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SYNTHESIS OF PHOSPHATIDYL-α-GLUCOSYL GLYCEROL CONTAINING A DIOLEOYL PHOSPHATIDYL MOIETY. APPLICATION OF THE TETRAISOPROPYLDISILOXANE-1,3-DIYL (TIPS) PROTECTING GROUP IN SUGAR CHEMISTRY. PART III*

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Abstract - In this study we demonstrate that the tetraisopropyldisiloxane-1,3-diyl protecting group could be introduced, in a two step procedure, at the 3'- and 4'-hydroxyl functions of α-glucosyldiglyceride 3 to give derivative 6. Compound 6 could be selectively condensed with a suitably protected phosphatidyl part 9 at its primary hydroxyl function to afford the protected glycophospholipid 10a. The phosphatidyl part 9 was obtained by phosphorylation of optically pure 1,2-di-O-oleoyl-sn-glycerol (8a) with phosphoditriazolide 7b. Finally, the 2,4-dichlorophenyl and TIPS protecting groups were removed from 10a by syn-4-nitro-benzaldoximate and fluoride ions, respectively, to afford glycophospholipid 10c.

Introduction

Glycolipids and glycophospholipids are important membrane components which have been identified in virtually all kinds of organisms. A well established[1,2] class of glycolipids consists of glycosyldiglycerides which have been isolated in particular from plants and bacteria. The latter membrane substances are carbohydrate derivatives of 1,2-diacyl-sn-glycerol in which the carbohydrate part is joined to the 3-hydroxyl of the sn-glycerol moiety by a glycosidic bond.

The glycophospholipids in Fig. 1 (e.g. II, IV and V) are closely related to glycolipids (e.g. I, III and VI) but they differ in one important aspect: certain hydroxyl functions are joined by phosphodiester linkages to other diglyceride residues. Thus they form an intermediate class between the phospholipids and glycolipids.

It is of interest to review briefly the occurrence and mutual relationship of some glyco(phospho)-lipids. Compound I (see Fig. 1) is the basic glycolipid of glycophospholipids II and V and also of the glycolipids VIa and VIb, which have all been identified in Gram-positive Streptococci bacteria[1,2,3,4]. The glyco(phospho)lipids I, II, V, VIa and VIb have a metabolic relationship. Some of these glyco(phospho)lipids are precursors of the lipoteichoic acids. For instance, glycolipid VIb proved to be the precursor of lipoteichoic acid VII of Streptococcus Lactis Kiel 42172[5]. Further, glycolipids I and VIa have been found in Acholeplasma[6,7], while glycophospholipid II has been isolated from Pseudomonas diminuta[8]. Glycolipid III is the β-glucosyl analog of I and its occurrence has been established in Mycoplasma[9]. On the other hand glycophospholipid IV, which is the β-glucosyl analog of compound II, has not yet been isolated. In this paper we describe the synthesis of glycolipid I and glycophospholipid II. Furthermore, we wish to emphasize the use of a new bifunctional silyl protective group: i.e. the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPS) protective group[10,11].

The fatty acid composition of the naturally occurring glycophospholipid II has been established[12]: the main components are oleic and palmitic acids. It was for this reason that we decided to synthesize compound II (R^1=palmitoyl, R^2=oleoyl) having unsaturated acids in the phosphatidyl and satura-

ted acids in the α-glucosyldiglyceride moiety.

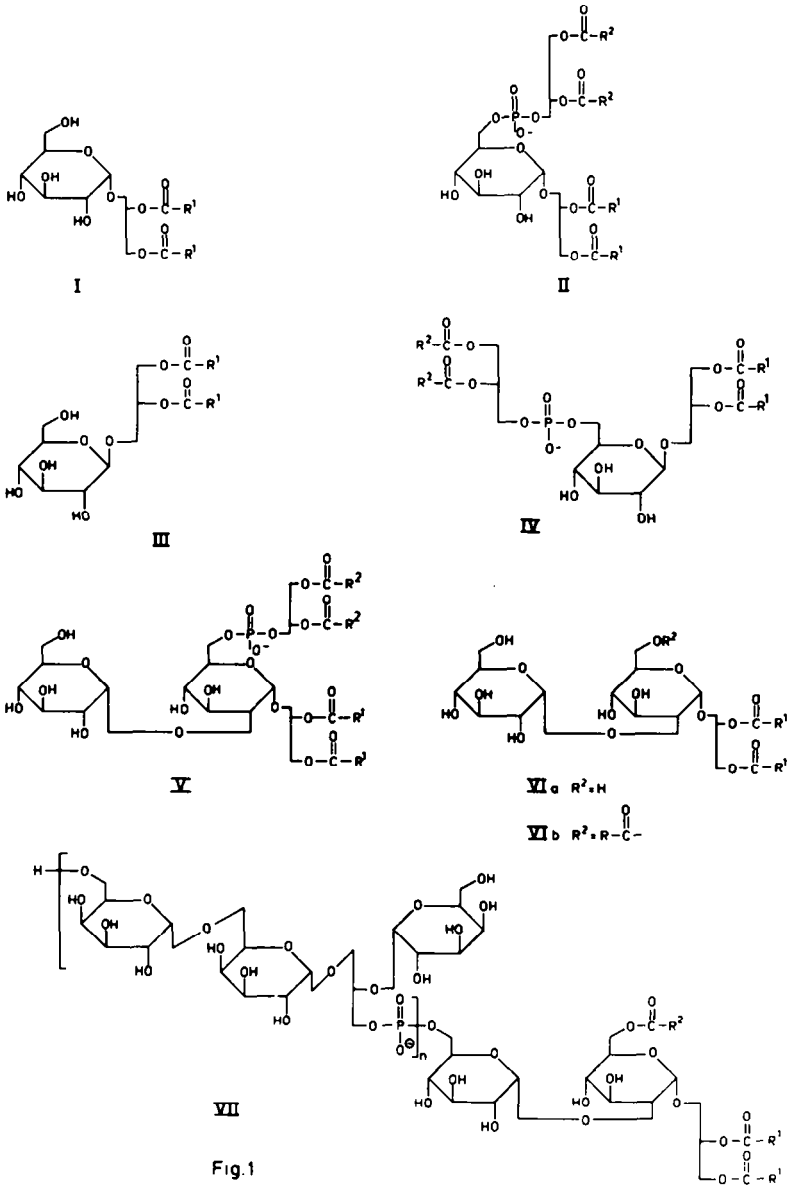


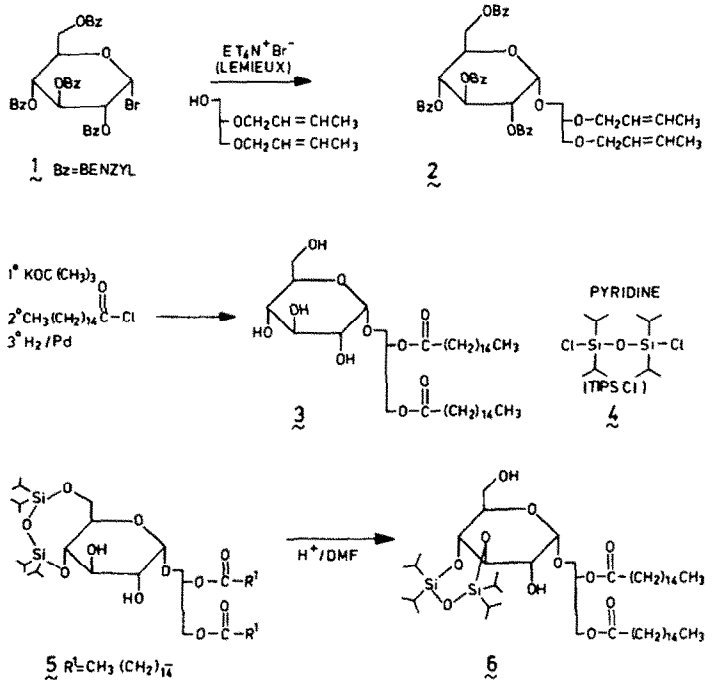
Fig.1

Synthesis of α-glucosyl diglyceride 3 (I)

For the preparation of the α-glucosyl diglyceride part 3 a suitably protected glycerol derivative was required. It has been shown that optically active 1,2-O-isopropylidene-sn-glycerol racemises[13], due to intramolecular migration of the isopropylidene group, under Koenigs-Knorr coupling conditions. We, therefore, decided to use the optically pure and very stable 1,2-di-O-(but-2-enyl)-sn-glycerol derivative which was prepared according to a method of R. Gigg[14] starting from 3,4-O-isopropylidene-D-mannitol[15]. The above mentioned 1,2-di-O-(but-2-enyl)-sn-glycerol was now condensed under the conditions of Lemieux[16] with 2,3,4,6-tetra-O-benzyl-glucopyranosyl bromide (1)[17,18] to afford fully protected 3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-1,2-di-O-(but-2-enyl)-sn-glycerol (2) as a syrup in 73% yield. Removal of the temporary but-2-enyl protecting groups was performed by treating 2 with potassium-t-butoxide in DMSO[19] to give 3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-sn-glycerol in 79% yield. The two hydroxyl groups present in the latter intermediate were acylated with palmitoyl chloride and in the next step, the benzyl groups were deblocked by hydrogenolysis over palladium on charcoal to afford α-glucosyl diglyceride 3 in 73% yield.

Preparation of suitably protected α-glucosyl diglyceride (i.e. compound 6 in Scheme 1)

R. Gigg et al. described[20], in a ten-step procedure starting from 1,6-anhydro-2,3,4-tri-O-benzyl-β-D-glucopyranose, the synthesis of 3-O-(2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-1,2-di-O-stearoyl-sn-glycerol and noted that this compound could be a suitable precursor for the synthesis of glycophospholipid II. However, in our case the presence of the benzyl groups excludes the introduction of a phosphatidyl part containing unsaturated fatty acids.

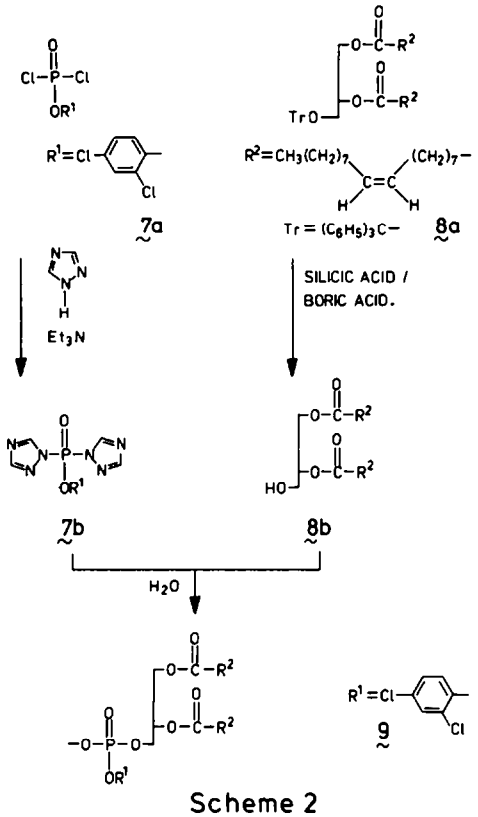


Scheme 1

Recently, we showed[11] that the tetraisopropyldisiloxane-1,3-diyl (TIPS) protective group[10], could be introduced selectively to protect the 4- and 6-hydroxyl functions of methyl-α-D-glucopyranoside. It was also demonstrated[11] that the 4,6-TIPS protected glucose derivative could be easily isomerized, in an acid catalysed reaction, into the 3,4-TIPS protected methyl-α-D-glucopyranoside. The same reaction sequence was now applied for the simultaneous protection of the 3'- and 4'-hydroxyl functions of α-glucosyldiglyceride 3. Compound 3 was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane 4 (TIPSCl)[21] to afford compound 5. Isomerization of compound 5 was accomplished in the presence of a catalytic amount of mesitylenesulfonic acid in DMF. After eighteen hours the reaction mixture was worked-up and purified by column chromatography to afford pure 3-O-[3,4-O-(tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranosyl]-1,2-di-O-palmitoyl-sn-glycerol (6) in 82% yield.

Preparation of 2,4-dichlorophenyl protected phosphatidyl part 9 (Scheme 2)

For the synthesis of a properly protected and optically pure phosphatidyl unit, we started from L-serine as the optically pure source. Thus, L-serine was converted into 3-O-trityl-sn-glycerol according to a slightly modified procedure described by Lok et al.[22]. The glycerol derivative was acylated with oleoyl chloride to afford, after column chromatography, pure 1,2-di-O-oleoyl-3-O-trityl-sn-glycerol (8a) in 89% yield. Removal of the trityl group from 8a under acidic conditions may lead, depending on the applied conditions, to extensive migration of 8b into the thermodynamically more stable 1,3-isomers[23]. For this reason, we attempted a recently developed method[24] which involves replacement of the trityl group from a diacyltritylglycerol by the action of trifluoroacetic acid in the presence of trifluoroacetic anhydride. The latter reagent had to protect immediately the released hydroxyl function, thereby preventing acyl migration. In a latter stage the trifluoroacetyl



Scheme 2

group can then be removed under very mild conditions[24]. Unfortunately, in our hands no removal of the trityl group occurred[25]. Fortunately, the removal of the trityl group from 8a could be performed by passing it through a silicic acid-boric acid column[26]. This deblocking procedure was not accompanied by acyl migration and afforded pure 1,2-di-O-oleoyl-sn-glycerol (8b) in high yield. For the protection of the phosphatidyl unit 9 (Scheme 2) we used the 2,4-dichlorophenyl group. Studies on model compounds[27] revealed that a 2,4-dichlorophenyl protected phosphotriester function of a fully protected phospholipid is quite stable and could be removed quantitatively by an oximate[28] treatment in dry THF. The synthesis of 9 was now accomplished by means of a phosphoditriazolide method[29,30]. Thus, 2,4-dichlorophenyl phosphorodichloridate[31] (7a) was reacted together with 1,2,-4-triazole and triethylamine in THF to afford a solution of phosphorylating agent 7b. Freshly prepared 1,2-di-O-oleoyl-sn-glycerol (8b) in pyridine was added dropwise to 7b.

When TLC analysis showed the reaction had gone to completion, water was added to hydrolyze the phosphotriazolide intermediates.

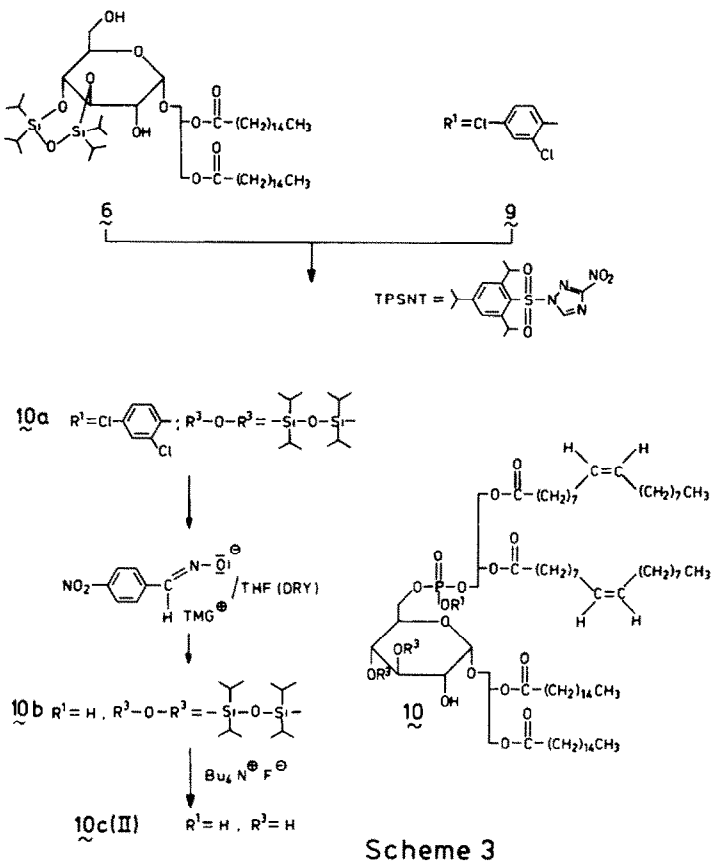
The excess of the 2,4-dichlorophenylphosphate was easily removed by high speed chromatography[32] of the crude material to give pure 1,2-dioleoyl-sn-glycero-3-phospho-(2,4-dichlorophenol) (9) in high yield (90%).

Preparation of protected glycophospholipid 10a (Scheme 3)

Compound 9 together with a slight excess of compound 6 were coupled in the presence of the activating agent 2,4,6-triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazole (TPSNT)[33] to afford pure 10a as a homogeneous oil (yield 70%). In this coupling process we observed, apart from the formation of 10a, a very lipophilic side product. The latter was isolated (yield 5% based on 9) and identified as the fully protected 2',6'-diphosphatidyl derivative of compound 6. The selective condensation of the phosphatidyl unit 9 with the primary hydroxyl function of 6 to give 10a was established by ^{13}C-NMR as well as by ^{31}P-NMR spectroscopy. The ^{31}P-NMR spectrum of 10a showed as expected, due to the presence of two diastereoisomers, two resonances in the proton decoupled spectrum.

Conversion of phosphotriester 10a into phosphatidyl-α-glucosyl-diacylglycerol 10c (II)

In order to obtain the fully-deprotected glycophospholipid 10c (II) the 2,4-dichlorophenyl and TIPS protecting groups had to be cleaved from 10a in the correct order. Firstly the 2,4-dichlorophenyl protective group had to be removed. Reversal of the deblocking process may lead to neighbouring group participation of the 4'-hydroxyl function on the phosphotriester[34]. Furthermore, deblocking of a TIPS protective group with fluoride ions from a molecule that also contains a phosphotriester unit leads to a non-specific removal[35] of the 2,4-dichlorophenyl protecting group[36]. Thus, the 2,4-dichlorophenyl group was deblocked selectively from compound 10a by the action of N^1,N^1,N^3,N^3-tetramethylguanidinium-syn-4-nitro-benzaldoximate[28] in dry THF[37] to give 10b in quantitative yield. Finally, the TIPS group was removed from compound 10b by the action of tetra-n-butylammonium fluoride[38] (TBAF) in the presence of pyridinium chloride[39] to afford 10c as a colourless



Scheme 3

waxy solid in 88% yield. The identity and homogeneity of compound 10c (i.e. compound II in Fig. 1, R^1=palmitoyl, R^2=oleoyl) were confirmed by TLC and GLC analysis as well as by ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectroscopy. TLC analysis of compound 10c revealed a single spot with an identical Rf-value as natural occurring glycophospholipid[12]. Further, compound 10c was treated with sodium methoxide in methanol-ether. GLC-analysis of the reaction mixture showed the presence of methylpalmitate and methyloleate in equimolar amounts.

The most solid structural evidence was obtained by ^{13}C-NMR spectroscopy. The proton decoupled ^{13}C-

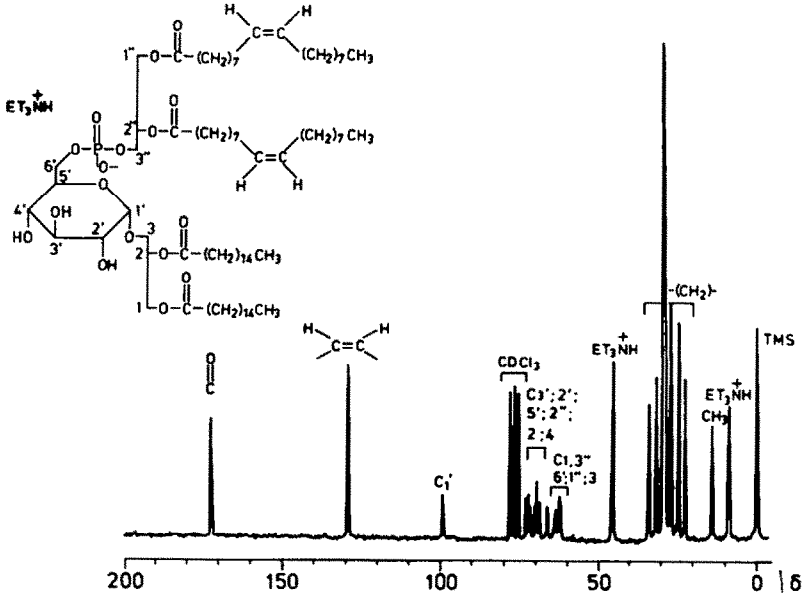


Figure 2: ^{13}C-NMR spectrum of compound 10c (II)

The most solid structural evidence was obtained by ^{13}C-NMR spectroscopy. The proton decoupled ^{13}C-NMR spectrum of 10c (Fig. 2) was completely consistent with its structure and all the carbon atoms were assigned by comparison with assignments made on the fragments 3 and 9 and with the aid of literature data[40-42]. Some important ^{13}C-nuclear resonances are: (i) the C_1'-resonance at 99.6 ppm, which is typically[42,44] for an α-substituted glucose derivative; (ii) the deshielded (~2 ppm) C_6'-carbon atom with a small ^{31}P-^{13}C coupling; (iii) the cis substituted double bond, the chemical shifts (129.7, 130.0 ppm) of which confirmed that no cis-trans isomerization had occurred. Finally, proton decoupled ^{31}P-NMR spectroscopy revealed the presence of only one resonance wich is a further evidence for the structural identity and purity of compound 10c (II).

Experimental

^1H-NMR spectra were measured at 100 MHz with a JEOL JNMPS 100 spectrometer; chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard. ^{13}C-NMR and ^{31}P-NMR spectra were measured at 25.15 MHz and 40.48 MHz, respectively, with a JEOL JNMPFT 100 spectrometer equipped with an EC-100 computer, operating in the Fourier transform mode. Proton noise decoupling was used. ^{13}C-chemical shifts are given in ppm (δ) relative to TMS as internal standard and ^{31}P-chemical shifts in ppm (δ) relative to 85% H_3PO_4 as external standard. Column chromatography was performed on Merck Kieselgel H or Kieselgel 60 (230-400 Mesh ASTM). TLC was performed on Silicagel (Schleicher & Schüll TLC-Ready Plastic Sheets F 1500 LS 254). Compounds were visualized by UV-light or by spraying with the appropriate reagents. Thus, compounds containing sugar moieties were visualized by spraying TLC-plates with 20% conc H_2SO_4 in methanol and charring at 140°C for a few minutes; lipids with molybdatophosphoric acid/ethanol (15% w/v); oleoyl esters with $KMnO_4$ in 5% aqueous K_2CO_3. Optical rotations were measured at 25°C with a Perkin Elmer 141 Polarimeter. GLC-analysis of the fatty acid methyl esters was carried out on a Becker 409 Multigraph with FID on Carbowax (glass-capillary column 18m x 0.3mm) at 150°C. Acetonitrile, tetrahydrofuran (THF), triethylamine, diisopropylethyl amine and pyridine were dried by heating with CaH_2, under reflux, for 16 hr and then distilled; pyridine was redistilled from p-toluenesulfonyl chloride (40g per liter). N,N-dimethylformamide was stirred with CaH_2 for 16 hr and then distilled under reduced pressure. Methylene chloride was washed with concentrated sulfuric acid, water and 10% aqueous $NaHCO_3$, dried on $CaCl_2$ and distilled from P_2O_5. All solvents were stored over molecular sieves 4Å. Methanol was dried by refluxing with magnesium methoxide, distilled and stored over molecular sieves 3Å. Petroleum-ether (b.p. 40-60°C) and ether were distilled before use. Tetraethylammonium bromide, mercuric cyanide, mercuric bromide, mesitylenesulfonic acid, pyridinium chloride and 1,2,4-triazole were dried before use, at 40°C, in vacuo over P_2O_5. All products were stored in the refrigerator at -20°C. Evaporations were carried out under reduced pressure (15 mm or 0.5 mm Hg) at bath temperature below 40°C.

3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-1,2-di-O-(but-2-enyl)-sn-glycerol (2)

p-Nitrobenzoyl-2,3,4,6-tetra-O-benzyl-α-D-glucopyranose[18] (5.6 g, 8 mmol) was treated with a saturated (ca. 0.6 N) solution of bromine-free hydrogen bromide in dichloromethane (250 ml) for 10 min. After removal of the p-nitrobenzoic acid by filtration the solvent was evaporated to afford glucosyl bromide 1 as a syrup. The syrup was dissolved in a mixture of dry dichloromethane (30 ml) and dry N,N-dimethylformamide (3 ml) containing tetraethylammonium bromide (1.72 g) and activated molecular sieves (4Å, 5 g). After 2 hr in the dark, the solution was filtrated under nitrogen and added to a mixture of 1,2-di-O-(but-2-enyl)-sn glycerol[14] (1.3 g, 6.5 mmol) and diisopropylethylamine (1.2 ml). The solution was stirred for 4 days under nitrogen in the dark. TLC analysis (ether/petroleum ether 1.5 : 1 v/v) revealed the presence of one major product together with some 1,2-di-O-(but-2-enyl)-sn-glycerol and decomposition products derived of bromide 4. The solution was diluted with chloroform (150 ml) and washed with aqueous $NaHCO_3$ (10%, 50 ml), aqueous $AgNO_3$ (5%, 10 ml) and finally with water. The dried ($MgSO_4$) organic layer was applied to a column of Kieselgel H (150 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 compound 2 as a syrup. Yield 3.44 g (73%); Rf = 0.80 (ether/petroleum ether, 1.5:1 v/v); $[\alpha]_D^{25}$ +36.2 (c 1, chloroform). ^1H-NMR ($CDCl_3$): δ

1.64 (d,6H,2xCH_3); 3.3-4.1 (m,15H,glucosylglycerol except H1',2xOCH_2 but-2-enyl); 4.1-5.1 (m,9H, H1',4xCH_2 Benzyl); 5.4-5.8 (c,4H,CH=CH); 7.3-7.4 (c,20H,4x5Harom).

3-O-(2,3,4,6-tetra-O-benzylglucopyranosyl)-sn-glycerol

Compound 2 (1.4 g, 1.9 mmol) was dissolved in dry dimethylsulfoxide (20 ml) and potassium t-butoxide (705 mg, 6.3 mmol) was added. The mixture was left for 3 hr at 60°C under nitrogen. TLC analysis (toluene/acetone, 1:1 v/v) indicated conversion of starting material 2 (Rf 0.96) into a fully debutenylated product (Rf 0.50) together with some minor impurities. The reaction mixture was diluted with ice-cold water (25 ml) and extracted with ether (3x150 ml). The combined extracts were washed with a saturated solution of KCl (50 ml), dried ($MgSO_4$) and evaporated to dryness. The crude material was applied to a column of Kieselgel H (100 g) suspended in chloroform/methanol (96:4, v/v). Elution with the same solvent mixture and evaporation of the appropriate fractions gave pure 3-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-sn-glycerol as an oil. Yield 943 mg (79%). ^{1}H-NMR ($CDCl_3$): δ 3.2-4.1 (m,11H,glucosylglycerol except H1'); 4.3-4.8 (m,8H,4xCH_2); 4.86 (d,1H,H1',J1'-2' =3.2 Hz); 7.3-7.4 (c,20H,4x5Harom).

3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-1,2-di-O-palmitoyl-sn-glycerol

To a solution of 3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-sn-glycerol (768 mg, 1.25 mmol) in dry methylene chloride (10 ml) and pyridine (2 ml) was added dropwise palmitoyl chloride (1.2 g, 4.4 mmol) in 5 ml methylene chloride. After stirring for 16 hr at 20°C, TLC analysis showed complete conversion into the diester. Water was added to the solution, which was stirred for another 3 hr. The solution was dissolved in chloroform (100 ml) and washed successively with 10% aqueous $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried ($MgSO_4$) and concentrated to an oil. The oil thus obtained was redissolved in ether/petroleum ether (3.5:2, v/v) and applied to a column of Kieselgel H (150 g) suspended in the same solvent. The column was eluted with this solvent to give after pooling of the appropriate fractions, 3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-1,2-di-O-palmitoyl-sn-glycerol. Yield 1.19 g (87%); Rf 0.67 (toluene/acetone; 10:1, v/v); ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (m,58H,2x$(CH_2)_{13}CH_3$); 2.24 (t,4H,2xCH_2COO,J=8 Hz); 3.4-4.0 (m,8H,2xH3,H2',H3', H4',H5',2xH6'); 4.0-5.0 (m,11H,H1',2xH3,4xCH_2 Benzyl); 5.2-5.4 (c,1H,H2); 7.3-7.4 (c,20H,4x5Harom). ^{13}C-NMR ($CDCl_3$): δ 14.1 (s,2xCH_3); 22.7 (s,2x$\underline{CH_2}CH_3$); 31.9 (s,2x$\underline{CH_2}CH_2CH_3$); 29.7, 29.5, 29.3, 29.2 (m,2x$(CH_2)_n$); 24.9 (s,2x$\underline{CH_2}CH_2$COO); 34.1, 34.2 (s,2x$\underline{CH_2}$COO); 62.4, 69.8, 66.4 (s,C1-C3 glycerol); 80.1, 81.8, 77.4, 70.6, 68.4 (s,C2'-C6', glucose); 97.7 (s,C1', glucose); 137.8, 138.2, 138.3, 138.8 (s,4xC1 Benzyl); 172.8, 173.1 (s,2xC=O).

1,2-Di-O-palmitoyl-3-O-α-D-glucopyranosyl-sn-glycerol (3) (I)

1,2-Di-O-palmitoyl-3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-sn-glycerol (1.0 g, 0.92 mmol) was dissolved in a mixture of methanol/ethylacetate/acetic acid (3:3:1, v/v; 50 ml) and hydrogenated over 10% palladium on charcoal (450 mg) at 4 atm for 1 day at 20°C. The catalyst was filtered off and washed thoroughly with pyridine/methanol (9:1, v/v, 40-60°C). After evaporation to dryness the crude material was coevaporated with toluene (2x50 ml) and applied to a column of Kieselgel H suspended in chloroform/methanol (92:8, v/v). Elution with the same solvent afforded α-glucosyl diglyceride 3 as a white solid. Yield 450 mg (73%). Rf 0.39 (chloroform/methanol, 9:1, v/v). $[\alpha]_D^{25}$ +44 (c 1, chloroform); m.p.: softening at 79°C and forming a meniscus at 141°C. ^{1}H-NMR ($CDCl_3$/CD_3OD): δ 0.8-1.8 (m,58H,2x$(CH_2)_{13}CH_3$); 2.24 (t,4H,CH_2COO,J=7.5 Hz); 3.2-4.0 (m,8H,2xH3,H2',H3', H4',H5',2xH6'); 4.0-4.5 (AB part of ABX,2H,2xH1); 4.82 (d,1H,H1',J1'-2'=3.2 Hz); 5.2-5.4 (c,1H,H2). ^{13}C-NMR ($CDCl_3$/CD_3OD): δ 14.1 (s,2xCH_3); 22.8 (s,2x$\underline{CH_2}CH_3$); 32.0 (s,2x$\underline{CH_2}CH_2CH_3$); 29.2, 29.4, 29.8 (m,2x$(CH_2)_n$); 25.0 (s,2x$\underline{CH_2}CH_2$COO); 34.2, 34.3 (s,2x$\underline{CH_2}$COO); 72.1, 73.9, 70.0, 72.1, 61.7 (s, C2'-6' glucose); 99.3 (s,C1' glucose); 62.6, 70.0, 66.3 (s,C1-C3 glycerol). Anal. calc. for $C_{41}H_{78}O_{10}$: C, 67.36; H, 10.75; O, 21.87, found C, 67.06; H, 11.02; O, 21.72.

3-O-[4,6-O-(tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranosyl]-1,2-di-O-palmitoyl-sn-glycerol (5)

To a stirred solution of compound 3 (490 mg, 0.67 mmol) in 6 ml dry pyridine was added dropwise, at -15°C (ice-salt), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[21] (TIPSCl 4, 0.28 ml) in pyridine (3 ml). After stirring for 3 hr at -15°C, TLC analysis indicated that the reaction was complete. Isopropanol (0.5 ml) was added and the reaction mixture was stirred for another hour at 20°C. The mixture was concentrated under reduced pressure to a small volume, which was dissolved in chloro-

form (100 ml). The organic phase was washed with 10% aqueous $NaHCO_3$ (50 ml) and water (50 ml). The dried ($MgSO_4$) organic layer was concentrated to an oil. TLC analysis (chloroform/acetone 94:6, v/v) of the crude oil revealed the presence of a major product (Rf 0.40, i.e. compound 5) but also a considerable amount of a more lipophilic product (Rf 0.90), presumably the 2',3'-4',6'-TIPS derivative of 3. The oil was dissolved in chloroform (3 ml) and applied to a column of Kieselgel H (60 g) suspended in chloroform. Pure product 5 was eluted from the column with chloroform/acetone (100:0 → 96:4, v/v) and concentrated to an oil. Yield 456 mg (70%). $[\alpha]_D^{25}$ +31.5 (c 1, chloroform). Rf 0.40 (chloroform/acetone 94:6, v/v). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (m,86H,2x$(CH_2)_{13}CH_3$,4xSiCH$(CH_3)_2$); 2.24 (t,4H,J=7.5 Hz); 3.3-4.0 (m,8H,2xH3,H2',H3',H4',H5',2xH6'); 4.0-4.5 (AB part of ABX,2H,2xH1); 4.84 (d,1H,H1',J=3.2 Hz); 5.2-5.4 (c,1H,H1). ^{13}C-NMR ($CDCl_3$): δ 12.5, 13.3, 13.6 (s,4xSi-C); 17.3 (s,8xCH_3 TIPS); 14.1, 22.7, 24.9, 29.4, 29.7, 31.9, 34.1, 34.3 (s,2x$(CH_2)_{13}CH_3$); 62.2, 69.8, 66.4 (C1-C3 glycerol); 72.6, 74.4, 69.0, 72.6, 60.6 (s,C2'-C6' glucose); 99.1 (C1' glucose); 173.0, 173.3 (s,2xC=O).

3-O-[3,4-O-(tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranosyl]-1,2-di-O-palmitoyl-sn-glycerol (6)

Compound 5 (450 mg, 0.46 mmol) was dissolved in dry N,N-dimethylformamide (6 ml). A catalytic amount of anhydrous mesitylenesulfonic acid was added (20 mg, 0.1 mmol) and the mixture was left at 20°C. TLC analysis (chloroform/acetone, 94:6, v/v) showed a slow conversion of starting material 5 (Rf 0.40) into isomer 6 (Rf 0.45). After 18 hr, when TLC analysis showed conversion of 5 into 6 for more than 90%, chloroform (100 ml) was added and the organic phase was washed with 10% aqueous $NaHCO_3$ and water, respectively. The dried ($MgSO_4$) organic layer was concentrated to an oil, which was redissolved in chloroform and applied to a column of Kieselgel H (60 g). The column was eluted with chloroform/acetone (100:0 → 97:3, v/v). Evaporation of the appropriate fractions gave pure 6 as an oil. Yield 370 mg (82%). Rf 0.45 (chloroform/acetone, 94:6, v/v). $[\alpha]_D^{25}$ +62.8 (c 1, chloroform). ^{1}H-NMR ($CDCl_3$): almost identical with compound 5. ^{13}C-NMR ($CDCl_3$): δ 12.2, 12.8, 13.0 (s, 4xSiC); 17.3 (s,8xCH_3 TIPS); 14.1, 22.7, 24.9, 29.2, 29.4, 29.7, 31.9, 34.1, 34.3 (s,2x$(CH_2)_{13}$-CH_3); 62.2, 69.8, 66.4 (s,C1-C3 glycerol); 72.3, 77.1, 72.5, 72.9, 62.0 (s,C2'-C6' glucose); 98.7 (s,C1' glucose); 173.0, 173.3 (s,2xC=O).

1,2-Dioleoyl-3-O-trityl-sn-glycerol (8a)

Oleoyl chloride was prepared by adding dropwise oleic acid (5.68 ml, 17.7 mmol), dissolved in hexane (2.6 ml) to freshly distilled oxalyl chloride (2.3 ml) in hexane (0.7 ml). After stirring for 0.5 hr at 40°C, the mixture was slowly heated and kept at the reflux temperature for 4 hr. The solvent and excess oxalyl chloride were removed at reduced pressure and the remaining oil was heated for 1 hr at 60-70°C in vacuo to remove the last traces of oxalyl chloride. The thus obtained light brown coloured oleoyl chloride was directly used in the next acylation procedure. Thus, to a stirred solution of 3-O-trityl-sn-glycerol[22] (3.0 g, 9 mmol) dissolved in a mixture of dry pyridine (5.0 ml) and dry methylene chloride (15 ml) was added, at 0°C (ice-water bath) under nitrogen, oleoyl chloride (6.0 g, 20 mmol) dissolved in methylene chloride. After 1 hr at 0°C, the ice-water bath was removed and the mixture was stirred for 48 hr at 20°C in the dark. TLC analysis (ether/petroleum ether 1:5) of the crude reaction mixture revealed the presence of one major product (Rf 0.74) together with some minor impurities. The reaction was stopped by the addition of water (1 ml) and the crude reaction mixture was dissolved in chloroform (200 ml) and washed with 10% $NaHCO_3$ (100 ml) and water (100 ml). The dried ($MgSO_4$) chloroform layer was evaporated to an oil, which was purified on a column of Kieselgel H (200 g) suspended in methylene chloride/petroleum ether (1:1.5, v/v). Elution of the column with the same solvent afforded pure 1,2-dioleoyl-3-O-trityl-sn-glycerol as a waxy oil. Yield 8.86 g (89%). Rf 0.74 (ether/petroleum ether). $[\alpha]_D^{25}$ +11.4 (c 1.2, chloroform). Literature data for 1-O-trityl-dioleoyl-sn-glycerol[5,6]: $[\alpha]_D^{25}$ -9.4 (c 1.23, chloroform).

1,2-Dioleoyl-sn-glycerol (8b)

The trityl protected derivative 8a was now detritylated on a silicic acid/boric acid column[26] (35 cm x 6 cm^2) which was prepared as follows: Silicic acid (Mallinckrodt, 45 g, 100 mesh) was washed 5 times with water and decanted. The silicic acid was filtrated and a hot solution of boric acid in water (30 g boric acid dissolved in 125 ml water at 100°C) was added. After three minutes the mixture was filtered and another portion of silicic acid (5 g) was added. The resulting mixture was

now activated for 24 hr at 120°C to give a dry white powder, which was suspended in petroleum ether and used to prepare the column. Compound 8b (2 g, 2.28 mmol) was dissolved in petroleum ether (10 ml) and applied to the silicic acid-boric acid column, which was eluted with the same solvent for 18 hr, and then with petroleum ether/ether (93:7, v/v) to remove the tritanol. After another 16 hr, 1,2-dioleoyl-sn-glycerol appeared and the appropriate fractions were collected and concentrated under reduced pressure to afford 1,2-dioleoyl-sn-glycerol 8b as an oil. Yield 1.21 g (86%). $[\alpha]_D^{25}$ -2.3 (c 3.5, chloroform). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (m,54H,4x$(CH_2)_6$ and 2xCH_3); 1.8-2.05 (c,8H,4x CH_2-C=C); 2.25 (c,4H,2xCH_2COO); 3.6-3.8 (c,2H,$\underline{CH_2}OH$); 4.2-4.4 (AB part of ABX,2H,CH_2OOCR); 5.1-5.4 (c,5H,HCOOCR,2xCH=CH).

1,2-Di-O-oleoyl-sn-glycero-3-phospho-(2,4-dichlorophenol) (9)

To an anhydrous solution of 1,2,4-triazole (283 mg, 4.1 mmol) in THF (11 ml) and triethylamine (0.56 ml, 4.1 mmol), was added at 0°C, 2,4-dichlorophenylphosphorodichloridate[31] 7a (560 mg, 2 mmol). After stirring for 30 min at room temperature, the reaction mixture was filtered to remove the triethylammonium hydrochloride salt. A solution of 8b (860 mg, 1.38 mmol) in dry pyridine (7 ml) was added dropwise, during 15 min, to the stirred filtrate containing the 2,4-dichlorophenyl-phosphoditriazolide 7b. After 2 hr at room temperature, TLC analysis (toluene/ether 8:2) indicated that no starting material 8b was present. Water (0.3 ml) was added to the reaction mixture and the mixture was concentrated under reduced pressure. The residue was dissolved in chloroform/methanol (95:5, v/v) and applied to a column of Kieselgel 60 suspended in the same solvent mixture. Elution of the column with chloroform/methanol (95:5 → 85:15, v/v) and concentration of the appropriate fractions afforded pure 9, which was dissolved in chloroform (200 ml) and extracted with TEAB (2x 30 ml, 1M, pH 7.5). The organic layer was concentrated to give the triethylammonium salt of 9 as a waxy compound. Yield 1.18 g (90%). Rf 0.45 (chloroform/methanol/25% ammonia, 80:20:2, v/v). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (m,63H,4x$(CH_2)_6$,2xCH_3 oleoyl,3xCH_3 triethylammonium); 1.8-2.05 (c,8H,4xCH_2-C=C); 2.25 (c,4H,CH_2COO); 3.05 (q,6H,3xCH_2 triethylammonium); 4.0-4.5 (m,4H,CH_2OP,CH_2OOCR); 5.05-5.5 (c,5H,HCOOCR,CH=CH); 7.0-7.7 (m,3H,2,4-dichlorophenyl). ^{13}C-NMR ($CDCl_3$): δ 8.6 (s,$\underline{CH_3}CH_2N$); 14.1 (s,2xCH_3); 22.7 (s,2x$\underline{CH_2}CH_3$); 31.9 (s,2x$\underline{CH_2}CH_2CH_3$); 24.9 (s,2x$\underline{CH_2}CH_2COO$); 34.1, 34.2 (s,2x $\underline{CH_2}CO$); 27.2 (s,4x$\underline{CH_2}C$=C); 29.2-29.8 (m,$(CH_2)_n$); 45.8 (s,$CH_3\underline{CH_2}N$); 62.4 (s,C1 glycerol); 64.3 (d, C3 glycerol, ^{2}Jc-p=4.2 Hz); 70.2 (d,C2 glycerol, ^{3}Jc-p=8.4 Hz); 129.7, 129.9 (s,2xC=C cis); 148.2 (d,C1 2,4-dichlorophenyl, ^{2}Jc-p=6.1 Hz); 125.7 (d,C2 2,4-dichlorophenyl, ^{3}Jc-p=7.6 Hz), 129.4, 127.7, 127.5, 122.2 (m,C3-C6 2,4-dichlorophenyl); 172.8, 173.2 (s,2xC=O). ^{31}P-NMR ($CDCl_3$): δ 5.94 (s).

Fully protected phosphatidyl-α-glucosyldiglyceride 10a

2,4,6-Triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazole TPSNT (145 mg, 0.38 mmol) was added to an anhydrous solution of the triethylammonium salt of 9 (322 mg, 0.34 mmol) and compound 6 (350 mg, 0.36 mmol) in pyridine (2 ml) at 20°C. After 1.5 hr, water was added and the products were dissolved in chloroform (100 ml) and washed with 10% aqueous $NaHCO_3$ (30 ml) and water (30 ml), respectively. The dried ($MgSO_4$) organic layer was concentrated under reduced pressure to give an oil. TLC analysis (chloroform/acetone, 96:4, v/v) of the crude oil indicated the presence of a major product (Rf 0.43) together with some alcohol 6 and a lipophilic byproduct (Rf 0.58) which turned out to be the diphosphatidyl derivative of 6 (5%). The oil was chromatographed on a column of Kieselgel H (40 g). Elution of the column with chloroform/acetone (100:0 → 99:1, v/v) and evaporation of the appropriate fractions gave the desired product 10a. Yield 421 mg (70%). Rf 0.43 (chloroform/acetone, 96:4, v/v). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (m,140H,2x$(CH_2)_{13}CH_{13}$,4x$(CH_2)_6$ and 2x(H3 oleoyl,4xSiCH-$(CH_3)_2$), 1.8-2.05 (c,8H,4xCH_2-C=C); 2.25 (c,8H,4xCH_2COO); 3.3-4.0 (m,6H,2xH3,H2',H3',H4',H5', glucosylglycerol); 4.0-4.5 (m,8H,2xCH_2OP,2xCH_2OOCR); 4.76 (dd,1H,H1 glucose,J1'2'=3.2 Hz,2 diastereoisomers); 5.05-5.5 (m,6H,2xHCOOCR,2xCH=CH); 7.0-7.5 (c,3H,2,4-dichlorophenyl). ^{13}C-NMR ($CDCl_3$): δ 12.2, 12.8, 13.0 (s,4xSiC); 17.3 (s,8xSiCH$\underline{CH_3}$); 14.1, 22.7, 32.0 (s,4x$CH_2CH_2CH_3$); 24.9, 34.1, 34.3 (s,$\underline{CH_2}\underline{CH_2}CO$); 29.2-29.8 (m,$(CH_2)_n$); 27.2 (s,4x$\underline{CH_2}$C=C); 62.2, 69.8, 66.6 (s,C1-C3 glucosyldiglyceride); 61.6, 69.1, 66.4 (m,C1''-C3'' phosphatidyl); 67.7 (broad,C6' glucose); 70.9 (d,C5' glucose, ^{3}Jc-p=6.1 Hz); 72.3, 77.0 (C2',C4' glucose); 98.7 (C1' glucose); 129.7 (s,C3 2,4-dichlorophenyl); 126.3 (d,C2 2,4-dichlorophenyl,^{3}Jc-p=6.9 Hz); 145.3 (d,C1 2,4-dichlorophenyl,^{2}Jc-p=5.4 Hz); 172.6, 172.9, 173.0, 173.2 (4xC=O). ^{31}P-NMR ($CDCl_3$): δ -6.85; -7.03: two diastereoisomers.

Phosphatidyl-α-glucosyldiglyceride 10c (II)

To a solution of phosphotriester derivative 10a (364 mg, 0.205 mmol) in dry THF (2 ml) was added syn-4-nitrobenzaldoxime (165 mg, 1 mmol) and N^1,N^1,N^3,N^3-tetramethylguanidine (0.115 ml, 0.9 mmol). After 3 hr at 20°C, when TLC analysis (chloroform/acetone 96:4) revealed quantitative removal of the 2,4-dichlorophenyl group, the solution was neutralized with acetic acid (60 µl) and the solvent was removed under reduced pressure. The crude material was chromatographed on a small bed of silicagel ($CHCl_3$/MeOH, 100:0 → 90:10) to give, after collection of the appropriate fractions, compound 10b (352 mg). Compound 10b (352 mg) was redissolved in dry THF together with some pyridine-HCl (46 mg, 0.4 mmol) and tetrabutylammonium fluoride (TBAF) in dry THF (1 ml, 0.8 M) was added. After 1.5 hr at 20°C, TLC analysis (chloroform/acetone/methanol/acetic acid/water, 30:40:10:10:5, v/v) indicated complete conversion of compound 10b (Rf 0.79) into the desired glycophospholipid 10c (Rf 0.49). Dowex 50W cation-exchange resin (100-200 mesh, ammonium-form, 2 g) was added together with methanol (2 ml). The resin was removed, after 10 min, by filtration and washed with THF/methanol (3:1, v/v). The combined filtrates were concentrated and the resulting oil was fractionated by short column chromatography on Kieselgel (15 g). The glycophospholipid 10c was eluted from the column with chloroform/methanol (99:1 → 92:8, v/v) and the appropriate fractions were collected and after TEAB extraction (2x20 ml, pH 7.5, 1 M) the organic layer was evaporated to afford the triethylammonium salt of 10c as an oil. Yield 260 mg (88%). $[\alpha]_D^{25}$ +16.5 (c 1, chloroform). Rf 0.53 (chloroform/methanol/25% ammonia, 60:30:3, v/v). Rf 0.78 (chloroform/acetone/methanol/acetic acid/water, 50:10:20:10:5, v/v). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (m,121H,2x$(CH_2)_{13}CH_3$,4x$(CH_2)_6$,2xCH_3 oleoyl, 3xCH_3 triethylammonium); 1.8-2.05 (c,8H,4xCH_2C=C); 2.25 (c,8H,4xCH_2COO); 3.05 (q,6H,3xCH_2 triethylammonium); 3.3-4.0 (m,6H,2xH3,H2',H3',H4',H5' glucosylglycerol); 4.0-4.5 (m,8H,2xCH_2OP,2xCH_2OOCR); 4.84 (d,1H,H1',J1'2'=3.4 Hz); 5.05-5.5 (m,6H,2xHCOOCR,2xCH=CH). ^{13}C-NMR ($CDCl_3$): δ 8.7 (s,$\underline{CH_3}CH_2N$); 14.1, 22.7, 32.0 (s,4x$CH_2CH_2CH_3$); 24.9, 34.1, 34.2 (s,$\underline{CH_2}CH_2CO$); 29.2-29.8 (m,$(CH_2)_n$); 27.2 (s,4x$\underline{CH_2}$C=C); 45.8 (s,$CH_3\underline{CH_2}N$); 62.7, 69.8, 66.4 (s,C1-C3 glucosyldiglyceride); 62.4, 63.7 (m,C1",C3" phosphatidyl); 70.3 (d,C2" phosphatidyl,$^3J_{C-P}$=7.6 Hz); 72.4, 73.3, 68.9 (s,C2',C3',C4' glucose); 63.4 (d,C6' glucose); 72.1 (d,C5' glucose); 129.7, 130.0 (s,2xC=C cis); 172.7, 173.0 (s,4xC=O). ^{31}P-NMR ($CDCl_3$): δ 1.74 (s).

References and Notes

*. For part I and II see references 11 and 39, respectively.
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