A Divergent Synthesis of Lipid A and Its Chemically Stable Unnatural Analogues

Wen-Chi Liu, Masato Oikawa, Koichi Fukase, Yasuo Suda, and Shoichi Kusumoto*

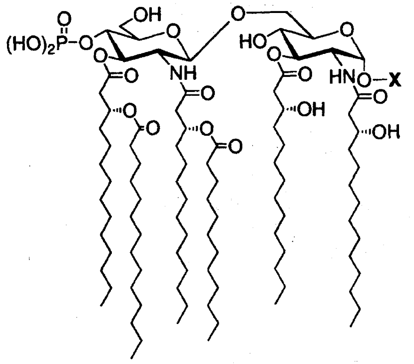
Department of Chemistry, Graduate School of Science, Osaka University, Machikaneyama-cho 1-1, Toyonaka, Osaka 560-0043

(Received December 14, 1998)

Lipid A and its two chemically stable analogues, wherein the glycosidic phosphoryl groups in lipid A is replaced with 2-(phosphonooxy)ethyl or carboxymethyl groups, have been synthesized by an improved and divergent route via a common allyl glycoside intermediate in which the 4-hydroxy group was protected as a benzyl ether. The total yields were more than 20% for 11 or 12 steps starting from allyl 4,6-*O*-benzylidene-2-deoxy-2-(trichloroethoxycarbonylamino)-D-glucopyranoside. These synthetic chemically stable analogues induce interleukin-6 and tumor necrosis factor α in human peripheral whole blood cells with potencies comparable to those by natural-type synthetic lipid A. The *Limulus* activities of both analogues were found to be even stronger than the activity of the natural-type one.

Lipopolysaccharide (LPS) is a ubiquitous glycoconjugate located in the outer membrane of Gram-negative bacteria, and is also called endotoxin. The endotoxic activities of LPS include pyrogenicity and induction of hypotension and lethal shock, whereas beneficial effects such as antitumor activity and enhancement of immunological responses are also caused by the same LPS molecule.[1] LPS is composed of a hydrophilic polysaccharide portion and a hydrophobic lipid part.[1,2] The lipid part was named lipid A; its chemical structure and the full endotoxic responsibility were established by our chemical synthesis of *Escherichia coli*-type lipid A **1**.[3,4] The typical fundamental structure of lipid A of many Gram-negative bacteria is a $\beta(1\rightarrow6)$ disaccharide of D-glucosamines having phosphate groups at the 1- and 4′-positions and long chain acyl groups (Chart 1).[2]

In the chemical structure of lipid A **1**, the phosphoryl group at the 1-position is quite unstable; it is readily cleaved even by its own acidic property, so particular precautions must be taken during the synthesis and purification. Chemically stable analogues have been eagerly required for this reason for the investigation of the mechanism of the biological action. In 1990, the 2-(phosphonooxy)ethyl (PE) derivative **2** was reported by Kusama et al. as a chemically stable analogue of lipid A **1**, and **2** was found to have activities indistinguishable from those of natural-type **1**. It was thus demonstrated that the glycosyl phosphate functionality is not strictly required to remain as it is, but may be modified without losing the endotoxic activity.[5] Kusama et al. also prepared a carboxymethyl (CM) analogue in which the phosphoryl group at the 1-position is replaced with a carboxymethyl group, but the CM analogue **3** with the same acylation pattern as lipid A **1** has not been synthesized.[6] In this paper we describe an improved divergent and efficient synthetic method for lipid A **1** as well as its chemically stable analogues **2** and **3** via a common allyl glycoside intermediate. A comparative study of the bioactivities for **1**, **2**, and **3** is also presented.



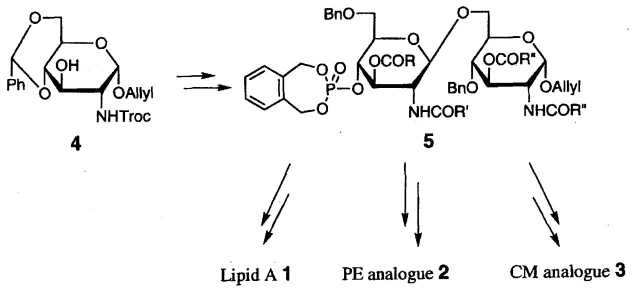
1 (X = $-P(O)(OH)_2$) : Lipid A from *Escherichia coli*
2 (X = $-CH_2CH_2OP(O)(OH)_2$) : PE analogue
3 (X = $-CH_2COOH$) : CM analogue

Chart 1.

Results and Discussion

Synthetic Plan. For the efficient synthesis of lipid A (**1**) and the PE and CM analogues (**2** and **3**), the allyl glycoside (**5**) (Scheme 1) was expected to be the most favorable common intermediate which has all the acyl and 4′-phosphate functionalities as required in the disaccharide, because the glycosidic allyl group can be chemoselectively converted by deprotective removal or oxidative cleavage to any of the glycosyl phosphate, PE and CM groups.

The protection strategy also plays a key role. From a preliminary experiment, the synthetic pathway successfully applied to our recent synthesis of lipid A analogues[7,8] was not applicable for the present purpose, because protection of the 4-hydroxy group turned out to be essential to avoid side



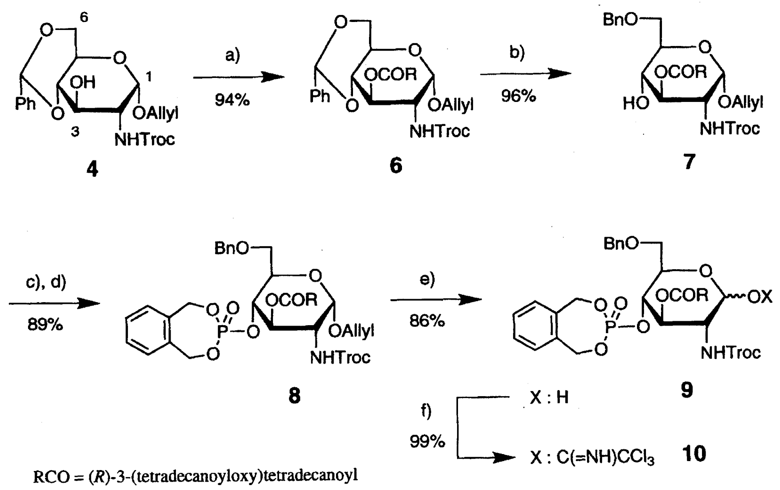
Scheme 1.

reactions during oxidation of the allyl group and the phosphorylation by the phosphoroamidite method (vide infra). The synthesis of highly pure materials in a large scale thus became possible by the following full protection strategy.

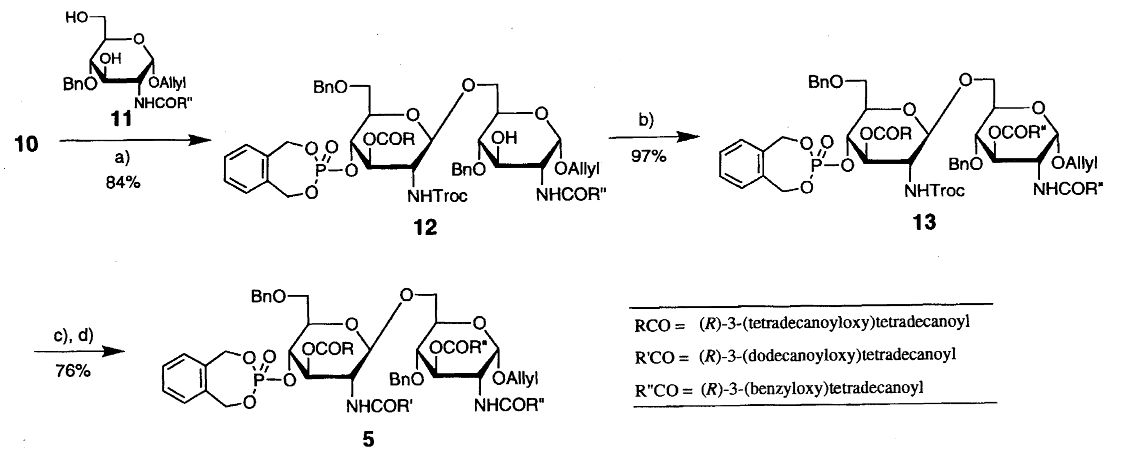
Synthesis of the Glycosyl Donor 10. The common glycosyl donor **10** for the synthesis of **1**, **2**, and **3** was prepared from the known compound **4**[7] as shown in Scheme 2. The alcohol **4** was acylated with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid[4b,9] using dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in 94% yield. Reductive opening of the benzylidene group was then effected by conventional sodium cyanotrihydroborate and HCl to give the 6-*O*-benzyl (Bn) ether **7** in 96% yield with complete regioselectivity. The transformation can also be achieved by dimethylamine-borane complex and diethyl ether-boron trifluoride, as we have recently reported.[10] But the latter reaction was found to be not applicable for a larger scale reaction as required in the present work, where the reductive opening leading to the common glycosyl donor **10** had to be performed with a quantity higher than 10 mmol scale. The remaining hydroxy group in **7** was successively phosphitylated[11] and oxidized to afford the protected phosphate **8** in 89% yield. Finally the 1-*O*-allyl group was deprotected by the 2 step sequence ([Ir(cod)$(MePh_2P)_2$]PF_6, then aqueous iodine)[12] which was followed by the trichloroacetimidate formation (trichloroacetonitrile and Cs_2CO_3) to give the glycosyl donor **10** in 86% yield. The total yield of **10** from **4** was satisfactorily 69% for 5 steps.

Disaccharide Formation and Synthesis of the Common Allyl Glycoside Intermediate 5. Coupling of the trichloroacetimidate **10** with the known glycosyl acceptor **11**[10b] was carried out in dichloromethane at −20 °C by the use of tin(II) trifluroromethanesulfonate ($Sn(OTf)_2$) as a catalyst to give the desired $\beta(1\rightarrow6)$ disaccharide **12** in 84% yield with complete regio- and stereoselectivity (Scheme 3). Acylation of the 3-position of **12** with (*R*)-3-(benzyloxy)-tetradecanoic acid[9,13] proceeded smoothly with the aid of DCC and DMAP to give **13** in 97% yield. Deprotection of *N*-2,2,2-trichloroethoxycarbonyl (Troc) group at the 2′-position of **13** (zinc–copper couple in aqueous acetic acid) was followed by *N*-acylation with (*R*)-3-(dodecanoyloxy)-tetradecanoic acid[4b,9] by using DCC to give the common intermediate **5** for the synthesis of **1**, **2**, and **3**.

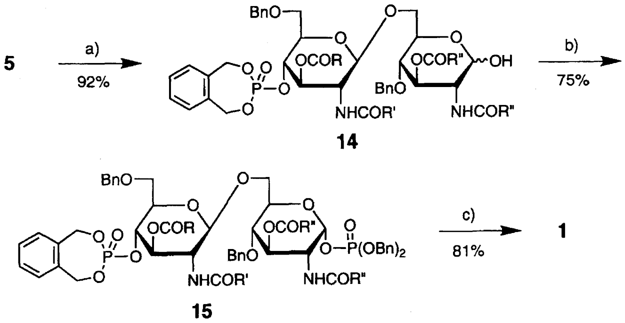
Synthesis of Natural *E. coli*-type Lipid A 1. For the introduction of the phosphoryl group at the 1-position, the 1-*O*-allyl group of **5** was removed as above to yield **14** in 92% yield (Scheme 4). The hydroxy group was then phosphorylated with tetrabenzyl diphosphate in the presence of lithium bis(trimethylsilyl)amide ($LiN(TMS)_2$) at −78 °C to give the 1-α-phosphate **15** in 75% yield.[14] Though 20% of **14** was recovered after purification by silica-gel column chromatography, the yield was apparently higher than our previous work[7,8] where an intermediate with two free hydroxy groups on the 1- and 4-positions was used. The improvement is due to the full protection strategy which allowed us to use an excess amount of the base and phosphorylation reagent. Finally, hydrogenolysis (7 kg cm^{-2} of hydrogen and Pd-black) of all the benzyl-type protecting groups furnished the desired **1** (81% yield), along with a 1-dephosphorylated product (16%) which was produced during the reaction but can be readily removed by liquid–liquid partition column chromatography on Sephadex® LH-20 gel.[7] The data of synthetic **1** were identical in all respects with those of authentic **1**.[4]



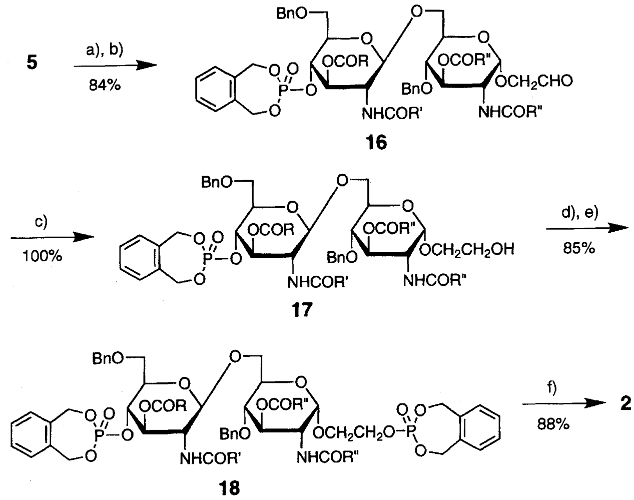
Scheme 2. Troc = 2,2,2-trichloroethoxycarbonyl. Bn = benzyl. a) (*R*)-3-(Tetradecanoyloxy)tetradecanoic acid, DCC, DMAP, CH_2Cl_2. b) Na[BH_3(CN)], HCl, THF. c) *N*,*N*-Diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine, 1*H*-tetrazole, CH_2Cl_2. d) *m*CPBA, −20 °C. e) [Ir(cod)$(MePh_2P)_2$]PF_6, THF; I_2, water. f) CCl_3CN, Cs_2CO_3, CH_2Cl_2.



Scheme 3. a) $Sn(OTf)_2$, MS4A, CH_2Cl_2, −20 °C. b) (*R*)-3-(Benzyloxy)tetradecanoic acid, DCC, DMAP, CH_2Cl_2. c) Zn–Cu, acetic acid. d) (*R*)-3-(Dodecanoyloxy)tetradecanoic acid, DCC, CH_2Cl_2.



Scheme 4. a) $[Ir(cod)(MePh_2P)_2]PF_6$, THF; I_2, water. b) $(TMS)_2NLi$, $((BnO)_2PO)_2O$, THF, −78 °C. c) H_2 (7 kg cm^{-2}), Pd black, THF.



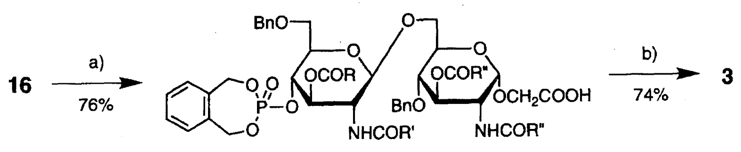
Scheme 5. a) OsO_4, NMO, THF/*t*-butyl alcohol/water (10 : 10 : 1). b) $Pb(OAc)_4$, benzene. c) $NaBH_4$, isopropyl alcohol/methanol/CH_2Cl_2 (5 : 1 : 1). d) *N*,*N*-Diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine, 1*H*-tetrazole, CH_2Cl_2. e) *m*CPBA, −20 °C. f) H_2 (7 kg cm^{-2}), Pd black, THF.

Synthesis of Chemically Stable Analogues 2 and 3. The synthetic scheme for the PE analogue **2** is illustrated in Scheme 5. Dihydroxylation of **5** using a catalytic amount of osmium(VIII) oxide (OsO_4) and 4-methylmorpholine *N*-oxide (NMO) in *t*-butyl alcohol and water followed by lead(IV) acetate ($Pb(OAc)_4$) oxidation afforded the aldehyde **16** in 84% yield. The use of ozone for this transformation unexpectedly resulted in the over-oxidation of ca. 10% of the benzyl groups on hydroxyacyl functions into the benzoyl groups, and the over-oxidized compounds could not be removed even after the final deprotecting step. Reduction of the aldehyde **16** was carried out with sodium tetrahydroborate ($NaBH_4$) to give the alcohol **17**, which was pure enough for the next reaction without further purification. Phosphitylation on **17** followed by oxidation[11] gave the bisphosphate **18** in 85% yield. In this transformation, the protection of the 4-hydroxy group was essential, as has been discussed above, because the hydroxy group was also reactive toward phosphitylation: If the 4-hydroxy group was not protected, the desired bisphosphate corresponding to **18** was obtained only in 22% yield by the use of 1 mol amt. of the phosphoroamidite, whereas the use of an excess amount of the reagent caused the reaction of both hydroxy groups. In that case the use of $LiN(TMS)_2$ and tetrabenzyl diphosphate, which have been proved to be effective for selective phosphorylation in our previous study,[10b] also turned out not to lead to the completion of the desired phosphorylation. In the presence of the free 4-hydroxy group, monophosphorylation is thus so far unsuccessful.

The final deprotection was accomplished by hydrogenolysis under the same conditions as those described for **1**. After the purification by liquid–liquid partition column chromatography, the chemically stable PE analogue **2** was obtained in 88% yield. The structure of synthetic **2** was confirmed by MS and NMR spectra, which were consistent with the reported data.[5]

For the synthesis of the CM analogue **3**, the aldehyde **16** was smoothly oxidized by the treatment with sodium chlorite ($NaClO_2$) (Scheme 6).[15] Successive deprotection under hydrogenolytic conditions, followed by liquid–liquid partition chromatographic purification, provided the desired CM analogue **3** in satisfactory yield (74%).

The Study on Biological Activity. Bioactivities of the



19

Scheme 6. a) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-butyl alcohol/water (4 : 1). b) H_2 (7 kg cm^{-2}), Pd-black, THF.

three pure synthetic preparations were directly compared for the first time. Cytokines inducing activity was tested first in heparinized human peripheral whole blood cells collected from an adult volunteer in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland). Incubation of the cells with synthetic compounds or LPS (*E. coli* 0111 : B4, Sigma Chemicals Co.) as a positive control was carried out under a 5% carbon dioxide atmosphere at 37 °C for 24 h.[16)]

Figure 1 shows the interleukin-6 (IL-6) inducing activity of **1**, **2**, **3**, and LPS. The levels of induced IL-6 were measured by means of enzyme-linked immunosorbent assay (ELISA). All experiments were done in duplicate, and the average values are used for the discussion. Positive activity was exhibited by all the samples tested. In addition, the use of blood from a different donor gave the same result (data not shown), thus indicating the reproducibility and high reliability of this experiment. The three synthetic compounds exhibited comparable effects at the concentration of 1 ng mL^{-1}, but at 0.1 ng mL^{-1} the inducing activity of the PE analogue **2** was slightly higher than that of **1** and **3**.

Induction of tumor necrosis factor α (TNF-α) was also measured by a procedure similar to that used for IL-6 by employing anti-TNF-α antibody for ELISA. The reliability of the experiment has been also confirmed by the use of blood from a different donor. As can be seen in Fig. 2, all the synthetic compounds have the comparable effects for the TNF-α induction. No TNF-α was induced at the concentration of 0.01 ng mL^{-1} of all samples. In both cytokine inducting experiments, the activities of the synthetic materials were higher at the high dose (10 ng mL^{-1}), but lower at the lower concentration (0.1 ng mL^{-1}) than the LPS from *E. coli* 0111 : B4 used as a positive control. This observation is quite consistent with our previous study.[16)]

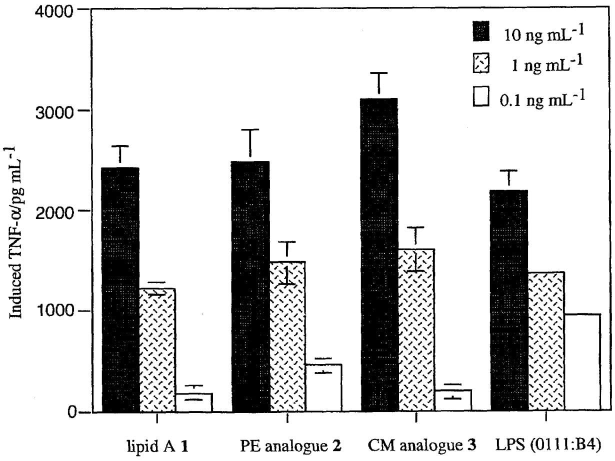


Fig. 2. TNF-α induction by **1**, **2**, **3**, and LPS (*E. coli* 0111 : B4) in heparinized human peripheral whole blood cells. The blood donor was Y. S.

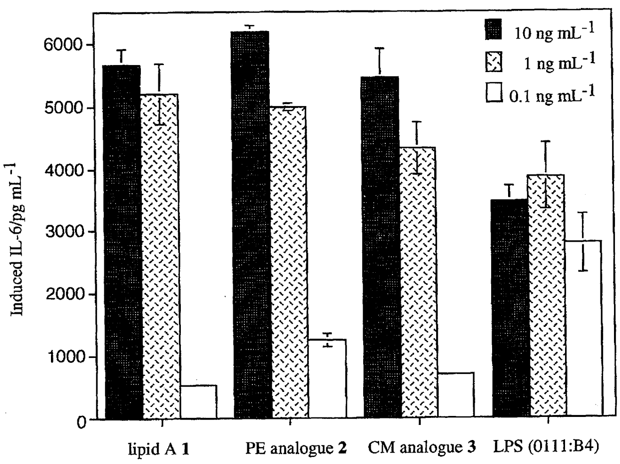


Fig. 1. IL-6 induction by **1**, **2**, **3**, and LPS (*E. coli* 0111 : B4) in heparinized human peripheral whole blood cells. The blood donor was W.-C. L.

The *Limulus* activity of **1**, **2**, **3**, and LPS was measured by the activations of factor *C* at various concentrations by means of Endospecy Test® (Seikagaku Corporation, Tokyo). The results are summarized in Table 1. All the samples were found to exhibit strong positive activity, and for the full activation of factor *C* 10 pg mL^{-1} of **2**, **3**, and LPS was sufficient, whereas 100 pg mL^{-1} is required for **1**. The minimal concentration for 50% activation of factor *C* (ED_{50}) is 6 pg mL^{-1} for **1**, 3 pg mL^{-1} for LPS, 2 pg mL^{-1} for **2**, and 1 pg mL^{-1} for **3**. The *Limulus* activity of the new analogue **3** was thus found to be 6 times as potent as **1**, 3 times as potent as the reference LPS employed, and 2 times as potent as **2**.

Conclusions

We have successfully completed an efficient divergent synthesis of lipid A and its chemically stable analogues. The total steps for **1**, **2**, and **3** from 4,6-*O*-benzylidene-*N*-Troc-D-glucosamine **4** were 11, 12, and 11 steps in the total yields of 24%, 28%, and 20%, respectively. This efficient method has also allowed us to synthesize a tritium-labeled **2** in a highly

Table 1. *Limulus* Activity of **1**, **2**, **3**, and LPS (*E. coli* 0111 : B4) As Tested by Endospecy Test® (Seikagaku Corporation, Tokyo)

	Activation	ED_{50} (pg mL^{-1})
E. coli lipid A **1**	+	6
PE analogue **2**	+	2
CM analogue **3**	+	1
LPS (*E. coli* 0111:B4)	+	3

pure state.[17)]

The CM analogue **3** was found to exhibit the same order of cytokines inducing activity as lipid A **1** and the PE analogue **2** do, indicating again that the phosphate group can be substituted with other acidic moiety without losing the endotoxic activity. In addition to this, the chemically stable analogues **2** and **3** exhibit apparently stronger *Limulus* activity than lipid A **1**, showing their stronger activation of factor *C*.

One of the today's major issues of LPS research is to identify the possible receptors for lipid A on competent animal cells and to elucidate their manner of interaction at the molecular level. These chemically stable analogues **2** and **3** with endotoxic activities, thus obtained in a pure state by the present synthetic pathway, will provide a new access for this purpose to understand the biological events initiated by LPS of Gram-negative bacteria.

Experimental

^{1}H NMR spectra were measured with JEOL JNM-LA500 or Varian UNITYplus 600 spectrometers. The chemical shifts are given in δ values from tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL JMS-SX 102 and a Perkin–Elmer SCIEX API III mass spectrometer, or a Mariner™ Biospectrometry™ Workstation (PerSeptive Biosystem). Specific rotations were measured on a Perkin–Elmer 241 polarimeter. Elemental analyses were performed by the staff members of our department. Recycling preparative HPLC was carried out with LC908 (Japan Analytical Industry). Silica-gel column chromatography was carried out using Kieselgel 60 (E. Merck. 0.040—0.063 mm) at medium-pressure (2—4 kg cm^{-2}). Anhydrous dichloromethane (CH_2Cl_2) and chloroform ($CHCl_3$) were distilled from calcium hydride. Anhydrous tetrahydrofuran (THF) and benzene was purchased from Kanto Chemicals, Tokyo. Molecular sieves 4A were activated by heating at 350 °C in vacuo for 3 h. Unless otherwise noted, each nonaqueous reaction was carried out under a nitrogen atmosphere.

Allyl 4,6-*O*-Benzylidene-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-*α*-D-glucopyranoside (6). (*R*)-3-(Tetradecanoyloxy)tetradecanoic acid[4b,9)] (9.91 g, 21.8 mmol), DCC (4.97 g, 23.6 mmol), and DMAP (222 mg, 1.82 mmol) were added to a solution of allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-*α*-D-glucopyranoside (**4**)[7)] (8.77 g, 18.2 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred at room temperature for 24 h. Then methanol (2.0 mL) and acetic acid (1.0 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 ethyl acetate (200 mL), and washed successively with saturated aqueous $NaHCO_3$ (100 mL×2) and brine (100 mL). The ethyl acetate solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (600 g, toluene/ethyl acetate = 40 : 1) to give **6** as a colorless powder (15.7 g, 94%). $[\alpha]_D^{25}$ = +31.4 (*c* 1.25, $CHCl_3$); FAB-MS (positive) *m/z* 918 [(M+H)$^+$]; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.45—7.32 (m, 5 H), 5.89 (m, 1 H), 5.52 (s, 1 H), 5.39 (d, *J* = 9.6 Hz, 1 H), 5.33 (dd, *J* = 10.0, 9.8 Hz, 1 H), 5.31 (dd, *J* = 17.2, 1.6 Hz, 1 H), 5.24 (dd, *J* = 10.0, 1.6 Hz, 1 H), 5.15 (m, 1 H), 4.94 (d, *J* = 3.6 Hz, 1 H), 4.75 (d, *J* = 11.9 Hz, 1 H), 4.68 (d, *J* = 11.9 Hz, 1 H), 4.29 (dd, *J* = 10.0, 5.0 Hz, 1 H), 4.21 (dd, *J* = 12.9, 5.3 Hz, 1 H), 4.06—3.98 (m, 2 H), 3.93 (ddd, *J* = 10.0, 9.5, 5.0 Hz, 1 H), 3.77 (t, *J* = 10.0 Hz, 1 H), 3.70 (t, *J* = 9.5 Hz, 1 H), 2.69 (dd, *J* = 15.4, 6.8 Hz, 1 H), 2.51 (dd, *J* = 15.4, 5.7 Hz, 1 H), 2.16 (t, *J* = 7.8 Hz, 2 H), 1.56—1.49 (m, 4 H), 1.33—1.15 (m, 38 H), 0.88 (t, *J* = 6.9 Hz, 6 H). Found: C, 61.28; H, 8.08; N, 1.52%. Calcd for $C_{47}H_{74}Cl_3NO_{10}$: C, 61.40; H, 8.11; N, 1.52%.

Allyl 6-*O*-Benzyl-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)-tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-*α*-D-glucopyranoside (7). To a solution of **6** (14.5 g, 15.8 mmol) in anhydrous THF (150 mL) were added sodium cyanotrihydroborate (9.92 g, 158 mmol) and dry hydrogen chloride in THF (20% (w/v), 35 mL). After stirring for 10 min, saturated aqueous $NaHCO_3$ (110 mL) and acetone (75 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 (500 mL) and washed successively with saturated aqueous $NaHCO_3$ (300 mL) and brine (300 mL). The ethyl acetate solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (600 g, toluene/ethyl acetate = 8 : 1) to give **7** as a colorless syrup (13.9 g, 96%). $[\alpha]_D^{25}$ = +37.2 (*c* 1.04, $CHCl_3$); FAB-MS (positive) *m/z* 920 (M$^+$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.38—7.24 (m, 5 H), 5.88 (m, 1 H), 5.40 (d, *J* = 9.8 Hz, 1 H), 5.29 (dd, *J* = 17.2, 1.3 Hz, 1 H), 5.21 (dd, *J* = 10.3, 1.3 Hz, 1 H), 5.17—5.09 (m, 2 H), 4.92 (d, *J* = 3.6 Hz, 1 H), 4.75 (d, *J* = 11.9 Hz, 1 H), 4.66 (d, *J* = 11.9 Hz, 1 H), 4.63 (d, *J* = 12.1 Hz, 1 H), 4.58 (d, *J* = 12.1 Hz, 1 H), 4.20 (dd, *J* = 12.8, 5.6 Hz, 1 H), 4.00 (dd, *J* = 12.8, 5.6 Hz, 1 H), 3.96 (ddd, *J* = 10.2, 10.0, 3.6 Hz, 1 H), 3.86—3.81 (m, 1 H), 3.80—3.71 (m, 3 H), 3.23 (s, 1 H), 2.56 (dd, *J* = 15.0, 7.8 Hz, 1 H), 2.49 (dd, *J* = 15.0, 3.9 Hz, 1 H), 2.28 (t, *J* = 7.8 Hz, 2 H), 1.65—1.54 (m, 4 H), 1.32—1.21 (m, 38 H), 0.88 (t, *J* = 6.9 Hz, 6 H). Found: C, 61.11; H, 8.35; N, 1.52%. Calcd for $C_{47}H_{76}Cl_3NO_{10}$: C, 61.26; H, 8.31; N, 1.52%.

Allyl 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*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-*α*-D-glucopyranoside (8). To a solution of **7** (10.2 g, 11.1 mmol) in anhydrous CH_2Cl_2 (100 mL) were added *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (4.00 g, 16.7 mmol)[11)] and 1*H*-tetrazole (1.56 g, 22.2 mmol). The mixture was stirred at room temperature for 30 min and then cooled to −20 °C. *m*CPBA (70%, 5.49 g, 22.2 mmol) was added, and stirring was continued for another 40 min. The solution was quenched with saturated aqueous $NaHCO_3$ (100 mL) and extracted with ethyl acetate (100 mL). The ethyl acetate solution was washed successively with saturated aqueous $NaHCO_3$ (80 mL×2) and brine (80 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (500 g, toluene/ethyl acetate = 8 : 1) to give **8** as a colorless syrup (10.9 g, 89%). $[\alpha]_D^{25}$ = +28.3 (*c* 1.02, $CHCl_3$); FAB-MS (positive) *m/z* 1101 (M$^+$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.41—7.24 (m, 5 H), 7.23—7.12 (m, 4 H), 5.88 (m, 1 H), 5.37 (dd, *J* = 10.5, 9.5 Hz, 1 H), 5.32 (d, *J* = 9.8 Hz, 1 H), 5.29 (dd, *J* = 17.7, 1.3 Hz, 1 H), 5.22 (dd, *J* = 10.2, 1.3 Hz, 1 H), 5.20—5.17 (m, 1 H), 5.16—5.01 (m, 4 H), 4.95 (d, *J* = 3.6 Hz, 1 H), 4.75 (d, *J* = 11.9 Hz, 1 H), 4.74—4.69 (m, 1 H), 4.66 (d, *J* = 11.9 Hz, 1 H), 4.65 (d, *J* = 11.9 Hz, 1 H), 4.58 (d, *J* = 11.9 Hz, 1 H), 4.21 (dd, *J* = 12.6, 5.1 Hz, 1 H), 4.06—3.96 (m, 3 H), 3.79 (dd, *J* = 11.0, 1.9 Hz, 1 H), 3.72 (dd, *J* = 11.0, 5.0 Hz, 1 H), 2.75 (dd, *J* = 17.0, 6.5 Hz, 1 H), 2.57 (dd, *J* = 17.0, 6.5 Hz, 1 H), 2.24 (t, *J* = 7.9 Hz, 2 H), 1.64—1.51 (m, 4 H), 1.34—1.20 (m, 38 H), 0.88 (t, *J* = 6.9 Hz, 6 H). 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%.

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*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (9). To a degassed solution of **8** (1.96 g, 1.72 mmol) in THF (25 mL) was added [bis(methyldiphenylphosphine)]-(1,5-cyclooctadiene)iridium(I) hexafluorophosphate (101 mg, 119 μmol). After activation of the iridium catalyst with hydrogen for 10 s, the mixture was stirred under a nitrogen atmosphere at room temperature for 20 min. Then iodine (873 mg, 3.44 mmol) and water (20 mL) were added and the reaction mixture was stirred for additional 20 min. To the mixture was added 5% aqueous $Na_2S_2O_3$ (100 mL), and the solution was extracted with ethyl acetate (100 mL). The extract was washed successively with 5% aqueous $Na_2S_2O_3$ (75 mL×2) and brine (75 mL), and dried over Na_2SO_4. After removal of the solvent in vacuo, the crude product was purified by silica-gel flash chromatography (180 g, toluene/ethyl acetate = 10 : 1) to give **9** as a colorless syrup (1.57 g, 86%). $[\alpha]_D^{25} = +4.9$ (*c* 1.10, $CHCl_3$); FAB-MS (positive) *m/z* 1086 $[(M+K)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ = 7.39—7.16 (m, 9 H), 5.46 (d, J = 9.8 Hz, 1 H), 5.40 (t, J = 10.5 Hz, 1 H), 5.30 (t, J = 1.8 Hz, 1 H), 5.24—5.17 (m, 1 H), 5.17—4.99 (m, 4 H), 4.69 (s, 2 H), 4.66—4.60 (m, 1 H), 4.63 (d, J = 11.9 Hz, 1 H), 4.56 (d, J = 11.9 Hz, 1 H), 4.22 (ddd, J = 10.7, 5.9, 1.8 Hz, 1 H), 3.96 (ddd, J = 10.5, 9.8, 3.6 Hz, 1 H), 3.78 (dd, J = 10.7, 1.8 Hz, 1 H), 3.72 (br s, 1 H), 3.70 (dd, J = 10.7, 5.9 Hz, 1 H), 2.72 (dd, J = 16.3, 6.5 Hz, 1 H), 2.56 (dd, J = 16.3, 6.5 Hz, 1 H), 2.24 (t, J = 7.9 Hz, 2 H), 1.61—1.53 (m, 4 H), 1.32—1.20 (m, 38 H), 0.88 (t, J = 6.9 Hz, 6H). Found: C, 58.94; H, 7.61; N, 1.44%. Calcd for $C_{52}H_{79}Cl_3NO_{13}P$: C, 58.73; H, 7.49; N, 1.32%.

6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl Trichloroacetimidate (10). To a solution of **9** (1.87 g, 1.76 mmol) in CH_2Cl_2 (20 mL) at room temperature were added Cs_2CO_3 (287 mg, 881 μmol) and trichloroacetonitrile (1.76 mL, 17.6 mmol). After stirring for 30 min, the reaction was quenched with saturated aqueous $NaHCO_3$ (40 mL), and the mixture was extracted with $CHCl_3$ (60 mL). The extract was washed with brine (20 mL) and dried over Na_2SO_4. Removal of the solvent in vacuo gave **10** as a pale yellow syrup (2.12 g, 99%), which was used for the subsequent glycosylation without further purification.

Allyl 4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-2-[(*R*)-3-(benzyloxy)-tetradecanoylamino]-2-deoxy-α-D-glucopyranoside (12). To a mixture of the imidate **10** (136 mg, 113 μmol), the acceptor **11**[10b] (60 mg, 96 μmol), and molecular sieves 4A (300 mg) in anhydrous CH_2Cl_2 (5 mL) at −20 °C was added $Sn(OTf)_2$ (5.0 mg, 12 μmol), and the mixture was stirred for 10 min. After removal of molecular sieves by filtration, the reaction mixture was neutralized with saturated aqueous $NaHCO_3$ (20 mL) and then extracted with ethyl acetate (50 mL). The ethyl acetate layer was washed successively with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), and dried over Na_2SO_4. After removal of the solvent in vacuo, the crude product was purified by silica-gel flash chromatography (50 g, toluene/ethyl acetate = 10 : 1) to give **12** as a colorless syrup (135 mg, 84%). $[\alpha]_D^{25} = +13.4$ (*c* 1.00, $CHCl_3$); FAB-MS (positive) *m/z* 1691 $[(M+Na)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ = 7.49—7.14 (m, 19 H), 6.58 (d, J = 8.0 Hz, 1 H), 5.67 (m, 1 H), 5.41 (t, J = 9.7 Hz, 1 H), 5.28 (d, J = 8.5 Hz, 1 H), 5.23 (m, 1 H), 5.15 (dd, J = 17.2, 1.5 Hz, 1 H), 5.11—4.96 (m, 5 H), 4.88 (d, J = 11.6 Hz, 1 H), 4.71 (d, J = 3.6 Hz, 1 H), 4.68 (d, J = 8.3 Hz, 1 H), 4.64 (d, J = 10.9 Hz, 1 H), 4.62—4.43 (m, 7 H), 4.12—4.03 (m, 2 H), 3.98 (dd, J = 12.8, 5.1 Hz, 1 H), 3.90—3.64 (m, 8 H), 3.50—3.41 (m, 2 H), 2.64 (dd, J = 16.0, 7.2 Hz, 1 H), 2.58 (dd, J = 16.0, 5.6 Hz, 1 H), 2.51 (dd, J = 15.0, 3.8 Hz, 1 H), 2.39 (dd, J = 15.0, 6.9 Hz, 1 H), 2.24 (t, J = 7.5 Hz, 2 H), 1.71—1.49 (m, 6 H), 1.47—1.13 (m, 56 H), 0.88 (t, J = 6.9 Hz, 9 H). Found: C, 63.95; H, 7.96; N, 1.67%. Calcd for $C_{89}H_{132}Cl_3N_2O_{19}P$: C, 63.83; H, 7.83; N, 1.62%.

Allyl 4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)-tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-α-D-glucopyranoside (13). (*R*)-3-(Benzyloxy)tetradecanoic acid[4b,9)] (264 mg, 789 μmol), DCC (177 mg, 857 μmol), and DMAP (8.5 mg, 69 μmol) were added to a solution of **12** (1.10 g, 658 μmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 4 h. Methanol (1.0 mL) and acetic acid (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 ethyl acetate (100 mL), and washed successively with saturated aqueous $NaHCO_3$ (50 mL×2) and brine (50 mL). The ethyl acetate solution was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica-gel flash chromatography (80 g, toluene/ethyl acetate = 5 : 1) to give **13** as a colorless powder (1.26 g, 97%). $[\alpha]_D^{25} = +19.6$ (*c* 1.01, $CHCl_3$); FAB-MS (positive) *m/z* 2008 $[(M+Na)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ = 7.46—7.16 (m, 24 H), 6.16 (d, J = 9.6 Hz, 1 H), 5.71 (m, 1 H), 5.42 (t, J = 9.8 Hz, 1 H), 5.36 (t, J = 9.1 Hz, 1 H), 5.33 (d, J = 9.6 Hz, 1 H), 5.27 (m, 1 H), 5.17 (dd, J = 17.4, 1.6 Hz, 1 H), 5.14—4.99 (m, 5 H), 4.76 (d, J = 3.6 Hz, 1 H), 4.69 (d, J = 8.2 Hz, 1 H), 4.67—4.41 (m, 11 H), 4.27 (td, J = 10.0, 3.6 Hz, 1 H), 4.06 (d, J = 10.8 Hz, 1 H), 4.00 (dd, J = 12.8, 5.2 Hz, 1 H), 3.88—3.63 (m, 9 H), 3.50—3.42 (m, 1 H), 2.67 (dd, J = 16.8, 6.9 Hz, 1 H), 2.62 (dd, J = 16.8, 5.3 Hz, 1 H), 2.56 (dd, J = 16.0, 7.5 Hz, 1 H), 2.40 (dd, J = 16.0, 4.7 Hz, 1 H), 2.30—2.24 (m, 4 H), 1.60—1.52 (m, 8 H), 1.39—1.20 (m, 74 H), 0.88 (t, J = 6.9 Hz, 12 H). Found: C, 66.46; H, 8.32; N, 1.41%. Calcd for $C_{110}H_{164}Cl_3N_2O_{21}P$: C, 66.50; H, 8.37; N, 1.48%.

Allyl 4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)-tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-α-D-glucopyranoside (5). To a solution of **13** (1.20 g, 603 μmol) in acetic acid was added zinc–copper couple (1.0 g), and the mixture was stirred at room temperature for 2 h. The insoluble materials were filtered off, and the filtrate was concentrated in vacuo. The residual solvent was coevaporated with toluene three times. The crude product was dissolved in ethyl acetate (100 mL) and washed successively with saturated aqueous $NaHCO_3$ (100 mL×2) and brine (100 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give the *N*-deprotected product (1.14 g), which was used without further purification for the following *N*-acylation reaction.

The crude amine thus obtained was dissolved in anhydrous CH_2Cl_2 (10 mL). To this solution were added DCC (248 mg, 1.20 mmol) and (*R*)-3-(dodecanoyloxy)tetradecanoic acid[4b,9)] (386 mg, 904 μmol). The mixture was stirred at room temperature for 3 d. The insoluble materials were filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in ethyl acetate (100 mL), and washed successively with saturated aqueous $NaHCO_3$ (50 mL×2) and brine (50 mL). The ethyl acetate solution was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica-gel flash chromatography (80 g, toluene/ethyl acetate = 10 : 1) to give **5** as a colorless syrup (1.01 g, 76%). $[\alpha]_D^{25} = +19.2$ (*c* 1.01, $CHCl_3$); FAB-MS (positive) *m/z* 2244 $[(M+Na)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ = 7.35—7.14 (m, 24 H), 6.35 (d, *J* = 9.4 Hz, 1 H), 6.20 (d, *J* = 7.2 Hz, 1 H), 5.66 (m, 1 H), 5.56 (dd, *J* = 10.6, 9.0 Hz, 1 H), 5.33 (dd, *J* = 10.8, 9.5 Hz, 1 H), 5.23 (m, 1 H), 5.15 (d, *J* = 8.2 Hz, 1 H), 5.13 (dd, *J* = 17.2, 1.6 Hz, 1 H), 5.09—4.91 (m, 6 H), 4.74 (d, *J* = 3.6 Hz, 1 H), 4.60—4.38 (m, 9 H), 4.27 (ddd, *J* = 10.8, 9.4, 3.6 Hz, 1 H), 4.01 (dd, *J* = 13.2, 1.8 Hz, 1 H), 3.98 (dd, *J* = 12.9, 5.5 Hz, 1 H), 3.84—3.61 (m, 9 H), 3.44 (m, 1 H), 2.63 (dd, *J* = 16.2, 4.7 Hz, 1 H), 2.57 (dd, *J* = 16.2, 7.6 Hz, 1 H), 2.52 (dd, *J* = 16.2, 7.0 Hz, 1 H), 2.34 (dd, *J* = 16.2, 5.1 Hz, 1 H), 2.28 (dd, *J* = 15.3, 5.0 Hz, 1 H), 2.27—2.19 (m, 6 H), 2.16 (dd, *J* = 15.3, 5.0 Hz, 1 H), 1.61—1.45 (m, 12 H), 1.34—1.09 (m, 108 H), 0.88 (t, *J* = 6.9 Hz, 18 H). Found: C, 71.92; H, 9.58; N, 1.26%. Calcd for $C_{133}H_{211}N_2O_{22}P$: C, 71.80; H, 9.62; N, 1.29%.

4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-*β*-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-D-glucopyranose (14). A degassed solution of **5** (250 mg, 112 μmol) in THF (10 mL) was treated with activated [bis(methyldiphenylphosphine)](1,5-cyclooctadiene) iridium(I) hexafluorophosphate (8 mg, 9 μmol) under a nitrogen atmosphere at room temperature for 20 min, and then iodine (57 mg, 0.22 mmol) and water (10 mL) for 20 min, as described above for the preparation of **9**. The usual work-up followed by purification by silica-gel flash chromatography (20 g, toluene/ethyl acetate = 10 : 1) gave **14** as a colorless syrup (220 mg, 92%). FAB-MS (positive) *m/z* 2202 $[(M+Na)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ = 7.41—7.10 (m, 24 H), 6.27 (d, *J* = 7.3 Hz, 1 H), 6.20 (d, *J* = 9.4 Hz, 1 H), 5.51 (d, *J* = 8.3 Hz, 1 H), 5.47 (dd, *J* = 10.3, 9.1 Hz, 1 H), 5.37 (dd, *J* = 10.6, 9.3 Hz, 1 H), 5.22 (m, 1 H), 5.17—4.89 (m, 5 H), 4.64—4.37 (m, 10 H), 4.17 (ddd, *J* = 10.1, 10.0, 3.6 Hz, 1 H), 4.06 (t, *J* = 9.0 Hz, 1 H), 3.88 (d, *J* = 12.3 Hz, 1 H), 3.84—3.61 (m, 6 H), 3.45 (m, 1 H), 3.35—3.24 (m, 2 H), 2.63 (dd, *J* = 16.0, 4.6 Hz, 1 H), 2.56 (dd, *J* = 16.0, 7.9 Hz, 1 H), 2.54 (dd, *J* = 16.4, 7.3 Hz, 1 H), 2.37 (dd, *J* = 15.9, 5.3 Hz, 1 H), 2.37—2.15 (m, 8 H), 1.67—1.40 (m, 12 H), 1.38—1.22 (m, 108 H), 0.86 (t, *J* = 5.9 Hz, 18 H). Found: C, 71.64; H, 9.69; N, 1.32%. Calcd for $C_{130}H_{207}N_2O_{22}P$: C, 71.59; H, 9.57; N, 1.28%.

4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-*β*-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-1-*O*-bis(benzyloxy)phosphoryl-2-deoxy-*α*-D-glucopyranose (15). To a solution of **14** (45 mg, 21 μmol) in anhydrous THF (5 mL) was added $LiN(TMS)_2$ in hexane (1 mol dm^{-3}, 61 μL, 61 μmol) at −78 °C. The mixture was stirred for 5 min. Tetrabenzyl diphosphate (44 mg, 82 μmol) was then added and the mixture was stirred at the same temperature for 10 min. The mixture was then allowed to warm gradually to room temperature, neutralized with saturated aqueous $NaHCO_3$ (15 mL), and extracted with ethyl acetate (30 mL). After the extract was dried over Na_2SO_4 and concentrated in vacuo, the residue was purified by silica-gel flash chromatography (15 g, $CHCl_3$/acetone = 30 : 1) to give **15** as a colorless syrup (38 mg, 75%) with recovery of **14** (10 mg, 22%). FAB-MS (positive) *m/z* 2462 $[(M+Na)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ = 7.39—7.14 (m, 34 H), 6.85 (d, *J* = 8.1 Hz, 1 H), 6.20 (d, *J* = 9.4 Hz, 1 H), 5.68 (t, *J* = 4.2 Hz, 1 H), 5.35 (t, *J* = 9.9 Hz, 1 H), 5.28 (t, *J* = 10.0 Hz, 1 H), 5.25 (m, 1 H), 5.11 (m, 1 H), 5.07—4.98 (m, 9 H), 4.62—4.45 (m, 7 H), 4.42 (d, *J* = 11.7 Hz, 1 H), 4.37 (d, *J* = 12.0 Hz, 1 H), 4.28 (m, 1 H), 4.04 (m, 1 H), 3.91 (dd, *J* = 12.2, 1.2 Hz, 1 H), 3.84—3.73 (m, 3 H), 3.72—3.65 (m, 2 H), 3.65—3.58 (m, 2 H), 3.54 (t, *J* = 9.7 Hz, 1 H), 2.62 (dd, *J* = 16.7, 6.9 Hz, 1 H), 2.60 (dd, *J* = 16.7, 5.5 Hz, 1 H), 2.52 (dd, *J* = 16.0, 7.5 Hz, 1 H), 2.40 (dd, *J* = 15.0, 6.9 Hz, 1 H), 2.36 (dd, *J* = 16.0, 5.5 Hz, 1 H), 2.27 (dd, *J* = 15.0, 5.1 Hz, 1 H), 2.24—2.16 (m, 5 H), 2.14 (dd, *J* = 14.7, 4.8 Hz, 1 H), 1.61—1.47 (m, 12 H), 1.33—1.16 (m, 108 H), 0.87 (t, *J* = 5.9 Hz, 18 H).

2-Deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-*β*-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-*α*-D-glucopyranose 1,4′-Bisphosphate (1). To a solution of **15** (35 mg, 14 μmol) in THF (4 mL) was added Pd-black (114 mg). The mixture was stirred under 7 kg cm^{-2} of hydrogen at room temperature overnight. The reaction mixture was then neutralized with triethylamine (10 μL). After removal of the Pd catalyst by filtration, the solvent was evaporated in vacuo. The residue was purified by liquid–liquid partition column chromatography (20 g of Sephadex® LH-20, $CHCl_3$/methanol/water/isopropyl alcohol = 8 : 8 : 6 : 1), wherein the organic layer was the stationary phase and the aqueous layer was the mobile phase, to give **1** as a triethylammonium salt (white powder, 21 mg, 81%). The physical data were identical with the reported ones.[13] FAB-MS (negative) *m/z* 1797 $[(M-H)^-]$; ^1H NMR (600 MHz, $CD_3OD/CDCl_3$ = 1 : 1) δ = 5.47 (dd, *J* = 5.8, 2.9 Hz, 1 H), 5.24—5.16 (m, 3 H), 5.09 (t, *J* = 8.1 Hz, 1 H), 4.55 (d, *J* = 7.0 Hz, 1 H), 4.25 (dd, *J* = 16.0, 7.9 Hz, 1 H), 4.18 (m, 1 H), 4.13 (m, 1 H), 4.07 (d, *J* = 9.8 Hz, 1 H), 4.02 (dd, *J* = 11.2, 1.8 Hz, 1 H), 4.00 (m, 1 H), 3.94—3.87 (m, 2 H), 3.84 (dd, *J* = 10.0, 5.0 Hz, 1 H), 3.76 (d, *J* = 11.4 Hz, 1 H), 3.49 (t, *J* = 7.7 Hz, 1 H), 3.37 (m, 1 H), 2.71 (dd, *J* = 13.5, 6.2 Hz, 1 H), 2.64 (dd, *J* = 13.5, 4.3 Hz, 1 H), 2.51 (dd, *J* = 12.6, 7.0 Hz, 1 H), 2.50 (dd, *J* = 12.6, 3.7 Hz, 1 H), 2.43 (dd, *J* = 12.6, 4.0 Hz, 1 H), 2.41 (dd, *J* = 13.1, 7.7 Hz, 1 H), 2.35—2.29 (m, 4 H), 2.26—2.22 (m, 1 H), 2.25 (dd, *J* = 12.0, 8.0 Hz, 1 H), 1.67—1.40 (m, 12 H), 1.38—1.22 (m, 108 H), 0.89 (t, *J* = 5.9 Hz, 18 H).

Formylmethyl 4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-*β*-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-*α*-D-glucopyranoside (16). To a vigorously stirred solution of **5** (450 mg, 202 μmol) in THF/*t*-butyl alcohol/water (10 : 10 : 1, 12 mL) at room temperature were added 4-methylmorpholine *N*-oxide (NMO) (94 mg, 0.80 mmol) and OsO_4 in water (2.5%, 400 μL, 40 μmol). After 6 h, saturated aqueous $Na_2S_2O_3$ (50 mL) was added, and the mixture was extracted with ethyl acetate (50 mL). The ethyl acetate layer was washed successively with saturated aqueous $Na_2S_2O_3$ (50 mL × 2) and brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo to give the crude diol (458 mg), which was used without further purification for the following oxidation.

The crude diol thus obtained was dissolved in anhydrous benzene (10 mL). To this solution was added lead(IV) acetate ($Pb(OAc)_4$) (90% purity, 119 mg, 242 μmol). After 30 min, the mixture was filtered through a silica-gel column (3 g) using ethyl acetate as an eluent. After removal of the solvent in vacuo, the residue was purified by silica-gel flash chromatography (20 g, toluene/ethyl acetate = 5 : 1) to give **16** as a colorless syrup (377 mg, 84%). FAB-MS (positive) *m/z* 2244 [(M+Na)$^+$]; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 9.37 (s, 1 H), 7.39—7.15 (m, 24 H), 6.47 (d, *J* = 9.4 Hz, 1 H), 6.18 (d, *J* = 8.4 Hz, 1 H), 5.54 (t, *J* = 9.6 Hz, 1 H), 5.33 (t, *J* = 10.0 Hz, 1 H), 5.25 (m, 1 H), 5.14—4.96 (m, 6 H), 4.70 (d, *J* = 3.6 Hz, 1 H), 4.60—4.40 (m, 9 H), 4.29 (td, *J* = 10.8, 3.6 Hz, 1 H), 3.99 (d, *J* = 10.8 Hz, 1 H), 3.93—3.76 (m, 5 H), 3.75—3.63 (m, 4 H), 3.55 (t, *J* = 9.6 Hz, 1 H), 3.46 (m, 1 H), 2.63 (m, 1 H), 2.55 (dd, *J* = 15.7, 7.4 Hz, 1 H), 2.39 (dd, *J* = 16.0, 5.0 Hz, 1 H), 2.35—2.24 (m, 7 H), 2.21 (dd, *J* = 15.3, 5.4 Hz, 1 H), 1.67—1.42 (m, 12 H), 1.39—1.09 (m, 108 H), 0.88 (t, *J* = 6.9 Hz, 18 H).

2-Hydroxyethyl 4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-α-D-glucopyranoside (17). To a solution of **16** (150 mg, 67.5 μmol) in isopropyl alcohol/methanol/CH_2Cl_2 (5 : 1 : 1, 6 mL) at 0 °C was added $NaBH_4$ (1.3 mg, 34 μmol). After being stirred for 30 min, the reaction was quenched with saturated aqueous NH_4Cl (5 mL), and the mixture was extracted with $CHCl_3$ (50 mL). The extract was dried over Na_2SO_4 and concentrated in vacuo to give **17** as a colorless syrup (150 mg, 100%), which was pure enough for the next phosphorylation reaction. $[\alpha]_D^{25}$ = +14.9 (*c* 0.96, $CHCl_3$); FAB-MS (positive) *m/z* 2246 [(M+Na)$^+$]; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.39—7.15 (m, 24 H), 6.29 (d, *J* = 9.8 Hz, 1 H), 6.27 (d, *J* = 8.1 Hz, 1 H), 5.33 (dd, *J* = 10.5, 9.5 Hz, 1 H), 5.32 (dd, *J* = 9.8, 9.3 Hz, 1 H), 5.25 (m, 1 H), 5.14—4.96 (m, 6 H), 4.69 (d, *J* = 3.6 Hz, 1 H), 4.64—4.39 (m, 9 H), 4.24 (ddd, *J* = 10.7, 9.8, 3.6 Hz, 1 H), 4.05 (dd, *J* = 10.6, 1.6 Hz, 1 H), 4.00 (m, 1 H), 3.87—3.77 (m, 3 H), 3.74—3.65 (m, 2 H), 3.58 (dd, *J* = 10.6, 6.8 Hz, 1 H), 3.53—3.31 (m, 6 H), 2.63 (d, *J* = 6.8 Hz, 2 H), 2.54 (dd, *J* = 15.7, 6.5 Hz, 1 H), 2.38 (dd, *J* = 15.7, 5.5 Hz, 1 H), 2.38—2.19 (m, 8 H), 1.62—1.43 (m, 12 H), 1.34—1.17 (m, 108 H), 0.88 (t, *J* = 6.9 Hz, 18 H). Found: C, 71.31; H, 9.59; N, 1.30%. Calcd for $C_{132}H_{211}N_2O_{23}P$: C, 71.25; H, 9.56; N, 1.26%.

2-(1,5-Dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yloxy)ethyl 4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-α-D-glucopyranoside (18). To a solution of **17** (150 mg, 67.4 μmol) in CH_2Cl_2 (6 mL) at 0 °C were added *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (48 mg, 201 μmol) and 1*H*-tetrazole (14 mg, 0.20 mmol). The mixture was stirred at room temperature for 30 min and then cooled to −20 °C. *m*CPBA (70%, 50 mg, 0.20 mmol) was added, and stirring was continued for another 40 min. The reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL), and the mixture was extracted with $CHCl_3$ (40 mL). Working-up as described for the preparation of **8** followed by purification by silica-gel flash chromatography (6 g, toluene/ethyl acetate = 1 : 1) and recycling preparative HPLC (column: JAIGEL 2H (20×600 mm)×2; solvent: $CHCl_3$) gave **18** as a colorless syrup (138 mg, 85%). $[\alpha]_D^{25}$ = +17.8 (*c* 1.00, $CHCl_3$); FAB-MS (positive) *m/z* 2428 [(M+Na)$^+$]; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.40—7.10 (m, 24 H), 6.69 (d, *J* = 9.3 Hz, 1 H), 6.44 (d, *J* = 7.6 Hz, 1 H), 5.60 (dd, *J* = 10.3, 9.4 Hz, 1 H), 5.33 (dd, *J* = 10.6, 9.4 Hz, 1 H), 5.28—4.94 (m, 11 H), 4.70 (d, *J* = 3.6 Hz, 1 H), 4.63—4.39 (m, 9 H), 4.30 (ddd, *J* = 10.3, 9.9, 3.6 Hz, 1 H), 4.19 (m, 1 H), 4.10 (m, 1 H), 4.04 (d, *J* = 9.5 Hz, 1 H), 3.89 (m, 1 H), 3.86—3.78 (m, 3 H), 3.78—3.71 (m, 2 H), 3.71—3.62 (m, 2 H), 3.58 (t, *J* = 9.6 Hz, 1 H), 3.42 (m, 1 H), 3.33 (m, 1 H), 2.62 (d, *J* = 6.2 Hz, 2 H), 2.55 (dd, *J* = 15.7, 6.9 Hz, 1 H), 2.39 (dd, *J* = 15.7, 5.2 Hz, 1 H), 2.36—2.17 (m, 7 H), 1.64—1.39 (m, 12 H), 1.38—1.13 (m, 108 H), 0.88 (t, *J* = 6.9 Hz, 18 H). Found: C, 69.96; H, 9.21; N, 1.14%. Calcd for $C_{140}H_{218}N_2O_{26}P_2$: C, 69.85; H, 9.13; N, 1.16%.

2-(Phosphonooxy)ethyl 2-Deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-α-D-glucopyranoside (2). In a manner similar to that for the synthesis of **1**, **18** (149 mg, 62.2 μmol) was hydrogenolytically deprotected. The crude material was purified by liquid–liquid partition column chromatography (20 g of Sephadex® LH-20, $CHCl_3$/methanol/water/isopropyl alcohol = 15 : 15 : 15 : 2), wherein the organic layer was the stationary phase and the aqueous layer was the mobile phase, to give **2** as a white powder (100 mg, 88%). FAB-MS (negative) *m/z* 1840 [(M−H)$^-$]; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 1 : 1) δ = 5.23 (m, 1 H), 5.19 (t, *J* = 8.2 Hz, 1 H), 5.17 (m, 1 H), 5.14 (t, *J* = 8.2 Hz, 1 H), 4.82 (d, *J* = 3.0 Hz, 1 H), 4.56 (d, *J* = 7.4 Hz, 1 H), 4.23 (q, *J* = 8.0 Hz, 1 H), 4.18 (dd, *J* = 8.9, 3.0 Hz, 1 H), 4.08—3.93 (m, 5 H), 3.92—3.82 (m, 4 H), 3.80 (dd, *J* = 10.3, 4.5 Hz, 1 H), 3.74 (d, *J* = 10.6 Hz, 1 H), 3.63 (m, 1 H), 3.56 (t, *J* = 8.1 Hz, 1 H), 3.37 (m, 1 H), 2.72 (dd, *J* = 14.0, 6.6 Hz, 1 H), 2.64 (dd, *J* = 14.0, 4.5 Hz, 1 H), 2.52—2.46 (m, 2 H), 2.44—2.36 (m, 2 H), 2.36—2.27 (m, 6 H), 1.66—1.39 (m, 12 H), 1.38—1.20 (m, 108 H), 0.89 (t, *J* = 5.6 Hz, 18 H).

4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-α-D-glucopyranosyloxyacetic Acid (19). To a solution of **16** (138 mg, 62.1 μmol), NaH_2PO_4 (7.5 mg, 62 μmol), and 2-methyl-2-butene (19.6 mg, 279 μmol) in water/*t*-butyl alcohol (1 : 4, 10 mL) was added $NaClO_2$ (21 mg, 0.19 mmol) at room temperature. After being stirred for 6 h, the reaction mixture was acidified with hydrochloric acid (1 mol dm^{-3}) and extracted with $CHCl_3$ (10 mL×2). The combined extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (6 g, toluene/ethyl acetate = 1 : 1) to give **19** as a colorless syrup (106 mg, 76%). $[\alpha]_D^{25}$ = +15.4 (*c* 1.00, $CHCl_3$); FAB-MS (positive) *m/z* 2237 [(M+Na)$^+$]; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.40—7.09 (m, 24 H), 6.50 (d, *J* = 9.4 Hz, 1 H), 6.28 (d, *J* = 8.1 Hz, 1 H), 5.37 (t, *J* = 10.8 Hz, 1 H), 5.35 (t, *J* = 10.5 Hz, 1 H), 5.23 (m, 1 H), 5.14—4.96 (m, 5 H), 4.82 (d, *J* = 7.9 Hz, 1 H), 4.72 (d, *J* = 3.4 Hz, 1 H), 4.69 (m, 1 H), 4.62—4.40 (m, 8 H), 4.26 (td, *J* = 10.6, 3.6 Hz, 1 H), 4.08 (t, *J* = 10.6 Hz, 1 H), 4.01—3.94 (m, 2 H), 3.87—3.73 (m, 4 H), 3.72—3.64 (m, 3 H), 3.54 (dd, *J* = 11.0, 8.5 Hz, 1 H), 3.36 (t, *J* = 9.7 Hz, 1 H), 2.66 (dd, *J* = 16.5, 5.5 Hz, 2 H), 2.61 (dd, *J* = 16.5, 7.2 Hz, 1 H), 2.55 (dd, *J* = 16.0, 7.0 Hz, 1 H), 2.41—2.35 (m, 2 H), 2.34—2.26 (m, 5 H), 2.24 (t, *J* = 7.6 Hz, 2 H), 1.62—1.49 (m, 12 H), 1.33—1.19 (m, 108 H), 0.88 (t, *J* = 6.9 Hz, 18 H). Found: C, 70.71; H, 9.55; N, 1.32%. Calcd for $C_{132}H_{209}N_2O_{24}P$: C, 70.80; H, 9.40; N, 1.25%.

2-Deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-α-D-glucopyranosyloxyacetic Acid (3). In a manner similar to that for the synthesis of **1**, **19** (103 mg, 45.9 μmol) was hydrogenolytically deprotected. The crude material was purified by liquid–liquid partition column chromatography (20 g of Sephadex® LH-20, $CHCl_3$/methanol/water/isopropyl alcohol = 100 : 100 : 100 : 13), wherein the organic layer was the stationary phase and the aqueous layer was the mobile phase, to give **3** as a white powder (61 mg, 74%). FAB-MS (negative) *m/z* 1774 $[(M-H)^-]$; ^1H NMR (600 MHz, $CD_3OD/CDCl_3$ = 1 : 1) δ = 5.26—5.17 (m, 2 H), 5.23 (t, J = 8.4 Hz, 1 H), 5.16 (t, J = 9.1 Hz, 1 H), 4.78 (d, J = 3.1 Hz, 1 H), 4.65 (d, J = 6.9 Hz, 1 H), 4.20—4.14 (m, 2 H), 4.09 (d, J = 12.5 Hz, 1 H), 4.08—3.98 (m, 2 H), 3.94 (m, 1 H), 3.86 (d, J = 12.5 Hz, 1 H), 3.91—3.65 (m, 5 H), 3.52 (t, J = 8.0 Hz, 1 H), 3.36 (m, 1 H), 2.82 (dd, J = 13.7, 5.4 Hz, 1 H), 2.64 (dd, J = 13.7, 4.7 Hz, 1 H), 2.54—2.36 (m, 4 H), 2.36—2.24 (m, 6 H), 1.68—1.40 (m, 12 H), 1.39—1.21 (m, 108 H), 0.89 (t, J = 5.8 Hz, 18 H).

This work was supported by "Research for the Future" Program No. 97L00502 from the Japan Society for the Promotion of Science.

References

1) a) E. Th. Rietschel, T. Kirikae, F. U. Schade, U. Mamat, G. Schmidt, H. Loppnow, A. J. Ulmer, U. Zähringer, U. Seydel, F. D. Padova, M. Schreier, and H. Brade, *FASEB J.*, **8**, 217 (1994); b) E. Th. Rietschel and H. Brade, *Sci. Am.*, **267**, August, 26 (1992).

2) E. Th. Rietschel, T. Kirikae, F. U. Schade, A. J. Ulmer, O. Holst, H. Brade, G. Schmidt, U. Mamat, H.-D. Grimmecke, S. Kusumoto, and U. Zähringer, *Immunobiology*, **187**, 169 (1993).

3) O. Westphal and O. Lüderitz, *Angew. Chem.*, **66**, 407 (1954).

4) a) M. Imoto, H. Yoshimura, N. Sakaguchi, S. Kusumoto, and T. Shiba, *Tetrahedron Lett.*, **26**, 1545 (1985); b) M. Imoto, H. Yoshimura, T. Shimamoto, N. Sakaguchi, S. Kusumoto, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **60**, 2205 (1987).

5) a) T. Kusama, T. Soga, E. Shioya, K. Nakayama, H. Nakajima, Y. Osada, Y. Ono, S. Kusumoto, and T. Shiba, *Chem. Pharm. Bull.*, **38**, 3366 (1990); b) H. Heine, H. Brade, S. Kusumoto, T. Kusama, E. Th. Rietschel, H.-D. Flad, and A. J. Ulmer, *J. Endotoxin Res.*, **1**, 14 (1994).

6) T. Kusama, T. Soga, Y. Ono, E. Kumazawa, E. Shioya, K. Nakayama, K. Uoto, and Y. Osada, *Chem. Pharm. Bull.*, **39**, 3244 (1991).

7) M. Oikawa, A. Wada, H. Yoshizaki, K. Fukase, and S. Kusumoto, *Bull. Chem. Soc. Jpn.*, **70**, 1435 (1997).

8) W.-C. Liu, M. Oikawa, K. Fukase, Y. Suda, H. Winarno, S. Mori, M. Hashimoto, and S. Kusumoto, *Bull. Chem. Soc. Jpn.*, **70**, 1441 (1997).

9) M. Oikawa and S. Kusumoto, *Tetrahedron: Asymmetry*, **6**, 961 (1995).

10) a) M. Oikawa, W.-C. Liu, Y. Nakai, S. Koshida, K. Fukase, and S. Kusumoto, *Synlett*, **1996**, 1179; b) K. Fukase, Y. Fukase, M. Oikawa, W.-C. Liu, Y. Suda, and S. Kusumoto, *Tetrahedron*, **54**, 4033 (1998).

11) Y. Watanabe, Y. Komoda, K. Ebisuya, and S. Ozaki, *Tetrahedron Lett.*, **31**, 255 (1990).

12) J. J. Oltvoort, C. A. A. v. Boeckel, J. H. d. Koning, and J. H. v. Boom, *Synthesis*, **1981**, 305.

13) M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **60**, 2197 (1987).

14) M. Inage, H. Chaki, S. Kusumoto, and T. Shiba, *Chem. Lett.*, **1982**, 1281.

15) M. Isobe, Y. Ichikawa, and T. Goto, *Tetrahedron Lett.*, **27**, 963 (1986).

16) Y. Suda, H. Tochio, K. Kawano, H. Takada, H. Yoshida, S. Kotani, and S. Kusumoto, *FEMS Immun. Med. Microbiol.*, **12**, 97 (1995).

17) To be published separately.