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PHOSPHOTRIESTER APPROACH TO THE SYNTHESIS OF α-GLUCOSYLATED MONO- AND DIPHOSPHATIDYL GLYCEROLS: BACTERIAL CELL-WALL COMPONENTS

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Abstract—Two bifunctional phosphorylating agents, 2,2,2-tribromoethyl and 2-chlorophenyl phosphorodichloridate, were used with 1,2,4-triazole to assemble phosphotriester derivatives of protected α-glucosyl mono- and diphosphatidyl glycerols. Benzyl, allyl and pivaloyl groups were used for the protection of the hydroxyl functions of the glucose and glycerol moieties, respectively. The merits of the pivaloyl group are discussed. Finally, special attention is paid to the conversion of the phosphotriesters into the required phosphodiester functions.

In 1973 Peleg *et al.*[1] isolated α-glucosyl monophosphatidyl glycerol (compound **I** in Fig. 1) from moderately halophilic halotolerant Gram-negative bacteria. A few years later, Fischer *et al.*[2] identified a similar glycophospholipid α-glucosyl diphosphatidyl glycerol (compound **II** in Fig. 1) in group N Streptococci. Other related glycophospholipids contain neutral or amino sugars, which are joined via α or β-linkages to phosphatidyl glycerol,[3-6] or the more complex glyco(phospho)lipids from Streptococci. The latter class of compounds are biosynthetic intermediates or enzymatic digestion products[7-12] of the structurally related lipoteichoic acids.

At the moment, chemical synthesis presents the only way to obtain well-defined naturally occurring and modified glyco(phospho)lipids. Compounds of this type are not only indispensable for the elucidation of glycophospholipid function in membranes, but also for the investigation of their physiological properties. In this paper we wish to present in detail[13] an efficient synthesis of α-glucosylated mono- and diphosphatidyl glycerols (compounds **I** and **II**, respectively, in Fig. 1) via phosphotriester intermediates.

RESULTS AND DISCUSSION

The strategy we adopted for the synthesis of the glycophospholipids **I** and **II** consisted of the following steps: (i) preparation of properly 1,3(3,1)-dihydroxyl protected and optically pure glycerol units containing two different protecting groups (allyl and pivaloyl), which could be removed selectively in the presence of each other (compounds **2a,b** in Scheme 1); (ii) introduction of the α-glycosidic linkage between the secondary hydroxyl group of the suitably protected glycerol units (**2a,b**) and the 2,3,4,6 - tetra - O - benzyl - D - glucopyranosyl unit (compound **4** in Scheme 2); (iii) synthesis of phosphate protected phosphatidic acid derivatives (compounds **11** and **12** in Scheme 3). The latter phosphodiester derivatives were easily accessible by phosphorylation of 1,2-diacyl-sn-glycerol (compounds **9a,b** in Scheme 3) using the phosphoditriazolide method[14,15]; (iv) condensation of the phosphatidyl derivative **11** with the suitably protected α-glucosyl glycerol moiety **7b** or **7a**, to afford the monophosphotriesters **13a** and **13d**, respectively (Scheme 4). Furthermore, selective removal of the prop-1-enyl group from **13d**, followed by coupling with the phosphatidyl derivative **12** (Scheme 4), gave the diphosphotriester intermediate **14a** (Scheme 5); finally, stepwise removal of all protecting groups followed by identification of the deblocked compounds.

Synthesis of the α-glucosyl glycerol units (Schemes 1 and 2)

The synthesis of the protected glycerol derivatives, which are suitable for the condensation with 2,3,4,6 - tetra - O - benzyl - α - D - glucopyranosyl bromide **4** is outlined in Scheme 1. D-Mannitol was firstly converted into 1,2:5,6 - di - O - isopropylidene - D - mannitol,[16,17] which was then cleaved by oxidation with sodium periodate.[18] The resulting glyceraldehyde acetonide was reduced with sodium borohydride.[18] In this way,1,2-O-isopropylidene-sn-glycerol of high optical purity was obtained. The less easily available 2,3-O-isopropylidene-sn-glycerol unit was prepared, starting from L-serine, by a four-step procedure as described by Lok *et al.*[19] The glycerol acetonides thus obtained were converted[20] into 3-O-allyl and 1-O-allyl-sn-glycerols **1a** and **1b**, respectively. The well established allyl protecting group,[21] which performs a temporary blocking function, can be deprotected by a two-step procedure. Firstly, the allyl-ether is isomerized into the prop-1-enyl-ether function. The prop-1-enyl group can now be removed efficiently by acid or the reagent $HgCl_2$/HgO.

For the protection of the other primary hydroxyl group of the glycerol unit we chose the pivaloyl group.[22] This base labile protecting group had several attractive features. For instance, the pivaloyl group could be introduced selectively and removed under basic conditions without affecting the allyl-ether function. Furthermore, no migration[23] of the pivaloyl group was observed under the conditions of Lemieux, which were required for the introduction of the α-glycosidic linkage. Thus, treatment of 3-O-allyl-sn-glycerol (**1a**) or 1-O-allyl-sn-glycerol (**1b**) with pivaloyl chloride in dry pyridine at −10° gave, after purification by short column chromatography,[24] pure 1 - O - pivaloyl - 3 - O - allyl - sn - glycerol (**2a**) and 1 - O - allyl - 3 - O - pivaloyl - sn - glycerol (**2b**), respectively, in high yield (92%). The identify and homogeneity of both compounds was established by ^{1}H NMR and ^{13}C NMR spectroscopy (see Experimental). Owing to the small optical rotations of both enantiomeric compounds **2a** and

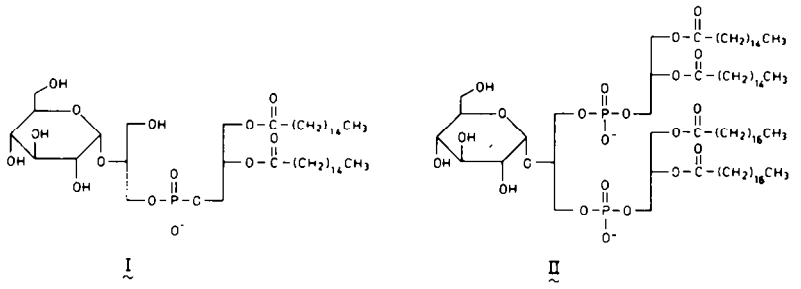
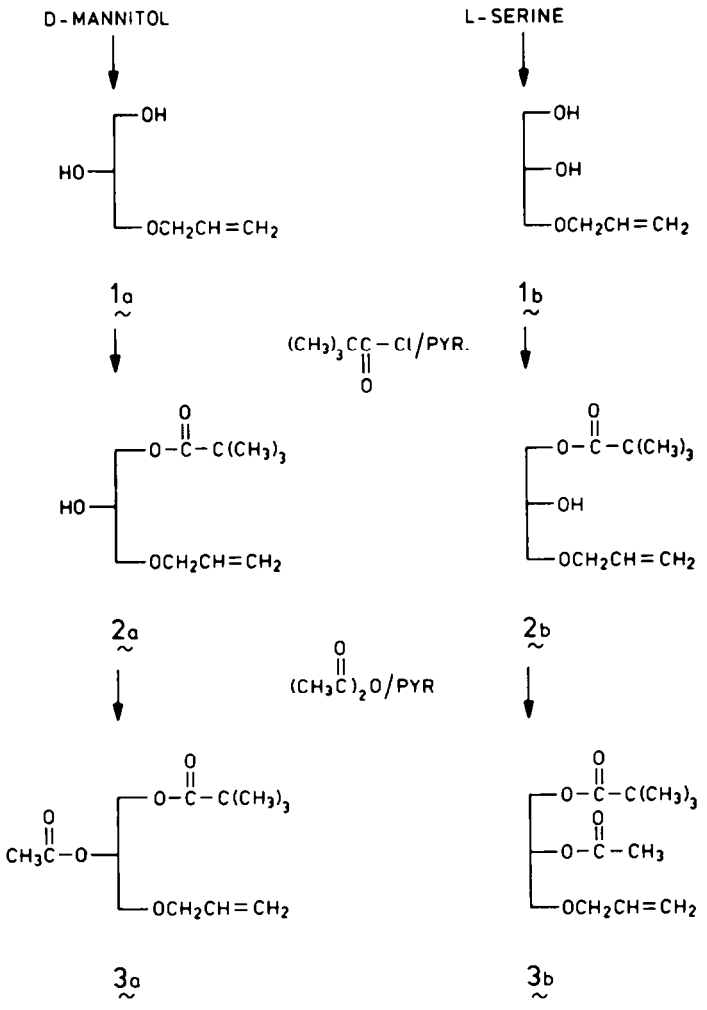


Fig. 1.



Scheme 1.

2b, we decided to determine the optical purity by means of the chiral shift reagent tris - (3 - heptafluorobutyryl - d - camphorato)praseodymium III (Pr(hfbc)$_3$).[25] Addition of the shift reagent to a racemic mixture of compounds **2a** and **2b** gave no satisfactory results. However, addition of the reagent to a sample containing equimolar amounts of the acetyl derivatives of **2a** and **2b** (compounds **3a** and **3b**) resulted in an enantiomeric splitting of the acetyl protons (see ^{1}H NMR spectra in Fig. 2). On the other hand, addition of Pr(hfbc)$_3$ to the separate enantiomers **3a** and **3b** revealed no splitting of the acetyl protons. From these observations we concluded that the optical purity of **2a** and **2b** should be at least 95%.

The above mentioned 1,3(3,1)-dihydroxyl protected glycerol molecules **2a** and **2b** could now be reacted together, under the conditions of Lemieux,[26] with 2,3,4,6 - tetra - O - benzyl - glucopyranosyl bromide (compound **4** in Scheme 2) to afford an α-glucosidic bond. Initially, bromide **4** was prepared[27] by treating p - nitrobenzoyl - 2,3,4,6 - tetra - O - benzyl - α - D - glucopyranose[28] with bromine-free hydrogen bromide in dichloromethane for 3 hr at room temperature. Under these conditions, we observed that a considerable amount of deblocking of the benzyl-ethers occurred. However, short bromination of the p-nitrobenzoyl derivative gave the bromide **4** (mixture of anomers) in excellent yield.

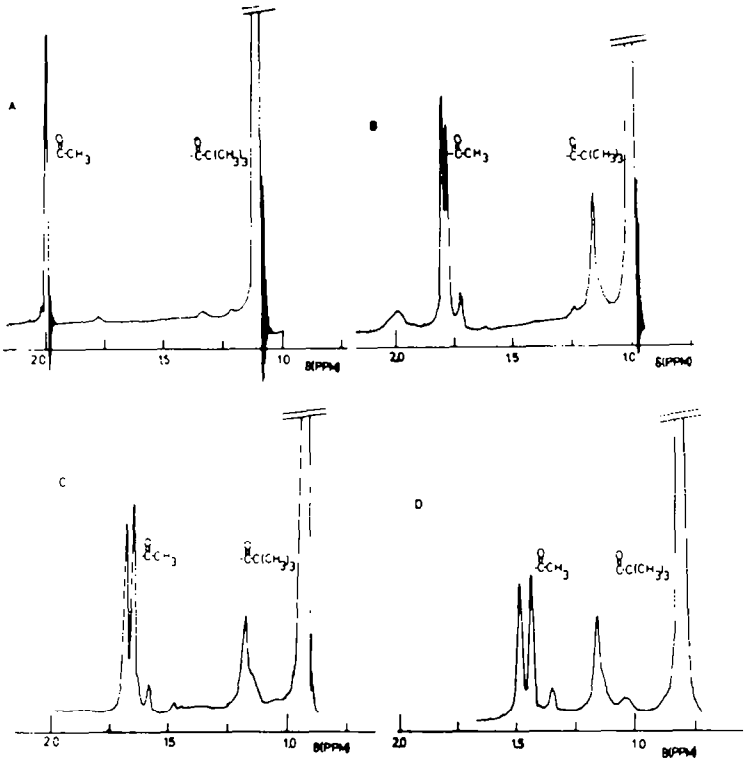


Fig. 2. ^{1}H NMR spectra of the acetyl and pivaloyl protons of an equimolar mixture of 1 - O - allyl - 2 - O - acetyl - 3 - O - pivaloyl - sn - glycerol (**3b**) and 1 - O - pivaloyl - 2 - O - acetyl - 3 - O - allyl - sn - glycerol (**3a**). (A) Without addition of the chiral shift reagent Pr(hfbc)$_3$. (B), (C) and (D): ^{1}H NMR spectra recorded after addition of different quantities of Pr(hfbc)$_3$ to an equimolar mixture of **3a** and **3b**: (B) molar ratio Pr(hfbc)$_3$/**3a**,**b** = 0.06; (C) molar ratio Pr(hfbc)$_3$/**3a**,**b** = 0.10; (D) molar ratio Pr(hfbc)$_3$/**3a**,**b** = 0.15.

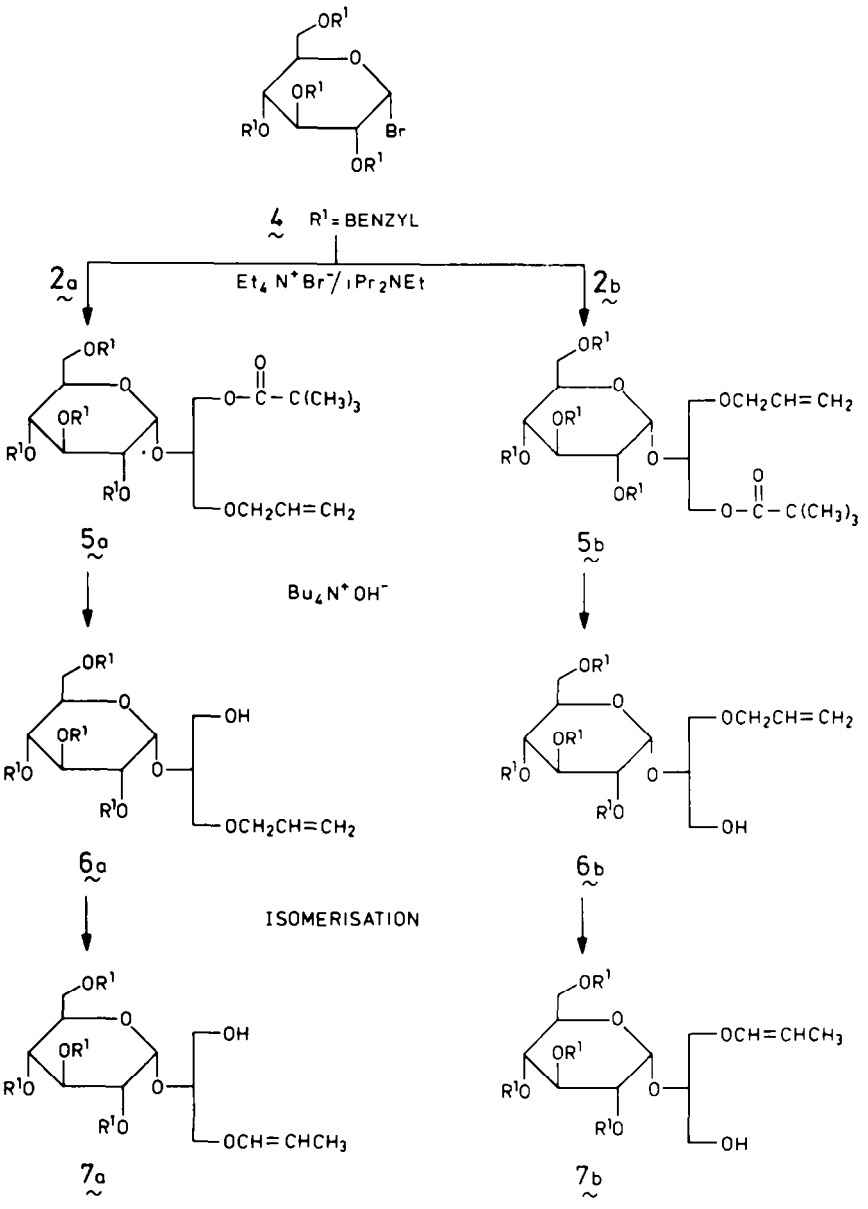
Coupling of the glycopyranosyl bromide **4** with the glycerol derivatives **2a** and **2b**, to afford **5a** and **5b**, respectively, was performed as follows. Compound **4** was dissolved in a mixture of dichloromethane and N,N-dimethylformamide to which was added tetraethylammonium bromide and activated molecular sieves (4 Å). The mixture was stirred for two hours in the dark, whereupon diisopropyylethylamine and alcohol **2a** were added. After stirring for four days at room temperature, the reaction mixture was worked-up and further purified by short column chromatography, to afford the fully protected 1 - O - pivaloyl - 2 - O - (2,3,4,6 - tetra - O - benzyl - α - D - glucopyranosyl) - 3 - O - allyl - sn - glycerol (**5a**) as an oil in 60% yield. The identity of **5a** was unambiguously confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy (see Experimental). In the same way, starting from **2b**, diastereoisomer **5b** (R^1 = benzyl) was obtained. Both diastereoisomers could be distinguished by ^{1}H NMR spectroscopy as well as by their specific rotations.

In the next step, the pivaloyl protecting group was removed from the fully protected α-glucosyl glycerol derivatives **5a** and **5b** by treatment with tetrabutylammonium hydroxide, to give **6a** and **6b**, respectively, in 87% yield. At this stage of the synthesis, it proved to be most convenient to isomerize the temporary allyl group into the prop-1-enylether, prior to the introduction of a rather base labile phosphotriester function. Initially, we performed the isomerization by the action of the rhodium catalyst tris(triphenylphosphine)rhodium chloride.[29,30] Thus, to a stirred solution of **6a** or **6b** and diazobicyclo{2,2,2}octane (DABCO) in ethanol/water was added at 85° a catalytic amount of tris(triphenylphosphine)rhodium chloride. After 2.5 hr, the reaction mixture was worked up, and purified by short column chromatography, to afford crude **7a** or **7b** in 96% yield. However, ^{1}H NMR spectroscopy of the crude products revealed the presence of *ca.* 10% of a propyl ether derivative. The formation of the latter side-product is due to concomitant reduction of the allyl(prop-1-enyl) protecting group.[31,32]

In a later stage of our synthetic study, we eliminated the formation of the propyl ether by using the iridium complex {Ir(cyclo - octa - 1,5 - diene(PMePh$_2$)$_2$}PF$_6$.[33,34] The use of the Ir-catalyst had the additional advantage that the allyl group was isomerized stereoselectively. The latter property enabled us to isolate **7a** and **7b** as crystalline compounds.[34]

Preparation of the protected phosphatidic acids (Scheme 3)

In our search for an easy and rapid phosphorylation of the diacylglycerols **9a** and **9b**, we established that phosphorylation was most easily accomplished by using a phosphoditriazolide derivative[14,15] (i.e. derivatives **10a**,**b**). Phosphorylation with the latter reagent had the following additional advantages; no acyl migration or formation of symmetrical phosphorylation products[14] was observed.



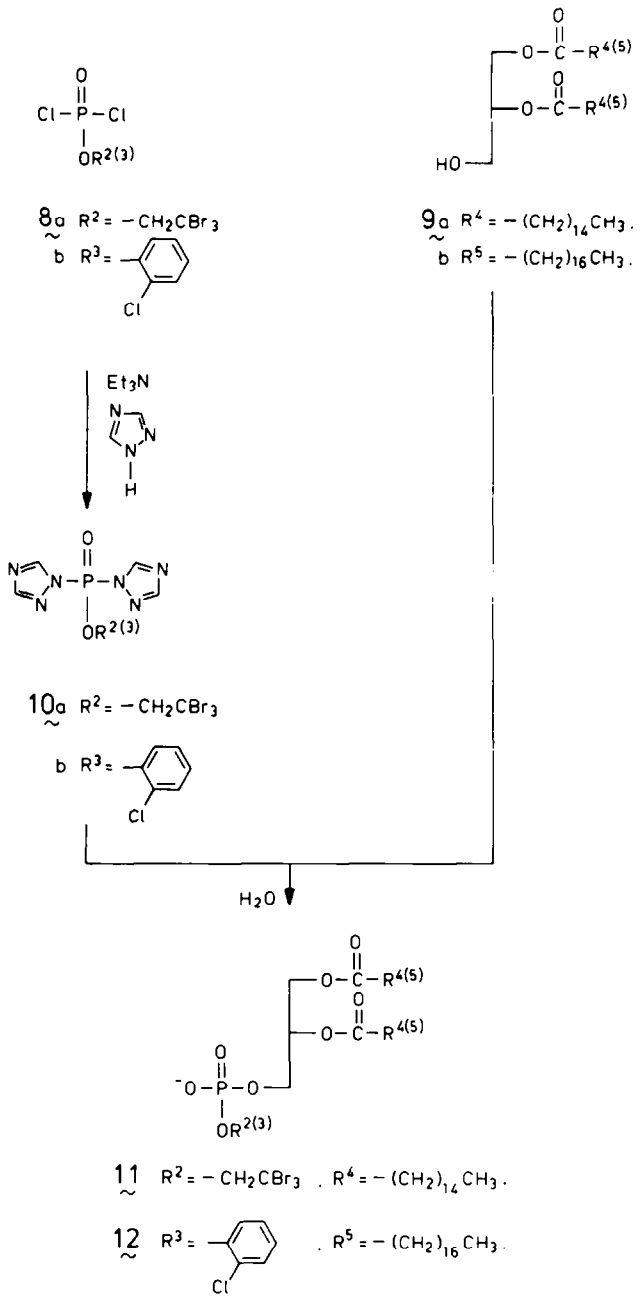
Scheme 2

The phosphorylating agent 2,2,2-tribromoethyl phosphoroditriazolide (**10a**), was prepared *in situ* by adding dropwise 2,2,2-tribromoethyl phosphorodichloridate[35] to an anhydrous solution of 1,2,4-triazole and triethylamine in tetrahydrofuran (THF). After 20 min, the triethylammonium hydrochloride salt was filtered off. A solution of 1,2-dipalmitoyl-sn-glycerol[36a,b] (**9a**) in pyridine was added dropwise to the filtrate. After 3 hr at room temperature, a small quantity of water was added to the reaction mixture to ensure hydrolysis of the intermediate phosphotriazolide and excess phosphoditriazolide. The crude product thus obtained was purified by short column chromatography, to afford the pure phosphodiester derivative **11**, which was converted by extraction with triethylammonium bicarbonate (TEAB; 2M, pH 7.5) in the homogeneous triethylammonium salt of **11** (yield 90% based on **9a**). The identity of **11** was ascertained by ^{1}H NMR and ^{13}C NMR spectroscopy (see Experimental).

The phosphatidyl unit **11** could now be coupled with the free hydroxyl group of **7b** or **7a**, to afford the relatively stable phosphotriester derivatives **13a** or **13d**, which, in turn, could be deprotected reductively with activated zinc dust in pyridine. In the same way, starting from 2-chlorophenyl phosphorodichloridate[37] (**8b**) and using 1,2-distearoyl-sn-glycerol[36] (**9b**) as the diglyceride, we obtained the triethylammonium salt **12** in 92% yield. The optical purity of the 1,2-diglycerieds **9a** and **9b** was ascertained, as described by Bus *et al.*,[38] by ^{1}H NMR spectroscopy in combination with the chiral shift reagent Pr(hfbc)$_3$.

Preparation of the fully protected glycophospholipids (Schemes 4 and 5)

The last step in the synthesis of the fully protected glycophospholipids **13a** or **13d** consisted of the introduction of phosphotriester bonds between the hydroxyl function of the glucosyl-glycerol parts of **7b** or **7a** and

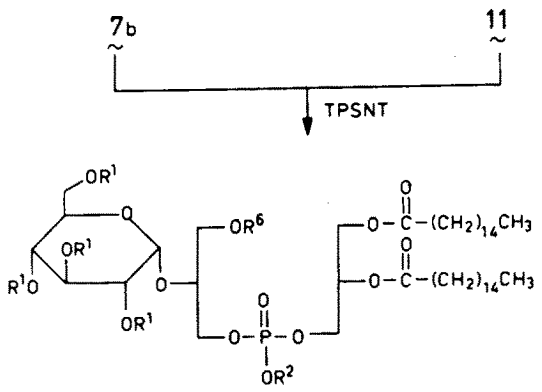


Scheme 3

the 2,2,2-tribromoethyl-protected phosphatidic acid unit **11**. In our opinion it was preferable, especially at this stage of the synthesis, to introduce a phosphotriester function which was not protected by a good leaving group such as a 2-chlorophenyl group. The reason for this was to prevent neighbouring group participation of a free primary hydroxyl group in the glycerol moiety of compounds **13b,e** (R^6 = H) with a neighbouring phosphotriester function. The latter process may not only occur during the removal of the prop-1-enyl group from compounds **13a,d** (R^6 = -CH=CHCH$_3$) to give derivatives **13b,e**, but also during the condensation of derivative **13e** (R^6 = H) with the phosphatidic acid **12** to afford the fully protected diphosphotriester compound **14a** (Scheme 5).

The condensation of **7a** or **7b** with **11** was easily accomplished using the activating agent 2,2,2 - triisopropylbenzenesulphonyl - 3 - nitro - 1,2,4 - triazole[39] (TPSNT). The latter agent has been proven to be not only very efficient in a phosphotriester approach to the synthesis of nucleic acids, but also of phospholipids.[14]

Thus, phosphodiester **11** was condensed with **7b** in pyridine under the influence of the coupling agent TPSNT. After 2 hr at room temperature, tlc-analysis indicated conversion of **7b** into the fully protected glycophospholipid **13a**, and the presence of a minor product which proved to be the sulphonylated derivative of **7b**. Work-up and purification by short column chromatography afforded phosphotriester **13a** as a waxy com-

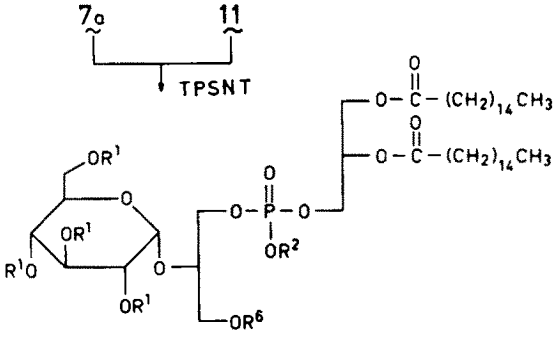


13a R^1 = BENZYL; $R^2 = -CH_2CBr_3$, $R^6 = -CH=CHCH_3$

b R^1 = BENZYL, $R^2 = -CH_2CBr_3$, $R^6 = -H$.

c R^1 = BENZYL $R^2 = -H$, $R^6 = -H$.

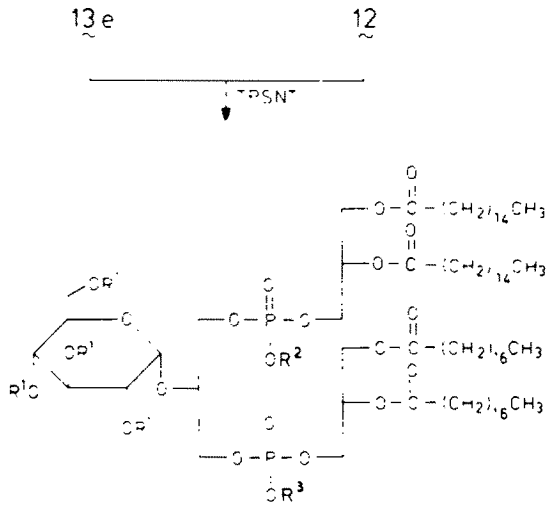
I $R^1 = -H$, $R^2 = -H$, $R^6 = -H$.



13d R^1 = BENZYL $R^2 = -CH_2CBr_3$ $R^6 = -CH=CHCH_3$.

13e R^1 = BENZYL $R^2 = -CH_2CBr_3$ $R^6 = -H$

Scheme 4



14a R^1 = BENZYL, $R^2 = -CH_2CBr_3$, R^3 = 2-chlorophenyl

b R^1 = BENZYL, $R^2 = -H$, R^3 = 2-chlorophenyl

c R^1 = BENZYL, $R^2 = -H$, $R^3 = -H$

II $R^1 = -H$, $R^2 = -H$, $R^3 = -H$

Scheme 5

pound in a yield of 83%. Compound **13a** thus obtained was, however, still contaminated with impurity **13a** ($R^6 = -CH_2CH_2CH_3$) which was previously introduced during the isomerization of the allyl into the prop-1-enyl ether function. Compound **13d** was prepared analogously by condensing compound **7a** with the phospholipid **11**.

In the next step, the prop-1-enyl group was removed from **13a** or **13d** by the action of $HgCl_2/HgO$[40] in aqueous acetone during 20 min at room temperature. The above mentioned impurity (compound **13a**; $R^6 = -CH_2CH_2CH_3$) was unaffected under these conditions. Fortunately, however, this impurity could easily be separated from the required product **13b** or **13e** ($R^6 = H$) by short column chromatography. The required phosphotriester derivative **13b** (**13e**) was isolated as a homogeneous colourless oil in 76% yield. The structure of **13b** (**13e**) was unambiguously ascertained by ^1H NMR and ^{13}C NMR spectroscopy (see Experimental). Having access to key intermediates **13b** or **13c**, we turned our attention to the introduction of the second phosphatidyl unit **12**.

For the protection of the second phosphatidic acid we selected the 2-chlorophenyl group. The reason for this is that the introduction of a second and differently protected phosphotriester function will facilitate the analysis of precursors and end-product by ^{31}P NMR spectroscopy. Furthermore, the specific combination of a 2,2,2-tribromoethyl and a 2-chlorophenyl protected phosphotriester functions enabled us to deblock one triester selectively in the presence of the other.

The assemblage of the fully protected glycophospholipid **14a** was performed according to the procedure described above for the preparation of **13a**. Thus, alcohol **13e** was condensed with **12** using TPSNT as the activating agent. After 4 hr at 20°, tlc analysis showed a quantitative conversion of **13e** into **14a**. The fully protected glucosyl diphosphatidyl glycerol **14a** thus obtained was purified by short column chromatography and isolated as a solid. The identity of **14a** was confirmed by ^1H NMR, ^{13}C NMR and ^{31}P NMR (see Fig. 3A) spectroscopy.

Conversion of phosphotriesters **13b** *and* **14a** *into the phosphodiesters* **I** *and* **II**

The last step in the synthesis involved stepwise deblocking of partially protected α-glucosylated monophosphatidyl glycerol **13b** and of fully protected α-glucosylated diphosphatidyl glycerol **14a**.

Reductive removal of the 2,2,2-tribromoethyl group from monophosphotriester **13b** (R^1 = benzyl; $R^2 = CH_2CBr_3$; $R^6 = H$), to give the phosphodiester **13c** (R^1 = benzyl; $R^2 = R^6 = H$), was easily accomplished, without affecting the benzyl ether functions, by the action of activated zinc[41] in the presence of 2,4,6-triisopropylben-

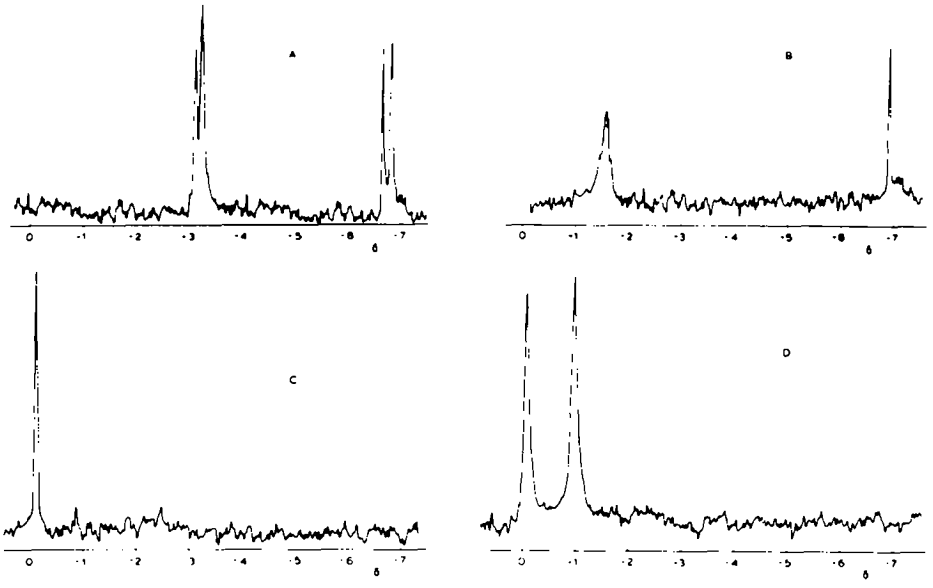


Fig. 3. (A) ^{31}P NMR spectrum of the fully protected α-glucosylated diphosphatidyl glycerol **14a** (R^1 = benzyl; $R^2 = CH_2CBr_3$; $R^3 = 2ClC_6H_4$); (B) ^{31}P NMR spectrum of compound **14b** (R^1 = benzyl; R^2 = H; $R^3 = 2ClC_6H_4$); (C) ^{31}P NMR spectrum of α-glucosylated monophosphatidyl glycerol (I); (D) ^{31}P NMR spectrum of α-glucosylated diphosphatidyl glycerol (II).

zenesulphonic acid (TPSOH). Thus, a stirred solution of phosphotriester **13b** in pyridine containing a few drops of pentane-2,4-dione,[42] activated zinc and TPSOH, was left for 10 min at 40°. When tlc-analysis showed the reaction to be complete, the excess zinc was filtered off and chloroform was added to the filtrate. The zinc ions were extracted from the organic phase with TEAB to give the triethylammonium salt of **13c** in quantitative yield.

In order to remove the benzyl groups, compound **13c** was converted into the sodium-form and hydrogenated over palladium on charcoal for 2 days at room temperature. Work-up of the reaction mixture, and fractionation by short column chromatography, afforded pure α-glucosyl-phosphatidyl glycerol **I**. Compound **I** was converted into its triethylammonium salt by the TEAB-extraction procedure and isolated as a waxy compound in 67% yield. The homogeneity and identity of **I** was confirmed by tlc analysis, ^1H NMR, ^{13}C NMR and ^{31}P NMR spectroscopy (see Fig. 3C).

Fully-deprotected α-glucosyl diphosphatidyl glycerol **II** was obtained after a three-step deblocking procedure of compound **14a** (R^1 = benzyl; $R^2 = CH_2CBr_3$; $R^3 = 2ClC_6H_4$), according to the following procedure; (i) selective removal of the 2,2,2-tribromoethyl group with activated zinc; (ii) deprotection of the 2-chlorophenyl group with oximate-ions;[43] (iii) hydrogenolysis of the benzyl protecting groups using palladium on charcoal as a catalyst.

Thus, addition of activated zinc dust and pentane-2,4-dione to a stirred solution of **14a** in the presence of TPSOH afforded, after work-up and TEAB-extraction, the triethylammonium salt of **14b** (R^1 = benzyl; R^2 = H; $R^3 = 2ClC_6H_4$) in quantitative yield. The selective removal of the 2,2,2-tribromoethyl group was confirmed by ^{31}P NMR (see Fig. 3B), ^1H NMR and ^{13}C NMR spectroscopy (see Experimental).

Secondly, the 2-chlorophenyl group could be removed selectively from **14b** with oximate ions in dry THF: the presence of water in the reaction mixture leads to a considerable amount[14] of hydrolysis of the ester functions. Thus, compound **14b** was dissolved in dry THF and treated with syn-4-nitrobenzaldoxime and N^1,N^1,N^2,N^2-tetramethylguanidine. After 6 hr at room temperature, tlc-analysis revealed complete conversion of **14b** into **14c** (R^1 = benzyl; $R^2 = R^3$ = H). Work-up of the reaction mixture followed by column chromatography and TEAB extraction afforded the triethylammonium salt of **14c** in 81% yield. The structure of the tetrabenzylglucosyl - diphosphatidyl - glycerol **14c** was ascertained by ^1H NMR, ^{13}C NMR and ^{31}P NMR spectroscopy (see Experimental). Finally, the remaining benzyl groups were removed by hydrogenolysis over palladium on charcoal. The crude deblocked glycolipid thus obtained was purified by silicagel chromatography and converted into its triethylammonium salt, to afford α-glucosylated diphosphatidyl-glycerol as a colourless waxy compound in 69% yield. The identify and homogeneity of **II** was confirmed by tlc analysis, ^1H NMR, ^{13}C NMR and ^{31}P NMR spectroscopy (see Fig. 3D). Furthermore, treatment of **II** with sodium methoxide in dry methanol, and analysis of the reaction mixture by glc analysis, showed the presence of methylpalmitate and methylstearate in equimolar amounts.

In conclusion, the data presented in this paper clearly show that the application of different groups (2-chlorophenyl and 2,2,2-tribromoethyl) for the protection of phosphotriester functions has the following advantages: (i) work-up of phosphotriester intermediates is not time consuming and allows easy purification by conventional column chromatography; (ii) removal of one protecting group in the presence of the other is selective; (iii) successive deblocking of the phosphotriester groups can

easily be monitored by tlc, and the conversion of triesters into diesters can unambiguously be ascertained by ^{31}P NMR spectroscopy. Finally, we believe that the phosphotriester methodology will be of great value for future preparations of complex glycophospholipids.

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EXPERIMENTAL

General methods and materials. Tetrahydrofuran, triethylamine and pyridine were dried by heating with CaH_2, under reflux, for 16hr and then distilled. Dimethylformamide was stirred with CaH_2 for 16 hr and then distilled under reduced pressure (*ca.* 14 mm Hg). All solvents were stored over molecular sieves 4 Å. Pyridine, used in phosphorylation and condensation reactions, was redistilled from p-toluenesulfonyl chloride (40 g per liter). Methylene chloride was washed with concentrated sulfuric acid, water and 10% aqueous $NaHCO_3$, dried on $CaCl_2$ and distilled from P_2O_5, and stored over molecular sieves 4 Å. Evaporations were carried out under reduced pressure (15 mm Hg) at 40°. ^1H NMR spectra were measured at 100 MHz with a Jeol JNMPS 100 spectrometer; chemical shifts are given in ppm(δ) relative to TMS as internal reference. ^{13}C and ^{31}P NMR spectra were measured with a Jeol JNMPS 100 spectrometer equipped with a EC-100 computer, operating in the Fourier Transform mode. Chemical shifts are given in ppm relatively to TMS as internal reference (^{13}C NMR), or to 85% H_3PO_4 as an external reference (^{31}P NMR). Compounds were visualized by UV-light, or by spraying with the appropriate reagents. Thus compounds containing sugar moieties were visualized by spraying with sulfuric acid/methanol (20%; v/v); phosphodiester functions with Zinzadze's reagent,[44] lipids with molybdatophosphoric acid/ethanol (15%; w/v). Tlc was performed on Silicagel (DC-Fertigfolien F1500 LS254, Schleicher and Schüll). Column chromatography was performed on Silicagel (Merck Kieselgel H). Optical rotations were measured at 25° with a Perkin Elmer 141 Polarimeter. Glc-analysis was carried out on a Becker 409 Multigraph with FID on Carbowax (glass-capillar column: 18 m × 8.0 mm) at 150°.

1-O-*Allyl*-3-O-*pivaloyl*-sn-*glycerol* (**2b**)

Pivaloyl chloride (1.81 g, 15 mmole) was added dropwise to a stirred solution of 1-O-allyl-sn-glycerol[20] (**1b**; 1.98 g, 15 mmole) in dry pyridine (20 ml) at −10°. After 1 hr, when tlc (toluene/acetone, 2:1, v/v) analysis indicated complete conversion of the starting material into the mono-pivaloyl derivative (R_f 0.66) together with a trace of the di-pivaloylated product (R_f 0.95), the reaction was stopped by the addition of water (2 ml). The solvent was evaporated off and the resulting oil was dissolved in chloroform (100 ml), washed with 5% aqueous $NaHCO_3$ (25 ml) and water (25 ml). After drying ($MgSO_4$), the organic layer was filtered and concentrated to an oil, which was applied to a column of Kieselgel H (70 g) suspended in chloroform/acetone (97.5:2.5, v/v). Elution of the column with the same solvent mixture gave, after evaporation of the appropriate fractions, pure **2b** as an oil. Yield 3.0 g (92%), R_f 0.66 (toluene/acetone, 2:1, v/v), b.p. 99° (0.8 mm Hg), $[\alpha]_D^{25} - 0.7$ (c 1, in chloroform), glc analysis: one peak. ^1H NMR ($CDCl_3$): $\delta = 1.20$ (s, 9 H, $C(CH_3)_3$); 2.76 (broad, 1 H, OH); 3.36–3.60 (m, 2 H, CH_2–O–allyl); 4.04 (m, 2 H, O–CH_2–C=C); 3.96–4.36 (m, 3 H, H–C–O, glycerol and CH_2–O–pivaloyl); 5.12–5.40 (m, 2 H, $=CH_2$); 5.72–6.12 ppm (m, 1 H, –CH=C). $^{13}C(^1H)$-NMR($CDCl_3$): $\delta = 27.2$ (s, $3 \times CH_3$); 38.8 (s, C_{quart}, t-butyl); 65.4 (s, CH_2–O–pivaloyl); 68.8 (s, CHOH); 71.0 (s, CH_2–O–allyl); 72.3 (s, $-CH_2-$, allyl); 117.3 (s, $=CH_2$); 134.3 (s, –CH=). 178.5 ppm (s, C=O). Found: C, 59.72; H, 9.38; Calc. for $C_{11}H_{20}O_4$ (216.777): C. 61.00, H, 9.32%.

1-O-Pivaloyl-3-O-allyl-sn-glycerol (**2a**) was obtained in the same way starting from **1a**.[20] Yield 3.0 g (92%), R_f 0.66 (toluene/acetone, 2:1, v/v), b.p. 99° (0.8 mm Hg), $[\alpha]_D^{25} + 0.7$ (c 1, in chloroform). Glc analysis: one peak.

1 - O - *Allyl* - 2 - O - *acetyl* - 3 - O - *pivaloyl* - sn - *glycerol* (**3a**) *and* 1 - O - *pivaloyl* - 2 - O - *acetyl* - 3 - O - *allyl* - sn - *glycerol* (**3b**)

A solution of **2a** (40 mg) or **2b** (40 mg) in dry pyridine (2 ml) was treated with acetic anhydride (2 ml). After 4 hr, the mixture was evaporated and the residue was coevaporated twice with toluene and absolute alcohol to give a mixture of **3a** and **3b** in quantitative yield. ^1H NMR ($CDCl_3$) $\delta = 1.20$ (s, 9 H, $C(CH_3)_3$); 2.05 (s, 3 H, $OCOCH_3$); 3.48 (d, 2 H, CH_2–O–allyl, J = 8 Hz); 3.92 (m, 2 H, O–CH_2–C=C); 4.0–4.36 (ABX, 2 H, CH_2–O–pivaloyl); 5.0–5.32 (m, 2 H, CH–O–acetyl and $C=CH_2$); 5.60–6.0 ppm (m, 1 H, –CH=C). Enantiomeric splitting of the acetyl proton signal was observed on addition of $Pr(hfbc)_3$: for ρ = $Pr(hfbc)_3$/substrate = 0.06 → $\Delta\Delta\delta = 0.015$, for $\rho = 0.10 \rightarrow \Delta\Delta\delta$ = 0.030, and for $\rho = 0.15 \rightarrow \Delta\Delta\delta = 0.048$. Addition of $Pr(hfbc)_3$ to **3a** or **3b** gave no change in the ^1H NMR spectra (see Fig. 2).

1 - O - *Allyl* - 2 - O - (2,3,4,6 - *tetra* - O - *benzyl* - α - D - *glucopyranosyl*) - 3 - O - *pivaloyl* - sn - *glycerol* (**5b**)

p-Nitrobenzoyl - 2,3,4,6 - tetra - O - benzyl - D - glucopyranose[28] (11 g, 16 mmole) was treated with a saturated (*ca.* 0.6 N) solution of hydrogen bromide in dichloromethane (340 ml) for 10 min. The p-nitrobenzoic acid was removed by filtration and after evaporation of the solvent the glucosyl bromide (**4**) was dissolved in a mixture of dry dichloromethane (50 ml) and dry N,N-dimethylformamide (10 ml) containing tetraethylammonium bromide (3 g) and activated molecular sieves (4 Å). After two hr in the dark, di-isopropyl-ethylamine (2 ml) and **2b** (2.16 g, 10 mmole) were added and the solution was kept at room temperature for 4 days in the dark. Tlc analysis (ether/petroleum ether, 1:1, v/v) indicated the presence of a major product (R_f 0.60), some minor impurities and a trace of **4** (R_f 0.75). The solution was diluted with chloroform (300 ml) and washed with aqueous $NaHCO_3$ (2.5%; 100 ml), twice with aqueous silver nitrate (2%; 100 ml). The dried ($MgSO_4$) organic layer was concentrated to an oil, which was applied to a column of Kieselgel H (200 g) suspended in ether/petroleum-ether (3:7, v/v). Elution of the column with the same solvent mixture and evaporation of the appropriate fractions afforded **5b** as a syrup. Yield 4.41 g (60%), R_f 0.60 (ether/petroleum-ether, 1:1, v/v), $[\alpha]_D^{25} + 31.6°$ (c 1, in chloroform). ^1H NMR ($CDCl_3$): $\delta = 1.20$ (s, 9 H, $C(CH_3)_3$); 3.4–4.4 (m, 11 H, glucosylglycerol except H_1'); 3.88–3.96 (m, 2 H, O–CH_2–C=C); 4.40–4.80 (m, 8 H, $4 \times CH_2$); 5.05 (d, 1 H, H_1', $J_{1',2'}$ = 3.1 Hz); 5.05–5.32 (m, 2 H, $C=CH_2$); 5.6–6.0 (m, 1 H, –CH=C); 7.30–7.40 ppm (m, 20 H, $4 \times 5\ H_{arom}$). ^{13}C (^1H) NMR ($CDCl_3$): $\delta = 27.2$ (s, $3 \times CH_3$); 38.8 (s, C_{quart}, t-butyl); 63.7 (s, CH_2–O–pivaloyl); 70.0 (s, CH_2–O–allyl); 72.3 (s, $-CH_2-$, allyl); 74.2 (s, H–C–O–glucosyl); 75.5, 74.9, 73.5, 73.0 (s, $4 \times CH_2$); 80.1, 81.8, 77.8 70.7, 68.9 (s, C_2'–C_6', glucose); 96.2 (s, C_1', glucose); 116.9 (s, $=CH_2$); 134.6 (s, –CH=); 138.6, 138.5, 138.3, 138.2 (s, $4 \times C_1$, benzyl); 177.9 ppm (s, C=O).

Compound **5a** was obtained in a similar way starting from **4** and **2a**. Yield 4.41 g (60%), R_f 0.60 (ether/petroleum ether, 1:1, v/v), $[\alpha]_D^{25} + 38.8°$ (c 1, in chloroform). ^1H NMR($CDCl_3$): The same as for **5b** except H_1'-glucose: $\delta = 5.18$ (d, 1 H, H_1', glucose, $J_{1',2'} = 3.1$ Hz).

1 - O - *Allyl* - 2 - O - (2,3,4,6 - *tetra* - O - *benzyl* - α - D - *glucopyranosyl*) - sn - *glycerol* (**6b**)

To a solution of **5b** (1.08 g, 1.46 mmole) in dry dioxane (50 ml) was added aqueous tetrabutylammonium hydroxide (40%; 18 ml). The reaction mixture was stirred for 4 hr at 20°. Tlc analysis (ether/petroleum ether, 2:1, v/v) indicated complete conversion of compound **5b** (R_f 0.95) into **6b** (R_f 0.34) together with a small amount of a less polar product (R_f 0.38). The reaction mixture was concentrated to an oil which was redissolved in chloroform (300 ml) and washed with aqueous $NaHCO_3$ (10%; 75 ml) and water (75 ml). The dried ($MgSO_4$) organic layer was evaporated to an oil, which was applied to a column of Kieselgel H (60 g) suspended in ether/petroleum ether (3:1, v/v). Elution of the column with the same solvent, afforded compound **6b** as a

colourless syrup. Yield 0.93 g (87%), R_f 0.34 (ether/petroleum ether, 2:1, v/v). ^{1}H NMR($CDCl_3$): δ = 2.6–3.0 (broad, 1 H, OH); 3.4–5.0 (m, 20 H, glucosylglycerol, 4 × $CH_2\phi$); 3.88–3.96 (m, 2 H, O–CH_2–C≡C); 5.0–5.32 (m, 2 H, C=CH_2); 5.6–6.0 (m, 1 H, =CH–); 7.3–7.4 ppm (m, 20 H, 4 × 5 H_{arom}).

Compound **6a** was obtained in the same way starting from **5a**. Yield 87%, R_f 0.34 (ether/petroleum ether, 2:1, v/v).

1 - O - (*Prop* - 1 - *enyl*) - 2 - O - (2,3,4,6 - *tetra* - O - *benzyl* - α - D - *glucopyranosyl*) - sn - *glycerol* (**7b**)

A solution of **6b** (660 mg, 1 mmole) and DABCO (40 mg) in ethanol/water (9:1, v/v), 15 ml was stirred at 85°. $(Ph_3P)_3RhCl$ (90 mg) was added to the boiling solution[19,20] and the reaction mixture was stirred for 2.5 hr at 85°. Tlc analysis (ether/petroleum ether, 5:2, v/v) of the reaction mixture indicated that **6b** (R_f 0.40) had been converted into a product having R_f 0.53. The solvents were evaporated and the residue was dissolved in chloroform (150 ml) and washed with aqueous $NaHCO_3$ (5%; 50 ml) and water (50 ml). The dried ($MgSO_4$) organic layer was concentrated and applied to a column of Kieselgel H (20 g) suspended in ether/petroleum ether (5:2, v/v) together with a few drops of methanolic ammonia (half saturated at 0°). Elution of the column with the same solvent mixture gave **7b** as an oil, which was contaminated with the propyl derivative of **7b** (*ca.* 10%); Yield 634 mg (96%), R_f 0.53 (ether/petroleum ether, 5:2, v/v). ^{1}H NMR($CDCl_3$): δ = 1.4–1.6 (m, 3 H, C=C–CH_3); 2.6–3.0 (broad, 1 H, OH); 3.4–5.16 (m, 21 H, glucosylglycerol, 4 × $CH_2\phi$, O–C=CH–C); 5.8–6.2 (m, 1 H, O–CH=C–C); 7.3–7.4 ppm (m, 20 H, 4 × 5 H_{arom}).

Compound **7a** was obtained in the same way, starting from **6a**. Yield 96%, R_f 0.53 (ether/petroleum ether, 5:2, v/v).

1,2 - *Di* - O - *palmitoyl* - sn - *glycero* - 3 - *phospho* - (2,2,2 - *tribromoethanol*) (**11**)

To an anhydrous solution of 1,2,4-triazole (144 mg, 2.2 mmole) and triethylamine (0.30 ml, 2.2 mmole) in tetrahydrofuran (6 ml) at 0° was added 2,2,2-tribromoethylphosphorodichloridate[35] (434 mg, 1.1 mmole). After stirring for 20 min at 0°, the reaction mixture was filtered to remove the triethylammonium hydrochloride salt. A solution of **9a**[36a,b] (400 mg, 0.70 mmole) in dry pyridine (5 ml) was added dropwise, during 30 min, to the stirred filtrate containing the 2,2,2-tribromoethylphosphoroditriazolide (**10a**). After 3 hr at room temperature, tlc analysis (chloroform/methanol/H_2O, 80:20:0.1, v/v) indicated the presence of a single product (R_f 0.65). Water (0.1 ml) was added to the reaction mixture, to hydrolyse the second triazolide, and the mixture was concentrated under reduced pressure. The residue was dissolved in chloroform/methanol (85:15, v/v) and applied to a column of Kieselgel H (10 g), suspended in chloroform/methanol (85:15, v/v). Elution of the column with the same solvent mixture and concentration of the appropriate fractions afforded pure **11**, which was dissolved in chloroform (200 ml) and extracted with TEAB (2 M, pH 7.5, 20 ml) and TEAB (1 M, 10 ml). The organic layer was concentrated to give the triethylammonium salt of **11** as a waxy compound. Yield 0.682 g (95.7%), R_f 0.6 (chloroform/methanol, 80:20, v/v). ^{1}H NMR($CDCl_3$): δ = 0.89 (t, 6 H, 2 × CH_3, J = 6 Hz); 1.26 (m, 2 × $(CH_2)_n$); 1.59 (m, 4 H, $\underline{CH_2}CH_2COO$); 2.20–2.40 (m, 4 H, 2 × CH_2COO); 4.08 (d.d, 2 H, CH_2–O–P, $^3J_{H-P}$ = 5.5 Hz); 4.08–4.5 (ABX, 2 H, CH_2–OOCR); 4.6 (d, 2 H, CH_2CBr_3, $^3J_{H-P}$ = 4 Hz); 5.16–5.36 ppm (m, 1 H, H–COOCR). ^{13}C{^{1}H} NMR($CDCl_3$): δ = 14.1 (s, 2 × CH_3); 22.7 (s, 2 × $\underline{CH_2}$–CH_3); 31.9 (s, 2 × $\underline{CH_2}$–CH_2–CH_3); 29.7, 29.5, 29.3, 29.2 (m, 2 × $(CH_2)_n$); 24.9 (s, 2 × $\underline{CH_2}$–CH_2COO); 34.1, 34.3 (s, 2 × $\underline{CH_2}$–COO); 40.4 (d, CBr_3, $^3J_{C-P}$ = 13 Hz; 62.5 (s, CH_2OOCR); 64.0 (d, CH_2–O–P, $^2J_{C-P}$ = 4 Hz); 70.3 (d, H–$\underline{C}$OOCR, $^3J_{C-P}$ = 9 Hz); 79.9 (d, $\underline{CH_2}CBr_3$, $^2J_{C-P}$ = 4 Hz); 173.2, 172.8 ppm (s, 2 × C=O).

1,2-*Di*-O-*stearoyl*-sn-*glycero*-3-*phospho*-(o-*chlorophenol*) (**12**)

Compound **12** was prepared in the same way as described for the synthesis of compound **11**, starting from **9b**,[36] to give the triethylammonium salt **12** as a white waxy compound. Yield 92%, R_f 0.6 (*chloroform/methanol*, 80:20, v/v). ^{1}H NMR($CDCl_3$): δ = 0.89 (t, 6 H, 2 × CH_3, J = 6 Hz); 1.26 (m, 2 × $(CH_2)_n$); 1.60 (m, 4 H, 2 × $\underline{CH_2}CH_2COO$); 2.2–2.4 (m, 4 H, CH_2–COO); 4.0–4.5 (m, 4 H, CH_2–O–P and CH_2OOCR); 5.15–5.40 (m, 1 H, HCOOCR); 7.2–7.6 ppm (m, 4 H_{arom}, o-chlorophenyl). ^{13}C{^{1}H}NMR($CDCl_3$): δ = 14.1 (s, 2 × CH_3); 22.7 (s, 2 × $\underline{CH_2}CH_3$); 31.9 (s, 2 × $\underline{CH_2}CH_2CH_3$); 29.7, 29.3, 29.1 (m, 2 × $(CH_2)_n$); 24.9 (s, $\underline{CH_2}CH_2COO$); 34.2, 34.1 (s, 2 × $\underline{CH_2}COO$); 62.5 (s, $\underline{C}H_2OOCR$); 64.3, 64.1 (d, CH_2–O–P, $^2J_{C-P}$ = 5.5 Hz); 70.4, 70.1 (d, H–$\underline{C}$OOCR, $^3J_{C-P}$ = 9.2 Hz; 149.3, 149 (d, C_1, o-chlorophenyl, $^2J_{C-P}$ = 5.5 Hz); 173.2, 172.8 ppm (s, 2 × C=O).

Fully protected α-glucosylated phosphatidylglycerol (**13a**)

A mixture of **7b** (308 mg, 0.47 mmole) and the triethylammonium salt of **11** (551 mg, 0.54 mmole) was dried by repeated co-evaporation with anhydrous pyridine (3 × 15 ml). TPSNT[39] (210 mg, 0.56 mmole) was added to the resulting viscous oil, and the reaction allowed to proceed at room temperature for 2 hr. Tlc analysis (ether/petroleum ether, 3:1, v/v) indicated complete conversion of **7b** (R_f 0.57) into the required product (R_f 0.67) and the presence of a minor product, which proved to be the sulphonylated derivative of **7b** (R_f 0.80). The solution was concentrated to an oil, which was dissolved in chloroform (100 ml), washed with aqueous $NaHCO_3$ (10%, 25 ml) and water (25 ml). The dried ($MgSO_4$) organic layer was concentrated to an oil, dissolved in ether/petroleum ether (1:2, v/v) and applied to a column of Kieselgel H (20 g). Elution with the same solvent mixture, followed by ether/petroleum ether (1:1, v/v) afforded **13a** as an oil. Yield 609 mg (83%), R_f 0.67 (ether/petroleum ether, 3:1, v/v). ^{1}H NMR($CDCl_3$): δ = 0.89 (t, 6 H, 2 × CH_3, J = 6 Hz); 1.26 (m, 2 × $(CH_2)_n$); 1.59 (m, 4 H, 2 × $\underline{CH_2}CH_2COO$); 1.48–1.56 (m, 3 H, –C≡C–CH_3); 3.5–5.0 (m, 24 H, phosphatidylglycerol except HCOOCR, glucosylglycerol except H_1', 4 × $CH_2\phi$, O–C=CH–C); 4.75 (m, 2 H, CH_2CBr_3); 5.1–5.4 (m, 1 H, HCOOCR); 5.04 (d, 1 H, H_1', glucose, $J_{1',2'}$ = 3 Hz); 5.8–6.2 (m, 1 H, OCH=C–C); 7.3–7.4 ppm (m, 20 H, 4 × 5 H_{arom}).

Compound **13d** was prepared analogously starting from **7a** and **11**, and had the same properties as described for **13a**. Yield 83%.

Partially protected α-glucosylated phosphatidylglycerol (**13b**)

Compound **13a** (380 mg, 0.25 mmole) was dissolved in acetone (6 ml) and water (0.4 ml). Mercuric chloride (71 mg, 0.26 mmole) and mercuric oxide (80 mg, 0.37 mmole) were added, and the solution was stirred at 20° for 30 min.[40] Tlc analysis (ether/petroleum ether, 3:1, v/v) indicated a major product (R_f 0.28) together with a minor product (R_f 0.65) that was obtained by condensation of the propyl derivative of **7b** with **11**. The mercuric oxide was removed by filtration, the acetone was evaporated and ether (100 ml) was added to the residue. The ether layer was washed with a half saturated aqueous solution of potassium iodide (10 ml), dried ($MgSO_4$) and the solvent was evaporated. The crude product thus obtained was dissolved in ether/petroleum ether (3:1, v/v) and applied to a column of Kieselgel H (10 g) suspended in the same solvent mixture. Elution of the column with this solvent and evaporation of the appropriate fractions gave **13b** as a viscous oil. Yield 281 mg (76%), R_f 0.28 (ether/petroleum ether, 3:1, v/v), $[\alpha]_D^{25}$ + 19.7° (c 1, in chloroform). ^{1}H NMR($CDCl_3$): δ = 0.89 (t, 2 × CH_3, J = 6 Hz); 1.26 (m, 2 × $(CH_2)_n$); 1.56 (m, 4 H, 2 × $\underline{CH_2}CH_2COO$); 2.2–2.4 (m, 4 H, CH_2COO); 2.8–3.2 (broad, 1 H, OH); 3.4–5.0 (m, 25 H, glucosylglycerol, phosphatidyl glycerol except HCOOCR, 4 × $CH_2\phi$); 4.76 (d.d, 2 H, CH_2CBr_3, $^3J_{H-P}$ = 6 Hz); 5.1–5.4 (m, 1 H, HCOOCR); 7.2–7.4 ppm (m, 20 H, 4 × 5 H_{arom}). ^{13}C{^{1}H}NMR($CDCl_3$): δ = 14.1 (s, 2 × CH_3); 22.7 (s, 2 × $\underline{CH_2}CH_3$); 31.9 (s, 2 × $\underline{CH_2}CH_2CH_3$); 29.7, 29.5, 29.3, 29.2 (m, 2 × $(CH_2)_n$); 24.8 (s, 2 × $\underline{CH_2}CH_2COO$); 34.1, 34.0 (s, 2 × CH_2COO); 35.1 (d, CBr_3); 61.5, 61.7 (s, CH_2OH, $\underline{C}H_2OOCR$); 66.3, 67.0 (d, 2 × CH_2OP); 69.3 (d, $\underline{C}HOOCR$); 78.7 (d, HCO–glucosyl); 79.6 (d, $\underline{C}H$ of Ps); 75.6, 75.1, 73.5, 73.1 (s, 4 × $CH_2\phi$); 79.8, 81.8, 77.8, 70.8, ... –C_6', glucose); 97.1 (s, C_1', glucose); 138.6, 138.0, 137.9, 137.5 (s, 4 × C_1, benzyl); 173.1, 172.7 ppm (s, 2 × C=O).

Compound **13e** was prepared in the same way starting from **13d**, and had nearly the same physical data as described for **13b**. Yield 76%, R_f 0.28 (ether/petroleum ether, 3:1, v/v).

Benzylated α-glucosyl phosphatidylglycerol (**13c**)

Compound **13b** (310 mg, 0.22 mmole) and TPSOH (10 mg) were stirred in a suspension of excess activated zinc dust[41] in pyridine (1.1 ml). A few drops of 2,4-pentane-di-one[42] were added. The temperature rose sharply and the suspension was stirred at 40°

for 10 min. Tlc analysis (ether/petroleum ether, 3:1, v/v) indicated complete conversion of the phosphotriester (R_f 0.3) into baseline material (R_f 0). Chloroform was added and the zinc dust was removed by filtration. The filtrate was diluted with chloroform (100 ml) and washed with TEAB (2 M, pH 7.5, 3 ml) and TEAB (1 M, 3 ml), filtered and the solvent was evaporated, to give **13c** as a light yellow oil in a quantitative yield. R_f 0.72 (chloroform/acetone/methanol/acetic acid/water, 50:20:10:10:5, v/v). $[\alpha]_D^{25}$ + 29.7 (c 1, in chloroform). ^{1}H NMR($CDCl_3$): δ = 0.89 (t, 6 H, 2 × CH_3, J = 6 Hz); 1.26 (m, 2 × $(CH_2)_n$); 1.56 (m, 4 H, 2 × $\underline{CH_2}CH_2COO$); 2.2–2.4 (m, 4 H, 2 × CH_2COO); 2.8–3.2 (broad, 1 H, OH); 3.4–5.0 (m, 23 H, glucosylglycerol except H$'_1$, phosphatidylglycerol except HCOOCR, and 4 × $CH_2\phi$); 5.06 (d, 1 H, H$'_1$, $J_{1',2'}$ = 3.1 Hz); 5.0–5.4 (m, 1 H, H–COOCR); 7.3–7.4 ppm (m, 20 H, 4 × H_{arom}). ^{13}C(^{1}H)NMR($CDCl_3$): δ = 14.1 (s, 2 × CH_3); 22.7 (s, 2 × $\underline{CH_2}CH_3$); 31.9 (s, 2 × $\underline{CH_2}CH_2CH_3$); 29.7, 29.5, 29.3, 29.2 (m, 2 × $(\overline{CH_2})_n$); 24.9 (s, 2 × $\overline{CH_2}CH_2COO$); 34.1, 34.0 (s, 2 × $\underline{CH_2}COO$); 61.6 (s, CH_2OH); 62.5 (s, $\underline{CH_2}OOCR$); 64.5, 64.4 (d, 2 × CH_2–O–P); 70.2 (d, H–$\underline{C}$OOCR); 77.5 (d, H–C–O–glucosyl); 75.6, 75.1, 73.4, 72.6 (s, 4 × $CH_2\phi$); 79.5, 81.8, 77.6, 70.8, 68.5 (s, C$'_2$–C$'_6$, glucose); 97.0 (s, C$'_1$, glucose); 138.8, 138.3, 138.2, 137.9 (s, 4 × C_1, benzyl); 172.9, 173.2 ppm (s, 2 × C=O).

α-Glucosylated phosphatidylglycerol (**I**)

Compound **13c** was converted into the sodium-form by running a solution of the triethylammonium salt of **13c** (128 mg, 0.095 mmole), dissolved in methanol/tetrahydrofuran (2:1, v/v) through a column (10 × 2 cm^2) of Dowex 50W cation-exchanger resin (100–200 mesh, sodium-form), suspended in the same solvent mixture. After concentration of the appropriate fractions the sodium salt of **13c** (120 mg, 0.095 mmole) was dissolved in a mixture of isopropanol/ethylacetate/acetic acid (6:3:1, v/v, 20 ml) and hydrogenated over 10% palladium on charcoal (350 mg) at 4 atm for two days at 20°.

Tlc-analysis (chloroform/acetone/methanol/acetic acid/water, 50:20:10:10:5, v/v) of the crude reaction mixture indicated *ca.* 90% conversion of the starting material into **I** (R_f 0.16). The catalyst was filtered off and washed thoroughly with methanol in pyridine (10%, 110 ml), and methanol in pyridine (20%, 100 ml). After evaporation to dryness the resulting oil was twice coevaporated with toluene (10 ml) and absolute alcohol (10 ml) and applied to a column of silicagel (8 g), suspended in chloroform/methanol/water (65:25:2, v/v). Elution of the column with the same solvent mixture and concentration of the appropriate fractions afforded pure **I**, which was dissolved in chloroform/methanol (4:1, v/v) and extracted with TEAB (2 M, 10 ml) and TEAB (1 M, 10 ml). Evaporation of the filtered organic layer afforded the triethylammonium salt of **I** as a white waxy solid. Yield 64 mg (69%), $[\alpha]_D^{25}$ + 21.1 (c 1, in chloroform). ^{1}H NMR($CDCl_3$/CD_3OD): δ = 0.89 (t, 6 H, 2 × CH_3, J = 6 Hz); 1.26 (m, 2 × $(CH_2)_n$); 1.56 (m, 2 H, 2 × $\underline{CH_2}CH_2COO$); 2.2–2.4 (m, 4 H, 2 × CH_2COO); 3.2–4.0 (m, 11 H, glucosylglycerol except H$'_1$); 3.96 (t, 2 H, CH_2OP, $^3J_{H-P}$ = 5.5 Hz); 4.0–4.6 (AB part of ABX, 2 H, CH_2OOCR); 5.08 (d, 1 H, H$'_1$, $J_{1',2'}$ = 3 Hz); 5.0–5.4 ppm (m, 1 H, HCOOCR); ^{13}C(^{1}H)NMR($CDCl_3$/CD_3OD): δ = 14.1 (s, 2 × CH_3); 22.8 (s, 2 × $\underline{CH_2}CH_3$); 31.9 (s, 2 × $\underline{CH_2}CH_2CH_3$); 29.7, 29.5, 29.3, 29.2 (m, 2 × $(\overline{CH_2})_n$); 24.9 (s, 2 × $\overline{CH_2}CH_2COO$); 34.3, 34.0 (s, 2 × $\underline{CH_2}COO$); 62.2 (s, CH_2OH); 62.7 (s, $\underline{CH_2}OOCR$); 63.7, 63.2 (d, 2 × CH_2–O–P); 70.4 (d, 70 (d, H$\underline{C}$OOCR); 72.7, 73.5, 70.1, 72.3, 62.2 (s, C$'_2$–C$'_6$, glucose); 77.6 (d, H–C–O–glucosyl); 99.0 (s, C$'_1$, glucose); 173.4, 173.0 ppm (s, 2 × C=O). ^{31}P NMR($CDCl_3$/CD_3OD): δ = − 0.13 (s, P–O) (see Fig. 3C).

Fully protected α-glucosylated diphosphatidylglycerol (**14a**)

A mixture of **13e** (230 mg, 0.152 mmole) and the triethylammonium salt of **12** (200 mg, 0.21 mmole) was dried by repeated coevaporation with anhydrous pyridine (3 × 15 ml). TPSNT (100 mg, 0.265 mmole) was added to the resulting viscous oil and the reaction was left for 4 hr at 20°. Tlc analysis (ether/petroleum ether, 3:1, v/v) indicated complete conversion of the starting compound (R_f 0.2) into a product with higher R_f value (R_f 0.8). The solution was evaporated and the residual oil was dissolved in chloroform (75 ml), washed with aqueous $NaHCO_3$ (10%, 20 ml) and water (20 ml). The dried ($MgSO_4$) organic layer was concentrated to an oil, which was dissolved in a small volume of ether/petroleum ether (2:1, v/v, 0.5 ml) and applied to a column of Kieselgel H (8 g) suspended in the same solvent mixture. Elution of the column with the same solvent and evaporation of the appropriate fractions gave the fully protected α-glucosyl diphosphatidylglycerol as a mixture of diastereomers. Yield 270 mg (75%).

The oil thus obtained was precipitated from ether/petroleum ether to give homogeneous **14a**. R_f 0.98 (chloroform/methanol/ammonia, 73:15:2, v/v) $[\alpha]_D^{25}$ + 15.1 (c 1.02, in chloroform). ^{1}H NMR($CDCl_3$): δ = 0.8 (t, 12 H, 4 × CH_3, J = 6 Hz); 1.26 (m, 4 × $(CH_2)_n$); 1.5–1.6 (m, 8 H, 4 × $\underline{CH_2}CH_2COO$); 2.2–2.4 (m, 8 H, 4 × CH_2COO); 3.5–4.9 (m, 27 H, 2× phosphatidylglycerol except 2 × H–COOCR, glucosylglycerol except H$'_1$, 4 × $CH_2\phi$); 4.75 (m, 2 H, CH_2CBr_3); 5.04–5.25 (m, 2 H, 2 × H–COOCR); 4.98 (d, 1 H, H$'_1$, $J_{1',2'}$ = 3 Hz); 7.0–7.5 ppm (m, 24 H, 4 × 5 H_{atom}, benzyl and 4 H_{arom}, o-chlorophenyl). ^{13}C(^{1}H)NMR($CDCl_3$): δ = 14.1 (s, 4 × CH_3); 22.7 (s, 4 × $\underline{CH_2}CH_3$); 31.9 (s, 4 × $\underline{CH_2}CH_2CH_3$); 29.7, 29.3, 29.1 (m, 4 × $(CH_2)_n$); 24.1 (s, 4 × $\underline{CH_2}COO$); 33.9 (s,4 × $\underline{CH_2}COO$); 36.0 (d, CBr_3, $^3J_{C-P}$ = 11.6 Hz); 61.5 (s, 2 × $\underline{CH_2}OOCR$); 67.1, 66.8, 66.5, 66.3 (m, 4 × CH_2–O–P); 69.2 (d, 2 × H$\underline{C}$OOCR) $^3J_{C-P}$ = 7.3 Hz); 78.2 (m, H–C–O–gluçosyl); 75.6, 75.0, 73.4, 73.0 (s, 4 × $CH_2\phi$); 79.6 (d, $\underline{CH_2}Br_3$, $^2J_{C-P}$ = 3.1 Hz); 79.5, 81.7, 77.8, 71.0, 68.3 (s, C$'_2$–C$'_6$, glucose); 97.2 (s, C$'_1$, glucose); 146.3, 146.1 (d, C_1, o-chlorophenyl, $^2J_{C-P}$ = 6.1 Hz); 138.6, 138.1, 137.9, 137.8 (s, 4 × C_1, benzyl); 173.0, 172.6 ppm (s, 4 × C=O). ^{31}P NMR($CDCl_3$): δ = − 3.1, − 3.2 (s, $POCH_2CBr_3$); − 6.7, − 6.8 ppm (s, PO–o-chlorophenyl) (see Fig. 3A). Found: C, 61.72; H, 8.13; P, 2.9; Calc. for $C_{119}H_{188}O_{22}P_2ClBr_3$(2307.919): C, 61.93; H, 8.21; P, 2.7.

Partially protected α-glucosylated diphosphatidylglycerol (**14b**)

Fully protected α-glucosyl diphosphatidylglycerol **14a** (130 mg, 0.056 mmole) and TPSOH (4.5 mg, 0.016 mmole) were stirred in a suspension of excess activated zinc dust[41] in pyridine (1 ml) as described for the synthesis of **13c**. Yield of **14b** as a mixture of diastereomers 120 mg (100%), tlc 0.69 (chloroform/acetone/methanol/acetic acid/water, 50:20:10:10:5, v/v), $[\alpha]_D^{25}$ + 12.5 (c, 1.128, in chloroform), ^{1}H NMR($CDCl_3$): δ = 0.8 (t, 12 H, 4 × CH_3, J = 6 Hz); 1.26 (m, 4 × $(CH_2)_n$); 1.5–1.6 (m, 8 H, 4 × $\underline{CH_2}CH_2COO$); 2.1–2.3 (m, 8 H, 4 × CH_2COO); 3.4–5.2 (m, 30 H, glucosylglycerol, 2 × phosphatidylglycerol, 4 × $CH_2\phi$); 7.0–7.5 ppm (m, 24 H, 4 × 5 H_{arom}, benzyl, 4 H_{arom}, o-chlorophenyl). ^{13}C(^{1}H) NMR($CDCl_3$): δ = 14.1 (s, 4 × CH_3); 22.6 (s, 4 × $\underline{CH_2}CH_3$); 31.9 (s, 4 × $\underline{CH_2}CH_2CH_3$); 29.7, 29.3, 29.1 (m, 4 × $(\overline{CH_2})_n$); 24.8 (s, 4 × $\underline{CH_2}\overline{CH_2}COO$); 34.0 (s, 4 × $\underline{CH_2}COO$); 62.1 (s, 2 × $\underline{CH_2}COOR$); 67.0–66.0 (m, 4 × CH_2–O–P); 69.4 (m, 2 × H–$\underline{C}$OOCR); 75.7, 74.9, 73.2, 72.2 (s, 4 × $CH_2\phi$); 78.2, 81.7, 77.8, 70.8, 68.3 (s, C$'_2$–C$'_6$, glucose); 99.3 (s, C$'_1$, glucose); 148.9, 148.2 (d, C_1, o-chlorophenyl); 138.7, 138.1, 137.9, 137.8 (s, 4 × C_1, benzyl); 173.1, 172.9, 172.8, 172.6 ppm (4 × C=O). ^{31}P NMR($CDCl_3$): δ = − 1.5 (broad, P–O$^-$); − 6.9 (s, P–O–o-chlorophenyl) (see Fig. 3B).

Benzylated α-glucosylated diphosphatidylglycerol (**14c**)

Compound **14b** (0.12 g, 0.056 mmole) was twice co-evaporated with dry dioxane (2 × 10 ml) and dissolved in dry tetrahydrofuran (1 ml). TMG (64 mg, 0.56 mmole) and syn-4-nitrobenzaldoxime (139 mg, 0.84 mmole) were added.[43] After 16 hr, tlc-analysis (chloroform/methanol/ammonia, 73:15:2, v/v) indicated complete conversion of the phosphotriester (R_f 0.80) into the phosphodiester (R_f 0.53). The reaction mixture was taken up in chloroform (60 ml), washed with water (2 × 250 ml), HCl (0.01 M, 25 ml) and TEAB (2 M, pH 7.5, 2 × 10 ml). The organic layer was concentrated to an oil which was dissolved in chloroform (1 ml) and applied to a column of silicagel (5 g) suspended in the same solvent. Elution of the column was started with chloroform, to remove by-products, and then with chloroform/methanol (85:15, v/v) to obtain pure **14c**, followed by extraction with TEAB (2 M, 2 × 10 ml). Evaporation of the filtered organic layer afforded **14c** as an oil. Yield 95.9 mg (81%), R_f 0.53 (chloroform/methanol/ammonia, 73:15:2, v/v), $[\alpha]_D^{25}$ + 17.7 (c, 1.246, in chloroform). ^{1}H NMR($CDCl_3$): δ = 0.89 (t, 12 H, 4 × CH_3, J = 6 Hz); 1.2 (m, 4 × $(CH_2)_n$); 1.5–1.6 (m, 8 H, 4 × $\underline{CH_2}CH_2COO$); 2.1–2.3 (m, 8 H, 4 × CH_2COO); 3.4–4.9 (m, 27 H, glucosylglycerol

except H_1', diphosphatidylglycerol except 2×H-COOCR, 4×$CH_2\phi$); 5.0 (d, 1 H, H_1', $J_{1'2'}$ = 3 Hz); 5.05–5.3 (m, 2 H, 2×H-COOCR); 7.0–7.15 ppm (m, 20 H, 4×5 H_{arom}, benzyl). $^{13}C(^1H)$NMR($CDCl_3$): δ = 14.1 (s, 4×CH_3); 22.7 (s, 4×$\underline{CH_2}CH_3$); 31.9 (s, 4×$\underline{CH_2}CH_3$); 29.7, 29.3, 29.2 (m, 4×$(CH_2)_n$); 24.9 (s, 4×$\underline{CH_2}CH_2\overline{COO}$); 34.3, 34.1 (s, 4×$\underline{CH_2}COO$); 62.8, 62.6 (s, 2×$\overline{CH_2}OOCR$); 65.2, 64.7, 63.6 (m, 4×$\overline{CH_2}$–O–P); 70.4 (m, 2×H–$\overline{COO}$CR); 75.7, 75.0, 73.7, 72.1 (s, 4×$CH_2\phi$); 79.7, 81.9, 77.8, 70.7, 68.5 (s, C_2'–C_6', glucose); 77.5 (m, H–C–O–glucosyl); 103.6 (s, C_1', glucose); 139.0, 138.6, 138.3, 137.9 (s, 4×C_1, benzyl); 173.3, 172.9 ppm (s, 4×C=O). ^{31}P NMR($CDCl_3$): δ = −0.6, −0.7 (s, 2×P–O^-).

α-Glucosylated diphosphatidylglycerol (II)

The sodium salt of **14c** (90.4 mg, 0.042 mmole) was dissolved in a mixture of isopropanol/ethylacetate/acetic acid (6:3:1, v/v, 20 ml) and hydrogenated over 10% palladium on charcoal (350 mg) as described for the synthesis of I. After two days, tlc analysis (chloroform/acetone/methanol/acetic acid/water, 60:20:10:10:5, v/v) indicated *ca.* 90% conversion of the starting compound (R_f 0.82) into II (R_f 0.21). Working up as described for I, afforded II in the triethylammonium form as a white waxy compound. Yield 52.3 mg (69%), R_f 0.21 (chloroform/acetone/methanol/acetic acid/water, 60:20:10:10:5, v/v); $[\alpha]_D^{25}$ + 14.4 (c 1, in chloroform). ^1H NMR($CDCl_3/CD_3OD$): δ = 0.9 (t, 12 H, 4×CH_3, J = 6 Hz); 1.2–1.4 (m, 4×$(CH_2)_n$); 1.5–1.6 (m, 8 H, 4×$\underline{CH_2}CH_2COO$); 2.2–2.4 (m, 8 H, 4×CH_2COO); 3.4–4.4 (m, 19 H, glucosylglycerol except H_1', diphosphatidylglycerol except 2×H-COOCR); 5.0 (d, 1 H, H_1', glucose, $J_{1'2'}$ = 3 Hz); 5.0–5.4 ppm (m, 2 H, H-COOCR); $^{13}C(^1H)$NMR($CDCl_3/CD_3OD$): δ = 14.1 (s, 4×CH_3); 22.8 (s, 4×$\underline{CH_2}CH_3$); 32.0 (s, 4×$\underline{CH_2}CH_2CH_3$); 29.8, 29.5, 29.3 (m, 4×$(CH_2)_n$); 25.0 (s, 4×$\underline{CH_2}CH_2COO$); 34.3, 34.2 (s, 4×CH_2COO); 58.2 (s, 2×$\underline{CH_2}OOCR$); 65.0, 63.6 (m, 4×CH_2–O–P); 70.3 (m, 2×H–$\underline{C}OOCR$); 73.9, 72.4, 71.6, 70.6, 62.8 (C_2'–C_6', glucose); 77.7 (m, H–C–O–glucosyl); 99.0 (s, C_1', glucose); 173.9, 173.5 ppm (s, 4×C=O). ^{31}P NMR($CDCl_3/CD_3OD$): δ = −0.06, −0.9 (s, 2×P–O^-).

Determination of the fatty acid content of compound II

Glycophospholipid II (19.3 mg) was treated at room temperature with 1 M NaOMe in MeOH (5 ml). After 2 hr, Dowex 50W cation-exchange resin (100–200 mesh, hydrogen-form; 5 g) was added. The resin was filtered off and the filtrate was contrated. The residue thus obtained was dissolved in chloroform (20 ml) and washed with water (10 ml). The dried ($MgSO_4$) organic layer was evaporated and the remaining methylesters were dissolved in tetra (3 ml). Glc analysis of the mixture showed the presence of solely methyl palmitate and methylstearate in equimolar amounts.

REFERENCES

[1]E. Peleg and A. Tietz, *Biochim. Biophys. Acta* **306**, 368 (1973).
[2]W. Fischer, *Ibid.* **487**, 74 (1977).
[3]Y. Ohno, I. Yano, T. Hiramatsu and M. Masui, *Ibid.* **424**, 337 (1976).
[4]J. A. F. Op den Kamp, P. P. M. Bonsen and L. L. M. van Deenen, *Ibid.* **176**, 298 (1969).
[5]J. C. MacDougall and P. J. R. Phizackerley, *Biochem. J.* **114**, 361 (1969).
[6]J. A. F. Op den Kamp, U. M. T. Houtsmuller and L. L. M. van Deenen, *Biochim. Biophys. Acta* **106**, 438. (1965).
[7]W. Fischer, *Lipids* (Edited by R. Paoletti, G. Porcellatti and G. Jacini), Vol. I, p. 255, Raven Press, New York (1976).
[8]W. Fischer, M. Nakano, R. A. Laine and W. Bohrer, *Biochim. Biophys. Acta* **528**, 288 (1978).
[9]W. Fischer, R. A. Laine, M. Nakano, D. Schuster and H. Egge, *Chem. Phys. Lipids* **21**, 103 (1978).
[10]W. Fischer, R. A. Laine and M. Nakano, *Biochim. Biophys Acta* **528**, 298 (1978).
[11]H. U. Koch and W. Fischer, *Biochemistry* **17**, 5275 (1978).
[12]W. Fischer, I. Ishizuka, H. R. Landgraf and J. Herrmann, *Biochim. Biophys. Acta* **296**, 527 (1973).
[13]C. A. A. van Boeckel and J. H. van Boom, *Tetrahedron Letters* 3561 (1979).
[14]C. A. A. van Boeckel and J. H. van Boom, *Tetrahedron Letters* 3705 (1980).
[15]N. Katagiri, K. Itakura and S. A. Narang, *J. Am. Chem. Soc.* **97**, 7332 (1975).
[16]B. T. Golding and V. P. Ioannou, *Synthesis* 423 (1977).
[17]G. J. F. Chittenden, *Carbohydr. Res.* **84**, 350 (1980).
[18]L. Lecocq and C. E. Ballou, *Biochemistry* **3**, 976 (1964).
[19]C. M. Lok, J. P. Ward and D. A. van Dorp, *Chem. Phys. Lipids* **16**, 115 (1976).
[20]P. A. Gent and R. Gigg, *J. Chem. Soc. Perkin I* 364 (1975).
[21]J. Gigg and R. Gigg, *J. Chem. Soc. (C)* 82 (1966).
[22]C. B. Reese, *Protective Groups in Organic Chemistry* (Edited by J. F. W. McOmie), p. 117. Plenum Press, New York (1973).
[23]J. Baker, M. Jarman and J. A. Stock, *J. Chem. Soc. Perkin Trans. I*, 665 (1973).
[24]B. J. Hunt and W. Rigby, *Chem. Ind. (London)* 1868 (1967).
[25]J. Briggs, G. H. Frost, F. A. Hart, G. R. Moss and M. L. Staniforth, *J. Chem. Soc. Chem. Comm.* 749 (1970).
[26]R. U. Lemieux, K. B. Hendriks, R. V. Stick and K. James, *J. Am. Chem. Soc.* **97**, 4056 (1975).
[27]T. Ishikawa and H. G. Fletcher, *J. Org. Chem.* **34**, 563 (1969).
[28]C. P. J. Glaudemans and H. G. Fletcher, *Methods Carbohydr. Chem.* **6**, 372 (1972).
[29]E. F. Corey and J. W. Suggs, *J. Org. Chem.* **38**, 3224 (1973).
[30]P. A. Gent and R. Gigg, *J. Chem. Soc. Chem. Comm.* 277 (1974).
[31]C. D. Warren and R. W. Jeanloz, *Carbodydr. Res.* **53**, 67 (1977).
[32]T. Nishiguchi, K. Tachi and K. Fukuzimi, *J. Org. Chem.* **40**, 237 (1975).
[33]D. Baudry, M. Ephritikhine and H. Felkin, *J. Chem. Soc. Chem. Comm.* 694 (1978).
[34]J. J. Oltvoort, C. A. A. van Boeckel, J. H. de Koning and J. H. van Boom, *Synthesis* 308 (1981).
[35]G. R. Owen, C. B. Reese, C. J. Ransom, J. H. van Boom and J. P. M. Herscheid, *Synthesis* 704 (1974).
[36a]J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.* **63**, 3244 (1941); [b]R. J. Howe and T. Malkin, *J. Chem. Soc.* 2663 (1951).
[37]Houben-Weyl, *Methoden der Organischen Chemie*, Band 12-2, p. 216 (1964). Georg Thieme Verlag, Stuttgart.
[38]J. Bus and C. M. Lok, *Chem. Phys. Lipids* **21**, 253 (1978).
[39]J. F. M. de Rooij, G. Wille-Hazeleger, P. H. van Deursen, J. Serdijn and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas* **98**, 93 (1979).
[40]R. Gigg and C. D. Warren, *J. Chem. Soc. (C)* 1903 (1968).
[41]J. H. van Boom, P. M. J. Burgers, G. van der Marel, C. H. M. Verdegaal and G. Wille, *Nucleic Acids Res.* **4**, 1074 (1977).
[42]R. W. Adamiak, E. Biala, W. T. Markiewicz, J. Stawinsky and M. Wiewiorowski, *Nucleic Acids Res.* **4**, 2321 (1977).
[43]C. B. Reese, R. C. Titmas and L. Yan, *Tetrahedron Letters* 2727 (1978).
[44]J. C. Dittmer and R. L. Lester, *J. Lipid Res.* **5**, 126 (1964).