Synthetic Study of Lipoteichoic Acid of Gram Positive Bacteria. II. Synthesis of the Proposed Fundamental Structure of *Enterococcus hirae* Lipoteichoic Acid[1)]

Koichi FUKASE, Takuya YOSHIMURA, Shozo KOTANI,† and Shoichi KUSUMOTO*
Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560
†Hyogo College of Medicine, Nishinomiya, Hyogo 663

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The proposed fundamental structure of *Enterococcus hirae* lipoteichoic acid (LTA) was synthesized in order to elucidate the chemical structure responsible for the cytokine-inducing activity described for the natural LTA fraction of this bacteria. Synthesis was accomplished by coupling of the glycolipid part with the poly(glycerol phosphate) (PGP) part by using a phosphoramidite method. The glycolipid part was constructed by coupling of the phosphatidic acid moiety with a kojibiosyl diacylglycerol which had been prepared by stepwise glycosidation with glycosyl fluorides. α-Selective glucosidations were effected by virtue of the 2,2,2-trichloroethoxycarbonyl (Troc) group introduced at the 6-hydroxyl function. *p*-Nitrobenzyl (NPM) and *p*-pivaloylaminobenzyl (PAB) groups were successfully applied to temporary protection of hydroxyl functions.

Lipoteichoic acids (LTAs) are amphiphilic cell-surface glycoconjugates characteristic of gram-positive bacteria. They consist of covalently bound glycolipid and hydrophilic parts. The latter is a (1→3)-phosphodiester-linked poly(glycerol phosphate) (PGP). Species-specific structural variations of the glycolipid part have been described.[2)] Recently, immunostimulating activity was found in LTAs isolated from several bacterial species.[3—9)] Usami et al. described the potent antitumor activity of *Streptococcus pyogenes* LTA based on an induced tumor necrosis factor (TNF). They also described the low toxicity of the TNF induced by streptococcal LTA compared with that induced by lipopolysaccharides (LPS) of gram-negative bacteria.[3—5)] Tsutsui et al. studied the biological activities of two sub-fractions of LTA (LTA-1 and LTA-2) separated from the LTA fraction of *Enterococcus hirae* ATCC 9790 by hydrophobic chromatography. They demonstrated that the more hydrophobic LTA-2 was more active than LTA-1 in inducing cytokines, but both LTAs showed similar antitumor potency in vivo.[6,7)]

The chemical structures of the above LTAs were proposed previously by Fischer et al. as shown in Fig. 1.[2,10,11)] LTA-1 of *E. hirae* is assumed to have the same structure as that proposed for *S. pyogenes* LTA. Its glycolipid part consists of an α(1→2) disaccharide of D-glucose (kojibiose) linked to 1,2-diacyl-*sn*-glycerol by an α-glycosidic bond. LTA-2 of *E. hirae* has a phosphatidic acid linked to the 6-position of glucose in the glycolipid part of LTA-1 or *S. pyogenes* LTA. The average chain length of the PGP part is deduced to be about $n=20$. The secondary hydroxyl groups of the glycerol moieties in the PGP part were reported to be substituted with (oligo)glycosyl and/or D-alanyl groups. However, the structure of the entity responsible for the immunostimulating activity has not been defined chemically, since natural LTAs were always obtained as heterogeneous mixtures of congeners. We then started a synthetic study of the above LTAs to confirm the proposed structure and elucidate the chemical entity responsible for their interesting activities.

In a previous study,[12)] we reported the synthesis of the structure proposed for the LTA of *S. pyogenes*, **1** ($n=3$), by coupling of the glycolipid with PGP and subsequent final deprotection. The PGP part was prepared by a simple repeating procedure using a phosphoramidite method for the construction of the 1,3-phosphodiester linkage. Since the contents of D-alanine or D-glucose linked to glycerol vary depending on various factors, and since alanine-free LTA was reported to retain the biological activity,[7)] we omitted these PGP substituents in our first synthesis. The glycolipid part of **1** was synthesized using appropriate glycosyl fluorides as donors, where the *p*-nitrobenzyl (*p*-nitrophenylmethyl, NPM) group was successfully applied to temporary protection of a hydroxyl function. Palmitic acid was used as the sole acyl moiety although palmitic and oleic acids proved to be the main components of fatty acids in the natural product. In this paper, we describe the synthesis of another more complex structure, **2** ($n=3$),

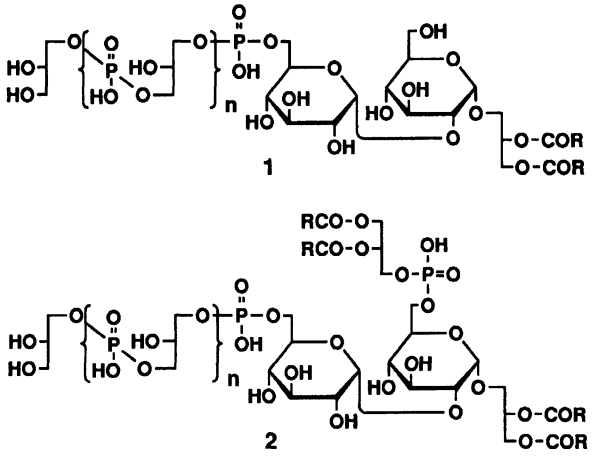


Fig. 1. The proposed structures of *Streptococcus pyogenes* LTA (**1**) and *Enterococcus hirae* LTA-2 (**2**).

proposed for LTA-2 of *E. hirae*.

Results and Discussion

The synthesis of *E. hirae* LTA-2 was carried out by a route similar to that employed for the synthesis of *S. pyogenes* LTA. The glycolipid was prepared by stepwise coupling of glucose units to a glycerol derivative. Glycosyl fluorides, whose 2-hydroxyl groups were protected as nonparticipating benzyl-type ethers, were used as glycosyl donors for the formation of α-glucosidic linkages. We used an NPM group for the temporary protection of the 2-hydroxyl group of the first glucosyl donor as well in the present case, since the NPM function proved to be versatile for the temporary protection of hydroxyl functions in our previous studies.[12—14] The NPM ether is stable under various reaction conditions, particularly in the presence of strong acids including Lewis acids, which are frequently used for the activation of glycosyl fluorides or trichloroacetimidates. The ether can be removed selectively via reduction to the *p*-aminobenzyl group followed by anodic oxidation or by 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) oxidation after *N*-acetylation.

The phosphatidyl residue in structure **2** was introduced after construction of the kojibiosyl diacylglycerol structure in order to avoid exposing the phosphotriester to acidic reaction conditions in the glycosidation steps. Therefore, the 6-position was temporarily protected with a *p*-pivaloylaminobenzyl (PAB) group, as we reported recently.[15] The PAB group can be selectively removed with DDQ in a manner similar to the cleavage of the *p*-methoxybenzyl (MPM) group in the presence of benzyl and acyl groups, but is stable under glycodisation conditions using Lewis acids. The MPM group is, in contrast, partially cleaved under such conditions. However, we first protected the 6-position with a 2,2,2-trichloroethoxycarbonyl (Troc) group, since we expected a possible influence of the 6-*O*-Troc group to increase the α-selectivity in the glycosidation step.[16] After the glycosidation of 1,2-di-*O*-allyl-*sn*-glycerol (**8**) with 6-*O*-Troc fluoride **7** and subsequent removal of the Troc group, a PAB group was introduced as shown in Scheme 1. The acyl moieties were also introduced after this glycosidation step in order to avoid their possible migration in 1,2-di-*O*-acyl-*sn*-glycerol during the glycosidation using an acid catalyst.

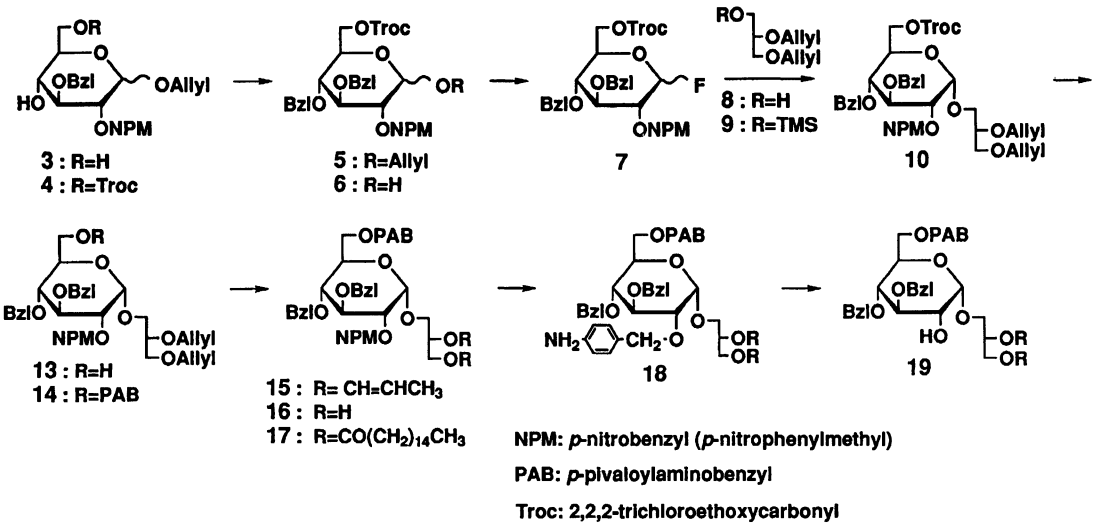
The 6-position of allyl 3-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)glucoside (**3**)[12] was selectively protected with the Troc group. The 4-position of product **4** was benzylated with benzyl trichloroacetimidate and trifluoromethanesulfonic acid (triflic acid, TfOH) to give **5**. The allyl group of **5** was then cleaved by isomerization to the 1-propenyl group with an iridium complex[17] followed by treatment with iodine and water[18] to give 1-hydroxy derivative **6**. Glycosyl fluoride **7** was obtained in a quantitative yield ($\alpha:\beta=1.3:1$)[19] by fluorination of **6** using 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (FMPT) and triethylamine.[20]

The glycosidation with 6-*O*-Troc fluoride **7** of 1,2-di-*O*-allyl-*sn*-glycerol (**8**) was carried out in ether by Noyori's procedure[21] using the 3-*O*-TMS derivative of the latter (**9**) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf). The desired glucosyl glycerol derivative **10** was obtained as a mixture of α- and β-anomers in an 81% yield with high α-selectivity ($\alpha:\beta=11.5:1$).[22] The results by other glycosidation procedures such as Mukaiyama's method using **8** and $SnCl_2$–$AgClO_4$[23] in ether (yield: 41%, $\alpha:\beta=4:1$) or Suzuki's method using **8** and $[ZrCl_2(Cp)_2]$–$AgClO_4$[24] in toluene (yield: 73%, $\alpha:\beta=6:1$) surpassed the above result in neither the yield nor the anomeric selectivity. In our previous synthetic study of *S. pyogenes* LTA,[12] the glycosidation of **9** with 6-*O*-Bzl fluoride **11** [3,4,6-tri-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α-D-glucopyranosyl fluoride] by Noyori's procedure gave the corresponding glycoside **12** [1,2-di-*O*-allyl-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α-D-glucopyranosyl]-*sn*-glycerol] in a ratio of $\alpha:\beta=3.4:1$. These results indicate that the α-selectivity was increased remarkably by the influence of the 6-*O*-Troc group as we expected.

The 6-*O*-Troc group of **10** was then removed with NaOMe to give a mixture of the desired α-glucosyl glycerol **13** and its β-anomer, which were completely separated by silica-gel column chromatography. The PAB group was introduced at the free 6-position of **13** by the use of *p*-pivaloylaminobenzyl trichloroacetimidate and TfOH in dichloromethane at −70 °C. PAB ether **14** was obtained in a 79% yield (recovery of **13**: 10%). The allyl groups of compound **14** were then isomerized, and the resulting 1-propenyl ethers in **15** were cleaved with 90% aqueous acetic acid at 50 °C to give diol **16** in a 63% yield. The free hydroxyl groups of **16** were acylated with palmitoyl chloride to give dipalmitoyl derivative **17**.

The NPM group in **17** was then removed according to the method mentioned above. The nitro group of **17** was reduced by the use of a combination of a zinc–copper couple and acetylacetone to give aminobenzyl ether **18** quantitatively.[12,14] The aminobenzyl group in **18** was selectively cleaved by anodic oxidation in 2-methyl-2-propanol (^{t}BuOH)–water (3:1) using perchloric acid as the supporting electrolyte and platinum electrodes under a constant current without any change of the PAB group, which was oxidatively removed later with DDQ. Compound **19** was thus obtained in a 72% yield with 15% recovery of **18**.

The glycosidation of acceptor **19** with the second glucosyl donors was then examined. In order to estimate the effect of the 6-*O*-Troc group for α-selectivity again, we used both 2,3,4,6-tetra-*O*-benzylglucosyl fluoride (**20**) and 2,3,4-tri-*O*-benzyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)glucosyl fluoride (**21**) as donors. Suzuki's procedure using $[ZrCl_2(Cp)_2]$ and $AgClO_4$ as an activator gave better results in the present glycosidation



Scheme 1.

reactions.

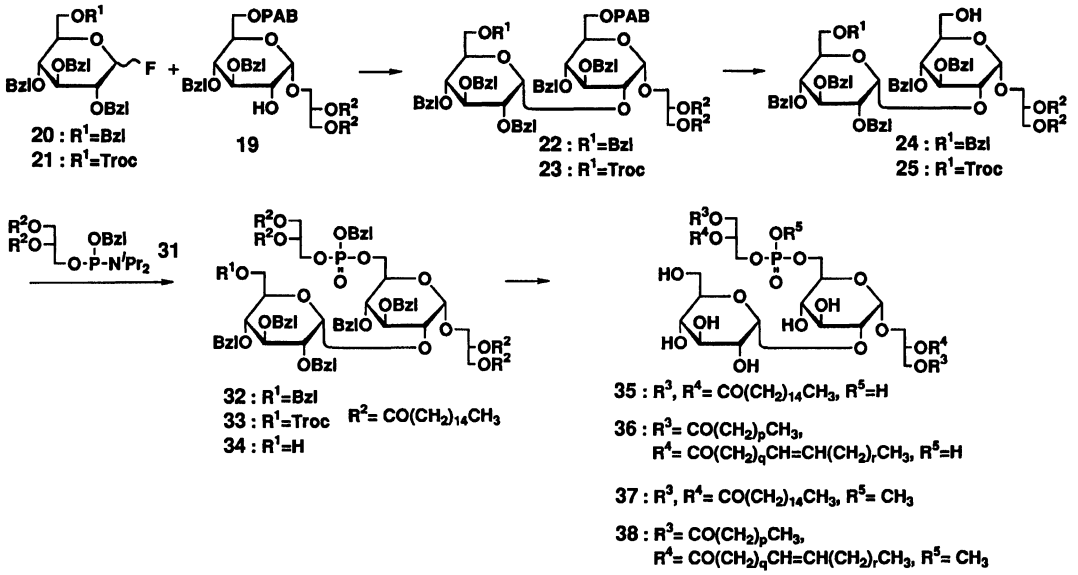
The glycosidation of **19** with 6-*O*-Bzl fluoride **20** afforded the desired α-anomer **22** (39%) and its β-anomer (17%), which were separated by silica-gel column chromatograpny ($\alpha:\beta=2.2:1$).[25] The PAB group of α-anomer **22** was then selectively removed by DDQ to obtain disaccharide derivative **24** to be used for the subsequent coupling with a phosphatidyl residue (Scheme 2). The glycosidation of **19** with 6-*O*-Troc fluoride **21** followed by the removal of the PAB group gave the desired α-anomer **25** (56%) and its β-anomer (8%) ($\alpha:\beta=7.3:1$).[26] The α-orienting effect of the 6-*O*-Troc group was clearly observed here again.[16]

Phosphoramidite reagent **31** to be used for the next coupling reaction as the phosphatidic acid component was synthesized as follows. Benzyloxybis(diisopropylamino)phosphine (**28**)[27] was prepared from benzyl alcohol and chlorobis(diisopropylamino)phosphine (**27**), which was prepared in situ using dichloro(diisopropylamino)phosphine (**26**) and diisopropylamine. The reaction of **27** with 1,2-di-*O*-palmitoyl-*sn*-glycerol (**30**) using 1*H*-tetrazole as a catalyst gave amidite **31** (Scheme 3). Since amidite **31** was contaminated by a small amount of dibenzyloxy(diisopropylamino)phosphine **29**, a by-product formed during the preparation of **28**, **31** was purified by extraction of **29** with acetonitrile from a hexane–triethylamine (40:1) solution of the crude mixture and subsequent silica-gel column chromatography with eluents containing triethylamine. The absence of racemization owing to 1,3-acyl migration under the weakly acidic reaction conditions (pH 5.0) was assured by the fact that the optical rotation of **30** did not change at all even after prolonged contact with 1*H*-tetrazole.

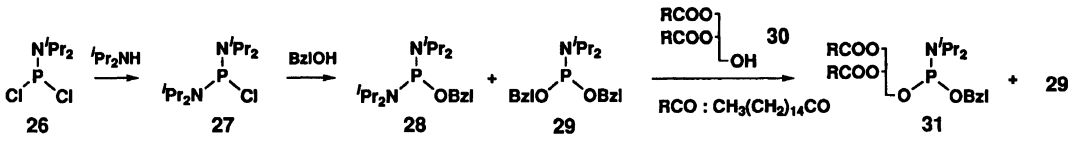
The coupling reactions of purified amidite reagent **31** with 6′-*O*-benzyl derivative **24** or 6′-*O*-Troc derivative **25** proceeded smoothly in dichloromethane and acetonitrile in the presence of 1*H*-tetrazole. The resultant phosphites were oxidized with *m*-chloroperbenzoic acid (mCPBA) without isolation to give glycolipid parts **32** and **33**, respectively, in good yields.

All benzyl groups of glycolipid **32** were then removed by catalytic hydrogenation with Pd-black to give free glycolipid **35**, which was purified by column chromatography on silica-gel and Sephadex LH-20. Since a glyceroglycolipid with the identical architecture is expected to be present in the cell membrane of the bacteria,[12] we then planned to identify the synthetic glycolipid **35** by using the natural one, **36**, obtained from *E. hirae*.[28] Since the ^{1}H NMR spectrum of **35** showed broad signals that were unable to be assigned, **35** was converted to methyl ester **37** with diazomethane to obtain a better spectrum. The proton and ^{13}C-carbon signals of **37** were unambigously assigned by analysis of the ^{1}H–^{1}H and ^{13}C–^{1}H COSY NMR spectra. Comparison of the ^{1}H NMR spectra of **37** and methyl ester **38** derived from natural glycolipid **36** clearly showed that the natural glycolipid is identical to the synthetic one except for the existence of double bonds in the acyl moieties of the former. Consequently, the structure of the glycolipid of *E. hirae* was strictly confirmed in the present study with the exception of the positions of unsaturation. Synthesis of the saturated glycolipid of *E. hirae* was also described previously by van Boeckel et al. by the use of a bifunctional silyl protective group, but the synthetic glycolipid was not compared with the natural one except by TLC analysis.[29]

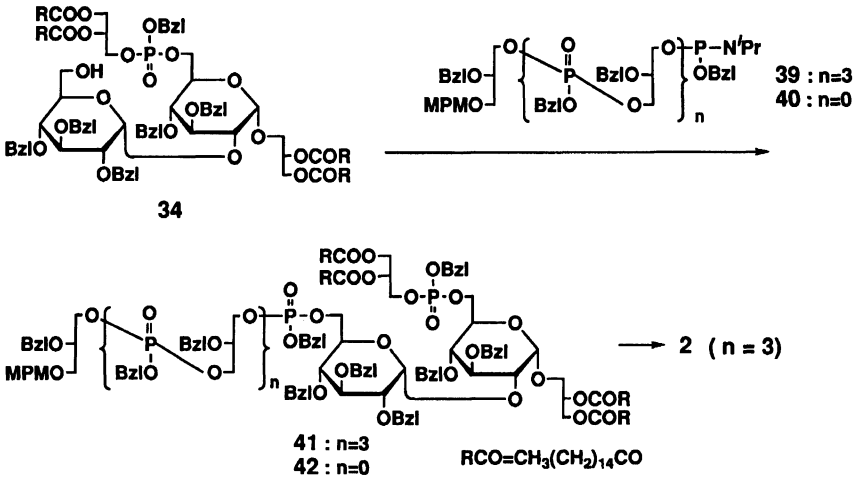
For the coupling with the glycerol phosphate component, the 6′-*O*-Troc group of protected glycolipid **33** was then removed by zinc and acetic acid. The coupling reaction of the resultant **34** and the phosphoramidite of glycerol phosphate tetramer **39**[12] was then investigated (Scheme 4). When the reaction was carried out in dichloromethane and acetonitrile in a manner similar to that described above for the introduction of the phosphatidyl moiety, the coupling reaction did not proceed at all. We therefore examined various solvents using the



Scheme 2.



Scheme 3.



Scheme 4.

glycerol phosphate monomer **40** as a model substrate. The reaction proceeded most smoothly in a mixture of 1,2-dichloroethane and acetonitrile among the solvents tested and the coupling product was formed in a quantitative yield. Therefore, we carried out the coupling of **34** with tetramer **39** in this solvent system. The reaction proceeded, though the rate was much slower than in the case of monomer **40**. The crude resultant phosphite was oxidized by mCPBA to afford protected *E. hirae* LTA **41** in a 27% yield with 66% recovery of glycolipid part **34**. Finally, all benzyl-type protective groups were removed by catalytic hydrogenation under 6.0 kg cm^{-2} of H_2. The synthetic LTA-2, **2** (n=3), thus obtained could be effectively purified by gel-filtration on Sephadex LH-20 using H_2O–tetrahydrofuran (THF) as eluents. The structure of product **2** was confirmed by negative FAB-mass spectra where the M^- ion was observed at m/z 2139.2.

As described, we succeeded in the synthesis of the proposed fundamental structure of *E. hirae* LTA-2, **2** (n=3), and synthetically confirmed the structure of its glycolipid part. The results of biological tests of these LTAs and their glycolipid parts revealed, however, that neither antitumor activity nor induction of cytokines

such as TNF or IL-6 were observed in the synthetic compounds.[30] This may suggest that another structural component not involved in the synthetic LTAs is important for the biological activities or a chemical species different from LTA investigated by Fischer is responsible for the activities. Recent results of our study on natural *E. hirae* LTA indicated the latter possibility might be the case.[31] We are continuing our efforts to identify the chemical entity responsible for the biological activity described for LTA, and the details will be reported elsewhere.

Experimental

All melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were measured on JEOL JNM-GSX 270 or 400 spectrometers for $CDCl_3$ solutions unless otherwise noted. The chemical shifts are given in δ values with tetramethylsilane (TMS) on ^{1}H NMR and with $CDCl_3$ (77.02 ppm) on ^{13}C NMR, respectively, as the internal standards. FAB-MS spectra were obtained with a JEOL JMS-SX-102 mass spectrometer. Specific rotations were measured on a Perkin–Elmer 241 polarimeter. HPLC was carried out with a Shimadzu LC-6AD liquid chromatograph. Silica-gel column chromatography was carried out using Merck Kieselgel 60 (0.040—0.063 mm) at medium-pressure (2—4 kg cm^{-2}). Silica-gel TLC was carried out using Merck Kieselgel 60 F_{254}. Organic solutions were dried over $MgSO_4$ and evaporated in vacuo.

Allyl 3-*O*-Benzyl-2-*O*-(*p*-nitrobenzyl)-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α- and β-D-glucopyranoside (4). To a solution of allyl 3-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α- and β-D-glucopyranoside[12] (**3**) (16.0 g, 35.9 mmol) and pyridine (5.8 ml, 72 mmol) in CH_2Cl_2 (500 ml) was added dropwise a solution of 2,2,2-trichloroethyl chloroformate (TrocCl) (5.93 ml, 43.1 mmol) in CH_2Cl_2 (300 ml) at −20 °C over 3 h, and excess TrocCl was quenched with MeOH (50 ml). After the solution was concentrated, the residue was dissolved in AcOEt. The solution was washed with 1 M HCl (1 M=1 mol dm^{-3}), a saturated $NaHCO_3$ solution, and brine, and worked up as usual. The residue was crystallized from AcOEt–hexane to give colorless needles: Yield 18.6 g (83.2%); ^{1}H NMR (270 MHz) δ=8.15—6.99 (9H, m, aromatic H), 6.00—5.86 (1H, m, CH_2=C<u>H</u>–CH_2O), 5.39—5.24 (2H, m, C<u>H$_2$</u>=CH–CH_2O), 4.98—4.73 (6H, m, C_6H_5C<u>H$_2$</u>–, $NO_2C_6H_4C$<u>H$_2$</u>–, and Cl_3CC<u>H$_2$</u>OCO–), and 4.46—4.38 (2H, m, H-6).

Allyl 3,4-Di-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α- and β-D-glucopyranoside (5). To a solution of **4** (3.02 g, 4.86 mmol) in Et_2O (90 ml) were added benzyl 2,2,2-trichloroacetimidate (1.81 ml, 9.72 mmol) and TfOH (43 μl, 0.49 mmol), successively, under N_2 atmosphere. The mixture was stirred at room temperature for 12 h. To the mixture were added a saturated $NaHCO_3$ solution (20 ml) and Et_2O (100 ml). The organic layer was washed with a saturated $NaHCO_3$ solution and brine, and worked up as usual. The residue was purified by silica-gel column chromatography (180 g, toluene–AcOEt 40:1→10:1) three times to give colorless crystals: Yield 3.45 g (99.7%); mp 65—67 °C. Anal. ($C_{33}H_{34}O_{10}NCl_3$) C, H, N, Cl.

3,4-Di-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α- and β-D-glucopyranose (6). To a solution of **5** (6.67 g, 9.39 mmol) in THF (250 ml) was added $[Ir(cod)(PMe(C_6H_5)_2)_2]PF_6$ (cod: cyclooctadiene) (Ir-complex) (220 mg) under N_2 atmosphere. The N_2 in the system was replaced with H_2, and the solution was stirred at room temperature for 30 s. The system was evacuated until the color of the solution changed from yellow to light red, and then filled again with N_2, and the solution was stirred for 5 min at room temperature. To the solution were added H_2O (70 ml) and I_2 (4.77 g, 18.8 mmol), and the mixture was stirred for 10 min at room temperature. After excess I_2 was quenched with a 10% $Na_2S_2O_3$ solution (180 ml), the solution was concentrated and worked up as usual. The residue was purified by silica-gel column chromatography (180 g, toluene–AcOEt 7:1) to give **6** as a syrup: Yield 5.20 g (82.7%); $[\alpha]_D^{22}$ +44.9° (*c* 1.14, $CHCl_3$). Anal. ($C_{30}H_{30}O_{10}NCl_3$) C, H, N, Cl.

3,4-Di-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α- and β-D-glucopyranosyl Fluoride (7). To a solution of **6** (9.44 g, 14.1 mmol) in CH_2Cl_2 (400 ml) were added 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (FMPT) (7.97 g, 28.1 mmol) and Et_3N (7.90 ml, 53.5 mmol) at 0 °C. The mixture was stirred at room temperature for 12 h, then diluted with AcOEt, washed with a saturated $NaHCO_3$ solution and brine, and worked up as usual. The residue was passed through a short column of silica gel (20 g, toluene–AcOEt 20:1) to give **7** as a syrup: Yield 9.34 g (98.6%); ^{1}H NMR (270 MHz) δ=8.18—7.24 (14H, m, aromatic H), 5.64 (0.57H, dd, *J*=2.7 and 52.9 Hz, H-1(α)), 5.27 (0.43H, dd, *J*=6.7 and 52.4 Hz, H-1(β)), 4.93—4.60 (8H, m, C_6H_5C<u>H$_2$</u>–, $NO_2C_6H_4C$<u>H$_2$</u>–, and Cl_3CC<u>H$_2$</u>OCO–), 4.49—4.36 (2H, m, H-6), 4.08—4.00 (2H, m, H-5 and H-4), and 3.77—3.49 (2H, m, H-3 and H-2).

1,2-Di-*O*-allyl-3-*O*-[3,4-di-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α- and β-D-glucopyranosyl]-*sn*-glycerol (10). To a solution of **7** (1.39 g, 2.06 mmol) and 1,2-di-*O*-allyl-3-*O*-trimethylsilyl-*sn*-glycerol[12] (**9**) (904 mg, 3.09 mmol) in anhydrous Et_2O (15 ml) was added TMSOTf (388 μl, 2.06 mmol) dropwise at 0 °C. The mixture was stirred at 5 °C for 3 d. After addition of a saturated $NaHCO_3$ solution, the organic layer was washed with water and worked up as usual. The residue was purified by silica-gel column chromatography (80 g, toluene–AcOEt 20:1) to give an oily product (1.37 g, 80.6%) as a mixture of the α- and β-anomers (α:β=11.5:1): ^{1}H NMR (270 MHz) δ=8.16—7.24 (14H, m, aromatic H), 5.95—5.81 (2H, m, CH_2=C<u>H</u>–CH_2–O–), 5.30—5.10 (4H, m, C<u>H$_2$</u>=CH–CH_2–O–), 4.95 (0.92H, d, *J*=3.46 Hz, Glc H-1(α)), 4.93—4.60 (8H, m, C_6H_5C<u>H$_2$</u>–, $NO_2C_6H_4C$<u>H$_2$</u>–, and Cl_3CC<u>H$_2$</u>OCO–), 4.46 (0.08H, d, *J*=7.67 Hz, Glc H-1(β)), 4.40—4.39 (2H, d, *J*=3.22 Hz, Glc H-6), and 4.16—3.48 (9H, m, C<u>H$_2$</u>=CH–CH_2–O–, Glc H-2—5, and Gro H-1—3). Anal. ($C_{39}H_{44}O_{12}NCl_3$) C, H, N, Cl.

1,2-Di-*O*-allyl-3-*O*-[3,4-di-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α-D-glucopyranosyl]-*sn*-glycerol (13). A solution of **10** (1.37 g, 1.66 mmol) in 0.1 M MeONa (70 ml) was allowed to stand at 0 °C for 12 h and then neutralized with AcOH. After the mixture was concentrated, the residue was purified by silica-gel column chromatography (80 g, toluene–AcOEt 3:1) to give syrupy α-anomer **13** and the β-anomer.

13: 802 mg (74.2%); $[\alpha]_D^{27}$ +69.3° (*c* 1.56, $CHCl_3$);

^{1}H NMR (270 MHz) δ=8.16—7.23 (14H, m, aromatic H), 5.96—5.81 (2H, m, CH_2=C<u>H</u>–CH_2–O–), 5.30—5.10 (4H, m, C<u>H_2</u>=CH–CH_2–O–), 4.96 (1H, d, J=3.5 Hz, Glc H-1), 4.91—4.64 (6H, m, $C_6H_5C\underline{H_2}$– and $NO_2C_6H_4C\underline{H_2}$–), 4.21—3.48 (15H, m, C<u>H_2</u>=CH–CH_2–O–, Glc H-2—6, and Gro H-1—3), and 1.63 (1H, bs, OH). Anal. ($C_{36}H_{43}O_{10}N$), C, H, N.

The β-Anomer of 13: 70.0 mg (6.7%); ^{1}H NMR (270 MHz) δ=8.12—7.25 (14H, m, aromatic H), 5.93—5.79 (2H, m, CH_2=C<u>H</u>–CH_2O–), 5.29—5.11 (4H, m, C<u>H_2</u>=CH–CH_2–O–), 5.03—4.63 (6H, m, $C_6H_5C\underline{H_2}$– and $NO_2C_6H_4C\underline{H_2}$–), 4.68 (1H, d, J=7.92 Hz, Glc H-1), 4.13—3.34 (15H, m, C<u>H_2</u>=CH–CH_2–O–, Glc H-2—6, and Gro H-1—3), and 1.80 (1H, bs, OH).

1,2-Di-*O*-allyl-3-*O*-[3,4-di-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-6-*O*-(*p*-pivaloylaminobenzyl)-α-D-glucopyranosyl]-*sn*-glycerol (14). To a mixture of **13** (2.20 g, 3.40 mmol) and *p*-pivalolaminobenzyl 2,2,2-trichloroacetimidate (1.79 g, 5.10 mmol) in CH_2Cl_2 (20 ml) was added TfOH (90.0 μl) at −70 °C under N_2 atmosphere. The mixture was stirred at −70 °C for 2 h, neutralized with a saturated $NaHCO_3$ solution, diluted with Et_2O, and worked up as usual. The residue was purified by silica-gel column chromatography (180 g, hexane–AcOEt 4:1) three times to give a colorless syrup: Yield 2.24 g (78.6%); recovery of **13**: 220 mg (10%). **14**: $[\alpha]_D^{27}$ +55.3° (*c* 1.28, $CHCl_3$); ^{1}H NMR (270 MHz) δ=8.14—7.11 (19H, m, aromatic H and PivN<u>H</u>–), 5.95—5.80 (1H, m, CH_2=C<u>H</u>–CH_2O–), 5.30—5.09 (2H, m, C<u>H_2</u>=CH–CH_2O–), 4.97 (1H, d, J=3.5 Hz, Glc H-1), 4.85—4.39 (8H, m, $C_6H_5C\underline{H_2}$–, $NO_2C_6H_4C\underline{H_2}$–, and $PivNHC_6H_4C\underline{H_2}O$–), 4.16—3.47 (15H, m, C<u>H_2</u>=CH–CH_2–O–, Glc H-2—6, and Gro H-1—3), and 1.35 (9H, s, CH_3). Anal. ($C_{48}H_{58}O_{11}N_2$) C, H, N.

3-*O*-[3,4-Di-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-6-*O*-(*p*-pivaloylaminobenzyl)-α-D-glucopyranosyl]-*sn*-glycerol (16). The allyl groups of **14** (517 mg, 617 mmol) were isomerized to 1-propenyl groups with Ir-complex (17 mg) in THF (30 ml) as described for the preparation of **6**. The reaction mixture was concentrated, and a solution of the residue in 90% acetic acid (30 ml) was heated at 70 °C for 3 h. After concentration, the residue was purified by silica-gel column chromatography (25 g, benzene–AcOEt 1:4) to give **16** as a syrup: Yield 295 mg (63.0%); $[\alpha]_D^{27.5}$ +46.1° (*c* 1.05, $CHCl_3$); ^{1}H NMR (270 MHz) δ=8.18—7.14 (19H, m, aromatic H and PivN<u>H</u>–) and 1.31 (9H, s, CH_3). Anal. ($C_{42}H_{50}O_{11}N_2\cdot0.5H_2O$) C, H, N.

3-*O*-[3,4-Di-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-6-*O*-(*p*-pivaloylaminobenzyl)-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (17). To a solution of **16** (1.42 g, 1.87 mmol) in CH_2Cl_2 (50 ml) were added palmitoyl chloride (3.3 ml, 19.1 mmol), pyridine (3.2 ml, 40 mmol), and 4-dimethylaminopyridine (DMAP) (24 mg, 0.2 mmol) at room temperature. The mixture was stirred at room temperature for 2 h and then at 40 °C for 2 h. To the mixture were again added palmitoyl chloride (2.5 ml, 14.5 mmol) and pyridine (5.0 ml, 62.5 mmol), and the mixture was stirred at 40 °C for 3 h. After excess palmitoyl chloride was quenched with MeOH (20 ml), the solution was concentrated. The residue was dissolved in Et_2O (250 ml), and washed with 1 M HCl and brine. To the organic solution was added an ether solution of diazomethane. The solvent was evaporated, and the residue was purified by silica-gel column chromatography (70 g, toluene→toluene–AcOEt 4:1) to give an amorphous solid: Yield 2.19 g (94.8%); $[\alpha]_D^{23}$ +38.6° (*c* 0.420, $CHCl_3$); ^{1}H NMR (270 MHz) δ=8.18—7.12 (19H, m, aromatic H and PivN<u>H</u>–), 5.28—5.19 (1H, m, Gro H-2), 4.90 (1H, d, J=3.5 Hz, Glc H-1), 2.39—2.22 (4H, m, –COC<u>H_2</u>–), 1.69—1.51 (4H, m, $-COCH_2C\underline{H_2}$–), 1.37 (9H, s, Piv), 1.24 (24H, bs, CH_2), and 0.88 (6H, t, J=7.09 Hz, CH_3).

3-*O*-[2-*O*-(*p*-Aminobenzyl)-3,4-di-*O*-benzyl-6-*O*-(*p*-pivaloylaminobenzyl)-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (18). To a solution of **17** (2.19 g, 1.77 mmol) in THF (67 ml) were added Zn–Cu (2.0 g) and acetylacetone (10 ml). The mixture was stirred for 15 min at room temperature, and the insoluble materials were removed by filtration. The filtrate was concentrated, and excess acetylacetone was removed by lyophilization from dioxane. The residue was purified by silica-gel column chromatography (70 g, benzene–AcOEt 4:1) to give **18** as a colorless syrup: Yield 2.13 g (quantitative).

3-*O*-[3,4-Di-*O*-benzyl-6-*O*-(*p*-pivaloylaminobenzyl)-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (19). A solution of **18** (2.13 g, 1.87 mmol) and $HClO_4$ (440 μl, 5.08 mmol) in the mixture of 2-methyl-2-propanol (80 ml) and water (20 ml) was placed in an undivided cell equipped with Pt electrodes (each 3 cm^2). The mixture was electrolyzed at room temperature under a constant current of 13 mA cm^{-2} until 25.2 F mol^{-1} of electricity was passed. The solution was neutralized with a saturated $NaHCO_3$ solution (20 ml) and diluted with AcOEt (100 ml). The organic layer was washed with a saturated $NaHCO_3$ solution and brine and worked up as usual. The residue was purified by silica-gel column chromatography (70 g, benzene–AcOEt 4:1) to give an amorphous solid: Yield 1.40 g (71.8%); recovery of **18**: 320 mg (15%).

19: $[\alpha]_D^{27.5}$ +50.5° (*c* 1.00, $CHCl_3$); ^{1}H NMR (270 MHz) δ=7.48—7.11 (15H, m, aromatic H and PivN<u>H</u>–), 5.29—5.20 (1H, m, Gro H-2), 2.37—2.24 (4H, m, –COC<u>H_2</u>–), 2.10 (1H, bs, OH), 1.62—1.53 (4H, m, $-COCH_2C\underline{H_2}$–), 1.31 (9H, s, Piv), 1.25 (24H, bs, CH_2), and 0.88 (6H, t, J=7.1 Hz, CH_3). Anal. ($C_{67}H_{105}O_{11}N$) C, H, N.

3-*O*-[3,4-Di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-6-*O*-(*p*-pivaloylaminobenzyl)-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (22). To a mixture of **19** (386 mg, 351 μmol), dichlorobis (η^5-cyclopentadienyl)zirconium [$ZrCl_2(Cp)_2$] (113 mg, 386 μmol), $AgClO_4$ (160 mg, 772 μmol), and molecular sieves 4A (100 mg) was added a solution of fluoride **20** (210 mg, 386 μmol) in toluene (20 ml) at −10 °C. The mixture was stirred at the same temperature for 15 min, neutralized with a saturated $NaHCO_3$ solution, and diluted with AcOEt. After removal of the insoluble materials by filtration, the organic layer was washed with a saturated $NaHCO_3$ solution and brine and worked up as usual. The residue was purified by silica-gel column chromatography (40 g, toluene–AcOEt 6:1) to give α-anomer **22** and the β-anomer as syrups.

22: 221 mg (38.8%); $[\alpha]_D^{22}$ +61.6° (*c* 1.13, $CHCl_3$); ^{1}H NMR (400 MHz) δ=7.47—7.03 (35H, m, aromatic H and PivN<u>H</u>–), 5.18—5.12 (1H, m, Gro H-2), 5.00 (1H, d, J=3.5 Hz, Glc H-1′), 4.98 (1H, d, J=3.3 Hz, Glc H-1), 4.95—4.39 (14H, m, $C_6H_5C\underline{H_2}$–), 4.31 (1H, dd, J=12.1 and 3.3 Hz, Gro H-1), 4.16 (1H, dd, J=12.1 and 6.2 Hz, Gro H-1), 4.03 (Glc H-3′), 3.95 (Glc H-3), 3.94 (Glc H-5′), 3.76 (Glc H-2), 3.74 (Gro H-3), 3.68 (Glc H-4′), 3.60 (Glc H-6′), 3.58 (Glc H-6′),

3.58 (Glc H-2′), 3.56 (Gro H-3), 3.42 (Glc H-4), 2.28—2.20 (4H, m, $-COCH_2-$), 1.60—1.52 (4H, m, $-COCH_2CH_2-$), 1.31 (9H, s, Piv), 1.25 (24H, bs, CH_2), and 0.88 (6H, t, J=6.28 Hz, CH_3). Anal. ($C_{101}H_{139}O_{16}N\cdot0.5H_2O$) C, H, N.

The β-Anomer of 22: 100 mg (17.5%); ^{1}H NMR (270 MHz) δ=7.37—7.09 (35H, m, aromatic H and PivNH–), 5.27—5.23 (1H, m, Gro H-2), 5.01 (1H, d, J=3.46 Hz, Glc H-1), 4.51 (1H, d, J=7.42 Hz, Glc H-1′), 2.30—2.16 (4H, m, $-COCH_2-$), 1.56—1.52 (4H, m, $-COCH_2CH_2-$), 1.31 (9H, s, Piv), 1.24 (24H, bs, CH_2), and 0.88 (6H, t, J=6.28 Hz, CH_3).

3-*O*-[3,4-Di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (24). To a solution of **22** (184 mg, 114 μmol) in CH_2Cl_2 (5.3 ml) were added water (0.3 ml) and DDQ (27.1 mg, 119 μmol) at 0°C. The mixture was stirred at 0 °C for 4 h, and then another portion of DDQ (13.5 mg, 59.5 μmol) was added. After the solution was stirred at the same temperature for 12 h, excess DDQ was quenched with a solution of ascorbic acid (70 mg), citric acid (126 mg), and NaOH (92 mg) in water (10 ml). The mixture was diluted with AcOEt, washed with a saturated $NaHCO_3$ solution and brine, and worked up as usual. The residue was purified twice by silica-gel column chromatography (23 g, $CHCl_3$–acetone 50:1) to give an oil: Yield 137 mg (83.8%); $[\alpha]_D^{22}$ +55.7° (c 1.22, $CHCl_3$); ^{1}H NMR (270 MHz) δ=7.39—7.04 (30H, m, aromatic H), 5.18—5.12 (1H, m, Gro H-2), 5.05 (1H, d, J=3.5 Hz, Glc H-1′), 4.98 (1H, d, J=3.5 Hz, Glc H-1), 4.92—4.42 (12H, m, $C_6H_5CH_2-$), 4.31 (1H, dd, J=12.1 and 3.3 Hz, Gro H-1), 4.16 (1H, dd, J=12.1 and 6.2 Hz, Gro H-1), 2.34—2.20 (4H, m, $-COCH_2-$), 1.62—1.52 (4H, m, $-COCH_2CH_2-$), 1.28—1.20 (24H, m, CH_2), and 0.88 (6H, t, J=6.8 Hz, CH_3). Anal. ($C_{89}H_{124}O_{15}\cdot H_2O$) C, H.

3-*O*-[3,4-Di-*O*-benzyl-2-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α-D-glucopyranosyl]-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (25). The coupling reaction of **19** (275 mg, 250 μmol) with 6-*O*-Troc fluoride **21**[12] (236 mg, 375 μmol) was carried out by using $[ZrCl_2(Cp)_2]$ (95.0 mg, 325 μmol), $AgClO_4$ (135 mg, 650 μmol), and molecular sieves 4A (100 mg) in toluene (6 ml) at −5 °C for 10 min as described for the preparation of **22**. To the solution of the crude product in CH_2Cl_2 (8 ml) were added water (0.5 ml) and DDQ (85.0 mg, 375 μmol) at 0 °C, and the mixture was stirred at 5 °C for 24 h. Excess DDQ was quenched with a solution of ascorbic acid (70 mg), citric aicd (126 mg), and NaOH (92 mg) in water (10 ml). The mixture was worked up as usual, and the residue was purified by silica-gel column chromatography (40 g, hexane–AcOEt 2:1) to give α-anomer **25** and the corresponding β-anomer.

25: 213 mg (56.3%); $[\alpha]_D^{23}$ +65.0° (c 0.686, $CHCl_3$); ^{1}H NMR (270 MHz) δ=7.33—7.09 (25H, m, aromatic H and PivNH–), 5.16—5.12 (1H, m, Gro H-2), 4.96 (1H, d, J=3.0 Hz, Glc H-1′), 4.92 (1H, d, J=3.2 Hz, Glc H-1), 2.31—2.21 (4H, m, $-COCH_2-$), 1.68—1.55 (4H, m, $-COCH_2CH_2-$), 1.25 (24H, bs, CH_2), and 0.88 (6H, t, J=6.3 Hz, CH_3). Anal. ($C_{85}H_{119}O_{17}Cl_3$) C, H.

The β-Anomer of 25: 29.0 mg (7.7%); ^{1}H NMR (270 MHz) δ=7.50—7.11 (30H, m, aromatic H and PivNH–), 5.35—5.27 (1H, m, Gro H-2), 4.58 (2H, d, J=8.9 Hz, Glc H-6′), 2.34—2.20 (4H, m, $-COCH_2-$), 1.56 (4H, m, $-COCH_2CH_2-$), 1.25 (24H, bs, CH_2), and 0.88 (6H, t, J=6.43 Hz, CH_3).

Benzyloxybis(diisopropylamino)phosphine (28).[27] To a solution of dichloro(diisopropylamino)phosphine (**26**) (20.0 g, 99.0 mmol) in dry hexane (200 ml) was added dropwise diisopropylamine (27.7 ml, 198 mmol) under N_2 atmosphere. The misture was refluxed for 3 d under N_2 atmosphere. After the mixture was cooled, Et_3N (13.8 ml, 99.0 mmol) and benzyl alcohol (10.2 ml, 99.0 mmol) were added successively to the mixture at 0 °C. The mixture was stirred at room temperature for 3 h, filtered, and concentrated to give **28** as an oil.

Benzyloxo(diisopropylamido)[1,2-di-*O*-palmitoyl-*sn*-glycero(3)]phosphorus (31). To a solution of 1,2-di-*O*-palmitoyl-*sn*-glycerol (**30**) (279 mg, 491 μmol) in dry $ClCH_2CH_2Cl$ was added benzyloxybis(diisopropylamino)-phophine (**28**) (249 mg, 737 μmol) and 1*H*-tetrazole (27.5 mg, 393 μmol) under N_2 atmosphere. After the solution was stirred at room temperature for 1 h, CH_2Cl_2 and a saturated $NaHCO_3$ solution were added. The organic layer was then washed with a saturated $NaHCO_3$ solution and worked up as usual. The residue was dissolved in hexane (40 ml) and Et_3N (1 ml). This solution was washed with CH_3CN (10 ml×25) and evaporated. The product was purified by silica-gel column chromatography (40 g, hexane–Et_3N=40:1) to give a syrup: Yield 318 mg (80.3%); ^{1}H NMR (270 MHz) δ=7.34—7.27 (5H, m, aromatic H), 5.23—5.14 (1H, m, H-2), 4.78—4.58 (2H, m, $C_6H_5CH_2$), 4.38—4.12 (2H, m, H-1), 3.83—3.56 (4H, m, H-3 and –N–CH–), 2.31—2.25 (2H, m, $-COCH_2-$), 1.62—1.42 (2H, m, $-COCH_2CH_2-$), 1.25 (24H, m, CH_2), and 0.88 (6H, t, J=6.9 Hz, CH_3).

3-*O*-[2-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-6-[1,2-di-*O*-palmitoyl-*sn*-glycero(3)-benzylphospho]-3,4-di-*O*-benzyl-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (32). To a solution of disaccharide **24** (107 mg, 74.6 μmol) and phosphoramidite **31** (168 mg, 210 μmol) in CH_2Cl_2 (6 ml) was added 1*H*-tetrazole (16.8 mg, 238 μmol) in CH_3CN (2 ml) under N_2 atmosphere. The mixture was stirred at room temperature for 5.5 h. To the mixture were added **31** (126 mg, 156 μmol) in CH_2Cl_2 (3 ml) and 1*H*-tetrazole (11.6 mg, 166 μmol) in CH_3CN (1 ml). After the mixture was stirred for an additional hour, 80% mCPBA (51.4 mg, 238 μmol) was added. This mixture was stirred for 30 min, diluted with CH_2Cl_2, washed with an aqueous $NaHCO_3$ solution and brine, and worked up as usual. The residue was purified by silica-gel column chromatography (40 g, toluene–AcOEt 8:1) and then by column chromatography on Sephadex LH-20 ($CHCl_3$–MeOH 1:1). The fractions containing **32** were concentrated to give a colorless syrup: Yield 139 mg (86.5%); $[\alpha]_D^{23}$ +44.0° (c 1.10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.4—7.05 (35H, m, $C_6H_5CH_2$×7), 5.18 (1H, m, Gro(b) H-2), 5.13 (1H, m, Gro(a) H-2), 5.06 (2H, m, $C_6H_5CH_2$–OP), 5.01 (0.5H, d, J=3.5 Hz, Glc(b) H-1), 4.99 (0.5H, d, J=3.5 Hz, Glc(b) H-1), 4.94 (1H, d, J=3.6 Hz, Glc(a) H-1), 4.98—4.24 (12H, m, $C_6H_5CH_2$×6), 4.34—4.25 (2H, Gro(b) H-1, 3), 4.29 (1H, Gro(a) H-1), 4.29—4.15 (2H, Glc(a), H-6, 6′), 4.15 (1H, Gro(a) H-1′), 4.12 (1H, Gro(b) H-3′), 4.11 (1H, Gro(b) H-1′), 4.02 (1H, dd, J=9.3 and 9.3 Hz, Glc(b) H-3), 3.96 (1H, Glc(a) H-3), 3.95 (1H, Glc(b) H-5), 3.77 (1H, Glc(a) H-5), 3.70 (1H, Glc(a) H-2), 3.69 (1H, Gro(a) H-3), 3.68 (1H, Glc(b) H-4), 3.58 (1H, dd, J=3.5 and 9.7 Hz, Glc(b) H-2), 3.52 (1H, Gro-

(a) H-3′), 3.50 (1H, Glc(a) H-4), 3.48 (1H, m, Glc(b) H-6), 3.38 (1H, m, Glc(b) H-6′), 2.3—2.19 (8H, m, $OCOC\underline{H}_2\times4$), 1.6—1.45 (8H, m, $OCOCH_2C\underline{H}_2\times4$), 1.35—1.15 (96H, m, $OCOCH_2CH_2(C\underline{H}_2)_{12}CH_3\times4$), and 0.88 (12H, t, J=6.9 Hz, $CH_3\times4$); ^{13}C NMR (100.4 MHz) δ=96.53 (Glc(a) C-1), 95.54 (Glc(b) C-1), 95.54 (Glc(b) C-1), 82.09 (Glc(b) C-3), 80.26 (Glc(a) C-3), 79.25 (Glc(b) C-2), 77.60 (Glc(b) C-4), 77.22 (Glc(a) C-4), 76.03 (Glc(a) C-2), 75.90, 75.56, 75.08, 74.85, 73.34, 72.96 ($C_6H_5\underline{C}H_2CH_2O\times6$), 70.61 (Glc(b) C-5), 69.87 ($^3J_{PC}$=8.8 Hz, Glc(a) C-5), 69.83 (Gro(a) C-2), 69.52 ($^2J_{PC}$=5.9 Hz, $C_6H_5\underline{C}H_2$–OP), 69.34 ($^3J_{PC}$=7.4 Hz, Gro(b) C-2), 68.09 (Glc(b), C-6), 66.63 (Glc(a), C-3), 66.37 (m, Glc(a) C-6), 65.42 (m, Gro(b), C-3), 62.66 (Gro(a) C-1), and 61.70 (Gro(b) C-1); where Glc(a) and Glc(b) represent the reducing side and distal glucose residues, respectively; Gro(a) stands for the glycosyl glycerol residue and Gro(b) for the glycerol residue of the phosphatidyl moiety. Anal. ($C_{131}H_{197}O_{22}P$) C, H.

3-*O*-[2-*O*-[2,3,4-Tri-*O*-benzyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α-D-glucopyranosyl]-6-[1,2-di-*O*-palmitoyl-*sn*-glycero(3)-benzylphospho]-3,4-di-*O*-benzyl-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (33). Disaccharide **25** (270 mg, 178 μmol) was treated with phosphoramidite **31** (362 mg, 448 μmol) and 1*H*-tetrazole (37.6 mg, 529 μmol) in CH_2Cl_2 (5 ml) and CH_3CN (2 ml) as described for the preparation of **32**. The product was purified by repeated column chromatography on silica gel (35 g, hexane–AcOEt=4:1 and benzene–AcOEt=10:1) to give a colorless syrup: Yield 283 mg (71.1%). $[\alpha]_D^{22}$ +55° (*c* 0.142, $CHCl_3$). Anal. ($C_{127}H_{192}O_{24}Cl_3P\cdot H_2O$) C, H.

3-*O*-[2-*O*-(2,3,4-Tri-*O*-benzyl-α-D-glucopyranosyl)-6-[1,2-di-*O*-palmitoyl-*sn*-glycero(3)-benzylphospho]-3,4-di-*O*-benzyl-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (34). To a solution of **33** (253 mg, 113 μmol) in acetic acid (6.5 ml) was added Zn powder (500 mg, 7.65 mmol), and the mixture was stirred at room temperature for 20 min. AcOEt (50 ml) was added to the mixture, and insoluble materials were filtered off. The filtrate was washed with water, a saturated $NaHCO_3$ solution, and brine, and worked up as usual to give a syrup: Yield 228 mg (97.9%): $[\alpha]_D^{24}$ +46.0° (*c* 1.21, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ=7.4—7.05 (30H, m, $C_6\underline{H}_5CH_2\times6$), 5.25—5.1 (2H, m, Gro H−2×2), 5.06 (2H, m, $C_6H_5C\underline{H}_2$–OP), 5.05—4.4 (12H, $C_6H_5C\underline{H}_2\times5$ and Glc H−1×2), 4.4—3.82 (11H, Gro(a) H-1, 1′, Gro(b) H-1, 1′, 3, 3′, Glc(a) H-6, 6′, and Glc H×3), 3.82—3.40 (8H, Gro(a) H-3, 3′, Glc(b) H-6, 6′, Glc H×3), 2.4—2.2 (8H, m, $OCOC\underline{H}_2\times4$), 1.8—1.4 (8H, m, $OCOCH_2C\underline{H}_2\times4$), 1.4—1.0 (96H, m, $OCOCH_2CH_2(C\underline{H}_2)_{12}CH_3\times4$), and 0.88 (12H, t, J=6.7 Hz, $CH_3\times4$). Anal. ($C_{124}H_{191}O_{22}P\cdot H_2O$) C, H.

3-*O*-[2-*O*-(α-D-Glucopyranosyl)-6-[1,2-di-*O*-palmitoyl-*sn*-glycero(3)-phospho]-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (35). To a solution of perbenzylated glycolipid **32** (76.6 mg, 35.5 μmol) in THF (6 ml) was added Pd black (126 mg). The mixture was stirred under 6 kg cm^{-2} of H_2 at room temperature for 4 h. The catalyst was filtered off and the filtrate was neutralized with triethylammonium hydrogenecarbonate (1 M in water). The product was purified by column chromatographies on silica gel (6 g, $CHCl_3$–MeOH=4:1→1:1) and then on Sephadex LH-20 (12 mm×25 cm, $CHCl_3$–MeOH=1:1) to give a colorless solid: Yield 25.5 mg (47.0%). A part of the product was desalted to obtain a sample for elemental analysis as follows. The product (3.1 mg) was dissolved in $CHCl_3$–MeOH (1 ml, 4:1). The solution was washed with 0.01 M hydrochloric acid (1 ml) and water (1 ml). The organic layer was evaporated, and the residue was lyophilized from water to give a white powder: Yield 2.8 mg (87.0%); $[\alpha]_D^{27}$ +43° (*c* 0.072, $CHCl_3$). FAB-MS (negative). Found: *m/z* 1522.8. Calcd for $C_{82}H_{155}O_{22}P$: M, 1523.1. Anal. ($C_{82}H_{155}O_{22}P\cdot5H_2O$) C, H.

The Methyl Ester of Free Glycolipid 35. To a solution of desalted **35** (9 mg, 6 μmol) in $CHCl_3$ (10 ml) was added CH_2N_2 in ether. After evaporation of the solvent, the residue was purified by silica-gel column chromatography (800 mg, $CHCl_3$–MeOH=14:1) to give methyl ester **37**: Yield 6 mg (70%); ^{1}H NMR (400 MHz, $CDCl_3$–CD_3OD=5:1) δ=5.26 (1H, m, Gro(b) H-2), 5.23 (1H, m, Gro(a) H-2), 4.99 (1H, d, J=3.5 Hz, Glc(a) H-1), 4.95 (1H, d, J=3.9 Hz, Glc(b) H-1), 4.43 (1H, dd, J=3.5 and 12.1 Hz, Gro(a) H-1), 4.40—4.34 (2H, m, Gro(b) H-1, 3), 4.27—4.22 (2H, m, Glc(a) H-6, 6′), 4.21 (1H, Gro(a) H-1′), 4.20—4.14 (2H, m, Gro(b) H-1′, 3′), 3.88 (1H, m, Glc(b) H-5), 3.85 (1H, Glc(b) H-6), 3.83 (1H, dd, J=5 and 11 Hz, Gro(a) H-3), 3.800 (1.5H, d, $^3J_{PH}$=11.2 Hz, $OCH_3\times1/2$), 3.796 (1.5H, d, $^3J_{PH}$=11.2 Hz, $OCH_3\times1/2$), 3.79 (1H, dd, J=9 and 10 Hz, Glc(a) H-3), 3.71 (1H, Glc(b) H-6′), 3.70 (1H, m, Glc(a) H-5), 3.70 (1H, Glc(b) H-3), 3.66 (1H, dd, J=5 and 11 Hz, Gro(a) H-3′), 3.59 (1H, dd, J=3.5 and 9.7 Hz, Glc(a) H-2), 3.44 (1H, dd, J=3.9 and 9.7 Hz, Glc(b) H-2), 3.41 (1H, m, Glc(a) H-4), 3.34 (1H, dd, J=9 and 10 Hz, Glc(b) H-4), 2.4—2.3 (8H, m, $OCOC\underline{H}_2\times4$), 1.7—1.5 (8H, m, $OCOCH_2C\underline{H}_2\times4$), 1.4—1.2 (96H, m, $OCOCH_2CH_2(C\underline{H}_2)_{12}CH_3\times4$), and 0.89 (12H, t, J=6.9 Hz, $CH_3\times4$); ^{13}C NMR (100.4 MHz) δ=173.82 (CO), 173.41 (CO), 173.29 (CO), 172.94 (CO), 96.69 (Glc(b) C-1), 96.45 (Glc(a) C-1), 76.45 (Glc(a) C-2), 73.46 (Glc(b) C-3), 72.01 (Glc(b) C-5), 71.75 (Glc(b), C-2), 71.49 (Glc(a) C-3), 70.22 (Glc(a) C-5), 70.13 (Glc(b) C-4), 69.61 (Gro(b) C-2), 69.21 (Gro(a) C-2), 69.05 (Glc(a) C-4), 66.69 (m, Gro(b), C-3), 65.83 (Gro(a), C-3), 65.36 (Glc(a) C-6), 62.45 (Gro(a), C-1), 61.49 (Gro(b) C-1, Glc(b) C-6), 54.32 (m, OMe), 33.91 ($OCO\underline{C}H_2$), 33.80 ($OCO\underline{C}H_2$), 33.69 (acyl CH_2), 31.56 (acyl CH_2), 31.39 (acyl CH_2), 29.85 (acyl CH_2), 29.32 (acyl CH_2), 29.16 (acyl CH_2), 28.99 (acyl CH_2), 28.78 (acyl CH_2), 24.59 ($OCOCH_2\underline{C}H_2$), 24.52 ($OCOCH_2\underline{C}H_2$), 22.29 (acyl CH_2), and 13.55 (CH_3).

Isolation and Methyl Esterification of Natural Glycolipid from Cells of *E. hirae*. A suspension of wet cells of *E. hirae* ATCC 9790 (400 g) in a mixture of $CHCl_3$ (500 ml), MeOH (1 dm^3), and aqueous CH_3COONa buffer (pH 4.7) (500 ml) was stirred at 60—70 °C overnight. The organic layer from a total 2.2 kg of wet cells was evaporated to give a mixture of membrane lipids (17.0 g). A part of the mixture (10.8 g) was then subjected to silica-gel column chromatography (180 g, $CHCl_3$–MeOH=4:1). The fractions containing the glycolipid identified with synthetic specimen **35** on silica-gel TLC ($CHCl_3$–acetone–MeOH–AcOH–H_2O=50:20:10:10:0.5, R_f=0.4) was concentrated to give a syrup (3.99 g). A part of this fraction (310 mg) was applied to repeated column chromatography on Sephadex LH-20 (2.6×114 cm, $CHCl_3$–MeOH=1:1) four times to give pure natural glycolipid **36**: Yield 11 mg. Methyl esterification of **36** (9 mg) was carried out in the same manner as described

in the preparation of **37** to give methyl ester **38**: Yield 5 mg (60%); ^{1}H NMR (400 MHz, $CDCl_3$–CD_3OD=5:1) δ=5.38—5.32 (4H, m, acyl –CH=CH–), 2.1—1.9 (8H, m, CH_2–CH=CH–CH_2), 1.40—1.20 (84H, m, acyl CH_2), 0.892 (6H, t, J=6.8 Hz, $CH_3\times2$), and 0.888 (6H, t, J=6.8 Hz, $CH_3\times2$). All the other proton signals were identical to those of synthetic methyl ester **37**.

3-*O*-[2-*O*-[6-[3-[3-[3-[2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)-benzylphospho]-2-*O*-benzyl-*sn*-glycero(1)-benzylphospho]-2-*O*-benzyl-*sn*-glycero(1)-benzylphospho]-2-*O*-benzyl-*sn*-glycero(1)-benzylphospho]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-6-[1,2-di-*O*-palmitoyl-*sn*-glycero(3)-benzylphospho]-3,4-di-*O*-benzyl-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (41). To a mixture of **34** (38 mg, 18.4 μmol) and 1*H*-tetrazole (2.2 mg, 31.4 μmol) was added a solution of 3-[3-[3-[3-[2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)-benzylphospho]-2-*O*-benzyl-*sn*-glycero(1)-benzylphospho]-2-*O*-benzyl-*sn*-glycero(1)-benzylphospho]-2-*O*-benzyl-*sn*-glycero(1)-benzyloxo(diisopropylamido)phosphorus[12] (**39**) (45 mg, 29.2 μmol) in $ClCH_2CH_2Cl$ (1 ml) and CH_3CN (0.5 ml) under N_2 atmosphere. The mixture was stirred at room temperature for 90 min. To the mixture was added a solution of **39** (45 mg, 29.2 μmol) in $ClCH_2CH_2Cl$ (1 ml), and the mixture was stirred at the same temperature for 16 h. To the mixture was added 80% mCPBA (10 mg, 58.4 μmol) at 0 °C and the mixture was stirred for 10 min, diluted with AcOEt (30 ml), washed with an aqueous $NaHCO_3$ solution and brine, and then worked up as usual. The residue was purified by silica-gel column chromatography (8 g, benzene–AcOEt=3:1→1:1→1:2) to give **41** as a syrup: Yield 17.3 mg (26.7%); recovery of **34**: 25 mg (66%); ^{1}H NMR (270 MHz, $CDCl_3$) δ= 7.38—7.0 (72H, m, $C_6H_5CH_2\times14$ and $CH_3OC_6H_4CH_2$ *o*-protons), 6.83 (2H, m, $CH_3OC_6H_4CH_2$ *m*-protons), 5.18 (1H, m, Gro of the glycolipid part H-2), 5.08 (1H, m, Gro of the glycolipid part H-2), 5.06 (2H, m, $C_6H_5CH_2$–OP), 5.0—4.38 (30H, $C_6H_5CH_2\times13$, $CH_3OC_6H_4CH_2$, and Glc H−1×2), 4.36—3.82 (27H, Gro –CH_2–OP×8, Gro –CH_2–O–acyl×2, Glc H-6, 6′×2, and Glc H×3), 3.82—3.38 (13H, MPMOCH_2, OCH_2CH(OBzl)$CH_2O\times4$, Gro(a) H-3, 3′, Glc H×5), 3.76 (3H, s, CH_3O), 2.3—2.15 (8H, m, $OCOCH_2\times4$), 1.7—1.4 (8H, m, $OCOCH_2CH_2\times4$), 1.4—1.1 (96H, m, $OCOCH_2CH_2(CH_2)_{12}CH_3\times4$), and 0.88 (12H, t, J=6.7 Hz, $CH_3\times4$).

3-*O*-[2-*O*-[6-[3-[3-[3-[*sn*-Glycero(1)-phospho]-*sn*-glycero(1)-phospho]-*sn*-glycero(1)-phospho]-*sn*-glycero(1)-phospho]-α-D-glucopyranosyl]-6-[1,2-di-*O*-palmitoyl-*sn*-glycero(3)-phospho]-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (2). Catalytic hydrogenation of compound **41** (12.0 mg, 3.41 μmol) was carried out with Pd black (30 mg) under H_2 (6 kg cm^{-2}) in THF (2 ml) as described for the preparation of **35**. The product was purified by column chromatography on Sephadex LH-20 (5 mm×10 cm, $CHCl_3$–MeOH 1:1) to give a colorless solid: Yield 7.1 mg (93%). TLC ($CHCl_3$–MeOH–M_2O–Et_3N= 50:25:4:10, Merck silica gel 60 F_{254}, Art.5554): R_f 0.15. FAB-MS (negative). Found: m/z 2139.2. Calcd for $C_{94}H_{183}O_{42}P_5$: M, 2139.1.

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26) Since the PAB group of disaccharide **23** was partly cleaved by the Friedel–Crafts reaction with toluene in the glycosidation step, the PAB group was removed by DDQ without isolation of **23**. The structure of α-anomer **25** was confirmed by the presence of α-anomeric proton signals at 4.96 ppm (J_{12}=3.0 Hz) and 4.92 ppm (J_{12}=3.2 Hz) in 270 MHz ^{1}H NMR. Another product having a disaccharide structure was assumed to be the β-anomer, although the anomeric proton signals were not identified by 270 MHz ^{1}H NMR.

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