Bull. Chem. Soc. Jpn., 70, 1435—1440 (1997)

New Efficient Synthesis of a Biosynthetic Precursor of Lipid A

Masato Oikawa, Akira Wada, Hiroaki Yoshizaki, Koichi Fukase, and Shoichi Kusumoto*

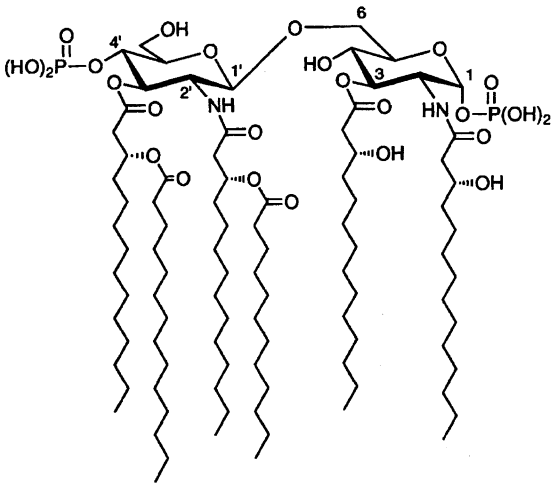
Department of Chemistry, Graduate School of Science, Osaka University, Machikaneyama, Toyonaka, Osaka 560

(Received February 20, 1997)

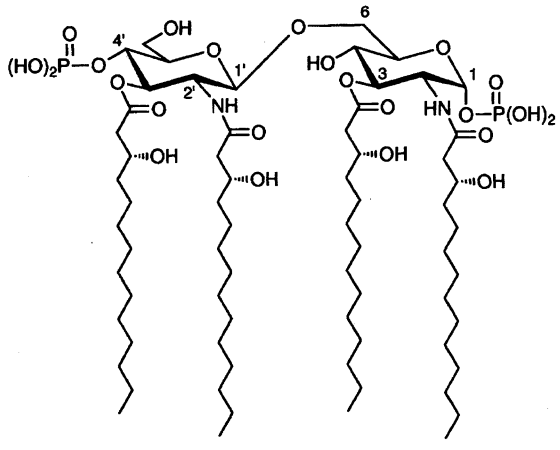
A biosynthetic precursor of lipid A has been synthesized by an improved efficient method. Two appropriately modified acyl-substituted glucosamine units were synthesized from D-glucosamine using (*R*)-3-(benzyloxy)tetradecanoic acid and then coupled by the Lewis acid-promoted glycosidation via the corresponding trichloroacetimidate. Glycosyl phosphorylation and hydrogenolytic deprotection, followed by purification by liquid–liquid partition chromatography, afforded the target compound in 2.9% total yield through 13 steps from *N*-Troc-D-glucosamine.

Lipopolysaccharide (LPS) is a characteristic component of the cell surface layer of Gram-negative bacteria. LPS, also called endotoxin, exhibits various activities such as lethal toxicity, pyrogenicity, induction of hypotension and sepsis syndrome, enhancement of immunological responces, and antitumor activity.[1] The activity is both toxic and beneficial for humans, depending strongly on the concentration of particular mediater substances (e.g., tumor necrosis factor-α or the interleukins),[2] which are released mainly from macrophages by the action of endotoxin, though the signal transduction pathway is still unknown. Generally, endotoxin appears in the order of the O-specific polysaccharide, core oligosaccharide, and lipid A from the non-reducing to the reducing end. The O-specific polysaccharide and core oligosaccharide are hydrophilic, and lipid A is the lipophilic component, respectively. In the middle of the 1980's we established the structure **1** of lipid A from *Escherichia coli* by total synthesis (Chart 1).[3] That work unequivocally showed that lipid A **1** is the active principle of endotoxin.[4] The typical basic structure of lipid A from many Gram-negative bacteria is composed of: 1) a β (1→6) disaccharide of two D-glucosamines, 2) (*R*)-3-hydroxyalkanoyl groups bound at the 2- and 3-positions of both sugar units, and 3) phosphono groups at the reducing end and the 4-position of the non-reducing sugar.

The tetraacyl analogue **2** which lacks the dodecanoyl and tetradecanoyl moieties of **1** was isolated and characterized as a biosynthetic precursor of lipid A.[5—7] Its chemical structure was also unequivocally determined by our previous chemical synthesis.[8] The biosynthetic precursor **2** shows weaker but definite endotoxic activity against murine cells. Quite interestingly, however, the same compound was found to act as an antagonist in human systems: **2** clearly inhibits the cytokine inducing potency of lipid A and LPS.[9] This finding prompted intensive biological studies, particularly those related to the elucidation of action mechanism of lipid A. In order to supply sufficient pure preparation of **2** for such studies, a new chemical synthesis was eagerly required. We complied with this demand by completing a new efficient synthetic procedure based on the knowledge accumulated during our previous work.



1 : Lipid A from *Escherichia coli*

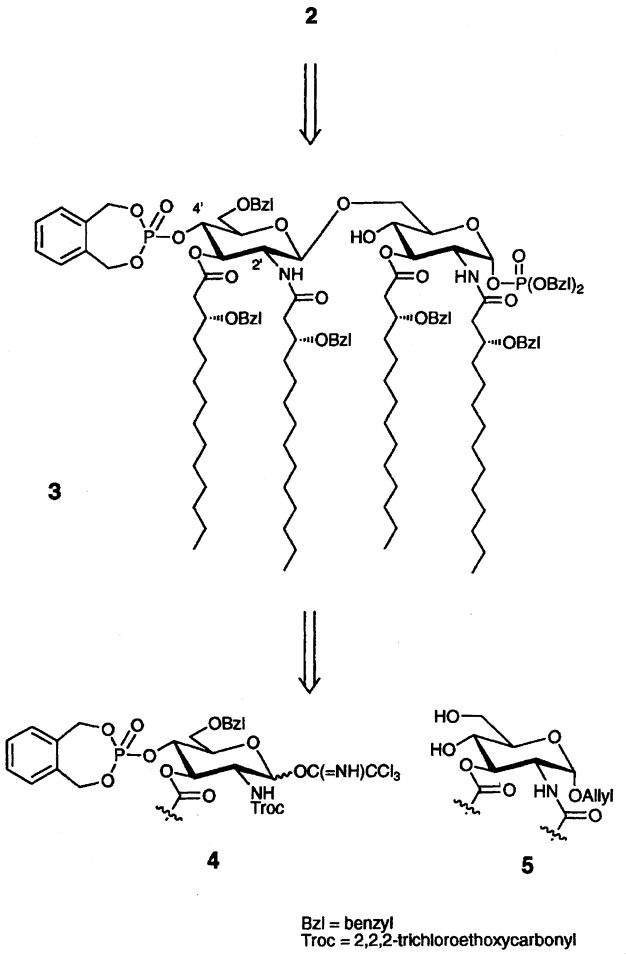


2 : Biosynthetic precursor of lipid A

Chart 1.

Results and Discussion

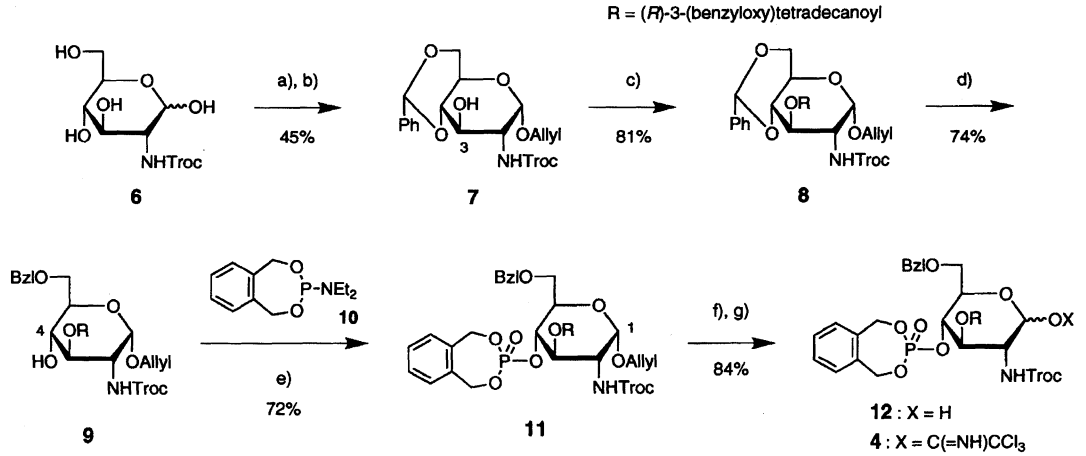
Synthetic Plan. The retrosynthesis of the biosynthetic precursor of lipid A **2** is outlined in Scheme 1. The final deprotection step was designed to be hydrogenolytic cleavage of benzyl (Bzl)-type groups, which has been already proved to be effective and satisfactory in our previous total synthesis of **2**.[8] In that study, the phosphono group at the 4′-position was protected as its diphenyl ester,[3,8] which was stable and met all synthetic requirements. In that route, however, a tedious two-step deprotection sequence (H_2/Pd black for benzyl groups, then H_2/PtO_2 for diphenoxyphosphoryl group) was required at the final steps. The long deprotection procedure also caused spontaneous cleavage of the glycosyl phosphono group, which reduced the yield of the product. So we employed in the present work Watanabe's cyclic protecting group[10] for the 4′-phosphono group instead. In this manner, the deprotection in one-step hydrogenolysis (H_2, Pd black) and removal of the catalyst, followed by purification, was expected to give the desired product **2** more readily.



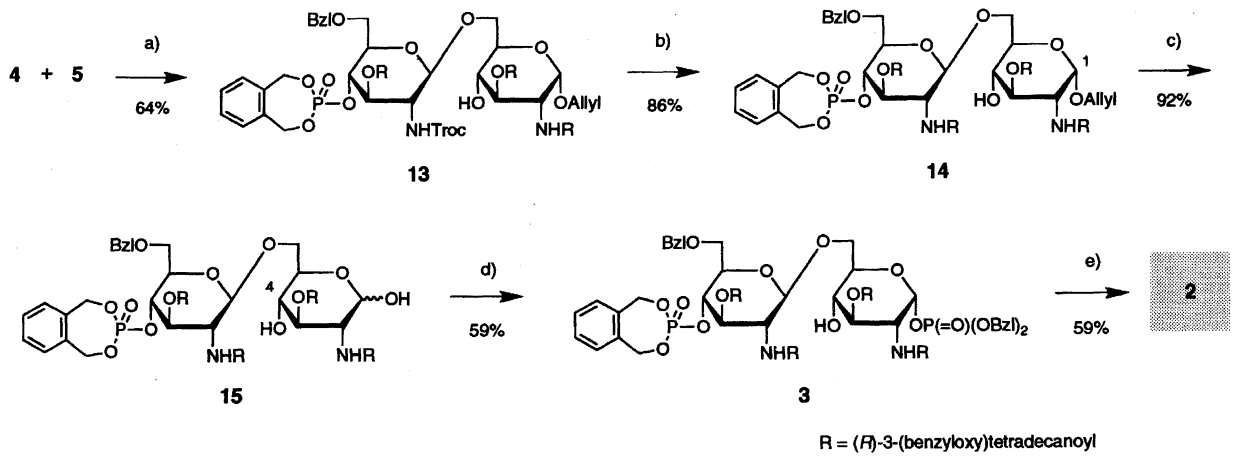
Scheme 1. Retrosynthesis of biosynthetic precursor **2**.

The protected form **3** of the target was divided into two parts, i.e., the imidate **4** as the glycosyl donor and the acceptor **5**,[3] retrosynthetically. The key β-selective condensation was expected by the assistance of the neighboring 2′-(2,2,2-trichloroethoxycarbonyl) (Troc) group, as achieved in the previous synthesis.[3] The 2′-amino functionality was planned to be acylated after glycosidation to avoid oxazoline formation or other side reactions.[11] Both **4** and **5**[3] can be obtained from D-glucosamine and (*R*)-3-(benzyloxy)tetradecanoic acid.

Synthesis of the Glycosyl Donor 4. As shown in Scheme 2, *N*-Troc-D-glucosamine (**6**) was converted into **7** by standard manipulations: i) hydrogen chloride and allyl alcohol, ii) benzaldehyde dimethyl acetal and camphorsulfonic acid in 45% yield. The remaining 3-hydroxy group was then acylated with (*R*)-3-(benzyloxy)tetradecanoic acid[8] in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) to give the 3-*O*-acylated product **8** in 81% yield. The reductive opening of the benzylidene acetal of **8** proceeded quite selectively using sodium cyanotrihydroborate and hydrogen chloride in THF, providing the 6-*O*-benzyl ether **9** without any formation of the isomeric 4-*O*-benzyl ether. The free 4-hydroxy group was phosphitylated by Watanabe's reagent **10**[10] and 1*H*-tetrazole, and succes-



Scheme 2. a) 2% HCl in allyl alcohol, reflux, 20 min. b) CSA, $PhCH(OMe)_2$, THF, reflux, 3 h. c) (*R*)-3-(benzyloxy)tetradecanoic acid, DCC, DMAP, CH_2Cl_2, 12 h. d) $Na[BH_3(CN)]$, HCl, THF, 1 h. e) 1*H*-tetrazole, CH_2Cl_2, 20 min; *m*CPBA, −20 °C, 5 min. f) $[Ir(cod)(MePh_2P)_2]PF_6$, THF, 30 min; I_2, H_2O, 30 min. g) CCl_3CN, Cs_2CO_3, CH_2Cl_2, 1 h.



Scheme 3. a) TMSOTf, MS4A, CH_2Cl_2, −20 °C, 30 min. b) Zn-Cu, AcOH, 2 h; (*R*)-3-(benzyloxy)tetradecanoic acid, DCC, CH_2Cl_2, 12 h. c) $[Ir(cod)(MePh_2P)_2]PF_6$, THF, 30 min; I_2, H_2O, 30 min. d) *n*-BuLi, $((BzlO)_2PO)_2O$, THF, −78→23 °C, 1 h. e) H_2 (7 kg cm^{-2}), Pd black, THF, 4 h.

sive addition of *m*-chloroperbenzoic acid (*m*CPBA) to the reaction mixture furnished the phosphate **11** in 72% yield. Finally, the allyl group at the 1-position was deprotected by treatment with the iridium complex ($[Ir(cod)(MePh_2P)_2]PF_6$)[12] and then with aqueous iodine. Subsequent treatment with trichloroacetonitrile and Cs_2CO_3 furnished the glycosyl donor **4** in a good yield.

Disaccharide Formation and the Synthesis of the Biosynthetic Precursor 2. The acceptor **5** was synthesized as already reported.[3] With the donor **4** in hand, the coupling reaction of **4** and the acceptor **5** was examined (Scheme 3). Reaction of **4** and **5** smoothly proceeded at −20 °C in dichloromethane on employing a catalytic amount of trimethylsilyl trifluoromethanesulfonate[13] as a Lewis acid to provide the disaccharide **13** as a sole product in 64% yield. All the functional groups in **4** and **5** including the protected phosphono group survived intact. The product **13** had the β-glycosidic bond as judged by ^{1}H NMR ($J_{1',2'}$ = 7.9 Hz), and its (1→6) linkage was confirmed at a later stage of **14** after two step conversions. The use of crystalline tin(II) trifluoromethanesulfonate as a stable Lewis acid also gave a comparable yield (69%) for this glycosidation. Deprotection of the *N*-Troc group (aqueous AcOH and zinc–copper couple)[14] was followed by *N*-acylation with (*R*)-3-(benzyloxy)tetradecanoic acid[8] (DCC, CH_2Cl_2) to give the fully acylated disaccharide **14** in 86% yield. ^{1}H NMR spectrum of **14** revealed the presence of the free 4-hydroxy group (δ = 3.43 ppm, d, J = 7.1 Hz), confirming the (1→6) linkage. The 1-*O*-allyl group was then removed also as described above to yield the reducing disaccharide **15** in 92% yield.

Finally, the bis(benzyloxy)phosphoryl group was introduced into **15** selectively at the 1-hydroxy group by the treatment with butyllithium and tetrabenzyl diphosphate in THF at −78 °C→r.t. The product **3** was not stable enough to the acidity of silica gel during its chromatographic purification, so that the yield of **3** (59%) was moderate and 20% of **15** which lost the phosphoryl group was recovered. Subsequently, all the protecting groups for the three hydroxy and two phosphono groups were removed in one step by hydrogenolysis (7 kg cm^{-2} of H_2, Pd black, THF, overnight). The crude material was purified by liquid–liquid partition column chromatography using Sephadex® LH-20 furnishing the desired lipid A precursor **2** in a moderate yield. In our recent works, synthetic lipid A analogues were purified by the use of centrifugal partition chromatography (CPC).[15] The present product **2** was also successfully purified by CPC in an almost identical yield (54%).[16] For the purification of a large quantity of **2** (> 100 mg), however, the partition chromatography was more satisfactory because its operation was simpler and more convenient than that of CPC.

Conclusion

By employing the glycosyl imidate for the glycosylation reaction in place of the glycosyl bromide used in our previous work,[3] the use of cyclic benzyl-type protection for the 4′-phosphono group became possible. Mainly by virtue of this major improvement, the total yield of 2.9% was attained in 13 steps starting from *N*-Troc-D-glucosamine. Thus, together with the facile purification by partition chromatography, a sufficient quantity of **2** has now become available for further detailed biological studies. The present synthetic route will also be applicable to the preparation of a variety of lipid A analogues which are important for the future research toward the elucidation of action mechanism of endotoxin.

Experimental

The experimental techniques and the characterizing apparatuses used are summarized in our previous paper.[17]

Allyl 4,6-*O*-Benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (7). A solution of 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucose (*N*-Troc-D-glucosamine) (**6**) (20.0 g, 0.056 mol) in allyl alcohol (160 mL) containing 2% (w/v) of dry hydrogen chloride was heated under reflux for 20 min. The mixture was cooled, and the solvent was removed in vacuo and the residue coevaporated with toluene. The residue was dissolved in anhydrous THF (80 mL). To this solution were added (+)-camphor-10-sulfonic acid (672 mg, 5 mmol) and benzaldehyde dimethyl acetal (17 mL, 0.11 mol), and the mixture was refluxed for 3 h. The solution was neutralized with saturated

aqueous $NaHCO_3$ (300 mL) and extracted with EtOAc (300 mL). The organic extract was successively washed with brine (500 mL) and water (250 mL×2), dried over Na_2SO_4, and concentrated in vacuo. The residue was recrystallized from MeOH to give **7** as colorless crystals (12.3 g, 45%). Mp 168 °C. $[\alpha]_D^{23}$ = +59.5 (*c* 1.04, $CHCl_3$). FAB-MS (positive) *m/z* 482 [$(M+H)^+$]. Found: C, 47.12; H, 4.42; N, 2.91%. Calcd for $C_{19}H_{22}Cl_3NO_7$: C, 47.27; H, 4.59; N, 2.90%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.50—7.34 (m, 5H), 5.89 (m, 1H), 5.55 (s, 1H), 5.35—5.29 (m, 2H), 5.24 (d, *J* = 10.3 Hz, 1H), 4.92 (d, *J* = 3.2 Hz, 1H), 4.81 (d, *J* = 11.9 Hz, 1H), 4.68 (d, *J* = 11.9 Hz, 1H), 4.26 (dd, *J* = 10.3, 4.8 Hz, 1H), 4.19 (dd, *J* = 12.8, 5.2 Hz, 1H), 4.01 (dd, *J* = 12.8, 6.4 Hz, 1H), 3.98—3.91 (m, 2H), 3.85 (ddd, *J* = 10.3, 8.9, 4.8 Hz, 1H), 3.75 (dd, *J* = 10.3, 10.3 Hz, 1H), 3.56 (dd, *J* = 8.9, 8.9 Hz, 1H), 2.77 (s, 1H).

Allyl 4,6-*O*-Benzylidene-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (8). (*R*)-3-(Benzyloxy)tetradecanoic acid (1.00 g, 3.0 mmol), DCC (1.57 g, 8.71 mmol), and DMAP (37 mg, 0.30 mmol) were added to a solution of **7** (1.44 g, 3.0 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at room temperature for 12 h. Then MeOH (1.0 mL) and AcOH (0.2 mL) were added, and the mixture was stirred for 10 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 $NaHCO_3$ (50 mL ×3) and brine (50 mL). The EtOAc layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (180 g, toluene/EtOAc=50 : 1) to give **8** as a colorless solid (2.90 g, 81%). $[\alpha]_D^{22}$ = +38.5 (*c* 1.02, $CHCl_3$). FAB-MS (positive) *m/z* 799 [$(M+H)^+$]. Found: C, 60.12; H, 6.80; N, 1.52%. Calcd for $C_{40}H_{54}Cl_3NO_9$: C, 60.11; H, 6.81; N, 1.75%. ^{1}H NMR (270 MHz, $CDCl_3$) δ = 7.42—7.25 (m, 10H), 5.97—5.83 (m, 1H), 5.47 (s, 1H), 5.46—5.23 (m, 4H), 4.94 (d, *J* = 3.6 Hz, 1H), 4.72 (d, *J* = 11.9 Hz, 1H), 4.58 (d, *J* = 11.9 Hz, 1H), 4.50 (d, *J* = 11.9 Hz, 1H), 4.38 (d, *J* = 11.9 Hz, 1H), 4.31—4.19 (m, 2H), 4.07—3.91 (m, 3H), 3.82—3.67 (m, 3H), 2.67 (dd, *J* = 15.5, 6.6 Hz, 1H), 2.43 (dd, *J* = 15.5, 5.9 Hz, 1H), 1.48—1.44 (m, 2H), 1.25 (m, 18H), 0.88 (t, *J* = 6.6 Hz, 3H).

Allyl 6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (9). To a solution of **8** (200 mg, 0.25 mmol) in anhydrous THF (8 mL) were added $Na[BH_3(CN)]$ (157 mg, 2.5 mmol) and dry hydrogen chloride (20% w/v THF solution, 3 mL) at 25 °C. After stirring for 1 h, EtOAc (10 mL) was added and the mixture was washed successively with saturated aqueous $NaHCO_3$ (10 mL×3) and brine (10 mL). The EtOAc layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (15 g, $CHCl_3$) to give **9** as a colorless oil (149 mg, 74%). $[\alpha]_D^{21}$ = +38.6 (*c* 1.05, $CHCl_3$). FAB-MS (positive) *m/z* 799 (M^+). Found: C, 59.94; H, 7.07; N, 1.81%. Calcd for $C_{40}H_{56}Cl_3NO_9$: C, 59.96; H, 7.04; N, 1.75%. ^{1}H NMR (270 MHz, $CDCl_3$) δ = 7.33—7.26 (m, 10H), 5.96—5.82 (m, 1H), 5.33—5.11 (m, 4H), 4.90 (d, *J* = 3.6 Hz, 1H), 4.68 (m, 2H), 4.52 (m, 2H), 3.98—3.63 (m, 7H), 3.02 (bs, 1H), 2.62 (dd, *J* = 14.5, 7.8 Hz, 1H), 2.49 (dd, *J* = 14.5, 4.6 Hz, 1H), 1.69—1.55 (m, 2H), 1.26 (s, 18H), 0.88 (t, *J* = 6.6 Hz, 3H).

Allyl 6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (11). To a solution of **9** (1.01 g, 1.3 mmol) in CH_2Cl_2 (20 mL) at room temperature were added *N,N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (**10**, 753 mg, 3.2 mmol) and 1*H*-tetrazole (442 mg, 6.3 mmol). After stirring for 20 min, the solution was cooled to −20 °C, and *m*CPBA (80%, 1.36 g, 6.3 mmol) was added. The mixture was stirred for 5 min and quenched with saturated aqueous $NaHCO_3$ (20 mL). The mixture was extracted with EtOAc (50 mL). The EtOAc layer was successively washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (70 g, $CHCl_3$/acetone=100 : 1) to give **11** as a colorless syrup (894 mg, 72%). $[\alpha]_D^{25}$ = +32.5 (*c* 0.99, $CHCl_3$). 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%. ^{1}H NMR (270 MHz, $CDCl_3$) δ = 7.40—7.10 (m, 14H), 5.93—5.83 (m, 1H), 5.42—5.23 (M, 4H), 5.08—4.92 (m, 5H), 4.81—4.49 (m, 7H), 4.26—4.21 (m, 1H), 4.07—3.71 (m, 6H), 2.74 (dd, *J* = 16.8, 7.6 Hz, 1H), 2.54 (dd, *J* = 16.8, 4.3 Hz, 1H), 1.56—1.45 (m, 2H), 1.26 (m, 18H), 0.88 (t, *J* = 6.6 Hz, 3H).

6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (12). To a degassed solution of **11** (397 mg, 0.40 mmol) in THF (8 mL) was added [bis(methyldiphenylphosphine)](1,5-cyclooctadiene) iridium(I) hexafluorophosphate (24 mg, 0.03 mmol). After activation of the iridium catalyst with hydrogen (30 s), the mixture was stirred under nitrogen at room temperature for 30 min. Iodine (154 mg, 0.61 mmol) and water (5 mL) were added and the reaction mixture was stirred for an additional 30 min. After addition of 10% aqueous $Na_2S_2O_3$ (10 mL), the mixture was extracted with EtOAc (10 mL). The organic layer was successively washed with saturated aqueous $NaHCO_3$ (10 mL×2) and brine (20 mL), and then dried over $MgSO_4$. After removal of the solvent in vacuo, the crude product was purified by silica-gel flash chromatography (10 g, $CHCl_3$/acetone = 50 : 1) to give **12** as a pale yellow solid (321 mg, 84%). $[\alpha]_D^{25}$ = +12.5 (*c* 1.00, $CHCl_3$). 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%. ^{1}H NMR (270 MHz, $CDCl_3$) δ = 7.39—7.17 (m, 14H), 5.51—4.97 (m, 7H), 4.69—4.53 (m, 7H), 4.20 (m, 1H), 4.11 (m, 2H), 3.99—3.93 (m, 1H), 3.79—3.64 (m, 2H), 2.67 (dd, *J* = 15.5, 6.6 Hz, 1H), 2.43 (dd, *J* = 16.2, 5.7 Hz, 1H), 1.48—1.44 (m, 2H), 1.25 (m, 18H), 0.88 (t, *J* = 6.9 Hz, 3H).

6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl Trichloroacetimidate (4). To a solution of **12** (100 mg, 0.11 mmol) in CH_2Cl_2 (5 mL) at room temperature were added Cs_2CO_3 (17 mg, 0.05 mmol) and trichloroacetonitrile (0.106 mL, 1.1 mmol). After stirring for 1 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL) and the solution was extracted with $CHCl_3$ (80 mL). The extract was washed with brine (20 mL), and dried over $MgSO_4$. Removal of the solvent in vacuo gave crude **4** as a pale yellow solid (401 mg, 100%), which was used for the next glycosidation reaction without further purification.

Allyl 6-*O*-[6-*O*-Benzy-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-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). To a mixture of **4** (1.22 g, 1.1 mmol), allyl 3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)-tetradecanoylamino]-2-deoxy-α-D-glucopyranoside (**5**) (956 mg, 1.1 mmol), and molecular sieves 4A (500 mg) in CH_2Cl_2 (20 mL) was added trimethylsilyl trifluoromethanesulfonate (22 μL, 0.11

mmol) at −20 °C. After stirring for 30 min, the insoluble materials were filtered off, and EtOAc (50 mL) was added to the filtrate. The solution was washed successively with saturated aqueous $NaHCO_3$ (20 mL×2) and brine (20 mL×2), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (80 g, $CHCl_3$/acetone = 50 : 1) to give **13** as a colorless oil (1.28 g, 64%). $[\alpha]_D^{21}$ = +15.5 (*c* 0.99, $CHCl_3$). FAB-MS (positive) *m/z* 1778 $[(M+H)^+]$. ^{1}H NMR (270 MHz, $CDCl_3$) δ = 7.42—7.11 (m, 24H), 6.22 (d, *J* = 9.2 Hz, 1H), 5.80—5.72 (m, 1H), 5.70—5.41 (m, 1H), 5.22—4.92 (m, 8H), 4.84 (d, *J* = 7.9 Hz, 1H), 4.76 (d, *J* = 3.6 Hz, 1H), 4.74—4.46 (m, 11H), 4.31—4.32 (m, 1H), 4.08—3.99 (m, 1H), 3.91—3.57 (m, 11H), 3.42—3.32 (m, 1H), 2.67 (m, 2H), 2.63 (dd, *J* = 15.1, 8.0 Hz, 1H), 2.46 (dd, *J* = 15.1, 4.8 Hz, 1H), 2.33—2.26 (m, 2H), 1.59 (s, 6H), 1.26 (s, 54H), 0.88 (t, *J* = 6.6 Hz, 9H).

Allyl 6-*O*-[6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-α-D-glucopyranoside (14). To a solution of **13** (100 mg, 0.056 mmol) in AcOH (1.5 mL) was added zinc-copper couple (300 mg), and the mixture was stirred at room temperature for 2 h. The insoluble materials were filtered off, and the filtrate was concentrated in vacuo by coevaporation with toluene three times. The residue was dissolved in EtOAc (50 mL) and was washed successively with saturated aqueous $NaHCO_3$ (20 mL×2) and brine (20 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give a crude *N*-deprotected product (86 mg).

The crude product thus obtained was dissolved in CH_2Cl_2 (5 mL). To this were added DCC (24 mg, 0.12 mmol) and (*R*)-3-(benzyloxy)tetradecanoic acid (40 mg, 0.12 mmol). The mixture was stirred at room temperature for 12 h. The insoluble materials were filtered off, and the filtrate was concentrated in vacuo. The residue was redissolved in EtOAc (20 mL), washed successively with saturated aqueous $NaHCO_3$ (20 mL×2) and brine (20 mL), and dried over $MgSO_4$. The organic layer was concentrated in vacuo and the residue was purified by silica-gel flash chromatography (5 g, toluene/EtOAc=2:1) to give **14** as a colorless oil (93 mg, 86%). $[\alpha]_D^{22}$ = +12.7 (*c* 1.02, $CHCl_3$). FAB-MS (positive) *m/z* 1941 $[(M+Na)^+]$. ^{1}H NMR (270 MHz, $CDCl_3$) δ = 7.43—7.10 (m, 29H), 6.28—6.18 (m, 2H), 5.77—5.63 (m, 1H), 5.39—5.32 (m, 1H), 5.19—4.87 (m, 8H), 4.74 (d, *J* = 3.6 Hz, 1H), 4.70—4.42 (m, 11H), 4.39—4.22 (m, 1H), 4.03—3.63 (m, 13H), 3.37—3.35 (m, 1H), 2.68 (dd, *J* = 16.4, 7.2 Hz, 1H), 2.59 (dd, *J* = 15.1, 7.3 Hz, 1H), 2.57 (dd, *J* = 16.4, 3.9 Hz, 1H), 2.42 (dd, *J* = 15.1, 5.0 Hz, 1H), 2.30—2.27 (m, 2H), 2.27—2.21 (m, 2H), 1.95—1.42 (m, 8H), 1.25 (s, 72H), 0.88 (t, *J* = 6.6 Hz, 12H).

6-*O*-[6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)teradecanoylamino]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-D-glucopyranose (15). To a degassed solution of **14** (64 mg, 33 μmol) in THF (5 mL) was added [bis(methyldiphenylphosphine)](1,5-cyclooctadiene)-iridium(I) hexafluorophosphate (3 mg, 3 μmol). After the activation of the catalyst with hydrogen for 10 s, the mixture was stirred under the same conditions and worked up as described in the synthesis of **12**. The crude product was purified by silica-gel flash chromatography (15 g, $CHCl_3$/acetone = 10 : 1) to give **15** as a colorless oil (58 mg, 92%). $[\alpha]_D^{22}$ = +5.3 (*c* 1.03, $CHCl_3$). FAB-MS (positive) *m/z* 1901 $[(M+Na)^+]$. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.39—7.19 (m, 27H), 7.12 (d, *J* = 7.6 Hz, 1H), 6.78 (d, *J* = 7.3 Hz, 1H), 6.31 (d, *J* = 7.8 Hz, 1H), 6.23 (d, *J* = 9.6 Hz, 1H), 5.44 (dd, *J* = 10.3, 9.4 Hz, 1H), 5.05—4.89 (m, 7H), 4.69—4.41 (m, 13H), 4.15 (m, 1H), 4.00 (d, *J* = 10.3 Hz, 1H), 3.88—3.77 (m, 6H), 3.73—3.69 (m, 2H), 3.62 (dd, *J* = 12.1, 8.0 Hz, 1H), 3.50 (dd, *J* = 18.3, 8.0 Hz, 1H), 3.28 (ddd, *J* = 14.0, 9.6, 4.6 Hz, 1H), 2.69 (dd, *J* = 16.5, 7.3 Hz, 1H), 2.59 (dd, *J* = 14.9, 9.6 Hz, 1H), 2.57 (dd, *J* = 16.5, 4.8 Hz, 1H), 2.43 (dd, *J* = 14.9, 5.0 Hz, 1H), 2.35—2.24 (m, 2H), 2.26 (dd, *J* = 15.0, 3.5 Hz, 1H), 2.18 (dd, *J* = 15.0, 7.9 Hz, 1H), 1.63—1.41 (m, 8H), 1.31—1.25 (m, 72H), 0.88 (t, *J* = 6.9 Hz, 12H).

6-*O*-[6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)teradecanoylamino]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-1-*O*-bis(benzyloxy)phosphoryl-2-deoxy-α-D-glucopyranose (3). To a solution of **15** (102 mg, 55 μmol) in anhydrous THF (5 mL) was added 15% *n*-BuLi in hexane (42 μL, 0.068 mmol) at −78 °C. After 5 min, tetrabenzyl diphosphate (38 mg, 0.071 mmol) was added and the solution was stirred at the same temperature for 1 h. The mixture was allowed to warm gradually to room temperature, and then neutralized with saturated aqueous $NaHCO_3$ (15 mL) and extracted with EtOAc (50 mL). After drying over $MgSO_4$ and removal of the solvent in vacuo, the crude material was purified by silica-gel flash chromatography (15 g, $CHCl_3$/acetone = 30 : 1) to give **3** as a colorless solid (69 mg, 59%). During the chromatographic purification the product was partly decomposed, so that 20% of **15** was recovered.

2-Deoxy-6-*O*-[2-deoxy-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-α-D-glucopyranose 1,4′-Bisphosphate (2). A mixture of **3** (261 mg, 0.12 mmol) and Pd-black (250 mg) in THF (15 mL) was stirred under 7 kg cm^{-2} of hydrogen at room temperature for 4 h. The mixture was then neutralized with triethylamine. After removal of the catalyst by filtration, the solvent was removed in vacuo. The crude product was purified by liquid–liquid partition column chromatography (40 g of Sephadex® LH-20, $CHCl_3$/MeOH/^{i}PrOH/H_2O/Et_3N = 20 : 20 : 2.5 : 22.5 : 0.001). The organic layer was the stationary phase, and the aqueous layer was the mobile phase in this chromatography. After removal of the solvent in vacuo, followed by lyophilization, **2** was obtained as a triethylammonium salt (colorless solid, 101 mg, 59%). The physical data were identical with the reported ones.[8]

This work was supported in part by the Grant-in-Aid for Scientific Research on Priority Areas No. 06240105 from the Ministry of Education, Science, Sports and Culture.

References

1) 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).

2) E. Th. Rietschel and H. Brade, *Sci. Am.*, **267**, August 26 (1992).

3) 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).

4) References cited in 3b).

5) Compound **2** was designated precursor Ia[6] or lipid IV_A.[7]

6) T. Hansen-Hagge, V. Lehman, and O. Lüderitz, *Eur. J. Biochem.*, **148**, 21 (1985).

7) C. R. H. Raetz, S. Purcell, M. V. Meyer, N. Qureshi, and K. Takayama, *J. Biol. Chem.*, **260**, 16080 (1985).

8) a) M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba, *Tetrahedron Lett.*, **25**, 2667 (1984); b) M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **60**, 2197 (1987).

9) 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).

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

11) a) S. Kusumoto, H. Yoshimura, M. Imoto, T. Shimamoto, and T. Shiba, *Tetrahedron Lett.*, **26**, 909 (1985); b) C. Diolez, S. R. Sarfati, and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, **1985**, 57.

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

13) R. R. Schmidt, *Angew. Chem., Int. Ed. Engl.*, **25**, 212 (1986).

14) T. B. Windholz and D. B. R. Johnston, *Tetrahedron Lett.*, **27**, 2555 (1967).

15) K. Fukase, W.-C. Liu, Y. Suda, M. Oikawa, A. Wada, S. Mori, A. J. Ulmer, E. Th. Rietschel, and S. Kusumoto, *Tetrahedron Lett.*, **41**, 7455 (1995).

16) Purification of **2** by centrifugal partition chromatography was carried out using the same solvent system and elution manner with those in liquid–liquid partition column chromatography ($CHCl_3$/MeOH/^{i}PrOH/H_2O/Et_3N = 20 : 20 : 2.5 : 22.5 : 0.001, aqueous solvent as the mobile phase).

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