t, *J* = 7.9 Hz, 2H), 1.60–1.56 (m, 4H), 1.31–1.24 (m, 38H), 0.88 (t, *J* = 6.9 Hz, 6H).

Allyl 6-*O*-Benzyl-3-*O*-[(*S*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (13b**).** In a manner similar to that for the synthesis of **13a**, **12b** (1.62 g, 2.0 mmol) was reduced to yield **13b** as a colorless syrup (1.52 g, 94%). [α]_D²⁹ = +28.8 (*c* 0.98, CHCl₃). FAB-MS (positive) *m/z* 799 (*M*⁺). Found: C, 59.94; H, 7.07; N, 1.81%. Calcd for C₄₀H₅₆Cl₃NO₉: C, 59.96; H, 7.04; N, 1.75%. ¹H NMR (500 MHz, CDCl₃) δ = 7.65–7.25 (m, 10H), 5.88 (m, 1H), 5.34 (d, *J* = 9.8 Hz, 1H), 5.28 (dd, *J* = 17.1, 1.3 Hz, 1H), 5.20 (dd, *J* = 10.5, 1.1 Hz, 1H), 5.11 (dd, *J* = 10.2, 9.4 Hz, 1H), 4.92 (d, *J* = 3.6 Hz, 1H), 4.69 (d, *J* = 12.1 Hz, 1H), 4.61 (d, *J* = 12.1 Hz, 1H), 4.59 (d, *J* = 11.5 Hz, 1H), 4.55 (s, 2H), 4.50 (d, *J* = 11.5 Hz, 1H), 4.19 (dd, *J* = 12.8, 5.3 Hz, 1H), 4.00 (dd, *J* = 12.8, 5.9 Hz, 1H), 3.95 (ddd, *J* = 10.2, 9.8, 3.6 Hz, 1H), 3.85–3.78 (m, 2H), 3.66–3.62 (m, 2H), 3.57 (dd, *J* = 9.4, 9.4 Hz, 1H), 2.71 (dd, *J* = 14.2, 5.2 Hz, 1H), 2.48 (dd, *J* = 14.2, 5.2 Hz, 1H), 1.65–1.48 (m, 2H), 1.40–1.25 (m, 18H), 0.88 (t, *J* = 6.9 Hz, 3H).

Allyl 6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*S*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (14a**).** To a solution of **13a** (960 mg, 1.0 mmol) in anhydrous CH₂Cl₂ were added *N,N*-diethyl-1,5-dihydro-3H-2,4,3-benzodioxaphosphepin-3-amine (619 mg, 2.5 mmol) and 1H-tetrazole (363 mg, 5.2 mmol). The mixture was stirred at room temperature for 30 min at –20 °C for 20 min. *m*CPBA (893 mg, 5.2 mmol) was added, and stirring was continued for another 40 min. The solution was quenched with saturated aqueous NaHCO₃ (40 mL) and extracted with EtOAc (50 mL). The EtOAc solution was washed successively with saturated aqueous NaHCO₃ (30 mL $\times$ 2) and brine (30 mL), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (180 g, toluene/EtOAc = 10 : 1) to give **14a** as a colorless syrup (1.03

g, 90%). FAB-MS (positive) m/z 1101 (M^+). Found: C, 58.56; H, 7.45; N, 1.39%. Calcd for $C_{55}H_{83}Cl_3NO_{13}P$: C, 59.86; H, 7.58; N, 1.27%. 1H NMR (600 MHz, $CDCl_3$) δ = 7.38–7.24 (m, 5H), 7.23–7.17 (m, 4H), 5.88 (m, 1H), 5.36 (dd, J = 10.6, 9.4 Hz, 1H), 5.28 (dd, J = 17.3, 1.3 Hz, 1H), 5.24 (d, J = 9.8 Hz, 1H), 5.22 (dd, J = 10.2, 1.3 Hz, 1H), 5.20–5.17 (m, 1H), 5.15–5.02 (m, 4H), 4.95 (d, J = 3.6 Hz, 1H), 4.78 (d, J = 12.1 Hz, 1H), 4.76–4.70 (m, 1H), 4.67 (d, J = 12.1 Hz, 1H), 4.64 (d, J = 12.1 Hz, 1H), 4.58 (d, J = 12.1 Hz, 1H), 4.20 (dd, J = 12.6, 5.4 Hz, 1H), 4.02 (dd, J = 12.6, 6.3 Hz, 1H), 4.03 (ddd, J = 10.6, 9.9, 3.6 Hz, 1H), 3.99–3.97 (m, 1H), 3.79 (dd, J = 11.0, 1.9 Hz, 1H), 3.73 (dd, J = 11.0, 4.9 Hz, 1H), 2.68 (dd, J = 17.0, 5.4 Hz, 1H), 2.62 (dd, J = 17.0, 7.4 Hz, 1H), 2.28 (t, J = 7.9 Hz, 2H), 1.60–1.56 (m, 4H), 1.28–1.25 (m, 38H), 0.88 (t, J = 6.9 Hz, 6H).

Allyl 6-*O*-Benzyl-3-*O*-[(*S*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3 λ^5 -3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (14b). In a manner similar to that for the synthesis of **14a**, **13b** (313 mg, 0.39 mmol) was phosphorylated to yield **14b** as a colorless syrup (311 mg, 85%). $[\alpha]_D^{25}$ = +28.5 (c 0.94, $CHCl_3$). FAB-MS (positive) m/z 982 [($M+H$) $^+$]. Found: C, 58.60; H, 6.53; N, 1.56%. Calcd for $C_{48}H_{63}Cl_3NO_{12}P$: C, 58.63; H, 6.46; N, 1.42%. 1H NMR (500 MHz, $CDCl_3$) δ = 7.39–7.11 (m, 14H), 5.88 (m, 1H), 5.39 (dd, J = 9.6, 9.6 Hz, 1H), 5.30 (dd, J = 15.8, 1.1 Hz, 1H), 5.28 (d, J = 8.5 Hz, 1H), 5.22 (dd, J = 10.3, 1.1 Hz, 1H), 5.14 (d, J = 13.9 Hz, 1H), 5.10 (d, J = 16.0 Hz, 1H), 5.05 (d, J = 16.0 Hz, 1H), 5.00 (d, J = 13.9 Hz, 1H), 4.96 (d, J = 3.6 Hz, 1H), 4.76 (d, J = 12.1 Hz, 1H), 4.75 (dd, J = 9.2, 9.2 Hz, 1H), 4.65 (d, J = 11.4 Hz, 1H), 4.58 (d, J = 11.6 Hz, 2H), 4.47 (d, J = 11.4 Hz, 1H), 4.25 (d, J = 12.1 Hz, 1H), 4.21 (dd, J = 12.8, 5.2 Hz, 1H), 4.05 (dd, J = 12.8, 4.8 Hz, 1H), 4.03–3.98 (m, 2H), 3.88–3.83 (m, 1H), 3.81–3.72 (m, 2H), 2.75 (dd, J = 16.2, 6.2 Hz, 1H), 2.58 (dd, J = 16.2, 5.9 Hz, 1H), 1.59–1.50 (m, 2H), 1.37–1.24 (m, 18H), 0.88 (t, J = 6.9 Hz, 3H).

6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3 λ^5 -3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*S*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (15a). To a degassed solution of **14a** (920 mg, 0.93 mmol) in THF (15 mL) was added [bis(methyldiphenylphosphine)](1,5-cyclooctadiene)iridium(I) hexafluorophosphate (50 mg, 0.06 mmol). After activation of the iridium catalyst with hydrogen two times (each 10 s), the mixture was stirred under a nitrogen atmosphere at room temperature for 20 min. Then iodine (436 mg, 1.7 mmol) and water (20 mL) were added and the reaction mixture was stirred for additional 20 min. To the mixture was added 5% aqueous $Na_2S_2O_3$ and the solution was extracted with $CHCl_3$. The extract was successively washed with 5% aqueous $Na_2S_2O_3$ (50 mL $\times$ 2) and brine (50 mL), and dried over Na_2SO_4 . After removal of the solvent in vacuo, the crude product was purified by silica-gel flash chromatography (80 g, toluene/EtOAc = 5 : 1) to give **15a** (α : β = 1 : 1) as a colorless syrup (719 mg, 82%). $[\alpha]_D^{23}$ = +1.6 (c 0.63, $CHCl_3$). FAB-MS (positive) m/z 1086 [($M+K$) $^+$]. Found: C, 58.94; H, 7.61; N, 1.44%. Calcd for $C_{51}H_{77}Cl_3NO_{13}P$: C, 58.37; H, 7.39; N, 1.33%. 1H NMR (500 MHz, $CDCl_3$) δ = 7.38–7.24 (m, 9H), 5.40 (dd, J = 10.2, 9.4 Hz, 1H), 5.36 (d, J = 9.8 Hz, 1H), 5.30 (d, J = 7.1 Hz, 0.5H), 5.11 (d, J = 3.4 Hz, 0.5H), 5.19–5.17 (m, 1H), 4.75 (d, J = 12.1 Hz, 1H), 4.69 (d, J = 12.1 Hz, 1H), 4.65 (m, 1H), 4.63 (d, J = 12.1 Hz, 1H), 4.56 (d, J = 12.1 Hz, 1H), 4.22 (ddd, J = 10.7, 9.9, 5.9 Hz, 1H), 3.98 (ddd, J = 10.2, 9.8, 3.4 Hz, 1H), 3.78 (dd, J = 10.7, 1.6 Hz, 1H), 3.70 (dd, J = 10.7, 5.9 Hz, 1H), 3.42 (br s, 1H), 2.66–2.64 (m, 2H), 2.27 (t, J = 7.9 Hz, 2H), 1.60–1.56 (m, 4H), 1.28–1.22 (m, 38H), 0.88 (t, J = 6.9 Hz, 6H).

6-*O*-Benzyl-3-*O*-[(*S*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3 λ^5 -3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (15b). In a manner similar to that for the synthesis of **15a**, **14b** (620 mg, 0.63 mmol) was deprotected to yield **15b** as a colorless syrup (520 mg, 88%). $[\alpha]_D^{25}$ = +2.5 (c 1.03, $CHCl_3$). FAB-MS (positive) m/z 964 [($M+Na$) $^+$]. Found: C, 57.44; H, 6.40; N, 1.60%. Calcd for $C_{45}H_{59}Cl_3NO_{12}P$: C, 57.30; H, 6.30; N, 1.48%. 1H NMR (500 MHz, $CDCl_3$) δ = 7.38–7.10 (m, 14H), 5.41 (dd, J = 10.5, 9.4 Hz, 1H), 5.33 (d, J = 9.8 Hz, 1H), 5.27 (dd, J = 3.4, 3.4 Hz, 1H), 5.13–4.96 (m, 4H), 4.72 (d, J = 11.9 Hz, 1H), 4.66–4.61 (m, 1H), 4.62 (d, J = 12.1 Hz, 1H), 4.58 (d, J = 11.5 Hz, 1H), 4.55 (d, J = 12.1 Hz, 1H), 4.47 (d, J = 11.5 Hz, 1H), 4.29 (d, J = 11.9 Hz, 1H), 4.22 (m, 1H), 3.97 (ddd, J = 10.5, 9.8, 3.4 Hz, 1H), 3.88 (d, J = 3.4 Hz, 1H), 3.85–3.80 (m, 1H), 3.78 (dd, J = 10.7, 1.8 Hz, 1H), 3.70 (dd, J = 10.7, 6.2 Hz, 1H), 2.73 (dd, J = 16.2, 6.2 Hz, 1H), 2.57 (dd, J = 16.2, 5.7 Hz, 1H), 1.60–1.50 (m, 2H), 1.40–1.23 (m, 18H), 0.88 (t, J = 6.9 Hz, 3H).

6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3 λ^5 -3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*S*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl Trichloroacetimidate (16a). To a solution of **15a** (350 mg, 0.33 mmol) in CH_2Cl_2 (10 mL) at room temperature were added Cs_2CO_3 (56 mg, 0.17 mmol) and trichloroacetonitrile (0.340 mL, 3.4 mmol). After stirring for 30 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (30 mL), and the mixture was extracted with $CHCl_3$ (50 mL). The extract was washed with brine (20 mL) and dried over Na_2SO_4 . Removal of the solvent in vacuo gave **16a** as a pale yellow syrup (391 mg, 99%), which was used for the subsequent glycosidation without further purification.

6-*O*-Benzyl-3-*O*-[(*S*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3 λ^5 -3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl Trichloroacetimidate (16b). In a manner similar to that for the synthesis of **16a**, **15b** (343 mg, 0.36 mmol) was reacted with trichloroacetonitrile to yield crude **16b** as a pale yellow syrup (401 mg, 100%), which was used for the next glycosidation reaction without further purification.

Allyl 3-*O*-[(*S*)-3-(Benzyloxy)tetradecanoyl]-2-deoxy-4,6-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (18). In a manner similar to that for the synthesis of **12a**, **17** (640 mg, 1.5 mmol) was acylated with **9** to yield **18** as a colorless syrup (900 mg, 81%). $[\alpha]_D^{24}$ = +37.6 (c 0.74, $CHCl_3$). FAB-MS (positive) m/z 748 (M^+). Found: C, 58.66; H, 7.39; N, 1.94%. Calcd for $C_{36}H_{54}Cl_3NO_9$: C, 59.56; H, 7.25; N, 1.86%. 1H NMR (500 MHz, $CDCl_3$) δ = 7.32–7.23 (m, 5H), 5.88 (m, 1H), 5.32 (d, J = 9.8 Hz, 1H), 5.28–5.22 (m, 3H), 4.88–4.87 (d, J = 3.6 Hz, 1H), 4.70 (d, J = 12.1 Hz, 1H), 4.61 (d, J = 12.1 Hz, 1H), 4.58 (d, J = 11.4 Hz, 1H), 4.46 (d, J = 11.4 Hz, 1H), 4.18 (dd, J = 12.5, 5.2 Hz, 1H), 4.02–3.97 (m, 2H), 3.88 (m, 1H), 3.85–3.80 (m, 1H), 3.78–3.73 (m, 3H), 2.67 (dd, J = 15.2, 6.6 Hz, 1H), 2.42 (dd, J = 15.2, 5.9 Hz, 1H), 1.57–1.24 (m, 20H), 0.88 (t, J = 6.9 Hz, 3H).

Allyl 3-*O*-[(*S*)-3-(Benzyloxy)tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (19). The acetone **18** (955 mg, 1.3 mmol) was heated in aqueous AcOH (90%, 10 mL) at 95 °C for 10 min, then the mixture was concentrated in vacuo and the residual solvent was coevaporated with toluene three times. The residue was purified by silica-gel flash chromatography (50 g, $CHCl_3$ /acetone = 50 : 1) to give **19** as a colorless powder (830 mg, 92%). $[\alpha]_D^{25}$ = +29.1 (c 0.98, $CHCl_3$).

FAB-MS (positive) m/z 710 [(M+H)⁺]. Found: C, 55.63; H, 7.07; N, 2.08%. Calcd for C₃₃H₅₀Cl₃N₂O₉: C, 55.74; H, 7.09; N, 1.97%. ¹H NMR (500 MHz, CDCl₃) δ = 7.35–7.24 (m, 5H), 5.85 (m, 1H), 5.34 (d, J = 9.6 Hz, 1H), 5.27 (dd, J = 17.2, 1.4 Hz, 1H), 5.19 (dd, J = 10.3, 1.4 Hz, 1H), 5.06 (dd, J = 10.4, 9.4 Hz, 1H), 4.88 (d, J = 3.6 Hz, 1H), 4.68 (d, J = 12.1 Hz, 1H), 4.61 (d, J = 12.1 Hz, 1H), 4.58 (d, J = 11.0 Hz, 1H), 4.49 (d, J = 11.0 Hz, 1H), 4.16 (dd, J = 12.8, 5.3 Hz, 1H), 3.96 (dd, J = 12.8, 6.4 Hz, 1H), 3.89 (ddd, J = 10.4, 9.6, 3.6 Hz, 1H), 3.83–3.78 (m, 1H), 3.70–3.62 (m, 3H), 3.43–3.38 (m, 2H), 2.74 (dd, J = 13.8, 5.0 Hz, 1H), 2.43 (dd, J = 13.8, 5.0 Hz, 1H), 1.64–1.23 (m, 20H), 0.88 (t, J = 6.9 Hz, 3H).

Allyl 3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-2-deoxy- α -D-glucopyranoside (20). To a solution of **19** (453 mg, 0.60 mmol) in AcOH (10 mL) was added zinc–copper couple (500 mg), and the mixture was stirred at room temperature for 30 min. The insoluble material was filtered off, the filtrate was concentrated in vacuo, and the residual solvent coevaporated with toluene three times. The crude product was dissolved in EtOAc (40 mL) and washed successively with saturated aqueous NaHCO₃ (30 mL $\times$ 2) and brine (20 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo to give the *N*-deprotected product (338 mg, 98%), which was used without further purification for the following *N*-acylation reaction.

The crude amine thus obtained was dissolved in anhydrous CH₂Cl₂ (10 mL). To this solution were added DCC (130 mg, 0.72 mmol) and (S)-3-(benzyloxy)tetradecanoic acid (**9**) (200 mg, 0.60 mmol). The mixture was stirred at room temperature for 2 d, and then worked up in the same manner as described for the synthesis of **12a**. The crude product obtained was then purified by silica-gel flash chromatography (50 g, CHCl₃/acetone = 50 : 1) to give **20** as a colorless syrup (424 mg, 83%). [α]_D²⁰ = +21.1 (c 1.18, CHCl₃). FAB-MS (positive) m/z 852 [(M+H)⁺]. Found: C, 71.28; H, 9.63%. Calcd for C₅₁H₈₁N₂O₉: C, 71.88; H, 9.58%. ¹H NMR (500 MHz, CDCl₃) δ = 7.37–7.26 (m, 10H), 6.68 (d, J = 9.4 Hz, 1H), 5.67 (m, 1H), 5.17 (dd, J = 17.2, 1.6 Hz, 1H), 5.10 (dd, J = 10.2, 1.6 Hz, 1H), 5.05 (dd, J = 10.7, 9.1 Hz, 1H), 4.80 (d, J = 3.6 Hz, 1H), 4.59 (d, J = 11.2 Hz, 1H), 4.55 (d, J = 11.2 Hz, 1H), 4.50 (d, J = 11.2 Hz, 1H), 4.46 (d, J = 11.2 Hz, 1H), 4.24 (ddd, J = 10.7, 9.4, 3.6 Hz, 1H), 4.01 (dd, J = 12.8, 5.4 Hz, 1H), 3.83 (dd, J = 12.8, 6.6 Hz, 1H), 3.81–3.72 (m, 1H), 3.71–3.62 (m, 4H), 3.39 (ddd, J = 9.1, 9.1, 3.2 Hz, 1H), 3.36 (d, J = 3.2 Hz, 1H), 2.74 (dd, J = 13.7, 5.2 Hz, 1H), 2.41 (dd, J = 15.3, 4.4 Hz, 1H), 2.36 (dd, J = 15.3, 6.6 Hz, 1H), 2.35 (dd, J = 13.7, 4.6 Hz, 1H), 1.86 (m, 1H), 1.59–1.56 (m, 4H), 1.31–1.25 (m, 36H), 0.88 (t, J = 6.9 Hz, 6H).

Allyl 6-O-[6-O-Benzyl-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)-3-O-[(S)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)- β -D-glucopyranosyl]-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-2-deoxy- α -D-glucopyranoside (21a). The imide **16a** (370 mg, 0.31 mmol), the acceptor **20** (260 mg, 0.31 mmol), and molecular sieves 4A (370 mg) in anhydrous 1,2-dichloroethane (10 mL) were stirred at –20 °C for 5 min. To this mixture was added trimethylsilyl trifluoromethanesulfonate (12 μ L, 0.06 mmol) and the mixture was stirred at –20 °C for 10 min. After removal of molecular sieves by filtration, the reaction mixture was neutralized with saturated aqueous NaHCO₃ (20 mL) and then extracted with EtOAc (50 mL). The EtOAc layer was washed successively with saturated aqueous NaHCO₃ (20 mL) and brine (20 mL), and was concentrated in vacuo. The residue was purified by silica-gel flash chromatography (50 g, CHCl₃/acetone = 70 : 1) to give **21a** as a colorless syrup (581 mg, 82%). [α]_D²³ = +6.2 (c 0.65, CHCl₃). FAB-MS (positive) m/z

1919 [(M+Na)⁺]. Found: C, 64.61; H, 8.38; N, 1.61%. Calcd for C₁₀₃H₁₅₈Cl₃N₂O₂₁P: C, 65.19; H, 8.39; N, 1.48%. ¹H NMR (500 MHz, CDCl₃) δ = 7.37–7.17 (m, 19H), 6.67 (d, J = 9.6 Hz, 1H), 5.68 (m, 1H), 5.44 (dd, J = 9.1, 9.1 Hz, 1H), 5.36 (d, J = 7.6 Hz, 1H), 5.17 (dd, J = 17.2, 1.3 Hz, 1H), 5.17–5.16 (m, 1H), 5.13–5.07 (m, 2H), 5.06–4.98 (m, 4H), 4.82 (d, J = 8.2 Hz, 1H), 4.77 (d, J = 3.6 Hz, 1H), 4.64–4.40 (m, 9H), 4.24 (ddd, J = 10.7, 9.6, 3.6 Hz, 1H), 4.04 (d, J = 9.8 Hz, 1H), 4.00 (dd, J = 12.8, 5.2 Hz, 1H), 3.84–3.75 (m, 5H), 3.70–3.65 (m, 3H), 3.50–3.43 (m, 2H), 2.70–2.61 (m, 3H), 2.45 (dd, J = 14.4, 4.7 Hz, 1H), 2.38–2.31 (m, 2H), 2.28 (t, J = 7.6 Hz, 2H), 1.60–1.45 (m, 8H), 1.40–1.25 (m, 74H), 0.88 (t, J = 6.9 Hz, 12H).

Allyl 6-O-[6-O-Benzyl-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)- β -D-glucopyranosyl]-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- α -D-glucopyranoside (21b). In a manner similar to that for the synthesis of **21a**, **16b** (204 mg, 0.19 mmol) was reacted with **19** (134 mg, 0.19 mmol) to yield **21b** as a colorless syrup (227 mg, 74%). [α]_D²⁵ = +13.6 (c 0.72, CHCl₃). FAB-MS (positive) m/z 1655 [(M+Na)⁺]. Found: C, 56.99; H, 6.53; N, 1.81%. Calcd for C₇₈H₁₀₇Cl₆N₂O₂₀P: C, 57.25; H, 6.59; N, 1.71%. ¹H NMR (600 MHz, CDCl₃) δ = 7.32–7.12 (m, 19H), 5.86 (m, 1H), 5.47 (dd, J = 9.8, 9.8 Hz, 1H), 5.28 (d, J = 9.8 Hz, 1H), 5.27 (dd, J = 15.7, 1.3 Hz, 1H), 5.20 (dd, J = 10.2, 1.3 Hz, 1H), 5.17–4.97 (m, 6H), 4.88 (d, J = 3.6 Hz, 1H), 4.84 (d, J = 7.7 Hz, 1H), 4.70–4.49 (m, 11H), 4.16 (dd, J = 12.9, 5.2 Hz, 1H), 4.02 (d, J = 10.7 Hz, 1H), 3.96 (dd, J = 12.9, 6.3 Hz, 1H), 3.92 (ddd, J = 10.7, 9.8, 3.6 Hz, 1H), 3.89–3.81 (m, 3H), 3.77–3.74 (m, 1H), 3.72–3.70 (m, 3H), 3.50–3.48 (m, 1H), 3.36–3.34 (m, 1H), 3.14 (s, 1H), 2.74 (dd, J = 15.7, 7.1 Hz, 1H), 2.65 (dd, J = 14.3, 5.4 Hz, 1H), 2.57 (dd, J = 15.7, 5.2 Hz, 1H), 2.53 (dd, J = 14.3, 5.7 Hz, 1H), 1.62–1.49 (m, 4H), 1.36–1.24 (m, 36H), 0.88 (t, J = 6.9 Hz, 6H).

Allyl 6-O-[6-O-Benzyl-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)-2-[(S)-3-(dodecanoyloxy)tetradecanoylamino]-3-O-[(S)-3-(tetradecanoyloxy)tetradecanoyl]- β -D-glucopyranosyl]-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-2-deoxy- α -D-glucopyranoside (22a). In a manner similar to that for the synthesis of **20**, **21a** (223 mg, 0.12 mmol) was deprotected and acylated with **10** to yield **22a** as a colorless syrup (204 mg, 82%). [α]_D²³ = +13.1 (c 1.00, CHCl₃). FAB-MS (positive) m/z 2152 [(M+Na)⁺]. Found: C, 69.89; H, 9.65; N, 1.37%. Calcd for C₁₂₆H₂₀₅N₂O₂₂P: C, 71.02; H, 9.70; N, 1.31%. ¹H NMR (500 MHz, CDCl₃) δ = 7.36–7.18 (m, 19H), 6.79 (d, J = 9.4 Hz, 1H), 6.35 (d, J = 7.1 Hz, 1H), 5.66 (m, 1H), 5.53 (dd, J = 10.6, 9.0 Hz, 1H), 5.20 (d, J = 8.2 Hz, 1H), 5.15 (dd, J = 17.2, 1.6 Hz, 1H), 5.15–4.92 (m, 7H), 5.05 (dd, J = 11.3, 1.6 Hz, 1H), 4.78 (d, J = 3.6 Hz, 1H), 4.60–4.53 (m, 4H), 4.56 (d, J = 11.4 Hz, 1H), 4.52 (d, J = 11.4 Hz, 1H), 4.47 (d, J = 11.4 Hz, 1H), 4.37 (d, J = 11.4 Hz, 1H), 4.26 (ddd, J = 10.7, 9.4, 3.6 Hz, 1H), 3.98–3.97 (m, 1H), 3.84–3.66 (m, 8H), 3.64–3.60 (m, 2H), 3.36–3.31 (m, 1H), 2.64 (dd, J = 16.2, 7.3 Hz, 1H), 2.60–2.51 (m, 3H), 2.47–2.37 (m, 2H), 2.32–2.26 (m, 6H), 1.64–1.50 (m, 12H), 1.40–1.24 (m, 108H), 0.88 (t, J = 6.9 Hz, 18H).

Allyl 6-O-[6-O-Benzyl-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)- β -D-glucopyranosyl]-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-2-deoxy- α -D-glucopyranoside (22b). In a manner similar to that for the synthesis of **20**, **21b** (197

mg, 0.12 mmol) was deprotected and acylated with **9** to yield **22b** as a colorless syrup (164 mg, 78%). $[\alpha]_D^{25} = +13.3$ (c 1.14, CHCl₃). FAB-MS (positive) m/z 1940 [(M+Na)⁺]. Found: C, 69.86; H, 8.51; N, 1.55%. Calcd for C₁₁₄H₁₆₉N₂O₂₀P: C, 71.37; H, 8.88; N, 1.46%. ¹H NMR (500 MHz, CDCl₃) δ = 7.37–7.10 (m, 29H), 6.66 (d, J = 9.8 Hz, 1H), 6.54 (d, J = 8.3 Hz, 1H), 5.63 (m, 1H), 5.36 (dd, J = 9.1, 9.1 Hz, 1H), 5.15 (dd, J = 17.1, 1.3 Hz, 1H), 5.14 (m, 1H), 5.05 (dd, J = 10.6, 1.3 Hz, 1H), 5.03 (d, J = 13.7 Hz, 2H), 4.96 (d, J = 14.0 Hz, 2H), 4.73 (d, J = 3.6 Hz, 1H), 4.56 (d, J = 8.0 Hz, 1H), 4.65–4.37 (m, 11H), 4.24 (ddd, J = 10.4, 9.8, 3.6 Hz, 1H), 3.96 (dd, J = 12.8, 5.3 Hz, 1H), 3.92 (d, J = 8.9 Hz, 1H), 3.84–3.69 (m, 4H), 3.76 (dd, J = 12.8, 6.4 Hz, 1H), 3.68–3.61 (m, 6H), 3.56 (ddd, J = 9.4, 9.4, 4.8 Hz, 1H), 3.41 (d, J = 4.8 Hz, 1H), 2.69 (dd, J = 16.0, 6.4 Hz, 1H), 2.56 (dd, J = 16.0, 5.4 Hz, 1H), 2.52–2.51 (m, 2H), 2.31 (d, J = 4.8 Hz, 1H), 2.31 (d, J = 4.7 Hz, 1H), 2.22 (d, J = 3.9 Hz, 1H), 2.21 (d, J = 7.3 Hz, 1H), 1.64–1.28 (m, 80H), 0.88 (t, J = 6.0 Hz, 12H).

6-O-[6-O-Benzyl-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)-2-[(S)-3-(dodecanoyloxy)tetradecanoylamino]-3-O-[(S)-3-(tetradecanoyloxy)tetradecanoyl]- β -D-glucopyranosyl]-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-D-glucopyranose (23a**).** In a manner similar to that for the synthesis of **15a**, **22a** (150 mg, 0.072 mmol) was deprotected to yield **23a** as a colorless syrup (125 mg, 85%). FAB-MS (positive) m/z 2112 [(M+Na)⁺]. Found: C, 69.99; H, 9.79; N, 1.38%. Calcd for C₁₂₃H₂₀₁N₂O₂₂P: C, 70.66; H, 9.69; N, 1.34%. ¹H NMR (500 MHz, CDCl₃) δ = 7.39–7.16 (m, 19H), 6.50 (d, J = 6.9 Hz, 1H), 6.47 (d, J = 9.4 Hz, 1H), 5.78 (d, J = 8.2 Hz, 1H), 5.63 (dd, J = 10.3, 8.9 Hz, 1H), 5.18–4.87 (m, 8H), 4.83 (s, 1H), 4.63–4.43 (m, 7H), 4.19–4.15 (m, 1H), 3.97–3.67 (m, 8H), 3.30 (d, J = 7.1 Hz, 1H), 3.19–3.14 (m, 1H), 2.96–2.91 (m, 1H), 2.69–2.64 (dd, J = 15.6, 7.8 Hz, 1H), 2.61–2.53 (m, 2H), 2.50–2.26 (m, 9H), 1.62–1.50 (m, 12H), 1.40–1.24 (m, 108H), 0.88 (t, J = 6.9 Hz, 18H).

6-O-[6-O-Benzyl-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)- β -D-glucopyranosyl]-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-D-glucopyranose (23b**).** In a manner similar to that for the synthesis of **15a**, **22b** (110 mg, 0.057 mmol) was deprotected to yield **23b** as a colorless syrup (88 mg, 82%). $[\alpha]_D^{25} = +5.2$ (c 1.02, CHCl₃). FAB-MS (positive) m/z 1900 [(M+Na)⁺]. Found: C, 70.13; H, 8.88; N, 1.48%. Calcd for C₁₁₁H₁₆₅N₂O₂₀P: C, 70.97; H, 8.85; N, 1.49%. ¹H NMR (600 MHz, CDCl₃) δ = 7.39–7.11 (m, 29H), 6.48 (d, J = 8.8 Hz, 1H), 6.34 (d, J = 9.3 Hz, 1H), 5.39 (dd, J = 10.5, 9.0 Hz, 1H), 5.13–5.00 (m, 4H), 4.98 (d, J = 8.2 Hz, 1H), 4.94 (dd, J = 10.7, 9.1 Hz, 1H), 4.63 (d, J = 11.8 Hz, 1H), 4.62–4.57 (m, 1H), 4.58 (d, J = 11.8 Hz, 1H), 4.57 (d, J = 11.8 Hz, 1H), 4.54 (d, J = 11.0 Hz, 1H), 4.53 (d, J = 11.3 Hz, 1H), 4.47 (d, J = 11.8 Hz, 1H), 4.46 (d, J = 11.0 Hz, 1H), 4.45 (d, J = 11.3 Hz, 1H), 4.39 (d, J = 10.1 Hz, 1H), 4.31 (d, J = 10.1 Hz, 1H), 4.19 (d, J = 3.6 Hz, 1H), 3.97 (ddd, J = 10.7, 9.3, 3.6 Hz, 1H), 3.88–3.84 (m, 4H), 3.71–3.75 (m, 3H), 3.69–3.66 (m, 1H), 3.65–3.61 (m, 2H), 3.53 (dd, J = 12.9, 8.5 Hz, 1H), 2.98 (dd, J = 9.1, 9.1 Hz, 1H), 2.72 (dd, J = 16.2, 6.6 Hz, 1H), 2.62 (dd, J = 13.7, 5.2 Hz, 1H), 2.59 (dd, J = 16.2, 5.2 Hz, 1H), 2.38 (dd, J = 15.1, 6.9 Hz, 1H), 2.33 (dd, J = 15.1, 4.4 Hz, 1H), 2.29 (dd, J = 13.7, 5.0 Hz, 1H), 2.28 (dd, J = 15.7, 2.7 Hz, 1H), 2.23 (dd, J = 15.7, 8.1 Hz, 1H), 1.62–1.24 (m, 80H), 0.88 (t, J = 6.0 Hz, 12H).

6-O-[6-O-Benzyl-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)-2-[(S)-3-(dodecanoyloxy)-

tetradecanoylamino]-3-O-[(S)-3-(tetradecanoyloxy)tetradecanoyl]- β -D-glucopyranosyl]-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-1-O-bis(benzyloxy)phosphoryl-2-deoxy- α -D-glucopyranose (24a**).** To a solution of **23a** (65 mg, 0.03 mmol) in anhydrous THF (5 mL) was added *n*-BuLi in hexane (15%, 35 mL, 0.05 mmol) at -78°C . The mixture was stirred for 5 min. Tetrabenzyl diphosphate (23 mg, 0.04 mmol) was then added and the mixture was stirred at that temperature for 1.5 h. The mixture was then allowed to warm gradually to room temperature, neutralized with saturated aqueous NaHCO₃ (15 mL), and extracted with EtOAc (30 mL). After drying over Na₂SO₄ and removal of the solvent in vacuo, the residue was purified by silica-gel flash chromatography (15 g, CHCl₃/acetone = 30 : 1) to give **24a** as a colorless syrup (41 mg, 56%).

6-O-[6-O-Benzyl-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)- β -D-glucopyranosyl]-3-O-[(S)-3-(benzyloxy)tetradecanoyl]-2-[(S)-3-(benzyloxy)tetradecanoylamino]-1-O-bis(benzyloxy)phosphoryl-2-deoxy- α -D-glucopyranose (24b**).** In a manner similar to that for the synthesis of **24a**, **23b** (75 mg, 0.04 mmol) was phosphorylated to yield **24b** as a colorless syrup (39 mg, 46%). FAB-MS (positive) m/z 2161 [(M+Na)⁺].

2-Deoxy-6-O-[2-deoxy-2-[(S)-3-(dodecanoyloxy)tetradecanoylamino]-3-O-[(S)-3-(tetradecanoyloxy)tetradecanoyl]- β -D-glucopyranosyl]-3-O-[(S)-3-hydroxytetradecanoyl]-2-[(S)-3-hydroxytetradecanoylamino]- α -D-glucopyranose 1,4'-Bisphosphate (3**).** To a solution of **24a** (41 mg, 17 mmol) in THF (3 mL) was added Pd-black (60 mg). The mixture was stirred under 7 kg cm⁻² of hydrogen at room temperature overnight. The reaction mixture was then neutralized with Et₃N (0.2 mL). After removal of the Pd catalyst by filtration, the solvent was evaporated in vacuo. The residue was purified by centrifugal partition chromatography (CPC) (*n*-BuOH/THF/H₂O/Et₃N = 45 : 35 : 100 : 0.02, 1600 rpm, the organic phase as the mobile phase at 2 mL min⁻¹). After concentration in vacuo and lyophilization, **3** was obtained as a triethylammonium salt (white powder, 28 mg, 93%). FAB-MS (negative) m/z 1797 (M - H)⁻. ¹H NMR (500 MHz, CDCl₃) δ = 5.55 (m, 1H), 5.22–5.18 (m, 4H), 4.77 (d, J = 8.7 Hz, 1H), 4.27–4.21 (m, 2H), 4.09–4.03 (m, 2H), 3.90–3.75 (m, 6H), 3.55 (dd, J = 9.6, 9.6 Hz, 1H), 3.49–3.47 (m, 1H), 2.70 (dd, J = 16.7, 4.8 Hz, 1H), 2.62–2.55 (m, 2H), 2.49–2.47 (m, 2H), 2.43–2.35 (m, 2H), 2.34–2.27 (m, 4H), 2.26–2.22 (m, 1H), 1.66–1.54 (m, 8H), 1.53–1.42 (m, 4H), 1.40–1.24 (m, 108H), 0.88 (t, J = 6.9 Hz, 18H).

2-Deoxy-6-O-[2-deoxy-3-O-[(S)-3-hydroxytetradecanoyl]-2-[(S)-3-hydroxytetradecanoylamino]- β -D-glucopyranosyl]-3-O-[(S)-3-hydroxytetradecanoyl]-2-[(S)-3-hydroxytetradecanoylamino]- α -D-glucopyranose 1,4'-Bisphosphate (4**).** In a manner similar to that for the synthesis of **3**, **24b** (55.0 mg, 25.7 μ mol) was deprotected to yield **4** as a triethylammonium salt (white powder, 34 mg, 94%). FAB-MS (negative) m/z 1403 (M⁻).

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