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SYNTHESIS OF A LIPOTEICHOIC ACID-CARRIER FRAGMENT OF *Staphylococcus aureus**

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ABSTRACT

A lipoteichoic acid-carrier fragment containing three glycerol units and one glycolipid unit was synthesized by use of the bifunctional phosphorylating reagents bis(1-benzotriazolyl) 2,2,2-tribromoethyl and 2-chlorophenyl phosphates. Protection of the *sn*-glycerol derivatives was achieved by use of benzyl as a permanent, and allyl, 1-propenyl, and 4-oxovaleryl as temporary protecting groups; the glycolipid unit was protected by benzyl groups except for the single primary alcohol required for coupling. Two of the *sn*-glycerol units are connected by (1→3)-interglyceridic phosphoric diester bonds. The other *sn*-glycerol unit is linked by a (3→6)-phosphoric diester bond to the carbohydrate unit of the glycolipid unit.

INTRODUCTION

Membrane teichoic acids or lipoteichoic acids are important components of the cell wall of most Gram-positive bacteria[1–3] and are located in the inner region of the cell membrane and the wall. The antigenic[4] properties of membrane teichoic acids are not of primary physiological significance, and their most general property is the ability to bind cations[5], thus maintaining a correct balance of divalent cations at the surface of the membrane. Another important function of the membrane teichoic acids is their ability to act as a lipoteichoic acid carrier (LTC) in the biosynthesis of wall teichoic acids[6]. At present, the role of LTC in the biosynthesis of wall teichoic acids is controversial[7–10]. Fischer *et al.*[10] reported that the absence of a D-alanyl residue may block the LTC activity. In order to get a better insight in the function of LTC, a detailed knowledge of the chemical properties of pure LTC was required. The structure of a lipoteichoic acid of *Staphylococcus aureus* (see **1**) has been elucidated by Baddiley[2]; it consists of a glycolipid unit joined by a phosphodiester linkage to a glyceryl phosphate polymer. In a recent publication[11], we reported the synthesis of a small fragment (see **3**) of the lipoteichoic acid **1**. As part

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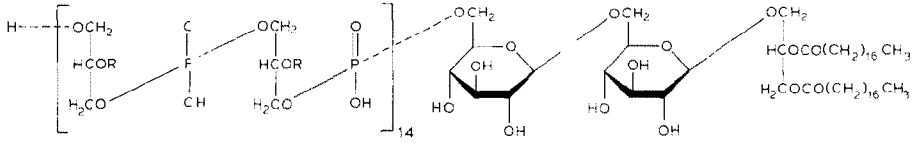
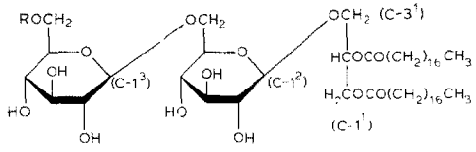
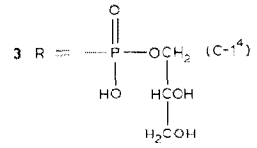
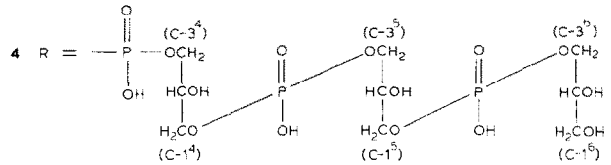


1 R = D-Alanyl
2 R = H



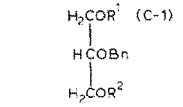




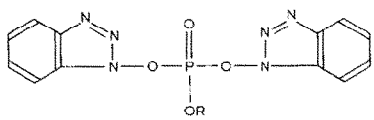
of our study to synthesize naturally occurring carbohydrate derivatives[11–14], we report herein the preparation of the LTC fragment **4** of *Staphylococcus aureus* in which the glycolipid unit is covalently linked to three phosphatidyl units lacking the D-alanyl residues

RESULTS AND DISCUSSION

The lipoteichoic acid fragment **4** contains a glycolipid unit linked at O-6^3 by a phosphoric diester bond to O-3^4 of the first glycerol unit that is part of a glycerol phosphate trimer, the three glycerol units of which are linked by interglyceridic (1→3) phosphoric diester bonds. The route followed for the assemblage of lipoteichoic acid fragment **4** consisted of linking by phosphorylation of OH-6^3 of the previously synthesized[11] glycolipid derivative **24** with OH-3^1 of the protected triglycerol diphosphate derivative **23**. Complete deblocking of the fully protected lipoteichoic acid fragment afforded **4**. For the preparation of the intermediate triglycerol bisphosphate **23**, three differently protected glycerol units were synthesized, *i.e.*, one terminal unit (**5**) having one free OH-3 and the other two hydroxyl groups protected with benzyl groups, and two nonterminal units (**6** and **7**) having OH-2 groups protected with benzyl groups. The OH-1 of the nonterminal unit (**6**) was protected with the temporary 1-propenyl group, whereas the OH-3 of **7** was protected with the temporary 5-oxovaleryl group. The properties of these protec-

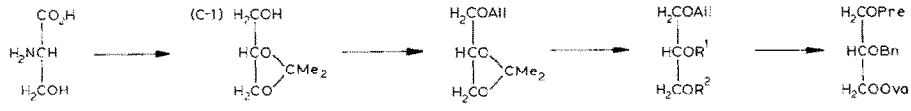


5 R^1 = Bn, R^2 = H
6 R^1 = Pre, R^2 = H
7 R^1 = H, R^2 = Ova



8 R = CH_2CBr_3
9 R = $C_6H_4Cl(2)$

All = $-CH_2-CH=CH_2$
Pre = $-CH=CH-CH_3$
Ova = $-CO-CH_2-CH_2-CO-CH_3$



10 11 12

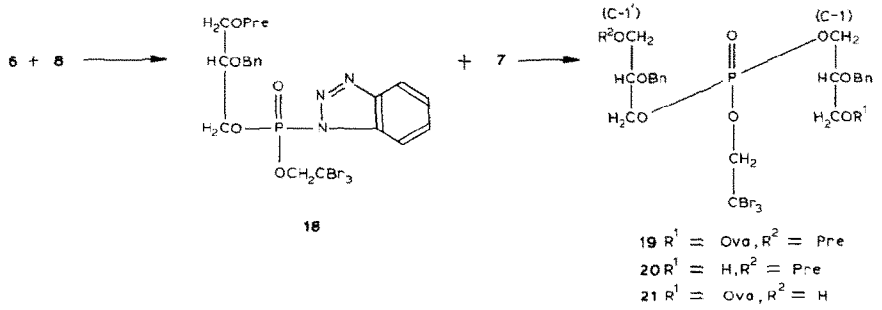
13 R^1 = R^2 = H
14 R^1 = H, R^2 = Tr
15 R^1 = Bn, R^2 = Tr
16 R^1 = Bn, R^2 = H

17

tive groups enabled the selective introduction, without the occurrence of neighboring group participation of the required (1→3) interglyceryl phosphoric triester linkages. For this purpose, the bifunctional phosphorylating reagents **8** and **9**, recently used for the synthesis of nucleic acid derivatives[15] as well as cell-wall components[16], were applied.

In addition to the correct protective groups and phosphorylating agents, it was also necessary to start from optically pure L-glycerol derivatives. Thus **5** was prepared[14,17] from 3,4-*O*-isopropylidene-D-mannitol[18], and **6** and **7** were obtained, from L-serine (**10**) as the chiral source, by conversion into 2,3-*O*-isopropylidene-*sn*-glycerol (**11**) in four steps by the procedure of Lok *et al.*[19]. Treatment of **11** with allyl bromide in the presence of sodium hydride[17] gave **12** which, after acid hydrolysis, afforded **13**. Tritylation with chlorotriphenylmethane[20], followed by benzylation of the crude **14** with benzyl bromide gave **15**. Removal of the trityl group with hydrochloric acid in methanol afforded the key intermediate **16**. The identity and homogeneity of **16** was established by ^{1}H- and ^{13}C-n.m.r. spectroscopy.

Two different routes for the synthesis of the two nonterminal glycerol derivatives **6** and **7** were investigated. In one approach, the allyl group in **16** was isomerized[21] with potassium *tert*-butoxide to afford the nonterminal unit **6**. Treatment of **6** with 4-oxovaleric anhydride[22] in the presence of 4-dimethylaminopyridine[23] gave intermediate **17**, and removal of the 1-propenyl group with mercuric chloride–mercuric oxide[24] led to the nonterminal unit **7**. The other approach differed from the previous one in that the allyl group of **16** was isomerized into the *trans*-1-propenyl group by use of the catalyst 1,5-cyclooctadienebis[methyl(diphenyl)phosphine]iridium hexafluorophosphate[25,26]. 4-Oxovalerylation afforded **17** in an excel-



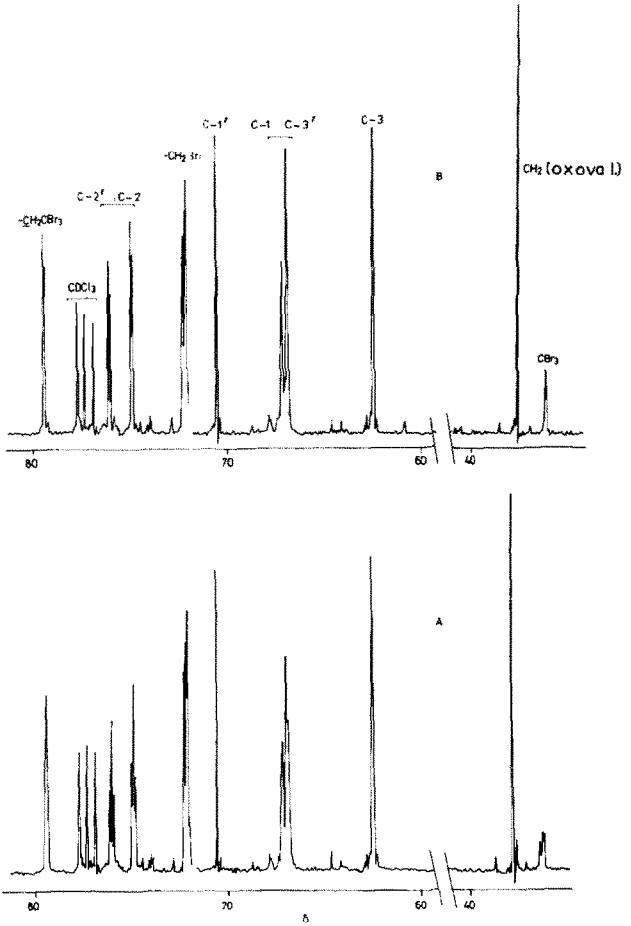
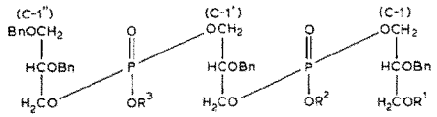


Fig. 1. Part of the ^{13}C-n.m.r. spectrum of **19**, recorded at 75 MHz in a probe with a double-tuned, decoupling coil for ^{1}H (300 MHz) and ^{31}P (121 MHz): (A) ^{1}H-Decoupled ^{13}C-n.m.r. spectrum. (B) ^{1}H-Decoupled and selectively ^{31}P-decoupled ^{13}C-n.m.r. spectrum. Decoupling was performed by external radiation (0.1 W) at the P resonance frequency.

lent yield. Removal of the 4-oxovaleryl group from **17** with hydrazine[27] gave the nonterminal unit **6**, whereas removal of the 1-propenyl group gave the nonterminal unit **7**. The nonterminal unit **6** was also prepared in excellent yield by isomerization of the allyl group of **16** with the aforementioned iridium catalyst.

The bifunctional phosphorylating reagents **8** and **9** that were used for the formation of the phosphoric triester function were obtained by treating the corresponding phosphoric dichloride derivatives[28,29] with two equivalents of 1-hydroxybenzotriazole in the presence of an equimolar amount of pyridine. In the first step of the synthesis leading to the partially protected glycerol trimer **23**, the two nonterminal units **6** and **7** were coupled with the phosphorylating reagent **8**, as will be explained later. Thus, treatment of the nonterminal unit **6** with an equimolar amount of **8** completely converted **6** into intermediate **18**. An excess of **7** and 1-methylimidazole were added to the crude reaction mixture to afford **19** in a high yield. The identity and homogeneity of dimer **19** was unambiguously ascertained by ^{1}H-, ^{31}P-, and ^{13}C-n.m.r. spectroscopy. The H-decoupled ^{13}C-n.m.r., and the H- and P-decoupled ^{13}C-n.m.r. spectra of **19** are illustrated in Fig. 1A and B, respectively. The resonance of the methylene carbon atom of the tribromoethyl group (δ 79.4) appeared as a broad singlet in spectrum 1A, but as two sharp singlets (diastereomeric mixture) in spectrum 1B. It is also evident that the ^{13}C-resonances of C-1, 3′, 2, and 2′, and CBr_3 are less complex in spectrum 1B than in spectrum 1A. Dimer **19** contains four different protective groups: two permanent (*i.e.*, benzyl and tribromoethyl) and two temporary (*i.e.*, 1-propenyl and 4-oxovaleryl). For the successful synthesis of the partially-protected trimer **23** and the fully-protected LTC fragment **25**, it was essential that (a) each of the two temporary protective groups be removed selectively in the presence of the others, and (b) the partially deblocked dimer **21** and trimer **23** having a free primary hydroxyl group be stable under the conditions required for the introduction of the other triester function. The 1-propenyl group was removed from dimer **19** with mercuric chloride–mercuric oxide[24] to afford **21**. The identity and homogeneity of **21** was ascertained by ^{1}H- and ^{31}P-n.m.r. spectroscopy. The 4-oxovaleryl group also could be split off from **19** with hydrazine to give **20**.

For the synthesis of the fully-protected trimer **22**, **5** was phosphorylated with reagent **9** to give a phosphoric triester intermediate of **5**. Dimer **21** and *N*-methylimidazole were added to the phosphoric triester to afford **22**, the ^{1}H- and ^{31}P-n.m.r. spectra of which were in complete accordance with the proposed struc-



22 R^1 = Ova, R^2 = CH_2CBr_3, R^3 = $C_6H_4Cl(2)$
23 R^1 = H, R^2 = CH_2CBr_3, R^3 = $C_6H_4Cl(2)$

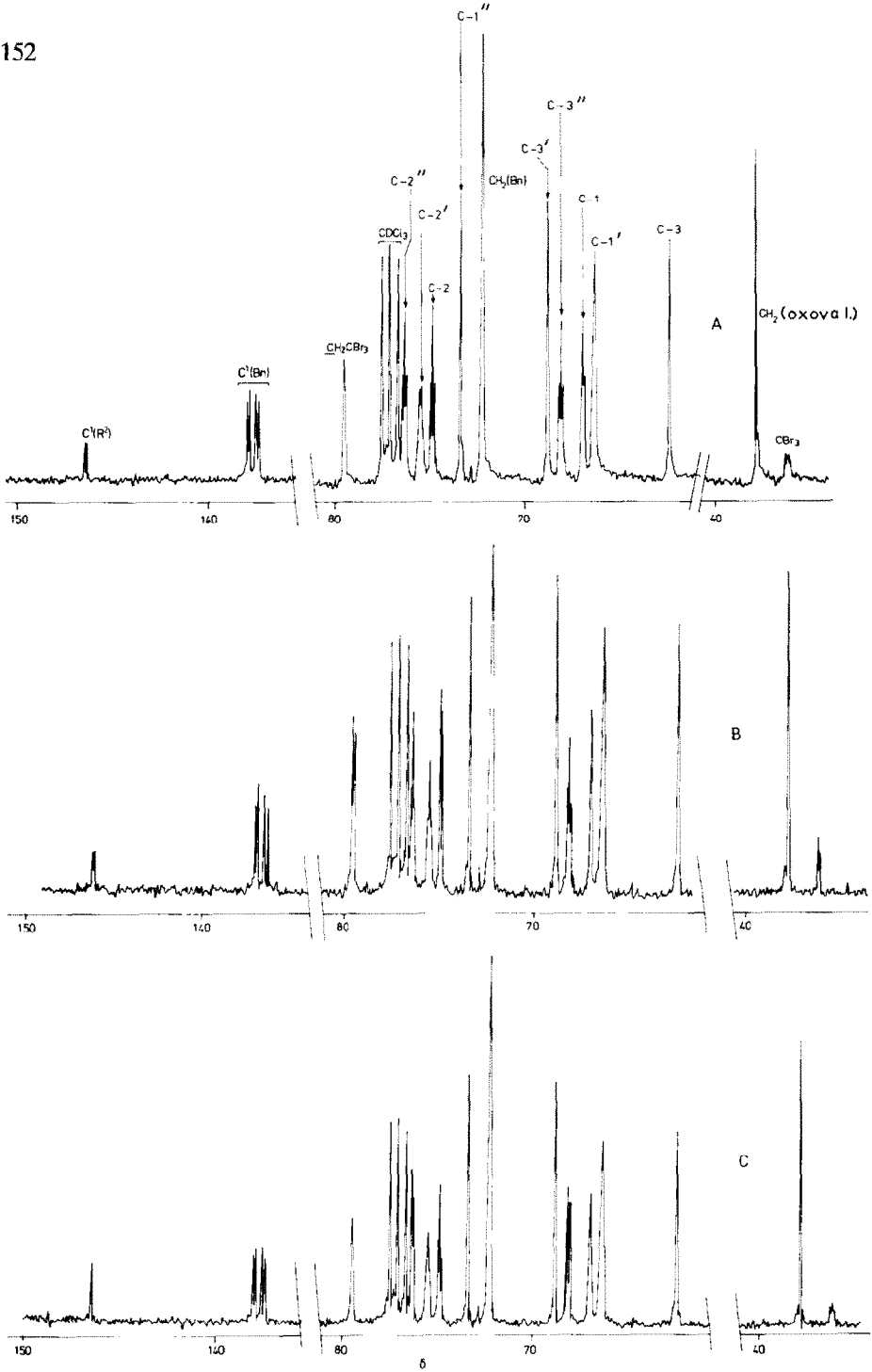
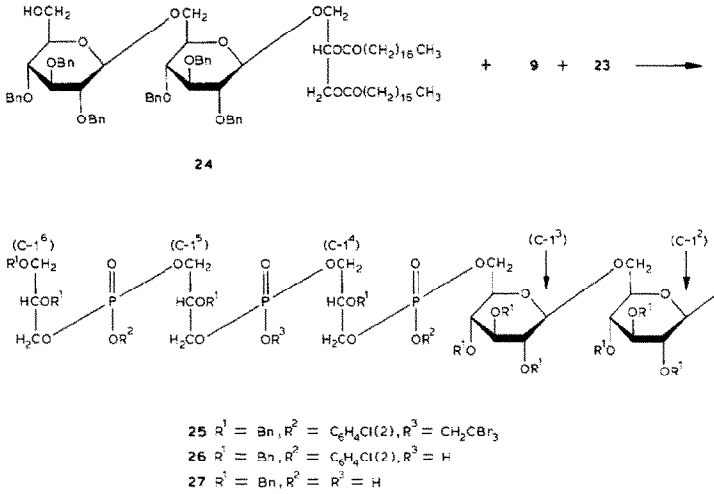


Fig. 2. Part of the ^{13}C-n.m.r. spectrum of **22**, recorded at 75 MHz in a probe with a double-tuned, decoupling coil for ^{1}H (300 MHz) and ^{31}P (121 MHz): (A) ^{1}H-Decoupled ^{13}C-n.m.r. spectrum. (B) ^{1}H-Decoupled and selectively ^{31}P-decoupled ^{13}C-n.m.r. spectrum; decoupling was performed by external radiation (0.1 W) at the P-OCH_2CBr_3 P resonance frequency. (C) ^{1}H-Decoupled and selectively ^{31}P-decoupled ^{13}C-n.m.r. spectrum; decoupling was performed by external radiation (0.1 W) at the P-OC_6H_4 Cl P resonance frequency.



ture. In the ^{13}C-n.m.r. spectrum (see Fig. 2A), selective P-decoupling of the tribromoethyl triester function (Fig. 2B) led to the appearance of less-complex resonances (Fig. 2A) for C-1,2,2′,3′, and carbon atoms of the tribromoethyl group. Following selective decoupling of the 2-chlorophenyl group (Fig. 2C), the spectrum contained less-complex resonances for C1′,2′,2″,3″, and C-2,6 of the 2-chlorophenyl group. The smooth formation of **22** showed that phosphorylation of **21** did not lead to neighboring group participation of OH-1′ with the tribromoethylphosphoric triester function. Short hydrazinolysis[27] of **22** afforded the partially-protected trimer **23** in an excellent yield for the synthesis of the fully-protected teichoic

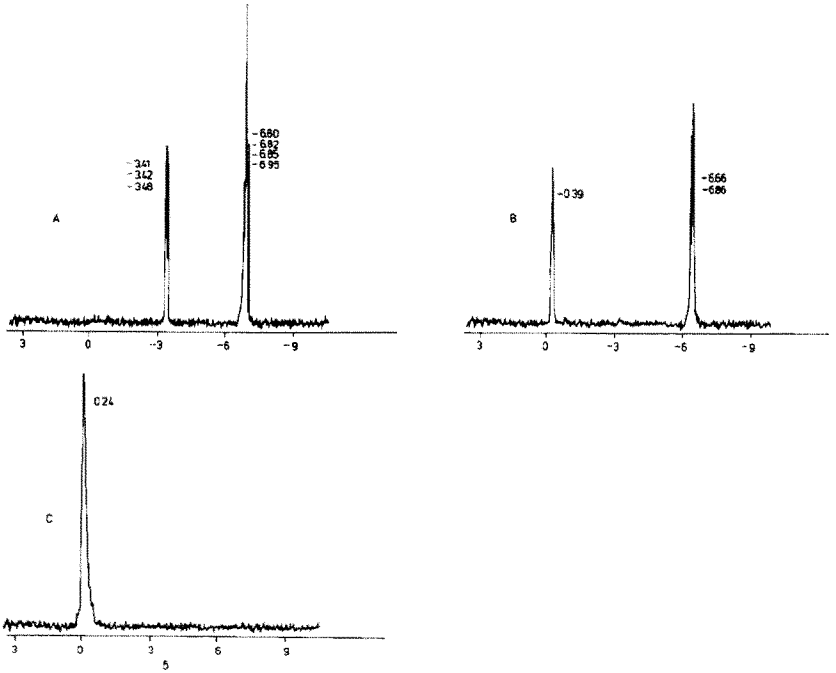


Fig. 3. ^{31}P-N.m.r. spectrum of **25** (A), **26** (B), and **4** (C).

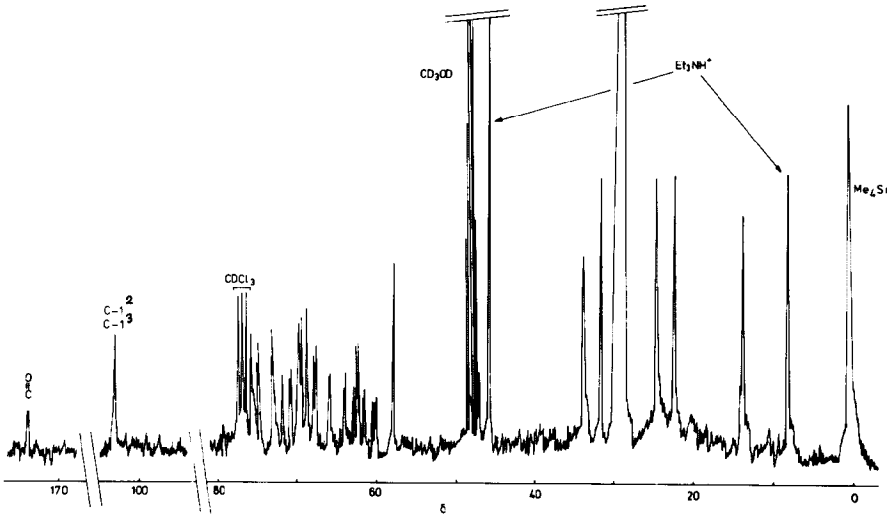


Fig. 4. ^{13}C-N.m.r. spectrum of **4**.

acid fragment **25**. The partially-protected glycolipid **24** was treated with phosphorylating agent **9**, followed by the addition of **23**. The ^{1}H-, ^{13}C- and ^{31}P-N.m.r. data were in complete agreement with the structure of **25**. The deblocking of the fully-protected LTC-fragment **25** was realized in three steps: (a) The tribromoethyl group was removed by treatment with activated Zn dust[30] in the presence of 2,4,6-triisopropylbenzenesulfonic acid to afford **26**. (b) The 2-chlorophenyl groups were removed from **26** under rigorous exclusion of moisture by treatment with (*E*)-pyridine-2-carbaldehyde oxime and N^1,N^1,N^3,N^3-tetramethylguanidine[31] to afford **27**. The complete, two-step deblocking process of the phosphate groups was monitored by ^{31}P-n.m.r. spectroscopy (see Fig. 3). (c) Finally, hydrogenolysis of **27** in the presence of palladium-on-carbon afforded the completely deblocked lipoteichoic acid fragment **4**, the ^{13}C-n.m.r. spectrum of which is illustrated in Fig. 4.

EXPERIMENTAL

*General methods**. — Optical rotations were measured with a Perkin–Elmer 141 polarimeter. ^{1}H-N.m.r. spectra were recorded at 100 MHz with a Jeol JNMPS-100 spectrometer or at 300 MHz with a Brucker WM-300 spectrometer, equipped with an ASPECT-2000 computer, operating in the Fourier-transform mode. Chemical shifts (δ) are given relative to the signal of tetramethylsilane (Me_4Si) as internal standard. ^{13}C- and ^{31}P-N.m.r. spectra were recorded at 25.15 MHz and 40.48 MHz, respectively, with a Jeol JNMFT-100 spectrometer equipped with an EC-100 com-

*The purity of most compounds described was not ascertained by elemental analysis (Editor).

puter, operating in the Fourier-transform mode. The H- and P-decoupled ^{13}C-n.m.r. chemical shifts (δ) are given relative to the signal of Me_4Si as internal standard. ^{31}P-N.m.r. chemical shifts (δ) are given relative to the signal of 85% H_3PO_4 as external standard. Column chromatography[32] was performed on Merck Kieselgel 60 (<230 mesh ASTM). Schleicher & Schüll DC Fertigfolien F 1500 LS 254 were used for t.l.c. analysis in several solvent systems. Compounds were detected with 1:4 H_2SO_4–methanol and charring at 140° for a few min, or with molybdatophosphoric acid (25 g) in 20:1 acetic acid–H_2SO_4, and $KMnO_4$ (1%) in K_2CO_3 (2%) when 1-propenyl or allyl ethers were present. Oxolane, 1,4-dioxane, and pyridine were dried by boiling under reflux in the presence of CaH_2 for 16 h, and then distilling. Pyridine was distilled in the presence of *p*-toluenesulfonyl chloride (60 g/L). Oxolane was redistilled in the presence of $LiAlH_4$ (5 g/L). Dichloromethane was washed successively with conc. H_2SO_4, water, and 10% aqueous $NaHCO_3$, dried ($CaCl_2$), boiled under reflux in the presence of CaH_2 and distilled. *N,N*-Dimethylformamide was stirred with CaH_2 for 16 h and distilled under reduced pressure. All solvents were stored over molecular sieves 4A. 1-Hydroxybenzotriazole was dried (P_2O_5) *in vacuo* for 70 h at 50°. HgO and $HgCl_2$ were dried (P_2O_5) *in vacuo* for a few hours at 40°. Evaporations were carried out under reduced pressure (2 kPa or 70 Pa, bath temperature <40°). All products were stored at −20°.

*1-O-Allyl-2-O-benzyl-*sn*-glycerol* (**16**). — To a suspension of sodium hydride (1.98 g, 82.5 mmol) and **14** (ref. 19, 8.0 g, 21.4 mmol) in dry *N,N*-dimethylformamide (30 mL) was added dropwise benzyl bromide (8.7 mL, 60 mmol) during 30 min at 0°. After 12 h at 20°, t.l.c. (1:3 ether–light petroleum) showed complete conversion of **14** (R_F 0.35) into **15** (R_F 0.44). Excess NaH was eliminated and the reaction mixture evaporated to dryness. Chloroform (100 mL) was added and the organic layer was washed with water (50 mL), dried ($MgSO_4$), and evaporated. The crude product was immediately detritylated with methanolic hydrogen chloride (200 mL, M HCl) in 1,4-dioxane (40 mL). After 20 min, t.l.c. analysis (chloroform) showed the reaction to be complete and triethylamine (30 mL) was added to make the reaction mixture neutral. After evaporation of the solvents, the residue was extracted with chloroform (250 mL), washed with water (75 mL), dried ($MgSO_4$), and evaporated. The crude product was purified on a column of Kieselgel (100 g) suspended in chloroform. The column was eluted with chloroform–methanol (10:0→9:1) and, after evaporation of the appropriate fractions, **16** was obtained as a light-yellow oil (3.78 g, 79%), $[\alpha]_D^{25}$ −20.8° (*c* 1, chloroform); R_F 0.18 (chloroform); ^1H-n.m.r. ($CDCl_3$): δ 3.2 (b, 1 H, OH), 3.5 (m, 5 H, glycerol), 3.9 (dd, 2 H, CH_2=CH-CH_2), 4.5 (s, 2 H, $C_6H_5CH_2$), 5.0–5.3 (m, 2 H, CH_2=CH-CH_2), 5.5–6.0 (m, 1 H, CH_2=CH-CH_2), 7.1–7.3 (s, 5 H, $C_6H_5CH_2$); ^{13}C-n.m.r.: δ 62.5 (s, CH_2OH), 70.1 (s, CH_2=CH-CH_2), 72.0, 72.2 (s, $C_6H_5CH_2$, CH_2O-allyl), 78.2 (s, HCO-Bn), 116.9 (s, CH_2=CH), 127.6–128.3 (s, C-2–C-6 Bn), 134.5 (s, CH_2=CH), 138.3 (C-1 Bn).

*2-O-Benzyl-1-O-(1-propenyl)-*sn*-glycerol* (**6**). — (*a*) *By isomerization of* **16** *with 1,5-cyclooctadienebis[methyl (diphenyl)phosphine]iridinium hexafluorophos-*

phate. To a solution of **16** (0.7 g, 3.2 mmol) in freshly distilled, peroxide-free oxolane was added the iridium catalyst (5 mg). The stirred solution was degassed, placed under dry and oxygen-free nitrogen, and degassed once more. The catalyst was activated by hydrogen during which operation the slightly red suspension became colorless. To effect isomerization, the solution was degassed again after 5 min, and kept for 2 h at 20° under an atmosphere of dry and oxygen-free nitrogen. T.l.c. (chloroform) showed complete conversion of the allyl ether **16** (R_F 0.18) into **6** (R_F 0.19). The solvent was evaporated, the residual oil dissolved in chloroform (50 mL), and the solution washed with 10% aqueous $NaHCO_3$ (10 mL), and then water (10 mL). The dried ($MgSO_4$) organic layer was evaporated and the residue applied to a column of Kieselgel (15 g) suspended in 49:1 chloroform–methanol. After elution and evaporation of the appropriate fractions, **6** was obtained as a colorless oil (0.65 g, 93%), $[\alpha]_D^{25}$ −27.3° (*c* 1, chloroform); R_F 0.19 (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.50–1.60 (dd, 3 H, J_{CH-CH} 6, $J_{CH=C-CH_3}$ 2 Hz, CH_3–C=C), 2.9 (b, 1 H, OH), 3.6–4.8 (m, 8 H, glycerol, $CH_2C_6H_5$ and C–CH=C), 5.8–5.9 (dd, 1 H, $J_{HC=CH}$ 13, $J_{CH=C-C}$ 2 Hz, C=CH–O), 7.1–7.3 (s, 5 H, $C_6H_5CH_2$); ^{13}C-n.m.r.: δ 9.25 (s, CH_3–C=C), 61.73 (s, C-3), 71.68 (s, $C_6H_5CH_2$), 72.17 (s, C-1), 78.39 (s, C-2), 100.99 (s, CH_3–C=C), 127.65–128.32 (s, C-2–C-6 Bn), 138.24 (s, C-1 Bn), 145.82 (s, O–C=C).

Anal. Calc. for $C_{13}H_{17}O_3$: C, 70.24; H, 7.71. Found: C, 70.04; H, 7.66.

(b) By isomerization of **16** *with potassium* tert*-butoxide.* To a solution of **16** (3.14 g, 14.1 mmol) in dimethyl sulfoxide (50 mL) was added potassium *tert*-butoxide (3.17 g, 28.3 mmol). After stirring for 2 h at 100°, t.l.c. (chloroform) showed conversion of **16** (R_F 0.18) into **6** (R_F 0.19). Water was added and the mixture extracted with ether (3 × 150 mL). The organic layer was washed with a saturated solution of NaCl (75 mL) and dried ($MgSO_4$). After evaporation of the solvent, the residue was dissolved in ether (2 mL) and applied to a column of Kieselgel (100 g) suspended in ether. After elution with the same solvent and evaporation of the appropriate fractions, **6** was obtained as a colorless oil (2.77 g, 89%), $[\alpha]_D^{25}$ −26.9° (*c* 1, chloroform); R_F 0.19 (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.5–1.6 (m, 3 H, CH_3–C=C), 2.5 (t, 1 H, $J_{H,OH}$ 6 Hz, OH), 3.5–4.7 (m, 8 H, glycerol, $C_6H_5CH_2$, and C–CH=C), 5.8–5.9 (m, 1 H, O–CH=C), 7.2 (s, 5 H, $C_6H_5CH_2$).

(c) By hydrazinolysis of **17**. To a solution of **17** (0.32 g, 1 mmol) in pyridine (10 mL) was added 12:8:1 pyridine–acetic acid–hydrazine hydrate (10 mL), and the mixture stirred for 10 min at 35°. Chloroform (100 mL) was added and the mixture washed with water (2 × 50 mL), 10% aqueous $NaHCO_3$ (50 mL), and water (50 mL). The organic layer was dried ($MgSO_4$), evaporated to an oil, and toluene (2 × 10 mL) and absolute ethanol (2 × 10 mL) were added and evaporated. Crude compound **6** was purified on a column of Kieselgel (10 g) suspended in 49:1 chloroform–methanol. After evaporation of the appropriate fractions, the product was obtained as a colorless oil (0.20 g, 91%). T.l.c. analysis, optical rotation, and n.m.r. spectra were in accordance with compound **6** obtained from **16** (*a* and *b*).

*2-O-Benzyl-3-O-(4-oxovaleryl)-1-O-(1-propenyl)-*sn*-glycerol* (**17**). — *(a)*

From **6**. Treatment of **6** (2.2 g, 10 mmol) with 4-oxovaleric anhydride in the same way as will be described in the next paragraph, afforded **17** (2.7 g, 86%), $[\alpha]_D^{25}$ −2.8° (*c* 1, chloroform); R_F 0.41 (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.5–1.6 (m, 3 H, CH_3–C=C), 2.1 (s, CH_3 oxoval.), 2.4–2.7 (m, 4 H, CH_2CH_2), 3.5–3.8 (m, 3 H, H-1,2), 4.2 (m, 2 H, H-3), 4.6 (s, 2 H, $C_6H_5CH_2$), 4.5–4.9 (m, 1 H, C–CH=C), 6.2–6.3 (m, 1 H, O–CH=C), 7.3 (s, 5 H, $C_6H_5CH_2$).

(b) From **16**. To a solution of **16** (1.62 g, 7.3 mmol) in pyridine (25 mL) were added M 4-oxovaleric anhydride[22] in 1,4-dioxane (17.4 mL) and a catalytic amount of 4-dimethylaminopyridine. After 3 h at 20°, water (5 mL) was added and the solvent evaporated to a small volume. Chloroform (100 mL) was added and the organic layer washed with 10% aqueous $NaHCO_3$ (50 mL), water (50 mL), dried ($MgSO_4$), and evaporated. The crude product was applied to a column of Kieselgel (40 g) and eluted with 1:4 ether–light petroleum to give 1-*O*-allyl-2-*O*-benzyl-3-*O*-(4-oxovaleryl)-*sn*-glycerol as an oil (2.0 g, 86%), $[\alpha]_D^{25}$ −3.4° (*c* 1, chloroform); R_F 0.40 (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 2.18 (s, 3 H, CH_3), 2.4–2.72 (m, 4 H, CH_2CH_2), 3.46–3.5 (d, 2 H, H-1),3.68–3.8 (dd, 1 H, H-2), 3.9–4.4 (dd, 2 H, CH_2–C=C), 4.12–4.24 (t, 2 H, H-3), 4.6 (s, 2 H, $C_6H_5CH_2$), 5.0–5.3 (m, 2 H, CH_2=C–), 5.7–6.1 (m, 1 H, –CH=C), 7.2–7.4 (s, 5 H, $C_6H_5CH_2$); ^{13}C-n.m.r.: δ 27.3 (s, CH_2CO), 29.0 (s, CH_3), 37.1 (s, CH_2CO_2), 63.4 (s, C-3), 69.0 (s, CH_2=CH), 71.5 (s, C-1), 71.6 (s, $C_6H_5CH_2$), 75.2 (s, C-2), 116.2 (s, CH_2=), 127.0–127.7 (s, C-2–C-6 Bn), 134.0 (s, -CH=), 137.8 (s, C-1 Bn), 171.8 (s, OC=O, 4-oxoval.), 205.6 (s, CH_3C=O, 4-oxoval.).

1-*O*-Allyl-2-*O*-benzyl-3-*O*-(4-oxovaleryl)-*sn*-glycerol (1.92 g, 6 mmol) was treated as described for the isomerization of **16**. After purification by short-column chromatography (eluent chloroform), **17** was obtained as a homogeneous oil (1.86 g, 97%), $[\alpha]_D^{25}$ −2.9° (*c* 1, chloroform); R_F 0.41 (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.5 (dd, 3 H, CH_3–C=C), 2.1 (s, 3 H, CH_3 4-oxoval.), 2.4–2.7 (m, CH_2CH_2), 3.5–3.8 (m, 3 H, H-1,2), 4.2 (m, 2 H, H-3), 4.6 (s, 2 H, $C_6H_5CH_2$), 4.5–4.9 (m, 1 H, =CH–CH_3), 6.2 (dd, 1 H, $J_{CH=CH}$ 12.8, $J_{CH=C-CH_3}$ 1.5 Hz, O–CH=), 7.36 (s, 5 H, $C_6H_5CH_2$); ^{13}C-n.m.r.: δ 9.1 (s, CH_3–CH=CH), 27.8 (s, CH_2=O), 29.7 (s, CH_3C=O), 37.8 (s, CH_2CO_2), 63.4 (s, C-3), 71.4 (s, $C_6H_5CH_2$), 72.3 (s, C-2), 75.6 (s, C-2), 101.4 (s, =CH–CH_3), 128.0–128.4 (s, C-2–C-6 Bn), 138.0 (s, O–CH=), 172.4 (s, OC=O), 206.2 (s, C=O).

2-O-Benzyl-3-O-(4-oxovaleryl)-sn-*glycerol* (**7**). — Compound **17** (1.60 g, 5 mmol) was dissolved in a mixture of acetone (60 mL) and water (4 mL). Mercuric oxide (1.08 g, 5 mmol) and mercuric chloride (1.73 g, 5 mmol) were added, and the solution was stirred for 30 min at 20°. T.l.c. (9:1 chloroform–acetone) indicated complete removal of the 1-propenyl group (R_F 0.7) to give **7** (R_F 0.3). Mercuric oxide was removed by filtration, acetone evaporated, and chloroform (100 mL) added to the residue. The chloroform layer was washed with a half saturated aqueous solution of KI (4 × 25 mL), dried ($MgSO_4$), and evaporated. The crude product was dissolved in ether and applied to a column of Kieselgel (25 g) suspended in the same solvent to afford **7** as a viscous oil (1.40 g, 100%), $[\alpha]_D^{25}$ −10.4° (*c* 1, chloro-

form); R_F 0.40 (ether); ^{1}H-n.m.r. ($CDCl_3$): δ 2.2 (s, 3 H, CH_3), 2.4–2.5 (b, 1 H, OH), 2.5–2.9 (m, 4 H, CH_2CH_2), 3.7 (m, 3 H, H-1,2), 4.3 (m, 2 H, H-3), 4.68 (d, 2 H, $C_6H_5CH_2$), 7.41 (s, 5 H, $C_6H_5CH_2$); ^{13}C-n.m.r.: δ 27.7 (s, CH_2CO), 29.6 (s, CH_3), 37.7 (s, CH_2CO_2), 61.5 (s, C-1), 63.1 (s, C-3), 71.9 (s, $C_6H_5CH_2$), 206.5 (s, C=O).

2-O-Benzyl-3-O-(4-oxovaleryl)-sn-*glycer-1-yl 2-O-benzyl-1-O-(1-propenyl)*-sn-*glycer-3-yl 2,2,2-tribromoethyl phosphate* (**19**). — 2,2,2-Tribromoethyl bis(1-hydroxybenzotriazolyl) phosphate (**8**) was prepared by adding dropwise a solution of 2,2,2-tribromoethylphosphoric dichloride[28] (3.20 g, 8 mmol) in dry oxolane (5 mL) to a mixture of 1-hydroxybenzotriazole (2.23 g, 26.5 mmol) and pyridine (1.33 mL, 16.5 mmol) in dry oxolane (35 mL). The mixture was stirred for 20 min at 0° and then for 1.5 h at 20°. The pyridinium chloride was filtered off to give a stock solution of reagent **8**. Compound **6** (0.89 g, 4 mmol) was dissolved in oxolane (5 mL) and pyridine (0.32 mL, 4 mmol), and **8** (20 mL, 4 mmol) was added. The mixture was stirred for 20 min at 20° when t.l.c. (9:1 chloroform–acetone) indicated complete conversion of **6** (R_F 0.4) into base-line material (the hydrolyzed derivative of **18**). To this solution was added **7** (1.53 g, 5.5 mmol) in oxolane (5 mL) and 1-methylimidazole (1 mL). The mixture was stirred for 2.5 h at 20° when t.l.c. (19:1 chloroform–acetone) showed a major product with R_F 0.49 together with two minor spots. The mixture was diluted with chloroform (150 mL) and washed twice with aqueous M triethylammonium hydrogencarbonate (50 mL, pH 7.5). The dried ($MgSO_4$) organic layer was evaporated and the residue applied to a column of Kieselgel (80 g) suspended in chloroform. Elution of the products with chloroform and 19:1 chloroform–acetone afforded **19** (3.09 g, 93%), $[\alpha]_D^{25}$ +1.7° (*c* 1, chloroform); R_F 0.49 (19:1 chloroform–acetone); ^{1}H-n.m.r. ($CDCl_3$): δ 1.55–1.61 (m, 3 H, CH_3CH=CH), 2.17 (s, 3 H, CH_3C=O), 2.55–2.78 (m, 4 H, CH_2CH_2), 3.7–4.8 (m, 17 H, 2 glycerol, 2 $C_6H_5CH_2$, CH_2CBr_3, and CH=C–O), 5.9–6.0 (m, 1 H, O-CH=C), 7.2–7.4 (b, 10 H, 2 $C_6H_5CH_2$); ^{13}C-n.m.r.: δ 9.26 (s, CH_3C=C), 27.72 (s, CH_2C=O), 29.71 (s, CH_3C=O), 36.40, 36.28, 36.16 (t, $^3J_{C-P}$ 9.06 Hz, CH_2CBr_3), 37.73 (s, CH_2CO_2), 62.43 (s, C-3), 67.15, 67.08, 66.93, 66.86 (2 dd, $^2J_{C-P}$ 5.0, $^2J_{C-P}$ 5.8 Hz, C-3′,1), 70.54 (s, C-1′), 72.28, 72.24, 72.15, 72.09 (s, 2 $C_6H_5CH_2$), 76.11, 76.02, 75.93, 74.79, 74.88, 74.79 (2 t, $^3J_{C-P}$ 7.1, $^3J_{C-P}$ 6.6 Hz, C-2′,2), 79.40 (b, CH_2CBr_3), 101.54 (s, C–C=), 127.63, 127.80, 128.23, 128.40 (m, 2 C-2–C-6 Bn), 137.55, 137 75 (s, 2 C-1 Bn), 145.56 (s, O–C=), 172.27 (s, OC=O), 206.26 (s, C=O); ^{31}P-n.m.r.: δ −3.18, −3.21 (s, 2 $POCH_2CBr_3$).

2-O-Benzyl-sn-*glycer-1-yl 2-O-benzyl-1-O-(1-propenyl)*-sn-*glycer-3-yl 2,2,2-tribromoethyl phosphate* (**20**). — Compound **19** (25 mg, 30 μmol) was treated with hydrazine hydrate in the same way as described for the synthesis of **6**. T.l.c. (19:1 chloroform–methanol) showed complete conversion of **19** (R_F 0.69) into **20** (R_F 0.62). The crude product was purified on a column of Kieselgel (1 g; eluent: 49:1 chloroform–methanol) to give **20** as an oil; ^{31}P-n.m.r.: ($CDCl_3$): δ −3.16, −3.11 (s, 2 $POCH_2CBr_3$).

2-O-Benzyl-sn-*glycer-3-yl 2-O-benzyl-3-O-(4-oxovaleryl)*-sn-*glycer-1-yl 2,2,2-tribromoethyl phosphate* (**21**). — To a solution of **19** (1.24 g, 1.5 mmol) in

acetone (20 mL) and water (1 mL) were added mercuric oxide (330 mg, 1.5 mmol) and mercuric chloride (360 mg, 1.5 mmol). The mixture was stirred for 45 min at 20° when t.l.c. (19:1 chloroform–methanol) revealed complete conversion of the starting material (R_F 0.69) into **21** (R_F 0.5). The mixture was processed as described for the synthesis of **7**. The crude product was purified by a colum chromatography on Kieselgel 60 (20 g) suspended in 39:1 chloroform–methanol. Elution with the same solvent mixture gave **21** as a viscous oil (1.15 g, 97%); R_F 0.5 (19:1 chloroform–methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 2.12 (s, 3 H, CH_3C=O), 2.4–2.8 (m, 4 H, CH_2CH_2), 3.6–4.8 (m, 17 H, 2 glycerol, 2 $C_6H_5CH_2$, CH_2CBr_3, and OH), 7.2–7.3 (b, 10 H, 2 $C_6H_5CH_2$); ^{31}P-n.m.r.: δ −2.81, −2.86 (s, 2 $POCH_2CBr_3$).

*2-O-Benzyl-3-O-(4-oxovaleryl)-*sn*-glycerol-[(1→3)-2,2,2-tribromoethylphospho]-2-O-benzyl-*sn*-glycerol-[(1→3)-2-chlorophenylphospho]-2,3-di-O-benzyl-*sn*-glycerol* (**22**). — To 1,2-di-*O*-benzyl-*sn*-glycerol (**5**; 0.55 g, 2.0 mmol) in oxolane (5 mL) was added, dropwise at 20°, a stock solution of phosphorylating agent **9** in oxolane (0.2M, 11 mL), which was prepared from 2-chlorophenylphosphonic dichloride[28,29] as described earlier for the synthesis of **8**. After 30 min, t.l.c. (9:1 chloroform–acetone) revealed complete conversion of the starting material (R_F 0.4) into baseline material (due to the hydrolysis of the benzotriazole function). To this solution was added dropwise **21** (0.7 g, 1 mmol) in oxolane (3 mL) and 1-methylimidazole (1 mL). After 1.5 h, t.l.c. (9:1 chloroform–acetone) showed the reaction to be complete. The mixture was diluted with chloroform (75 mL), and washed successively with aqueous M triethylammonium hydrogen carbonate (2 × 25 mL, pH 7.5) and water (25 mL). The organic layer was dried ($MgSO_4$) and evaporated to give an oil. The crude product was dissolved in chloroform (2 mL) and applied to a column of Kieselgel (20 g) suspended in 19:1 chloroform–acetone. Elution with the same solvent mixture gave **22** as a viscous oil (1.14 g, 92%), $[\alpha]_D^{25}$ −2° (*c* 1, chloroform); R_F 0.39 (9:1 chloroform–acetone); ^{1}H-n.m.r. ($CDCl_3$): δ 2.1 (s, 3 H, CH_2C=O), 2.4–2.7 (m, 4 H, CH_2CH_2), 3.4–4.6 (m, 25 H, 3 glycerol, 4 $C_6H_5CH_2$, CH_2CBr_3), 6.9–7.3 (m, 24 H; 4 $C_6H_5CH_2$ and C_6H_4Cl); ^{13}C-n.m.r.: δ 27.76 (s, CH_2C=O), 29.77 (s, CH_3C=O), 36.39, 36.31, 36.22, 36.14 (dd, $^3J_{C-P}$ 6.04 Hz, CBr_3), 37.80 (s, CH_2CO_2), 62.47 (s, C-3), 66.38 (b, C-1′,3′), 67.02 66.94 (b, C-1), 72.23, 73.33 (s, $C_6H_5CH_2$), 74.81, 74.90, 74.99 (t, $^3J_{C-P}$ 7.0 Hz, C-1), 75.48, 75.57 (bd, C-2′), 73.27, 73.36, 76.44 (t, $^3J_{C-P}$ 6.40 Hz, C-2″), 79.48 (b, CH_2CBr_3), 121.50 (b, C-6, C_6H_4Cl), 126.07–130.58 (m, C-2–C-6 Bn and C-2–C-5 ClC_6H_4), 137.38, 137.54, 137.89, 137.99 (s, 4 C-1 Bn), 146.41, 146.49 (d, $^2J_{C-P}$ 6.44 Hz, C-1, ClC_6H_4), 172.37 (s, OC=O), 206.36 (s, C=O); ^{31}P-n.m.r.: δ −3.16, −3.17 (s, $POCH_2CBr_3$), −6.55, −6.69, −6.70 (s, PO ClC_6H_4).

*2-O-Benzyl-*sn*-glycerol-[(1→3)-2,2,2-tribromoethylphospho]-2-O-benzyl-*sn*-glycerol-[(1→3)-2-chlorophenylphospho]1,2-di-O-benzyl-*sn*-glycerol* (**23**). — To a solution of **22** (0.72 g, 0.58 mmol) in pyridine (6 mL) was added 12:8:1 pyridine–acetic acid–hydrazine hydrate (6 mL), and the mixture stirred for 10 min at 35°. Chloroform (100 mL) was added, and the mixture washed successively with water

(2 × 50 mL), 10% aqueous $NaHCO_3$ (50 mL), and water (50 mL). T.l.c. (9:1 chloroform–acetone) showed complete conversion of the starting material **22** (R_F 0.39) into **23** (R_F 0.2). This was purified in a column of Kieselgel (15 g) suspended in 9:1 chloroform–acetone. Elution with the same solvent mixture gave **23** (602 mg, 91.4%), R_F 0.21 (9:1 chloroform–acetone); ^{1}H-n.m.r. ($CDCl_3$): δ 3.4–3.6 (m, 26 H, 3 glycerol, 4 $C_6H_5CH_2$, CH_2CBr_3, OH), 6.9–7.3 (m, 24 H, 4 $C_6H_5CH_2$ and C_6H_4Cl); ^{13}C-n.m.r.: δ 36.18 (b, CH_2CBr_3), 60.66 (s, C-3), 66.39, 66.58 (d, C-1',3'), 66.95, 67.24, (d, C-1), 68.15, 68.27, 68.37 (t, $^2J_{C-P}$ 6.9 Hz, C-3''), 68.72 (s, C-1''), 72.07, 72.12, 72.20, 72.28, 72.34, 73.40, (m, $C_6H_5CH_2$), 75.37, 75.47 (bd, C-2'), 76.21, 76.29, 76.39 (t, $^3J_{C-P}$ 6 Hz, C-2''), 77.42, 77.50, 77.58 (t, $^3J_{C-P}$ 6 Hz, C-2), 79.52 (b, CH_2CBr_3), 121.52 (b, C-6 C_6H_4Cl), 126.19–130.59 (m, C-2–C-6 Bn, C-2–C-5 C_6H_4Cl), 137.33, 137.76, 137.81, 137.92 (s, 4 C-1 Bn), 146.30, 146.38 (d, $^2J_{C-P}$ 6.24 Hz, C-1 C_6H_4Cl).

Anal. Calc. for $C_{45}H_{50}Br_3ClO_{13}P_2$: P, 5.45. Found: P, 5.30.

[*1,2-Di-*O*-stearoyl-*sn*-glycer-3-yl* O-*(2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-β-*D*-glucopyranoside*]-[*(6→3)-2-chlorophenylphospho*]-2-O-*benzyl*-sn-*glycerol*-[*(1→3)-2,2,2-tribromoethylphospho*]-2-O-*benzyl*-sn-*glycerol*-[*(1→3)-2-chlorophenylphospho*]-*1,2-di-*O*-benzyl*-sn-*glycerol* (**25**). — To a solution of **24** (0.89 g, 0.60 mmol) in oxolane (2 mL) was added dropwise at 20° 0.2M phosphorylating reagent **9** in oxolane (3.33 mL). After 45 min, t.l.c. (9:1 chloroform–acetone) indicated complete conversion of **24** (R_F 0.69) into baseline material (due to hydrolysis of the benzotriazole function). To this solution were added, dropwise, **23** (0.47 g, 0.41 mmol) in oxolane (3 mL) and 1-methylimidazole (0.5 mL). After 1.5 h, t.l.c. (9:1 chloroform–acetone) showed the disappearance of **23** and a major spot corresponding to **25** (R_F 0.65), together with some baseline material (excess of the phosphorylated compound **24**). The mixture was diluted with chloroform (75 mL) and washed with aqueous M triethylammonium hydrogencarbonate (2 × 25 mL, pH 7.5). The dried ($MgSO_4$) organic layer was evaporated and the crude residue applied to a column of Kieselgel (20 g) suspended in 39:1 chloroform–acetone. Elution with the same solvent mixture gave **25** as a wax (0,96 g, 83%), $[\alpha]_D^{25}$ +2.4° (*c* 1, chloroform); R_F 0.44 (39:1 chloroform–acetone); ^{1}H-n.m.r. ($CDCl_3$): δ 0.9 (t, 6 H, 2 CH_3), 1.2 [m, 56 H, 2 $(CH_2)_n$], 1.4–1.6 (m, 4 H, 2 $CH_2CH_2CO_2$), 2.1–2.3 (m, 4 H, 2 CH_2CO_2), 3.3–5.2 (m, 56 H, 3 glycerol, glyceryl diglucoside, 10 CH_2, CH_2CBr_3), 7.1–7.6 (m, 58 H, 10 $C_6H_5CH_2$, 2 C_6H_4Cl); ^{13}C-n.m.r.: δ 14.14 (s, 2 CH_3), 22.07 (s, 2 CH_3CH_2), 31.89 (s, 2 $CH_3CH_2CH_2$), 27.69–29.68 [m, 2 $(CH_2)_n$], 24.81 (s, 2 $CH_2CH_2CO_2$), 33.97, 34.16 (s, 2 CH_2CO_2), 36.05, 36.20, 36.35 (m, CH_2CBr_3), 66.36, 66.44, (b, C-1^3,3^2), 67.00, 67.38 (d, C-1^2), 67.89–68.47 (m, HCO_2CR, C-3^6,3^3), 68.77 (s, C-1^6), 62.58, 62.75, 69.96, 69 97, 72.15, 72.26, 72.32, 73.37, 74.60, 75.57, 77.68, 77.88, 81.79, 81.92, 84.43 (m, C-2^2,2^1,6^2,6^1, $C_6H_5CH_2$, HCO_2R, H_2CO_2R), 73.86, 74.75 74.93 (t, C-2^2), 76.27, 76.31, 76.40 (t, C-2^6), 76.84, 76.92 (b, C-2^5), 79.40, 79.47 (d, $^2J_{C-P}$ 5.62 Hz, CH_2CBr_3), 103.64, 103.82 (s, C-1^2,1^3), 121.48, 121.64 (d, $^3J_{C-P}$ 10.69 Hz, C-6 C_6H_4Cl), 125.16–130.53 (m, C-2–C-6 Bn and C-2–C-6 C_6H_4Cl), 137.34–138.43

(m, 10 C-1 Bn), 146.37, 146.46 (m, 2 C-1 C_4H_4Cl), 172.87, 173.11, 173.18 (m, 2 C=O); ^{31}P-n.m.r.: δ −3.41, −3.42 −3.48 (m, $POCH_2CBr_3$), −6.80, −6.82, −6.85, −6.90 (m, 2 POC_6H_4Cl).

[*1,2-Di*-O-*stearoyl*-sn-*glycer-3-yl* O-(*2,3,4-tri*-O-*benzyl*-β-D-*glucopyranosyl*)-(*1→6*)-(*2,3,4-tri*-O-*benzyl*-β-D-*glucopyranoside*]-[(*6→3*)-*2-chlorophenylphospho*]-2-O-*benzyl*-sn-*glycerol*-[(*1→3*)-*phospho*]-2-O-*benzyl*-sn-*glycerol*-[(*1→3*)-*2-chlorophenylphospho*]-*1,2-di*-O-*benzyl*-sn-*glycerol* (**26**). — Activated zinc[30] was added to a solution of **25** (0.48 g, 0.17 mmol) and 2,4,6-triisopropylbenzenesulfonic acid (5 mg) in pyridine (0.85 mL). After the addition of 2,4-pentanedione, the temperature rose and, after 5 min, the mixture was filtered to remove excess zinc. T.l.c. (24:1 chloroform–acetone) showed complete conversion of **25** (R_F 0.4) into baseline material. The filtrate was diluted with 9:1 chloroform–methanol (100 mL) and washed with aqueous M triethylammonium hydrogencarbonate (2 × 20 mL, pH 7.5). The organic layer was evaporated and the residue applied to a column of Kieselgel (10 g) suspended in 19:1 chloroform–methanol. Elution of the column with 19:1 → 9:1 chloroform–methanol afforded, after extraction with aqueous M triethylammonium hydrogencarbonate (pH 7.5), the triethylammonium salt of **26** as a colorless oil (0.37 g, 82%), $[\alpha]_D^{25}$ +2.7° (*c* 1, chloroform); R_F 0.44 (23:2 chloroform–methanol); ^{31}P-n.m.r. ($CDCl_3$–CD_3OD): δ −0.39 (b, phosphoric diester), −6.66, −6.86 (2 2-chlorophenyl-protected triesters).

[*1,2-Di*-O-*stearoyl*-sn-*glycer-3-yl* O-(*2,3,4-tri*-O-*benzyl*-β-D-*glucopyranosyl*)-(*1→6*)-(*2,3,4-tri*-O-*benzyl*-β-D-*glucopyranoside*]-[(*6→3*)-*phospho*]-*2*-O-*benzyl*-sn-*glycerol*-[(*1→3*)-*phospho*]-*2*-O-*benzyl*-sn-*glycerol*-[(*1→3*)-*phospho*]-*1,2-di*-O-*benzyl*-sn-*glycerol* (**27**). — Dry 1,4-dioxane (2 × 10 mL) was added to **26** (270 mg, 0.1 mmol) and evaporated, and the residue dissolved in dry oxolane (4 mL). N^1,N^1,N^3,N^3-Tetramethylguanidine (254 mL, 2 mmol) and 2-pyridinealdehyde oxime (366 mg, 3mmol) were added[31]. After 24 h, t.l.c. (23:2 chloroform–methanol) indicated nearly complete conversion of the starting compound (R_F 0.44) into baseline material. The mixture was taken up in 9:1 chloroform–methanol, and washed successively with water (25 mL), 10mM HCl (25 mL), and aqueous M triethylammonium hydrogencarbonate (25 mL, pH 7.5). The organic layer was evaporated to give an oil, which was applied to a column of Kieselgel (10 g) suspended in chloroform. Elution with 10:0 → 4:1 chloroform–methanol afforded **27** which was dissolved in 9:1 chloroform–methanol (50 mL) and extracted with aqueous M triethylammonium hydrogencarbonate (2 × 10 mL, pH 7.5). Evaporation of the filtered organic layer afforded the triethylammonium salt of **27** as a white foam (199 mg, 76%); ^{1}H-n.m.r. ($CDCl_3$): δ 0.9 (t, 6 H, *J* 6 Hz, 2 CH_3), 1.1 (t, 27 H, *J* 7 Hz, 9 CH_3CH_2N), 1.2–1.3 [m, 56 H, 2 $(CH_2)_n$], 1.4–1.6 (m, 4 H, 2 $CH_2CH_2CO_2$), 2.1–2.3 (m, 4 H, 2 CH_2CO_2), 2.9 (q, 18 H, *J* 7 Hz, 9 CH_3CH_2N), 3.3–5.2 (m, 54 H, 3 glycerol, glyceryl diglucoside, 10 $C_6H_5CH_2$), 7.1–7.3 (c, 50 H, 10 $C_6H_5CH_2$); ^{13}C-n.m.r. ($CDCl_3$–CD_3OD): δ 8.36 (s, 9 CH_3CH_2N), 14.15 (s, 2 CH_3), 22.74 (s, 2 CH_3CH_2), 31.98 (s, 2 $CH_3CH_2CH_2$), 29.20–29.77 [m, 2 $(CH_2)_n$], 24.90 (s, 2 $CH_2CH_2CO_2$), 34.12, 34.27 (s, 2 CH_2CO_2), 45.5 (s, 9 CH_3CH_2N),

103.71, 104.01 (s, C-1^2,1^1), 127.50–138.43 (m, C-2–C-6 Bn), 138.07–138.7 (m, C-1 Bn), 173.21, 173.48 (s, 2 C=O); ^{31}P-n.m.r. ($CDCl_3$–CD_3OD): δ −0.24 (b, 3 phosphoric diester).

*[1,2-Di-*O*-stearoyl-*sn*-glycer-3-yl* O-*β*-D-*glucopyranosyl)-(1→6)-β*-D-*glucopyranoside]-[(6→3)-phospho]*-sn-*glycerol-[(1→3)-phospho]*-sn-*glycerol-[(1→3)-phospho]*-sn-*glycerol* (**4**). — Compound **27**(180 mg, 70 μmol) was converted into the sodium form by passing a solution of the triethylammonium salt of **27** in 1:1 methanol–oxolane through a column (10 × 2 cm^2) of Dowex 50W cation-exchange resin (Na^+ 100–200 mesh) suspended in the same solvent mixture. After evaporation of the appropriate fractions, the sodium salt of **27** was dissolved in 2:6:1:1 2-propanol–ethyl acetate–formic acid–acetic acid (20 mL) and hydrogenolyzed in the presence of 10% palladium-on-charcoal (200 mg) at 0.4 MPa for 2 days at 20°. T.l.c. (5:4:1 chloroform–methanol–triethylammonium hydrogencarbonate) showed complete conversion of the benzylated compound (R_F 0.4) into baseline material. The catalyst was filtered off and washed thoroughly with 1:1 chloroform–methanol (100 mL) at 40°. The residue was dissolved in 17:3 chloroform–methanol (50 mL) and extracted with M triethylammonium hydrogencarbonate (10 mL, pH 7.5). Evaporation of the filtered organic layer afforded the triethylammonium salt of **4** as a white. waxy solid; ^{1}H-n.m.r. ($CDCl_3$–CD_3OD): δ 0.9–1.8 [m, 75 H, 2 CH_3, 3 $(CH_2)_{15}$. ($CH_3CH_2)_3$,NH)], 2.2–2.4 (m, 4 H, 2 CH_2CO_2), 3.1 (q, 6 H, *J* 7.5 Hz, 3 CH_2N), 3.0–4.5 (m, 31 H, glyceryl diglucoside except H-1^2,1^3 of glucose and H-2 of glycerol. 3 phosphatidylglycerol), 4.47, 4.49 (2 d, $J_{1^2,2^2}$, $J_{1^3,2^3}$ 6.0 Hz, H-1^1,1^2), 5.2–5.4 (m, 1 H, HCO_2CR); ^{13}C-n.m.r.: δ 8.1, 45.8 (s, 3 Et_3NH), 14.1 (s, 2 CH_3), 22.3 (s, 2 CH_3CH_2-), 31.6 (s, 2 CH_2CO_2), 57.9 (s, C-1^5), 63.0, 68.9, 69.5, 70.0, 72.3, 73.2, 73.3, 75.0, 75.6, 75.8 (s, C-2^2,6^2, 2^3,6^3), 67.7–70.0 (m, C-2^6,2^3,2^2), 62.0–64.7 (m, C-1^2,3^2,1^3,3^3,3^6), 68.0 (s, C-2^2), 62.5 (s, C-1^1), 69.9 (s, C-3^1), 103.29, 103.30 (s, C-1^2,1^1), 173.42, 173.47 (s, 2 C=O); ^{31}P-n.m.r.: δ −0.24 (b, 3 phosphoric diester).

Anal. Calc. for $C_{60}H_{114}Na_3O_{30}P_3$: P, 6.29. Found: P, 6.15.

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