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Synthetic Study on Lipoteichoic Acid of Gram Positive Bacteria. I. Synthesis of Proposed Fundamental Structure of *Streptococcus pyogenes* Lipoteichoic Acid[1)]

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Synthetic study on *Streptococcus pyogenes* lipoteichoic acid (LTA) which was reported to induce nontoxic TNF is described. The proposed fundamental structure of LTA was constructed by coupling of the glycolipid part with the poly(glycerol phosphate) (PGP) part and subsequent deprotection. The PGP part was prepared by a simple repeating procedure using the phosphoramidite method for the construction of 1,3-phosphodiester-linkage. The glycolipid part was synthesized using appropriate glycosyl fluorides as donors, where the *p*-nitrobenzyl group was successfully applied to temporary protection of a hydroxyl function.

Lipoteichoic acid (LTA) is a characteristic and widespread cell-surface constituent of gram-positive bacteria. It is an amphiphile consisting of covalently bound two distinct parts, i.e., a glycolipid and a hydrophilic poly(glycerol phosphate) (PGP). LTA exists with lipid part being anchored by hydrophobic interaction in the lipid bilayer of cell membrane. Considerable species specific structural variations of the glycolipid part have been described for LTA.[2)]

Recently, immunostimulating and antitumor activities were found for LTAs isolated from several bacterial species.[3–7)] Usami et al. described that the potent antitumor activity of *Streptococcus pyogenes* LTA is attributed to low-toxic tumor necrosis factor (TNF) induced.[3,4)] They also described that the LTA whose PGP part was partially hydrolyzed (1 M HCl, 95 °C, 20 min ,1 M=1 mol dm^{-3}) still showed TNF-inducing activity, but the alkali-treated (0.1 M KOH, 37 °C, 1 h) LTA lacking acyl moieties was devoid of the ability.[4)] These results suggest that the whole molecule of LTA is not necessary for TNF induction, but the glycolipid part including acyl groups is essential. The chemical structure of *S. pyogenes* LTA was proposed by Fischer et al. as shown in Fig. 1,[8)] although the detail of the structural determination had not been published. We thus started synthetic study on LTA for the confirmation of the proposed structure and the elucidation of the chemical entity responsible for its interesting activity.

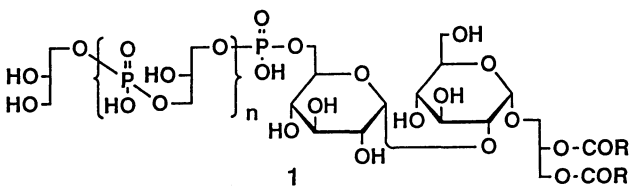

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Fig. 1. The proposed structure of *Streptococcus pyogenes* LTA.

Results and Discussion

For the synthesis of LTA, we employed a strategy to complete the desired LTA structure in the protected form by coupling of the glycolipid and PGP parts which had been constructed separately. After extensive purification, deprotection of all benzyl-type persistent protective groups by catalytic hydrogenolysis afforded the final product in one step. This strategy seemed to be generally recommended for the synthesis of amphiphilic compounds whose final purification is often difficult and tedious owing to their physical properties. In fact, lipid A and its derivatives were successfully synthesized according to the same principle in our previous studies.[9)]

The structure of the glycolipid part consists of D-glucose $\alpha(1\rightarrow2)$ disaccharide (kojibiose) linked to 1,2-diacyl-*sn*-glycerol through an α-glycosidic bond as shown in Fig. 1. For the present synthesis of the glycolipid part, we used palmitic acid as the sole acyl moiety although palmitic and oleic acids are proved to be the main component of fatty acids in the natural product. As for the synthetic route of this glycolipid, there exsit several possibilities concerning how to link the *O*-diacylglycerol and two glucose units. In the present study, the glycolipid was prepared by stepwise coupling of glucose units to the glycerol derivative. Glycosyl fluorides were used as donors for the formation of α-glucosidic linkages.

The hydrophilic poly(glycerol phosphate) (PGP) part is composed of a polymeric chain of 1,3-phosphodiester-linked *sn*-glycerols in which the 2-hydroxyl groups are partially esterified with D-alanine or linked to D-glucose by glycosidic bond. The average chain length of the repeating units is proposed to be about n=20. Since the contents of D-alanine or D-glucose linked to glycerol vary depending on the strains or growth conditions, and since alanine-free LTA still showed the activity,[6,7)] we chose the PGP part containing no substituents as the

first target of the synthesis. In the present study, we used glycerol phosphate tetramer **23** in which four glycerol units are linked by (1→3) phosphoric ester bonds.

Preparation of the PGP part. The PGP part was prepared by a simple repeating procedure. For the formation of phosphodiester, we applied the phosphoramidite procedure which has been frequently used in the nucleotide synthesis.[10] The benzyl group was used for the persistent protection of the phosphoric acid and the 2-hydroxyl group of glycerol according to the principle described above.

Thus, two optically pure 2-*O*-benzyl glycerols **6** and **9** protected with *p*-methoxybenzyl (MPM) group at 3-position and *t*-butyldimethylsilyl (TBDMS) group at 1-position, respectively, were prepared via two routes (Fig. 2). We first synthesized MPM ether **6** by reductive cleavage of a benzylidene derivative **5**, which was prepared from 1,2-*O*-isopropylidene-*sn*-glycerol (**2**). Desired isomer **6** was obtained preferentially (**6** : **7**=11 : 1)[11] by use of $Zn(BH_4)_2$ and trimethylsilyl chloride,[12] though the yield was unsatisfactory because of the partial cleavage of MPM group during the reduction. Compound **6** and its regioisomer **7** could not be separated completely by silica-gel column chromatography; even after repeated chromatography, **6** contained ca 2% of **7** as determined by ^{1}H NMR spectrum. Selective *t*-butyldimethylsilylation at the primary hydroxyl group was then carried out with partially purified **6** to give **8** in 97% yield without any contamination of the regioisomer derived from **7**. TBDMS group of compound **8** was removed with Bu_4NF to give pure MPM ether **6**, whereas MPM group of **8** was removed by 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) oxidation[13] to give TBDMS ether **9**.

We then attempted to synthesize **6** and **9** via another route using D-mannitol as a starting material for large scale synthesis. The primary hydroxyl group of 2,5-dibenzylated D-mannitol **10**[14] was benzoylated selectively to give **11** in 93% yield. Oxidative cleavage of **11** using HIO_4 gave aldehyde **12**. Since partial racemization (ca 5%) was observed during $NaBH_4$ reduction of **12**,[15] aldehyde **12** was reduced by $NaBH_3CN$ under acidic conditions to give optically pure glycerol derivative **13** in 98% yield.[16] Compound **13** was *p*-methoxybenzylated by use of trichloroacetimidate and (1*S*)-10-camphorsulfonic acid, and debenzoylation of the resulting **14** gave desired glycerol derivative **6**, which was readily converted to TBDMS ether **9** as described above.

The glycerol phosphate tetramer **21** was synthesized by the phosphoramidite method as shown in Fig. 3. Compound **6** was first allowed to react with 3 equivalent of phosphorodiamidite reagent (**15**)[17] using 1*H*-tetrazole as the catalyst in a mixture of dichloroethane and CH_3CN to afford phosphoramidite **16**. The single peak observed at δ=149 on ^{31}P NMR (162 MHz) of **16** indicated the exclusive formation of the desired phosphoramidite. The second glycerol unit **9** was then coupled with excess phosphoramidite **16** in the presence of 1*H*-tetrazole. The resulting phosphite was oxidized with *m*-chloroperbenzoic acid (mCPBA) to give glycerol phosphate dimer **17**, whose structure was confirmed by the phosphotriester signal at δ=−0.2 on ^{31}P NMR (162 MHz) and by an analysis of the ^{1}H NMR (270 MHz) spectrum. Compound **17** thus obtained was then treated with either Bu_4NF or DDQ to remove TBDMS or MPM group, respectively. Compound **18** and **19** thus obtained was then coupled by the same procedure to afford a glycerol phosphate tetramer **21**, whose structure was confirmed by ^{31}P NMR (162 MHz) and ^{1}H NMR (270 MHz). After removal of TBDMS group of compound **21**, the resulting alcohol **22** was coupled with phosphorodiamidite **15** to afford the tetramer phosphoramidite **23**, which was used for the condensation with the glycolipid part as described below. The phosphoramidite intermediates (**16**, **20**, **23**) obtained were effectively purified by silica-gel column chromatography without decomposition using eluents containing triethylamine. This purification step was essential to facilitate the purification of phosphotriesters formed

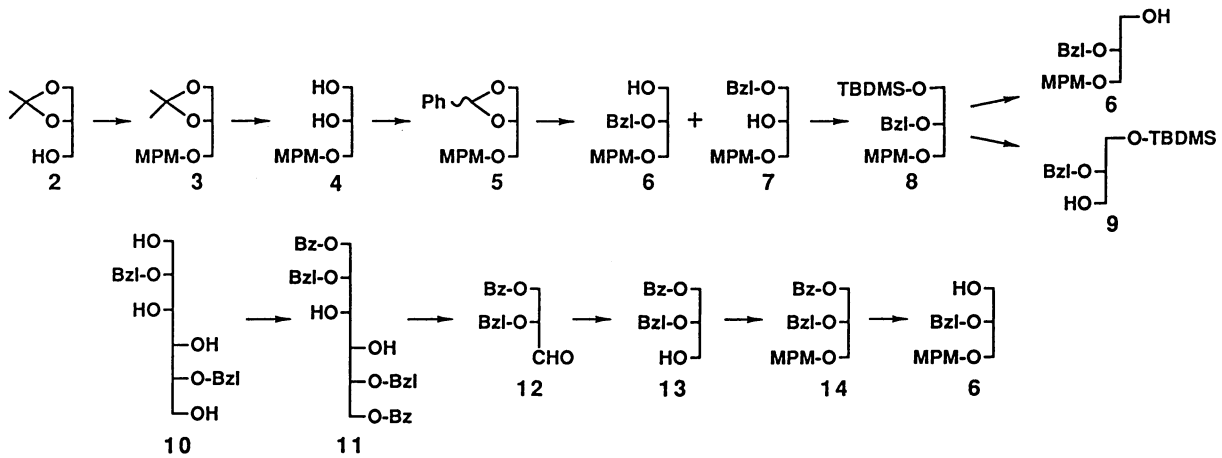

Fig. 2. Preparation of optically active glycerol derivatives.

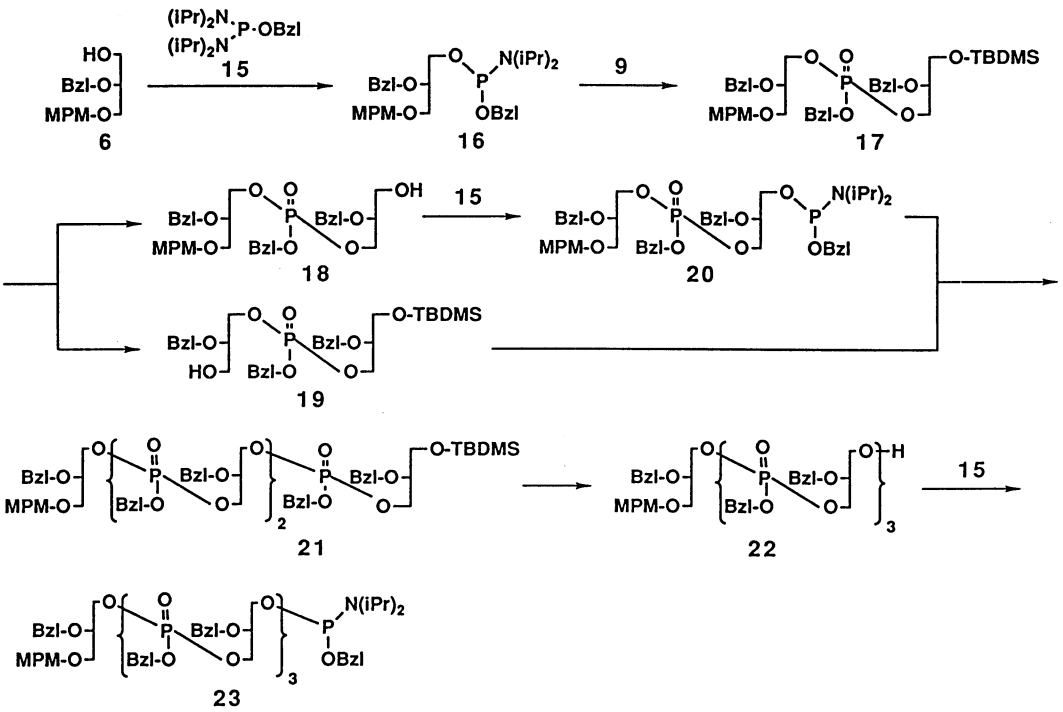

Fig. 3. The synthesis of the PGP part.

in the subsequent reactions. We could thus establish an efficient procedure for the synthesis of the PGP part. Although we used glycerol phosphate tetramer as the PGP part in the present LTA synthesis, we can expect that further repetition of the above procedure leads to the formation of the PGP part with longer chain length corresponding to that of native LTA.

Preparation of the Glycolipid Part. Glycosyl fluorides, whose 2-hydroxyl groups were protected as non-participating benzyl-type ethers, were used as glycosyl donors for the preparation of α-glucosidic linkage of the glycolipid part of LTA. Since strong Lewis acids are required for the activation of glycosyl fluorides, acid-stable *p*-nitrobenzyl (NPM) group was used for the protection of the 2-hydroxyl group of the first glycosyl donor. Although NPM ether had offered no particular advantages as a protecting group for hydroxyl functions, we recently found that NPM ether can be cleaved selectively in the presence of other hydroxyl-protecting groups such as the benzyl and the allyl ethers by the following two step procedure,[18,19] i.e., 1) selective reduction of the nitro group to form *p*-aminobenzyl ether, and 2) cleavage of the latter either by electrochemical oxidation or by *N*-acetylation and DDQ oxidation. This finding enabled the use of NPM group as a versatile temporary protecting group.

The acyl moieties of the glycolipid were introduced after the coupling of the first glucose unit with 1,2-di-*O*-allyl-*sn*-glycerol **36** and the removal of the allyl groups. This sequence was taken in order to avoid the possible migration of the acyl groups in 1,2-di-*O*-acyl-*sn*-glycerol during the acid-catalyzed glycosidation step. Synthesis of a similar glycolipid was described by Gent et al. using 2-butenyl group for protection of glycerol.[20]

O-Diisopropylideneglucose **24** was benzylated and then treated with allyl alcohol and hydrogen chloride to give a mixture of α- and β-allyl glycosides **26** (α:β=2.7:1).[21] After protection of the 4- and 6-hydroxyl functions of compound **26** by *O*-benzylidenation, the 2-hydroxyl group was *p*-nitrobenzylated with NPM bromide and silver oxide in benzene to give **28**.[22] Benzylation, after removal of the benzylidene group, with benzyl bromide and silver oxide[22] gave tribenzyl derivative **30** in 77% yield. The glycosidic allyl group of **30** was cleaved after isomerization to the 1-propenyl group using an iridium complex.[23,24] After acetylation of free 1-hydroxyl group, 1-acetate **32** was treated with 65% HF-pyridine to give the α-fluoride **33**.

The condensation of the fluoride **33** and 1,2-di-*O*-allyl-*sn*-glycerol **36** was carried out by Noyori's procedure[25] using 3-*O*-TMS derivative **37** of the latter and trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give the desired α-glucoside **38** preferentially (α:β=3.4:1), its α-configuration being confirmed by the coupling constant of H-1 signal (δ=4.98, $J_{1,2}$=3.5 Hz) on ^{1}H NMR. The allyl groups of compound **38** were then isomerized as above to give **39**, whose 1-propenyl groups were hydrolyzed with 90% aqueous acetic acid at 110 °C.[26] The resultant free hydroxyl groups were then acylated with palmitoyl chloride to give the glyceroglycolipid **42**.

The NPM group in **42** was then removed according to the principle outlined above to furnish an acceptor for the second glycosidation reaction. As we recently de-

scribed,[19] a combination of zinc–copper couple and acetylacetone reduced the nitro function of NPN group quite smoothly at room temperature to give *p*-aminobenzyl ether **43** in 99% yield. Anodic oxidation of **43** was carried out in ^{t}BuOH–H_2O (4 : 1) in the presence of $HClO_4$ with Pt electrodes to give **44** to be used for the introduction of the second glucosyl residue at the 2-position. *N*-Acetylation of **43** followed by DDQ oxidation of the resultant *p*-acetamidobenzyl ether also gave **44**; the yield was, however, lower than that by the former anodic oxidation probably owing to nonselective cleavage of some of the benzyl groups by DDQ.

The glycosyl fluoride **49** was prepared as the second glucosyl donor. The 6-hydroxyl group was portected with 2,2,2-trichloroethoxycarbonyl (Troc) group, which was to be removed later for the coupling of the PGP part. The allyl glucoside **47** was prepared by benzylation of **27** followed by reductive cleavage of benzylidene group and 6-*O*-trichloroethoxycarbonylation. After removal of the allyl group, the resultant 1-hydroxyl group was converted into the glycosyl fluoride **49** by use of 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (FMPT) and Et_3N.[27]

The glycosidation reaction of the donor **49** and the acceptor **44** was carried out by Mukaiyama's procedure using $SnCl_2$ and $AgClO_4$ in ether[28] at room temperature or by Suzuki's procedure using Cp_2ZrCl_2 and $AgClO_4$ in toluene[29] at −5 °C to afford α-glucoside **50**, preferentially. The α-selectivity by Suzuki's procedure (α/β =3.0/1) was higher than that by Mukaiyama's procedure (α/β=2.3/1).[30] The Troc group of **50** was removed with zinc and acetic acid to give a protected glycolipid part **51** to be used for the subsequent coupling with the PGP part.

Separately from the completion of the LTA structure, all benzyl groups of **51** were removed in order to obtain the free glyceroglycolipid **52**. Since glyceroglycolipids with the identical architecture is expected to present in cell membrane of the bacteria,[8] we planed to identify the synthetic compound with the corresponding natural specimen and to test their biological activities. The structure of **52** purified by silica-gel column chromatography was confirmed by FAB-MS [m/z 915 [(M+Na)$^+$]], elemental analysis, ^{1}H NMR and ^{13}C NMR, where proton and carbon signals were unambiguously assigned by analysis of the H–H and C–H COSY spectra. The synthetic **52** was then compared with the corresponding natural glycolipid isolated from *S. pyogenes* cells.[31] ^{1}H NMR spectra clearly showed that natural one was identical with the synthetic glycolipid except the existence of double bond in the acyl moieties of the former. Synthesis of this saturated glycolipid was also described by van Boeckel et al. by use of a bifunctional silyl protective group.[32]

Preparation of the Proposed Structure of *S. pyogenes* LTA. The protected glycolipid part **51** and the phosphoramidite of PGP part **23** obtained above were

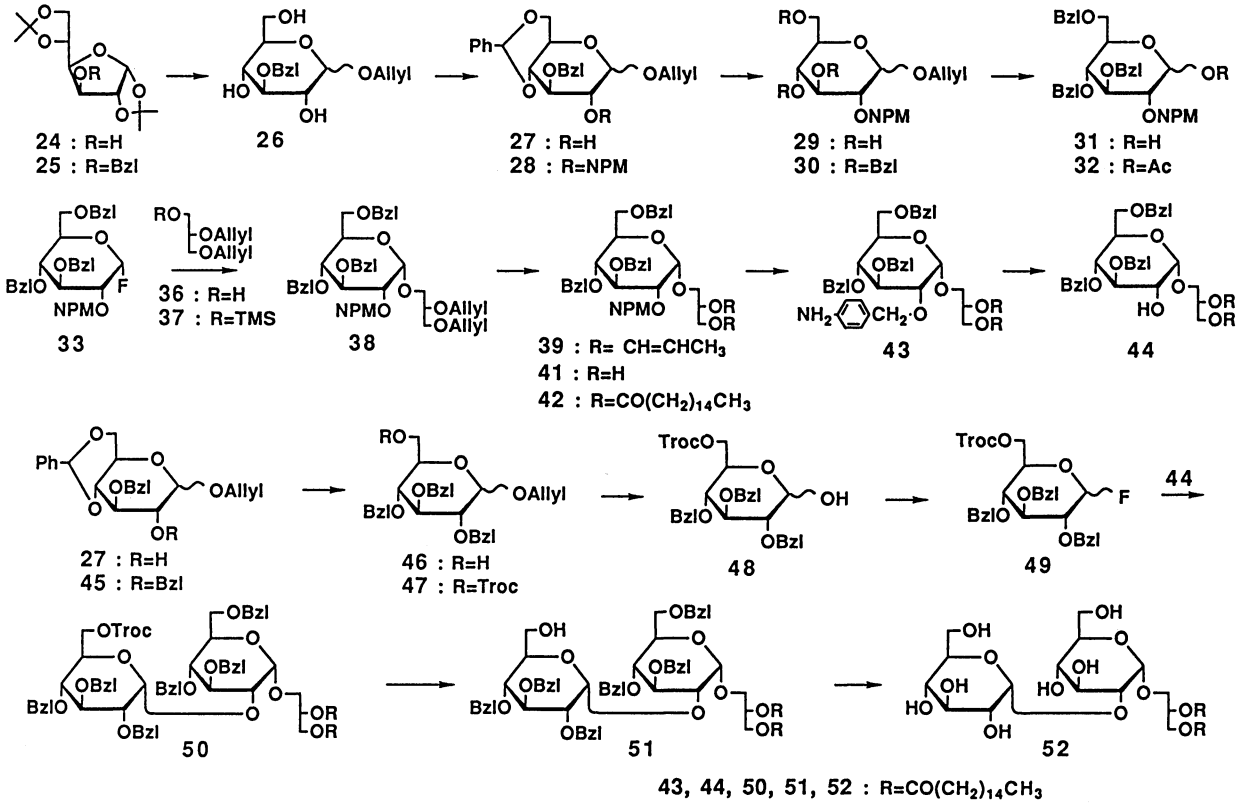

Fig. 4. The synthesis of Glycolipid part.

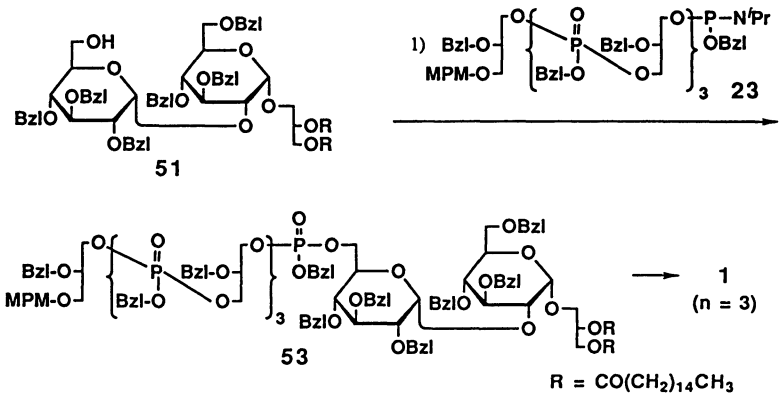

Fig. 5. The synthesis of *Streptococcus pyogenes* LTA.

coupled in the presence of 1*H*-tetrazole, and the resultant phosphite was oxidized by mCPBA to afford the fully protected fundamental structure of *S. pyogenes* LTA **53**. Finally, all benzyl-type protective groups were removed by catalytic hydrogenation under 6.0 kg cm^{-2} of H_2 to complete the synthesis. Since the solubility of the substrate greatly changed by cleavage of benzyl groups and liberation of hydroxyl and phosphate groups as the hydrogenation proceeded, successive addition of methanol and water to the initial THF solution was necessary to perform the complete deprotection. The synthetic LTA **1** (n=3) thus obtained could be effectively purified by HPLC on a reversed-phase column (Asahipak C4P-50) using a mixture of 50 mM ammonium carbonate buffer (pH=8.5) and CH_3CN as eluents. The purified product gave satisfactory result in elemental analysis and a pseudomolecular ion peak at m/z 1507.6 [(M–H)$^-$] in its negative FAB-mass spectrum.

As described in this paper, we succeeded in the synthesis of the proposed structure of *S. pyogenes* LTA (n=3) and obtained an evidence for the structure of the glycolipid part by its identification with a major component of the cell membrane lipid. The strategy given here proved to be very effective for the synthesis of related structures in general. Synthesis of *Enterococcus hirae* LTA[5–7] possessing more complicated structure[2] than that described here will soon be reported elsewhere. The biological activities of these LTA and their glycolipid parts are now being investigated.

Experimental

All melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL JNM-GSX 270 or 400 spectrometer for $CDCl_3$ solutions unless otherwise noted. The chemical shifts are given in δ values from tetramethylsilane (TMS) as the internal standard. ^{31}P NMR spectra were measured on a JEOL JNM-GSX 400 spectrometer for $CDCl_3$ solutions. The chemical shifts are given in δ values from 85% phosphoric acid as the external standard. FAB-MS spectra were obtained with JEOL 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 Kieselgel 60 (E. Merck) (0.063—0.2 mm) for the purification of compounds **26** and **35** or Kieselgel 60 (0.040—0.063 mm) at medium-pressure (2—4 kg cm^{-2}) for the purification of other compounds. Organic solutions were dried over $MgSO_4$ and evaporated in vacuo.

1,2-*O*-Isopropylidene-3-*O*-(*p*-methoxybenzyl)-*sn*-glycerol (3). To a solution of (*S*)-2,2-dimethyl-1,3-dioxolane-4-methanol (**2**) (10.0 g, 75.7 mmol) in DMF (100 ml) was added 60% NaH (oil dispersion, 3.33 g, 83.3 mmol) at 0 °C, and the mixture was stirred at room temperature for 30 min. *p*-Methoxybenzyl chloride (13.0 g, 83.3 mmol) was added to the mixture at 0 °C. After the mixture was stirred at room temperature for 1.5 h, AcOEt and brine were added to the mixture. The organic layer was separated, washed with water, and worked up as usual. The residue was purified by silica-gel column chromatography (180 g, 3×56 cm, toluene–AcOEt=20 : 1) to give a liquid: Yield 17.9 g (97.0%); $[\alpha]_D^{21}$ +21.8° (*c* 1.00, $CHCl_3$). Anal. ($C_{14}H_{20}O_4$) C, H.

3-*O*-(*p*-Methoxybenzyl)-*sn*-glycerol (4). The solution of **3** (18.2 g, 72.1 mmol) in 90% aqueous acetic acid (100 ml) was stirred at 50 °C for 2.5 h, and then concentrated in vacuo. Evaporation under reduced pressure after addition of toluene (600 ml) was repeated three times to give a colorless solid (15.1 g, 98.7%) which was subjected to the following reaction without purification.

1,2-*O*-Benzylidene-3-*O*-(*p*-methoxybenzyl)-*sn*-glycerol (5). To a solution of **4** (15.1 g, 71.2 mmol) in THF (60 ml) were added benzaldehyde dimethyl acetal (15.4 g, 101 mmol) and (1*S*)-10-camphorsulfonic acid (168 mg, 0.722 mmol). The solution was refluxed for 2.5 h and cooled to 25 °C. The solution was neutralized with a saturated aqueous $NaHCO_3$ solution and diluted with AcOEt (160 ml). The organic layer was washed with brine and worked up as usual. The residue was purified by silica-gel column chromatography (180 g, 3×56 cm, toluene–AcOEt=30 : 1) two times to give an oil: Yield 15.6 g (73.0%); $[\alpha]_D^{21}$ +28.1° (*c* 0.98, $CHCl_3$). Anal. ($C_{18}H_{20}O_4$) C, H.

Reductive Cleavage of Benzylidene Group of 5. To a solution of **5** (6.00 g, 20.0 mmol) in anhydrous ether (11 ml) was added 576 mM $Zn(BH_4)_2$ in ether (104 ml, 60 mmol) at −18 °C. After the mixture was stirred at −18 °C for 8 min, TMSCl (18.3 ml, 144 mmol) was added, and the mixture was stirred at the same temperature for 30 min. After excess hydride was decomposed by addition of water (100 ml) and

concd HCl (1 ml), the organic layer separated was washed with brine and worked up as usual. The residue was purified by repeated silica-gel column chromatography (180 g, 3×56 cm, toluene–AcOEt=2:1) to give an oily product as 50:1 mixture (4.97 g, 41.4%) of 2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycerol **6** and 1-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycerol **7**. ^{1}H NMR (270 MHz) δ=4.70 (0.98H, d, J=9.2 Hz, $C_6H_5C\underline{H}_2$ of **6**), 4.60 (0.98H, d, J=9.2 Hz, $C_6H_5C\underline{H}_2$ of **6**), 4.57 (0.04H, s, $C_6H_5C\underline{H}_2$ of **7**).

2-*O*-Benzyl-1-*O*-*t*-butyldimethylsilyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycerol (8). To a solution of the 50:1 mixture of compound **6** and **7** obtained above (2.35 g, 7.78 mmol) and imidazole (1.48 g, 21.8 mmol) in DMF (10 ml) was added a solution of TBDMSCl (2.35 g, 15.6 mmol) in DMF (12 ml) at −50 °C, and the solution was stirred at the same temperature for 2 h. After excess TBDMSCl was quenched with water (5 ml), the mixture was concentrated. The residue was dissolved in AcOEt, washed with brine, and worked up as usual. The residue was purified by silica-gel column chromatography (180 g, 3×56 cm, toluene–AcOEt=20:1) to give an oil: Yield 3.15 g (97.2%); $[\alpha]_D^{20}$ −1.3° (*c* 1.20, $CHCl_3$). Anal. ($C_{24}H_{36}O_4Si$) C, H.

2-*O*-Benzyl-1-*O*-*t*-butyldimethylsilyl-*sn*-glycerol (9). To a solution of **8** (1.80 g, 4.33 mmol) in CH_2Cl_2 (35 ml) were added water (1.9 ml) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (1.08 g, 4.76 mmol) at 5 °C. The mixture was stirred at 5 °C for 5 h, the insoluble materials were removed by filtration and the filtrate concentrated. The residue was purified by silica-gel column chromatography (180 g, 3×56 cm, toluene–AcOEt=20:1) to give a syrup: Yield 1.04 g (81.3%); $[\alpha]_D^{20}$ −20.8° (*c* 1.00, $CHCl_3$); ^{1}H NMR (270 MHz) δ=7.36—7.26 (5H, m, $C_6\underline{H}_5CH_2$), 4.71 (1H, d, J=11.8 Hz, $C_6H_5C\underline{H}_2$), 4.62 (1H, d, J=11.8 Hz, $C_6H_5C\underline{H}_2$), 3.79—3.57 (5H, m, H-1, H-2, H-3), 0.90 (9H, s, $SiC(CH_3)_3$), 0.06 (6H, s, $Si(CH_3)_2$). Anal. ($C_{16}H_{28}O_3Si$) C, H.

2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycerol (6). To a solution of **8** (3.10 g, 7.45 mmol) in anhydrous THF (30 ml) was added 1 M Bu_4NF in THF (8.94 ml, 8.94 mmol) at room temperature under Ar atmosphere. The mixture was stirred at the same temperature for 30 min, and then concentrated. The residue was dissolved in AcOEt, washed with brine, and worked up as usual. The residue was purified by silica-gel column chromatography (180 g, 3×56 cm, toluene–AcOEt=2:1) to give an oil: Yield 2.11 g (93.8%); $[\alpha]_D^{20}$ +18.1° (*c* 1.18, $CHCl_3$); ^{1}H NMR (270 MHz) δ=7.34—6.87 (9H, m, aromatic H) 4.70 (1H, d, J=11.8 Hz, $C_6H_5C\underline{H}_2$), 4.60 (1H, d, J=11.8 Hz, $C_6H_5C\underline{H}_2$), 4.46 (2H, s, $CH_3OC_6H_4C\underline{H}_2$), 3.80 (3H, s, CH_3O), 3.76—3.56 (5H, m, H-1, H-2, H-3). Anal. ($C_{18}H_{22}O_4$) C, H.

1,6-Di-*O*-benzoyl-2,5-di-*O*-benzyl-D-mannitol (11). To a solution of 2,5-di-*O*-benzyl-D-mannitol (**10**)[14] (17.2 g, 47.3 mmol) in pyridine (150 ml) was added dropwise a solution of benzoyl chloride (13 g, 95 mmol) in pyridine (40 ml) at −18 °C during 45 min. The mixture was stirred at the same temperature for 6 h, and at −5 °C overnight. To the mixture was added water (1.3 l), and the crystals precipitated was filtered, washed with water, and then dissolved in AcOEt. The organic solution was washed with 1 M HCl, a saturated aqueous $NaHCO_3$ solution, and brine, and worked up as usual. The crystalline residue was recrystallized from AcOEt and hexane: Yield 25.1 g (92.8%); mp 88—89 °C; $[\alpha]_D^{19}$ −19.0° (*c* 1.02, $CHCl_3$); ^{1}H NMR (270 MHz) δ=8.04—7.22 (20H, m, aromatic H), 4.80—4.50 (8H, m, $C_6H_5C\underline{H}_2$×2, H-1, H-6), 4.05 (2H, d, J=7.2 Hz, H-3, H-4), 3.92—3.86 (2H, m, H-2, H-5), 1.78 (2H, bs, OH). Anal. ($C_{34}H_{34}O_8$) C, H.

1-*O*-Benzoyl-2-*O*-benzyl-*sn*-glycerol (13). To a solution of **11** (12.1 g, 21.2 mmol) in EtOH (300 ml) was added $HIO_4 \cdot 2H_2O$ (5.31 g, 23.9 mmol), and the solution was stirred at room temperature for 1 h. To the solution were added a saturated aqueous $NaHCO_3$ solution and ether, and the organic layer was washed with brine and worked up as usual to give aldehyde **12**. To a solution of **12** in MeOH (200 ml), water (50 ml), and AcOH (60 ml) was added $NaBH_3CN$ (13.3 g, 212 mmol). The solution was stirred at room temperature for 1 h and then neutralized with a saturated aqueous $NaHCO_3$ solution. The aqueous mixture was extracted with AcOEt. The organic layer was washed with water and worked up as usual. The residue was purified by silica-gel column chromatography (45 g, 2×28 cm, hexane–AcOEt=1:1) to give an oil: Yield 11.9 g (98.7%); $[\alpha]_D^{15}$ −16.4° (*c* 1.15, $CHCl_3$); ^{1}H NMR (270 MHz) δ=8.05—7.26 (10H, m, aromatic H), 4.78 (1H, d, J=11.6 Hz, $C_6H_5C\underline{H}_2$), 4.60 (1H, d, J=11.6 Hz, $C_6H_5C\underline{H}_2$), 4.48 (2H, d, J=5.2 Hz, H-1), 3.90—3.63 (3H, m, H-2, H-3), 1.58 (1H, bs, OH). Anal. ($C_{17}H_{18}O_4$) C, H.

2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycerol (6). To a solution of **13** (11.9 g, 41.6 mmol) in CH_2Cl_2 (50 ml) were added *p*-methoxybenzyl trichloroacetimidate (27.0 g, 95.7 mmol) and (1*S*)-10-camphorsulfonic acid (966 mg, 4.16 mmol) at 0 °C under N_2 atmosphere. The mixture was stirred at the same temperature for 4 h, and CCl_3CONH_2 precipitated was filtered off. The filtrate was washed with a saturated aqueous $NaHCO_3$ solution and brine, and worked up as usual. The residue was purified by silica-gel column chromatography (170 g, 3×53 cm, hexane–AcOEt=9:1) to give 1-*O*-benzoyl-2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycerol (**14**) as syrup. To a solution of compound **14** in MeOH (150 ml) was added 0.1 M MeONa solution (150 ml). The solution was stirred at room temperature for 8 h, and then neutralized with Dry Ice. After the solvent was evaporated, the residue was dissolved in AcOEt, washed with brine, and worked up as usual. The residue was purified by silica-gel column chromatography (170 g, 3×53 cm, toluene–AcOEt=3:1): Yield 12.0 g (95.4%); $[\alpha]_D^{20}$ +18.5° (*c* 1.08, $CHCl_3$); Anal. ($C_{18}H_{22}O_4$) C, H.

[2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)]benzyl-oxo(diisopropylamido)pbosphorus (16). To a solution of **15** (7.70 g, 22.8 mmol) in anhydrous $ClCH_2CH_2Cl$ (50 ml) were added a solution of 1*H*-tetrazole (598 mg, 8.5 mmol) in anhydrous CH_3CN (20 ml) and a solution of **6** (2.15 g, 7.10 mmol) in anhydrous $ClCH_2CH_2Cl$ (30 ml) under Ar atmosphere. After the solution was stirred at 0 °C overnight, CH_2Cl_2 and a saturated aqueous $NaHCO_3$ solution were added. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and worked up as usual. The residue was purified by silica-gel column chromatography (160 g, 3×50 cm, hexane–Et_3N=10:1) to give a syrup: Yield 3.47 g (90.7%); ^{1}H NMR (270 MHz) δ=7.32—6.84 (14H, m, aromatic H), 4.69—4.65 (4H, m, $C_6H_5C\underline{H}_2$, $NC\underline{H}(CH_3)_2$), 4.46 (2H, s, $CH_3OC_6H_4C\underline{H}_2$), 3.79 (3H, s, CH_3O), 3.77—3.57 (5H, m, H-1, H-2, H-3), 1.18 (6H, d, J=6.9 Hz, $CH(C\underline{H}_3)_2$), 1.17 (6H, d, J=6.7 Hz, $CH(C\underline{H}_3)_2$); ^{31}P NMR (162 MHz) δ=149 (s). Anal. ($C_{31}H_{42}O_5NP$) C, H, N.

[2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)benzyl-phospho](3)-2-*O*-benzyl-1-*O*-*t*-butyldimethylsilyl-*sn*-glycerol (17). To a solution of **16** (1.31 g, 2.43 mmol) in anhydrous CH_2Cl_2 (1 ml) were added a solution of 1*H*-tetrazole (193 mg, 2.76 mmol) in anhydrous CH_3CN (5 ml) and a solution of 2-*O*-

benzyl-1-*O*-*t*-butyldimethylsilyl-*sn*-glycerol (**9**) (415 mg, 1.83 mmol) in anhydrous CH_2Cl_2 (1 ml) under Ar atmosphere. After the mixture was stirred at room temperature for 1.5 h, 80% mCPBA (591 mg, 2.75 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 30 min, and then CH_2Cl_2 and a 10% $NaHSO_3$ solution were added to it. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and brine and worked up as usual. The residue was purified by silica-gel column chromatography (150 g, 3×47 cm, toluene–AcOEt=5 : 1) to give a syrup: Yield 1.34 g (97.8%); $[\alpha]_D^{21}$ −1.6° (*c* 1.11, $CHCl_3$); ^{1}H NMR (270 MHz) δ=7.31—6.84 (19H, m, aromatic H), 5.01 (2H, d, $^3J_{PH}$=7.8 Hz, $C_6H_5C\underline{H}_2$-O-P(=O)), 4.66—4.54 (4H, m, $C_6H_5C\underline{H}_2$-OCH-×2), 4.40 (1H, s, $CH_3OC_6H_4C\underline{H}_2$), 4.39 (1H, s, $CH_3OC_6H_4C\underline{H}_2$), 4.19—4.03 (4H, m, CH_2-CH-$C\underline{H}_2$-O-P(=O)×2), 3.76 (3H, s, CH_3O), 3.74—3.46 (6H, m, Bzl-OC$\underline{H}$×2, MPM-O$C\underline{H}_2$, TBDMS-O$C\underline{H}_2$), 0.87 (9H, s, SiC($C\underline{H}_3$)$_3$), 0.018 (3H, s, $SiCH_3$), 0.015 (3H, s, $SiCH_3$); ^{31}P NMR (162MNz) δ=−0.2 (s). Anal. ($C_{41}H_{55}O_9PSi$) C, H.

[2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)benzylphospho] (3)-2-*O*-benzyl-*sn*-glycerol (18). TBDMS group of **17** (752 mg, 1.02 mmol) was removed in anhydrous THF (23 ml) using 1 M Bu_4NF in THF (1.23 ml, 1.23 mmol) as described for the preparation of **6**. The product was purified by silica-gel column chromatography (40 g, 2×25 cm, hexane–AcOEt=1 : 2) to give a syrup: Yield 627 mg (98.7%); $[\alpha]_D^{20}$ +11.5° (*c* 1.00, $CHCl_3$). Anal. ($C_{35}H_{41}O_9P \cdot 0.25H_2O$) C, H.

[2-*O*-Benzyl-*sn*-glycero(1)benzylphospho] (3)-2-*O*-benzyl-1-*O*-*t*-butyldimethylsilyl-*sn*-glycerol (19). To a mixture of **17** (715 mg, 0.974 mmol) in CH_2Cl_2 (9 ml) and water (500 μl) was added DDQ (243 mg, 1.07 mmol) at 5 °C, and the mixture was stirred at 5 °C for 3.5 h. Excess DDQ was quenched with a solution of ascorbic acid (350 mg), citric acid (630 mg), and NaOH (460 mg) in water (50 ml). The organic layer was separated, washed with water and worked up as usual. The residue was purified by silica-gel column chromatography (40 g, 2×25 cm, hexane–AcOEt=1 : 2) to give a syrup: Yield 503 mg (81.9%). Anal. ($C_{33}H_{47}O_8PSi \cdot 0.25H_2O$) C, H.

[2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)]benzyloxo(diisopropylamido)phosphorus (20). Phosphoramidite **20** was prepared from compound **18** (1.40 g, 2.20 mmol) and benzyloxybis(*N,N*-diisopropylamino)phosphine (**15**) (1.38 g, 4.08 mmol) using 1*H*-tetrazole (172 mg, 2.45 mmol) as a catalyst in $ClCH_2CH_2Cl$ (64 ml) and CH_3CN (15 ml) as described for the preparation of **16**. The product was purified by silica-gel column chromatography (70 g, 2×44 cm, toluene–TEA=10 : 1) to give a syrup: Yield 1.80 g (94.4%); ^{31}P NMR (162 MHz) δ=149.0 (s), 148.0 (s), −0.22 (s). Anal. ($C_{48}H_{61}O_{10}NP_2$) C, H, N.

[2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)benzylphospho] (3)-2-*O*-benzyl-1-*O*-*t*-butyldimethylsilyl-*sn*-glycerol (21). Coupling reaction of compound **19** (156 mg, 0.247 mmol) with phosphoramidite **20** (391 mg, 0.475 mmol) was carried out using 1*H*-tetrazole (36.6 mg, 0.522 mmol) as a catalyst in CH_2Cl_2 (3 ml) and CH_3CN (1 ml). After the solution was stirred at room temperature, the resulting phosphite was oxidized with 80% mCPBA (85.1 mg, 0.585 mmol) as described above. The product was purified by silica-gel column chromatography (70 g, 3×53 cm, toluene–AcOEt=1 : 1) to give a syrup: Yield 312 mg (89.0%); $[\alpha]_D^{21}$ +0.94° (*c* 1.07, $CHCl_3$); ^{1}H NMR (270 MHz) δ=7.28—6.83 (39H, m, aromatic H), 5.01—4.94 (6H, m, $C_6H_5C\underline{H}_2$-O-P(=O)), 4.59—4.56 (4H, m, $C_6H_5C\underline{H}_2$-OCH- ×2), 4.52—4.50 (4H, m, $C_6H_5C\underline{H}_2$-OCH-×2), 4.39 (1H, s, $CH_3OC_6H_4C\underline{H}_2$), 4.38 (1H, s, $CH_3OC_6H_4C\underline{H}_2$), 4.17—3.96 (12H, m, CH_2-CH-$C\underline{H}_2$-O-P(=O)×6), 3.75 (3H, s, CH_3O), 3.70—3.45 (8H, m, Bzl-OC$\underline{H}$-×4, MPM-O$C\underline{H}_2$, TBDMS-O$C\underline{H}_2$), 0.86 (9H, s, SiC($C\underline{H}_3$)$_3$), 0.011 (3H, s, $SiCH_3$), 0.007 (3H, s, $SiCH_3$); ^{31}P NMR (162 MHz) δ=−0.11—−0.27 (m). Anal. ($C_{75}H_{93}O_{19}P_3Si \cdot 0.5H_2O$) C, H.

[2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)benzylphospho] (3)-2-*O*-benzyl-*sn*-glycerol (22). Compound **21** (1.14 g, 0.801 mmol) was treated in anhydrous THF (40 ml) with a 1 M solution of Bu_4NF in THF (0.961 ml, 0.961 mmol) as described for the preparation of **6**. The product was purified by silica-gel column chromatography (180 g, 3×56 cm, $CHCl_3$–acetone=2 : 1) to give a syrup: Yield 1.02 g (88.0%).

[2-*O*-Benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)]benzyloxo(diisopropylamido)phosphorus (23). Phosphoramidite **23** was prepared from **22** (893 mg, 0.684 mmol) and benzyloxybis(*N,N*-diisopropylamino)phosphine (**15**) (938 mg, 2.78 mmol) using 1*H*-tetrazole (58.4 mg, 0.833 mmol) as a catalyst in $ClCH_2CH_2Cl$ (33 ml) and CH_3CN (5 ml) as described for the preparation of **16**. The product was purified by silica-gel column chromatography (70 g, 2×44 cm, toluene–TEA=10 : 1) to give a syrup: Yield 889 mg (84.2%); ^{1}H NMR (270 MHz) δ=7.30—6.83 (44H, m, aromatic H), 5.00—4.95 (6H, m, $C_6H_5C\underline{H}_2$O-P (=O)), 4.70—4.50 (10H, m, $C_6H_5C\underline{H}_2$-OCH-×4, $C_6H_5C\underline{H}_2$-O-P-N), 4.40 (1H, s, $CH_3OC_6H_4C\underline{H}_2$), 4.39 (1H, s, $CH_3OC_6H_4C\underline{H}_2$), 4.18—4.03 (14H, m, CH_2-CH-$C\underline{H}_2$O-P(=O) ×6, C$\underline{H}$(CH_3)$_2$×2), 3.76 (3H, s, CH_3O), 3.73—3.46 (8H, m, Bzl-OC$\underline{H}$-×4, MPM-O$C\underline{H}_2$, ^{i}Pr$_2$N-P-OCH_2), 1.15 (12H, d, *J*=6.9 Hz, CH($C\underline{H}_3$)$_2$).

3-*O*-Benzyl-1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose (25). To a solution of 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose[33] (**24**) (3.50 g, 13.5 mmol) in *N,N*-dimethylformamide (DMF) (17 ml) was added 60% NaH (592 mg, 14.8 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. To the mixture was added benzyl bromide (1.76 ml, 14.8 mmol), and the mixture was stirred at room temperature for 30 min and then concentrated in vacuo. The residue was dissolved in AcOEt, washed with water and brine, and worked up as usual. The residue was purified by silica-gel column chromatography (150 g, 3×47 cm, toluene–AcOEt=5 : 1) to give a syrup: Yield 4.46 g (94.3%); $[\alpha]_D^{18}$ −26.4° (*c* 1.88, $CHCl_3$). Anal. ($C_{19}H_{26}O_6$) C, H.

Allyl 3-*O*-Benzyl-α- and β-D-glucopyranoside (26). A solution of **25** (54.7 g, 156 mmol) in HCl/allyl alcohol (560 ml, 4.25%, w/v) was refluxed for 10 min and concentrated. Evaporation under reduced pressure after addition of toluene (100 ml) was repeated three times. The residue was purified by silica-gel column chromatography (230 g, 5×25 cm, toluene–AcOEt=1 : 2) to give a solid: Yield 42.9 g (88.6%); ^{13}C NMR (68 MHz) δ=102.0 (0.27C, Cβ-1), 97.9 (0.73C, Cα-1). Anal. ($C_{16}H_{22}O_6$) C, H.

Allyl 3-*O*-Benzyl-4,6-*O*-benzylidene-α- and β-D-glucopyranoside (27). To a solution of **26** (7.97 g, 25.7 mmol) in THF (20 ml) were added benzaldehyde dimethyl acetal (5.5 g, 100 mmol) and (1*S*)-10-camphorsulfonic acid (1.19 g, 5.14 mmol). The solution was refluxed for 2 h and cooled to

25 °C. The solution was neutralized with a saturated aqueous $NaHCO_3$ solution and concentrated in vacuo. The residue was dissolved in AcOEt, and washed with a saturated $NaHCO_3$ solution, brine, and worked up as usual. The crystalline residue was recrystallized from AcOEt–hexane: Yield 8.23 g (80.4%). The α- (1.53 g) and β-anomers (0.07 g) were partially separated by silica-gel column chromatography (70 g, 3×20 cm, toluene–AcOEt=2 : 1) of the product (4.96 g).

α-Anomer: Mp 135—136 °C; $[\alpha]_D^{21}$ +77.5° (*c* 1.11, $CHCl_3$); ^1H NMR (270 MHz) δ=7.67—7.24 (10H, m, aromatic H), 5.94 (1H, m, CH_2=C<u>H</u>–CH_2O), 5.57 (1H, s, $C_6H_5C\underline{H}$), 5.37—5.21 (2H, m, $C\underline{H_2}$=CH–CH_2O), 4.96 (1H, d, *J*=11.6 Hz, $C_6H_5C\underline{H_2}$), 4.96 (1H, d *J*=3.7 Hz, H-1), 4.80 (1H, d, *J*=11.6 Hz, $C_6H_5C\underline{H_2}$), 4.39—3.54 (8H, m, CH_2=CH–C<u>H_2</u>O, H-2~H-6). Anal. ($C_{23}H_{26}O_6 \cdot 0.25H_2O$) C, H.

β-Anomer: Mp 137—138 °C; $[\alpha]_D^{21}$ −36.7° (*c* 1.02, $CHCl_3$); ^1H NMR (270 MHz) δ=7.50—7.23 (10H, m, aromatic H), 5.94 (1H, m, CH_2=C<u>H</u>–CH_2O), 5.57 (1H, s, $C_6H_5C\underline{H}$), 5.37—5.21 (2H, m, $C\underline{H_2}$=CH–CH_2O), 4.96 (1H, d, *J*=11.6 Hz, $C_6H_5C\underline{H_2}$), 4.80 (1H, d, *J*=11.6 Hz, $C_6H_5C\underline{H_2}$), 4.46 (1H, d, *J*=7.4 Hz, H-1), 4.41—3.39 (8H, m, CH_2=CH–C<u>H_2</u>O, H-2~H-6). Anal. ($C_{23}H_{26}O_6$) C, H.

Allyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-(*p*-nitrobenzyl)-α- and β-D-glucopyranoside (28). To a solution of **27** (6.00 g, 15.1 mmol) in benzene (60 ml) were added molecular sieves 3A (2 g), NPM bromide (4.23 g, 19.6 mmol), and Ag_2O (4.55 g, 19.6 mmol), successively. The mixture was stirred at room temperature overnight. To the mixture were added NPM bromide (2.61 g, 12.1 mmol) and Ag_2O (2.80 g, 12.1 mmol), and the mixture was stirred at room temperature overnight. The insoluble solids were removed by filtration and the filtrate was concentrated. The residue was purified by silica-gel column chromatography (80 g, 2×50 cm, toluene–AcOEt =30 : 1) to give a colorless solid: Yield 7.52 g (93.5 %). Both α- and β-anomers partially separated were recrystallized from AcOEt and hexane.

α-Anomer: Mp 135—136 °C; $[\alpha]_D^{21}$ +16.9° (*c* 1.08, $CHCl_3$); ^1H NMR (270 MHz) δ=8.15—7.26 (14H, m, aromatic H), 5.94 (1H, m, CH_2=C<u>H</u>–CH_2O), 5.57 (1H, s, $C_6H_5C\underline{H}$), 5.40—5.24 (2H, m, $C\underline{H_2}$=CH–CH_2O), 4.98—4.74 (4H, m, $C_6H_5C\underline{H_2}$, $O_2NC_6H_4C\underline{H_2}$), 4.95 (1H, d, *J*=3.7 Hz, H-1), 4.32—3.52 (8H, m, CH_2=CH–C<u>H_2</u>O, H-2~H-6). Anal. ($C_{30}H_{31}O_8N$) C, H, N.

β-Anomer: Mp 141—141.5 °C; $[\alpha]_D^{21}$ −16.7° (*c* 1.06, $CHCl_3$); ^1H NMR (270 MHz) δ=8.12—7.26 (14H, m, aromatic H), 5.92 (1H, m, CH_2=C<u>H</u>–CH_2O), 5.58 (1H, s, $C_6H_5C\underline{H}$), 5.36—5.19 (2H, m, $C\underline{H_2}$=CH–CH_2O), 5.00—4.72 (4H, m, $C_6H_5C\underline{H_2}$, $O_2NC_6H_4C\underline{H_2}$), 4.57 (1H, d, *J*=7.7 Hz, H-1), 4.32—3.52 (8H, m, CH_2=CH–C<u>H_2</u>O, H-2~H-6). Anal. ($C_{30}H_{31}O_8N$) C, H, N.

Allyl 3-*O*-Benzyl-2-*O*-(*p*-nitrobenzyl)-α- and β-D-glucopyranoside (29). A mixture of **28** (51.0 g, 95.6 mmol) in 90% aqueous acetic acid (300 ml) was stirred at 70 °C for 2 h and then concentrated in vacuo. Evaporation under reduced pressure after addition of toluene (100 ml) was repeated three times. The residue was crystallized from hexane to give a colorless solid: Yield 39.8 g (93.4%). Anal. ($C_{23}H_{27}O_8N$) C, H, N.

Allyl 3,4,6-Tri-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α- and β-D-glucopyranoside (30). To a solution of **29** (2.00 g) in CH_2Cl_2 (50 ml) was added molecular sieves 3A (2 g), and the mixture was stirred for 20 min. To the mixture were added benzyl bromide (3.08 g, 18.0 mmol) and Ag_2O (4.18 g, 18.0 mmol), and the mixture was stirred at room temperature overnight. To the mixture were added molecular sieves 3A (2 g), benzyl bromide (3.08 g, 18.0 mmol), and Ag_2O (4.18 g, 18.0 mmol), and the mixture was stirred at room temperature overnight. The insoluble solids were removed by filtration and the filtrate was concentrated. The residue was purified by silica-gel column chromatography (170 g, 3×53 cm, toluene–AcOEt= 10 : 1→1 : 1) to give **30** (2.17 g, 77.2%), and a dibenzyl derivative (0.47 g, 19.6%) as syrup.

3,4,6-Tri-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α- and β-D-glucopyranose (31). To a solution of **30** (1.63 g, 2.60 mmol) in THF (120 ml) was added $[Ir(cod)(PMe(C_6H_5)_2)_2]PF_6$ (cod: cyclooctadiene) (81.5 mg, 96.4 μmol) under N_2 atmosphere. The N_2 in the system was replaced with H_2, and the solution was stirred at room temperature for 1 min. The system was evacuated until the color of the solution changed from yellow to light red, and then filled again with N_2. The solution was stirred at 50 °C for 1 h and cooled to room temperature. To the solution were added water (46 ml) and I_2 (1.32 g, 5.20 mmol). After the solution was stirred at room temperature for 30 min, a 5% aqueous $Na_2S_2O_3$ solution was added until the color of I_2 disappeared. AcOEt and a saturated aqueous $NaHCO_3$ solution were added to the mixture. The organic layer separated was washed with brine, worked up as usual and then concentrated. The residue was purified by silica-gel column chromatography (180 g, 3×56 cm, toluene–AcOEt=5 : 1) to give a solid: Yield 1.46 g (96.1%). Anal. ($C_{34}H_{35}O_8N$) C, H, N.

1-*O*-Acetyl-3,4,6-tri-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-D-glucopyranose (32). To a solution of **31** (1.79 g, 3.05 mmol) in CH_2Cl_2 (16 ml) were added pyridine (986 μl, 12.2 mmol) and acetic anhydride (1.15 ml, 12.2 mmol). The solution was stirred at room temperature overnight and concentrated. The residue was purified by silica-gel column chromatography (160 g, 3×50 cm, toluene–AcOEt=10 : 1) to give a syrup: Yield 1.81 g (94.3%). Anal. ($C_{36}H_{37}O_9N$) C, H, N.

3,4,6-Tri-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α-D-glucopyranosyl Fluoride (33). To a solution of compound **32** (670 mg, 0.937 mmol) in CH_2Cl_2 (14 ml) was added HF-pyridine (6 ml, 65%, v/v) at −20 °C. After the mixture was stirred at 0 °C for 1 h, AcOEt (50 ml) and a 10% aqueous KF solution were added. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and brine and worked up as usual. The residue was purified by silica-gel column chromatography (8 g, 1×14 cm, toluene–AcOEt=10 : 1) to give crystals: Yield 486 mg (88.3%); mp 88.0—89.0 °C; $[\alpha]_D^{18}$ +33.7° (*c* 0.420, $CHCl_3$); ^1H NMR (270 MHz) δ=8.15—7.13 (19H, m, aromatic H), 5.67 (1H, dd, $J_{1,2}$=2.6 Hz, $J_{1,F}$=55.9 Hz, H-1), 4.92—4.46 (8H, m, $C_6H_5C\underline{H_2}$×3, $NO_2C_6H_4C\underline{H_2}$), 4.03—3.48 (6H, m, H-2~H-6). Anal. ($C_{34}H_{34}O_7NF$) C, H, N.

1,2,5,6-Tetra-*O*-allyl-3,4-*O*-isopropylidene-D-mannitol (34). To a solution of 3,4-*O*-isopropylidene-D-mannitol[34] (15.7 g, 70.4 mmol) in DMF (500 ml) was added 60% NaH (14.1 g, 352 mmol) at 0 °C and the mixture was stirred at the same temperature for 30 min. After addition of allyl bromide (30.5 ml, 352 mmol), the mixture was stirred at room temperature for 24 h and then concentrated. The residue was dissolved in Et_2O (250 ml), washed with water and brine, and worked up as usual to give a colorless oil, which was used for the following reaction without purification: Yield 26.9 g (100%); ^1H NMR (270 MHz) δ=6.01—5.90 (4H, m, CH_2=C<u>H</u>–CH_2O), 5.36—5.18 (8H, m, $C\underline{H_2}$=CH–CH_2O), 4.29—3.59 (16H, m, $C\underline{H_2}$=CH–CH_2O, H-1~H-6), 1.43 (6H, s, CH_3).

1,2,5,6-Tetra-*O*-allyl-D-mannitol (35). A solution of **34**

(26.9 g, 70.4 mmol) in 90% aqueous AcOH (500 ml) was allowed to stand at 90 °C for 8 h and concentrated. The residue was purified by silica-gel column chromatography (500 g, 7×25 cm, toluene–AcOEt 4 : 1) to give an oil: Yield 22.6 g (93.8%); $[\alpha]_D^{28}$ −24.8° (*c* 1.37, $CHCl_3$); ^{1}H NMR (270 MHz) δ=5.97—5.81 (4H, m, CH_2=C<u>H</u>-CH_2O), 5.30—5.11 (8H, m, $C\underline{H}_2$=CH-CH_2O), 4.24—3.55 (16H, m, $C\underline{H}_2$=CH-CH_2O, H-1~H-6), 3.16 (2H, bs, OH). Anal. ($C_{18}H_{30}O_6$) C, H.

1,2-Di-*O*-allyl-*sn*-glycerol (36).[20] To a solution of **35** (22.6 g, 66.0 mmol) in 95% ethanol (500 ml) was added $HIO_4 \cdot 2H_2O$ (16.6 g, 72.6 mmol) at 0 °C, and the solution was stirred at the same temperature for 15 min. To the reaction mixture was added $NaBH_4$ (8.25 g, 218 mmol), and excess hydride was quenched with acetone (100 ml) after 1 h. The insoluble solids were removed by filtration and the filtrate was evaporated. The residue was dissolved in AcOEt (300 ml), washed with water and brine, and worked up as usual. The residue was purified by vacuum distillation (bp 72—76 °C/0.3 mmHg, 1 mmHg=133.322 Pa) to give a colorless liquid: Yield 16.0 g (70.5%); $[\alpha]_D^{29}$ −27.1° (*c* 1.16, $CHCl_3$). ^{1}H NMR (270 MHz) δ=6.00—5.82 (2H, m, CH_2=C<u>H</u>-CH_2O), 5.33—5.16 (4H, m, $C\underline{H}_2$=CH-CH_2O), 4.22—3.99 (4H, m, CH_2=CH-$C\underline{H}_2O$), 3.77—3.49 (5H, m, H-1, H-2, H-3). Anal. ($C_9H_{16}O_3 \cdot 0.35H_2O$) C, H.

1,2-Di-*O*-allyl-3-*O*-trimethylsilyl-*sn*-glycerol (37). To a solution of **36** (469 mg, 2.73 mmol) in THF (20 ml) were added Et_3N (1.14 ml, 8.17 mmol) and trimethylchlorosilane (888 mg, 8.17 mmol) at 0 °C. The mixture was stirred at room temperature for 20 min, and the insoluble materials were filtered off. The filtrate was concentrated, and evaporation after addition of benzene repeated three times to give **37** as a colorless oil. This was subjected to the following reaction without purification.

1,2-Di-*O*-allyl-3-*O*-[3,4-tri-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α-D-glucopyranosyl]-*sn*-glycerol (38). To a solution of fluoride **33** (800 mg, 1.36 mmol) and **37** (665 mg, 2.73 mmol) in anhydrous Et_2O (30 ml) was added TMSOTf (388 ml, 2.06 mmol) at 0 °C. After the mixture was stirred at 5 °C overnight, a saturated aqueous $NaHCO_3$ solution and EtOAc were added. The organic layer was washed with water, and worked up as usual. The residue was purified by silica-gel column chromatography (70 g, 2×44 cm, toluene–EtOAc =10 : 1) two times to give the α-anomer **38** as a syrup (616 mg, 61.0%) and the β-anomer as a syrup (82 mg, 18%).

38: $[\alpha]_D^{22}$ +60.1° (*c* 1.11, $CHCl_3$); ^{1}H NMR (270 MHz) δ=8.12—7.12 (19H, m, aromatic H), 5.95—5.80 (2H, m, CH_2=C<u>H</u>-CH_2O×2), 5.29—5.09 (4H, m, $C\underline{H}_2$=CH-CH_2O ×2), 4.98 (1H, d, *J*=3.5 Hz, Glc H-1), 4.86—4.44 (8H, m, $C_6H_5C\underline{H}_2$×3, $NO_2C_6H_4C\underline{H}_2$), 4.16—4.12 (2H, m, CH_2=CH-$C\underline{H}_2O$), 4.10—3.47 (13H, m, CH_2=CH-$C\underline{H}_2O$, Glc H-2~H-6, Gro H-1~H-3). Anal. ($C_{43}H_{49}O_{10}N$) C, H, N.

β-Anomer: ^{1}H NMR (270 MHz) δ=8.10—7.09 (19H, m, aromatic H), 5.93—5.79 (2H, m, CH_2=C<u>H</u>-CH_2O ×2), 5.28—5.10 (4H, m, $C\underline{H}_2$=CH-CH_2O×2), 5.04—4.52 (8H, m, $C_6H_5C\underline{H}_2$ ×3, $NO_2C_6H_4C\underline{H}_2$), 4.43 (1H, d *J*=7,.9 Hz, Glc H-1), 4.12—4.09 (2H, m, CH_2=CH-$C\underline{H}_2O$), 3.98—3.36 (13H, m, CH_2=CH-$C\underline{H}_2O$, Glc H-2~H-6, Gro H-1~H-3).

3-*O*-[3,4,6-Tri-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α-D-glucopyranosyl]-*sn*-glycerol (41). Compound **38** (800 mg, 1.08 mmol) was treated with Ir-complex (40 mg, 47.2 μmol) in THF (40 ml) as described for the preparation of **31**. The reaction mixture was concentrated, and a solution of the resultant **39** in 90% acetic acid (60 ml) was heated at 110 °C for 30 min. After the solution was concentrated, the residue was purified by silica-gel column chromatography (70 g, 2×44 cm, benzene–AcoEt=1 : 1) to give a syrup: Yield 650 mg (91.4%); ^{1}H NMR (270 MHz) δ=8.13—7.12 (19H, m, aromatic H), 4.91 (1H, d, *J*=3.7 Hz, Glc H-1), 4.86—4.46 (8H, m, $C_6H_5C\underline{H}_2$ ×3, $NO_2C_6H_4C\underline{H}_2$), 4.01—3.50 (11H, m, Glc H-1~H-6, Gro H-1~H-3).

3-*O*-[3,4,6-Tri-*O*-benzyl-2-*O*-(*p*-nitrobenzyl)-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (42). To a solution of **41** (344 mg, 0.522 mmol) in CH_2Cl_2 (4 ml) were added palmitoyl chloride (532 μl, 1.56 mmol), pyridine (250 μl, 3.13 mmol) and 4-dimethylaminopyridine (DMAP) (13.5 mg, 0.104 mmol) at room temperature, and the mixture was stirred at the same temperature for 1.5 h. To the mixture were added palmitoyl chloride (709 μl, 2.01 mmol) and pyridine (334 μl, 4.18 mmol), and the mixture was stirred for 3 h. After excess palmitoyl chloride was quenched with MeOH (2 ml), AcOEt and water were added. The organic layer was washed with 1 M HCl and brine and worked up as usual. The residue was purified by silica-gel column chromatography (40 g, 1.5×44 cm, benzene–AcOEt=20 : 1) two times to give a syrup: Yield 522 mg (88.1%); ^{1}H NMR (270 MHz) δ=8.15—7.12 (19H, m, aromatic H), 5.25 (1H, m, Gro H-2), 4.91 (1H, d, *J*=3.5 Hz, Glc H-1), 4.85—4.45 (8H, m, $C_6H_5C\underline{H}_2$×3, $NO_2C_6H_4C\underline{H}_2$), 4.43—3.53 (10H, m, Glc H-2~H-6, Gro H-1, H-3), 2.30—2.25 (4H, m, $OCOCH_2$×2), 1.59—1.55 (4H, m, $OCOCH_2C\underline{H}_2$×2), 1.25 (48H, s, $OCOCH_2CH_2(C\underline{H}_2)_{12}CH_3$×2), 0.88 (6H, t, *J*=6.4 Hz, CH_3×2).

3-*O*-[2-*O*-(*p*-Aminobenzyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (43). To a solution of **42** (1.20 g, 1.06 mmol) in THF (12 ml) were added Zn–Cu (1.0 g) and acetylacetone (4.0 ml, 39 mmol). The mixture was stirred at room temperature for 10 min, and the insoluble solids 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 (50 g, 2×31 cm, benzene–AcOEt= 5 : 1) to give syrup: Yield 1.16 g (99.2%).

3-*O*-[3,4,6-Tri-*O*-benzyl-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (44). A solution of **43** (1.16 g, 1.05 mmol) and $HClO_4$ (182 μl, 2.10 mmol) in a mixture of ^{t}BuOH (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. To the solution were added a saturated aqueous $NaHCO_3$ solution and AcOEt. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and brine and worked up as usual. The residue was purified by silica-gel column chromatography (70 g, 2×44 cm, benzene–AcOEt=5 : 1) to give **44** (852 mg, 81.2%) as a solid: Recovery of **43** (58 mg, 5.0%).

44: ^{1}H NMR (270 MHz) δ=7.39—7.13 (15H, m, $C_6\underline{H}_5$×3), 5.24 (1H, m, Gro H-2), 4.96—4.46 (7H, m, Glc H-1, $C_6H_5C\underline{H}_2$×3), 4.41—3.59 (10H, m, Glc H-2~H-6, Gro H-1, H-3), 2.33—2.27 (4H, m, $OCOCH_2$×2), 1.62—1.57 (4H, m, $OCOCH_2C\underline{H}_2$×2), 1.25 (48H, s, $OCOCH_2CH_2(C\underline{H}_2)_{12}CH_3$ ×2), 0.88 (6H, t *J*=6.4 Hz, CH_3×2).

Allyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α- and β-D-glucopyranoside (45). To a solution of **27** (9.37 g, 23.5 mmol) in DMF (60 ml) was added 60% NaH (13.9 g, 347 mmol) at 0 °C, and the mixture was stirred at room temperature for 30 min. Benzyl bromide (4.42 g, 25.9 mmol) was added to the mixture at 0 °C. After the mixture was stirred at room temperature

overnight, AcOEt and brine were added to the mixture. The organic layer was separated, washed with brine, and worked up as usual. The residue was purified by silica-gel column chromatography (70 g, 2×44 cm, toluene–AcOEt=25 : 1) to give a solid: Yield 10.6 g (92.0%); mp 85—86 °C. Anal. ($C_{30}H_{32}O_6$) C, H.

Allyl 2,3,4-Tri-*O*-benzyl-α- and β-D-glucopyranoside (46). To a solution of **45** (500 mg, 1.02 mmol) in dry Et_2O (3.5 ml) and dry CH_2Cl_2 (3.5 ml) was added $LiAlH_4$ (54.0 mg,1.43 mmol) with stirring. A solution of $AlCl_3$ (209 mg, 1.57 mmol) in dry Et_2O (3.5 ml) was added dropwise under reflux and the stirring was continued for 2 h. The mixture was cooled to room temperature, excess hydride was quenched with successive addition of AcOEt and then brine. The mixture was diluted with Et_2O (15 ml), and the insoluble material was filtered off. The organic layer was washed with brine and worked up as usual. The residue was purified by silica-gel column chromatography (40 g, 1.5×44 cm, toluene–AcOEt=5 : 1) to give a syrup: Yield 474 mg (94.6%). Anal. ($C_{30}H_{34}O_6 \cdot 0.5H_2O$) C, H.

Allyl 2,3,4-Tri-*O*-benzyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α- and β-D-glucopyranoside (47). To a solution of **46** (7.05 g, 14.4 mmol) and pyridine (3.50 ml, 43.2 mmol) in CH_2Cl_2 (200 ml) were added TrocCl (5.93 ml, 43.1 mmol) and DMAP (370 mg, 2.9 mmol) at 0 °C. After the solution was stirred for 1 h, excess TrocCl was quenched with MeOH (50 ml). The mixture was concentrated, and the residue was dissolved in AcOEt (650 ml). The organic solution was washed with 1 M HCl, a saturated aqueous $NaHCO_3$ solution, and brine and worked up as usual. The residue was purified by silica-gel column chromatography (180 g, 3×56 cm, hexane–AcOEt=6 : 1) to give a syrup: Yield 9.30 g (96.9%); ^{1}H NMR (270 MHz) δ=7.38—7.23 (15H, m, $C_6\underline{H}_5$×3), 5.93 (1H, m, CH_2=C<u>H</u>–CH_2O), 5.36—5.19 (2H, m, $C\underline{H}_2$=CH–CH_2O), 4.94—4.59 (9H, m), 4.38 (2H, m, H-6). Anal. ($C_{33}H_{35}O_8Cl_3$), C, H, Cl.

2,3,4-Tri-*O*-benzyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α- and β-D-glucopyranose (48). The allyl group of **47** (8.00 g, 12.0 mmol) was isomerized to 1-propenyl group with Ir-complex (200 mg) in THF (200 ml) and the resulting 1-propenyl group was cleaved with H_2O (30 ml) and I_2 (6.00 g, 24.0 mmol) as described for the preparation of **31**. The product was purified by silica-gel column chromatography (180 g, 3×56 cm, hexane–AcOEt=19 : 1) to give a syrup: Yield 6.63 g (88.2%); ^{1}H NMR (270 MHz) δ=7.38—7.23 (15H, m, $C_6\underline{H}_5$×3), 5.20 (1H, m, H-1). Anal. ($C_{30}H_{31}O_8Cl_3$) C, H, Cl.

2,3,4-Tri-*O*-benzyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α- and β-D-glucopyranosyl Fluoride (49). To a solution of **48** (4.00 g, 6.38 mmol) in CH_2Cl_2 (80 ml) were added 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (FMPT) (3.63 g, 12.8 mmol) and Et_3N (2.70 ml, 19.1 mmol) at 0 °C. The mixture was stirred at room temperature for 12 h, then diluted with AcOEt. The solution was washed with a saturated aqueous $NaHCO_3$ solution and brine, and worked up as usual. The residue was purified by silica-gel column chromatography (80 g, 2×50 cm, toluene–AcOEt=20 : 1) to give a syrup: Yield 3.80 g (95.0%).

3-*O*-[3,4,6-Tri-*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 (50). To a solution of **44** (88.4 mg, 88.4 μmol) in toluene (3 ml) were added zirconocene dichloride (Cp_2ZrCl_2) (25.8 mg, 88.4 μmol), $AgClO_4$ (36.7 mg, 177 μmol), and molecular sieves (MS) 4A (200 mg), and the mixture was stirred at −5 °C for 30 min. To the mixture was added a solution of fluoride **49** (61.0 mg, 97.2 μmol) in toluene (3 ml) at −10 °C. The mixture was stirred at the same temperature for 18 h, and Cp_2ZrCl_2 (7.8 mg, 27 μmol), $AgClO_4$ (11.0 mg, 53.0 μmol), molecular sieves (MS) 4A (100 mg), and fluoride **49** (16.7 mg, 26.5 μmol) were added again. After the mixture was stirred at 0 °C for 3 h, a saturated aqueous $NaHCO_3$ solution and AcOEt were added. The insoluble materials were removed by filtration and the organic layer was washed with a saturated aqueous $NaHCO_3$ solution and brine and worked up as usual. The residue was purified by silica-gel column chromatography (20 g, 1.5×25 cm, benzene–AcOEt=20 : 1) three times to give the α-anomer **50** (71.4 mg, 50.3%) and the β-anomer (23.8 mg, 16.7%) as syrup, respectively.

50: $[\alpha]_D^{22}$ +60.0° (*c* 1.05, $CHCl_3$); ^{1}H NMR (270 MHz) δ=7.36—7.09 (30H, m, $C_6\underline{H}_5$×6), 5.14 (1H, m, Gro H-2), 4.96—4.45 (16H, m, Glc H-1, Glc H-1′, $C_6H_5C\underline{H}_2$×6, $CCl_3C\underline{H}_2$), 4.34—4.29 (2H, m, Gro H-1), 4.21—3.52 (14H, m, Glc H-2~H-6, Glc H-2′~H-6′, Gro H-3), 2.29—2.20 (4H, m, $OCOC\underline{H}_2$×2), 1.55—1.50 (4H, m, $OCOCH_2C\underline{H}_2$×2), 1.33—1.24 (48H, m, $OCOCH_2CH_2(C\underline{H}_2)_{12}CH_3$×2), 0.87 (6H, t, *J*=6.7 Hz, CH_3×2). Anal. ($C_{92}H_{125}O_{17}Cl_3$) C, H, Cl.

β-Anomer: ^{1}H NMR (270 MHz) δ=7.35—7.09 (30H, m, $C_6\underline{H}_5$×6), 5.28 (1H, m, Gro H-2), 5.11—4.37 (16H, m, Glc H-1, Glc H-1′, $C_6H_5C\underline{H}_2$×6, $CCl_3C\underline{H}_2$), 4.25—4.21 (2H, m, Gro H-1), 4.20—3.45 (14H, m, Glc H-2~H-6, Glc H-2′~H-6′, Gro H-3), 2.33—2.26 (4H, m, $OCOC\underline{H}_2$×2), 1.61—1.56 (4H, m, $OCOCH_2C\underline{H}_2$×2), 1.33—1.25 (48H, m, $OCOCH_2CH_2(C\underline{H}_2)_{12}CH_3$×2), 0.87 (6H, t, *J*=6.7 Hz, CH_3×2).

3-*O*-[3,4,6-Tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (51). To a solution of **50** (439 mg, 273 μmol) in acetic acid (20 ml) was added Zn powder (2.41 g, 36.9 mmol), and the mixture was stirred at room temperature for 2 h. After insoluble materials were filtered off, the filtrate was concentrated. The solution of the residue in AcOEt was washed with water, a saturated aqueous $NaHCO_3$ solution, and brine, and worked up as usual. The residue was purified by silica-gel column chromatography (22 g, 1.5×28 cm, benzene–AcOEt=5 : 1) to give a syrup: Yield 323 mg (82.5%); $[\alpha]_D^{22}$ +70.2° (*c* 1.20, $CHCl_3$); ^{1}H NMR (270 MHz) δ=7.34—7.12 (30H, m, $C_6\underline{H}_5$×6), 5.15 (1H, m, Gro H-2), 4.98—4.45 (16H, m, Glc H-1, Glc H-1′, $C_6H_5C\underline{H}_2$×6, OH), 4.36—4.30 (2H, m, Gro H-1), 4.21—3.51 (14H, m, Glc H-2~H-6, Glc H-2′~H-6′, Gro H-3), 2.30—2.20 (4H, m, $OCOC\underline{H}_2$×2), 1.60—1.55 (4H, m, $OCOCH_2C\underline{H}_2$×2), 1.33—1.25 (48H, m, $OCOCH_2CH_2(C\underline{H}_2)_{12}CH_3$×2), 0.88 (6H, t, *J*=6.7 Hz, CH_3×2); ^{13}C NMR (68 MHz) δ=96.6 and 95.4 (C-1 and C-1′). Anal. ($C_{84}H_{124}O_{15}$) C, H.

3-*O*-[2-*O*-(α-D-Glucopyranosyl)-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (52). To a solution of **51** (157 mg, 110 μmol) in THF–water (10 : 1) (6 ml) was added Pd black (430 mg). The mixture was stirred under 6 kg cm^{-2} of H_2 at room temperature for 4 d. The catalyst was filtered off and the filtrate was concentrated. The residue was purified by silica-gel column chromatography (4 g, 1×11 cm, $CHCl_3$–MeOH=9 : 1) to give a colorless solid: Yield 77.8 mg (79.4%); $[\alpha]_D^{27}$ +86° (*c* 0.442, $CHCl_3$–MeOH=4 : 1); FAB-MS, *m/z* 915 $[(M+Na)^+]$; ^{1}H NMR (400 MHz, $CDCl_3$–CD_3OD=3 : 1) δ=5.23 (1H, m, Gro H-2), 4.98 (1H, d, *J*=3.6 Hz, Glc(a) H-1), 4.95 (1H, d, *J*=3.8 Hz, Glc(b) H-1), 4.43 (1H, dd, *J*=3.0, 12.1 Hz, Gro H-1), 4.20 (1H, dd, *J*=6.4, 12.1 Hz, Gro H-1′), 3.88

(1H, m, Glc(b) H-5), 3.86 (1H, m, Glc(b) H-6), 3.83 (1H, dd, J=5.8, 10.6 Hz, Gro H-3), 3.8—3.7 (2H, m, Glc(a) H-6, H-6'), 3.78 (1H, m, Glc(a) H-3), 3.70 (1H, dd, J=6.2, 12.4 Hz, Glc(b), H-6'), 3.69 (1H, dd, J=9.2, 9.5 Hz, Glc(b) H-3), 3.64 (1H, dd, J=5.3, 10.6 Hz, Gro H-3'), 3.58 (1H, dd, J=3.6, 9.6 Hz, Glc(a) H-2), 3.57 (1H, m, Glc(a) H-5), 3.44 (1H, dd, J=3.8, 9.5 Hz, Glc(b) H-2), 3.44 (1H, dd, J=9.2, 9.9 Hz, Glc(a) H-4), 3.34 (1H, dd, J=9.2, 9.5 Hz, Glc(b) H-4), 2.36—2.30 (4H, m, $OCOCH_2$×2), 1.65—1.50 (4H, m, $OCOCH_2CH_2$×2), 1.40—1.20 (48H, m, $OCOCH_2CH_2(CH_2)_{12}CH_3$×2), 0.890 (3H, t, J=7.1 Hz, CH_3×2); ^{13}C NMR (100.4 MHz) δ=173.9 (CO), 173.5 (CO), 96.6 (Glc(b) C-1), 96.4 Glc(a) C-1), 76.7 (Glc(a) C-2), 73.5 (Glc(b) C-3), 72.0 (Glc(b) C-5), 71.8 (Glc(a) C-4 or Glc(b) C-2), 71.7 (Glc(a) C-5), 71.6 (Glc(a) C-3), 70.2 (Glc(b) C-4), 69.8 (Glc(a) C-4 or Glc(b) C-2), 69.7 (Gro, C-2), 65.6 (Gro, C-3) , 62.5 (Gro, C-1), 61.5 (Glc(b) C-6), 61.2 (Glc(a) C-6), 33.9 ($OCOCH_2$), 33.8 ($OCOCH_2$), 33.6 (acyl CH_2), 31.6 (acyl CH_2), 29.4 (acyl CH_2), 29.1 (acyl CH_2), 29.0 (acyl CH_2), 28.8 (acyl CH_2), 24.61 ($OCOCH_2CH_2$), 24.55 ($OCOCH_2CH_2$), 22.3 (acyl CH_2), 13.6 (CH_3); Glc(a) means the glucose residue of the reducing side, whereas Glc(b) means that of the non-reducing end. Anal. ($C_{47}H_{88}O_{15}\cdot 2.5H_2O$) C, H.

^{1}H NMR of Natural Glycolipid from *Streptococcus pyogenes*. ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD=3 : 1) δ=5.38—5.32 (2H, m, acyl -CH=CH-), 2.1—1.95 (4H, m, CH_2-CH=CH-CH_2), 1.40—1.20 (44H, m, acyl CH_2), and other proton signals were identical with those of synthetic glycolipid **52**.

3-*O*-[3,4,6-Tri-*O*-benzyl-2-*O*-[2,3,4-tri-*O*-benzyl-[2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)benzylphospho] [(3)-2-*O*-benzyl-*sn*-glycero(1)-benzylphospho] (6)-α-D-glucopyranosyl]-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (53). To a mixture of glycolipid part **51** (100 mg, 65.4 μmol) and phosphoramidite **23** (151 mg, 98.1 μmol) in $ClCH_2CH_2Cl$ (0.3 ml) was added a solution of 1*H*-tetrazole (6.87 mg, 98.1 μmol) in CH_3CN (0.5 ml) at 0 °C under N_2 atmosphere. After the solution was stirred at room temperature for 2 h, phosphoramidite **25** (151 mg, 98.1 μmol) in $ClCH_2CH_2Cl$ (0.3 ml) was added. After 3 h, 1*H*-tetrazole (4.58 mg, 65.4 μmol) in CH_3CN (0.5 ml) was added. After 3.5 h, 80% mCPBA (21.2 mg, 98.1 μmol) was added to the mixture at 0 °C and the mixture was stirred for 4 h. AcOEt and a saturated aqueous $NaHCO_3$ solution were added, and the organic layer was washed with a saturated aqueous $NaHCO_3$ solution and brine. After usual work-up, the residue was purified by silica-gel column chromatography (17 g, 1.5×21 cm, benzene-AcOEt=1 : 1) to give a syrup: 167.3 mg (89.5%); $[\alpha]_D^{24}$ +34.6° (*c* 1.19, $CHCl_3$). Anal. ($C_{165}H_{208}O_{36}P_4\cdot H_2O$) C, H.

3-*O*-[2-*O*-[[*sn*-glycero(1)phospho] [(3)*sn*-glycero(1)phospho] [(3)*sn*-glycero(1)phospho] [(3)*sn*-glycero(1)phospho] (6)-α-D-glucopyranosyl]-α-D-glucopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol Tetraammonium Salt (1). To a solution of compound **53** (56.3 mg, 19.4 μmol) in THF (2 ml) was added Pd black (30 mg). The mixture was stirred under 6 kg cm^{-2} of H_2 at room temperature for 7 h. Pd-black (100 mg), THF (2 ml), and MeOH (0.5 ml) were added, and the mixture was hydrogenated under the same conditions for 40 h. To the mixture were added Pd-black (100 mg), MeOH (3 ml), and water (3 ml), and the mixture was further hydrogenated for 3 h. The catalyst was filtered off and the filtrate was neutralized with 0.1% aqueous ammonia. After the organic solvents were removed by evaporation, the solution was lyophilized. The crude product was purified by HPLC (column: Asahipak C4P-50, 8×250 mm; solvent: 50 mM ammonium carbonate-CH_3CN; gradient 35—65% CH_3CN (2% min^{-1}); flow rate: 2.0 ml min^{-1}; detection: UV at 205 nm; retention time: 17.6 min) and lyophilyzed to give **1** as a powder: Yield 18.2 mg (53.3%); $[\alpha]_D^{21}$ +42° (*c* 0.223, H_2O). FAB-MS (negative). Found: *m/z* 1507.6. Calcd for $C_{59}H_{115}O_{35}P_4$: M−H, 1507.6. Anal. ($C_{59}H_{116}O_{35}P_4\cdot 4NH_3\cdot 10H_2O$) C, H, N.

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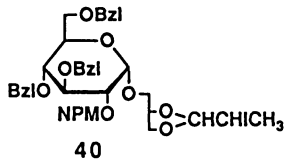

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