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

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Abstract - The preparation of phosphatidyl-β-glucosyl diglyceride 12c is described. The synthesis of glycophospholipid 12c was accomplished by using: (a) the levulinoyl group for the temporary protection of the glucose hydroxyl functions of 6b, which could then be converted into the dioleoyl substituted derivative 7c; (b) the tetraisopropyldisiloxane-1,3-diyl (TIPS) group to protect the 3'- and 4'-hydroxyl groups of 7c, in a two step procedure, to afford compound 8; (c) a 2,4-dichlorophenyl protected phosphatidic acid derivative 11. Compound 11 could be selectively coupled to the primary hydroxyl function of 8 to afford the fully protected glycophospholipid 12a. Finally, removal of the 2,4-dichlorophenyl and TIPS protecting groups from 12a was performed with syn-4-nitrobenzaldoximate and fluoride ions, respectively, to afford glycophospholipid 12c.

Introduction

Glucosyl diglycerides, in which a glucose residue is attached *via* an α or β linkage to 1,2-di-O-acyl-sn-glycerol, have been isolated from various microorganisms[1]. Phosphorylated derivatives of these glycolipids also occur in bacteria[2].

In this communication we wish to report the first synthesis of a phosphatidyl-β-glucosyldiglyceride (i.e. compound 12c, Scheme 4) having unsaturated oleoyl esters in the glucosyldiglyceride moiety. Glucophospholipid 12c is the β-glycosyl analog of phosphatidyl-α-glucosyldiglyceride from Streptococci, the synthesis of which we presented in a previous paper[3].

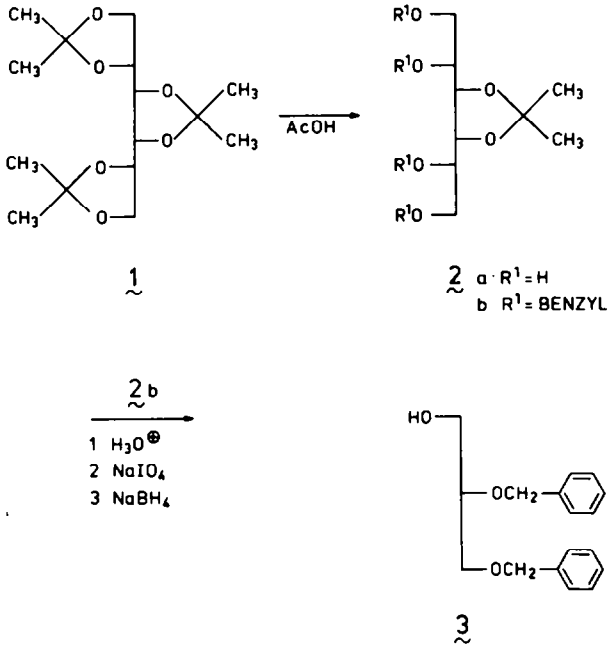
Up to now the β-glucophospholipid 12c has not been found in bacteria, however, its precursor, i.e. β-glucosyldiglyceride 7c has been isolated from mycoplasma neurolyticum[4].

The synthesis of compound 12c requires the introduction of a β-glycosidic bond between a glucose molecule and a glycerol unit containing unsaturated oleic acids (see compound 7c in Scheme 2). Furthermore, a saturated phosphatidyl part has to be linked, *via* a phosphodiester bond, to the primary 6'-hydroxyl function of the β-glucosyldiglyceride moiety 7c.

Synthesis of 3-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-sn-glycerol (5b)

For the synthesis of glycophospholipid 12c a suitable glucosyldiglyceride derivative is required having a β-glycosidic bond. Practically all the methods proposed for the introduction of a β-glucosidic bond require the presence of a participating ester group at the 2-hydroxyl function. As a consequence, base labile but-2-enyl[5] ether protection of the glycerol moiety cannot be used[3,5]. An alternative approach to the synthesis of a β-interglycosidic linkage is the method recently developed by Schmidt et al.[6,7]. A remarkable feature of this approach is that a non-participating benzyl group is present at the 2-hydroxyl group of glucose. However, the synthesis of properly protected β-glycosyl derivatives could also easily be achieved by condensing 2,3,4,6-tetra-O-acetyl-α-

D-glucopyranosyl bromide (4) with optically pure 1,2-di-O-benzyl-sn-glycerol (3) under well established Koenigs-Knorr conditions. In this respect it is noteworthy that the easily accessible and optically pure 1,2-O-isopropylidene-sn-glycerol racemises[8,9] during the Koenigs-Knorr condensation procedure.



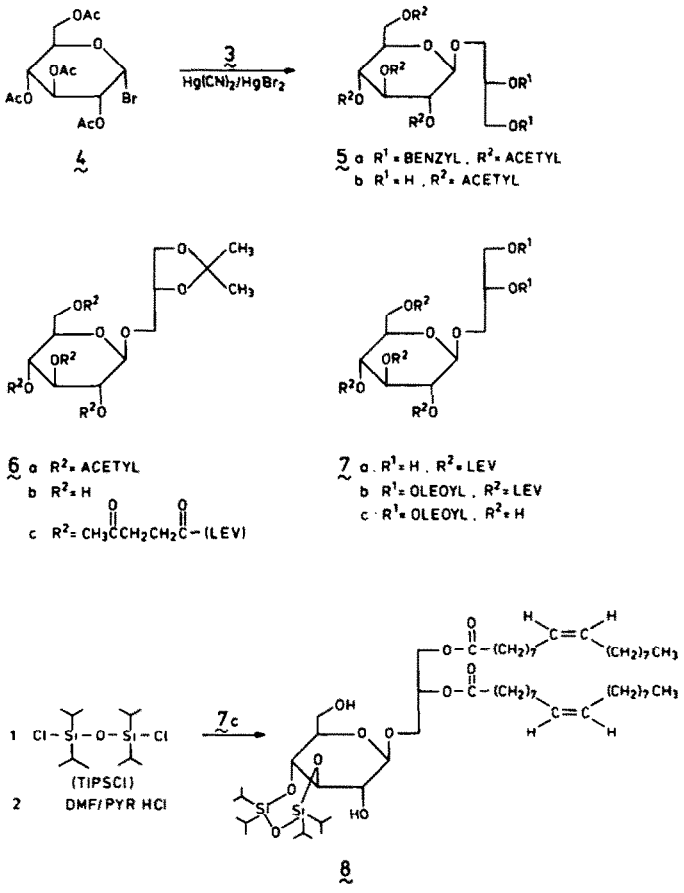
1 2 a R^1=H b R^1= BENZYL 3

Scheme 1

Optically pure 1,2-di-O-benzyl-sn-glycerol (3) was prepared starting from D-mannitol as the chiral source. D-mannitol was converted into 1,2-3,4-5,6-tri-O-isopropylidene-D-mannitol[11] (1) which, after a controlled aqueous acetic acid treatment, afforded 3,4-O-isopropylidene-D-mannitol (2a) as a crystalline solid[11]. The free hydroxyl functions of 2a were treated with benzyl chloride to give crude 2b. The isopropylidene blocking group of compound 2b was removed by acid treatment to give 1,2,5,6-tetra-O-benzyl-D-mannitol, which was then cleaved by oxidation with sodium periodate. Finally, reduction of the aldehyde function with sodium borohydride afforded, after purification by short column chromatography[10], 1,2-di-O-benzyl-sn-glycerol (3)[12] in 88% yield. Condensation of 1,2-di-O-benzyl-sn-glycerol (3) with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosylbromide (4) in dry acetonitrile, in the presence of $HgBr_2$ and $Hg(CN)_2$[13], gave glycosylglycerol 5a in excellent yield. Removal of the benzyl ethers of 5a by hydrogenolysis gave, after work-up, 3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-sn-glycerol (5b)[14] as a crystalline product.

Synthesis of 3-O-(β-D-glucopyranosyl)-1,2-di-O-oleoyl-sn-glycerol (7c)

We now, having a glucose protected β-glucosyl glycerol derivative (i.e. compound 5b) at our disposal, turned our attention to the conversion of 5b into the di-O-oleoyl derivative 7c. We solved this problem by protecting the glucose part of the β-glucosyl glycerol with levulinoyl[15] groups. The levulinoyl group, which has been used successfully in nucleotide synthesis[15,16] and sugar chemistry[17,18], can easily be removed under essentially neutral conditions without affecting the release of other esters. The introduction of the levulinoyl functions was performed in four steps. Firstly, the two hydroxyl functions of the glycerol moiety of 5b were simultaneously protected by the action of 2,2-dimethoxypropane in the presence of a catalytic amount of para-toluenesulfonic acid to give crystalline 3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1,2-O-isopropylidene-sn-glycerol (6a). Secondly, deblocking of the base-labile acetyl groups from 6a with a catalytic amount of sodium methoxide in dry methanol afforded compound 6b in quantitative yield. In the next step, 6b was levulinoylated with levulinic acid anhydride in the presence of 4-dimethylaminopyridine (DAP) as a catalyst to afford crude 6c. Finally, the isopropylidene group was removed from 6c by aqueous acetic acid treatment to give 3-O-(2,3,4,6-tetra-O-levulinoyl-β-D-glucopyranosyl)-sn-



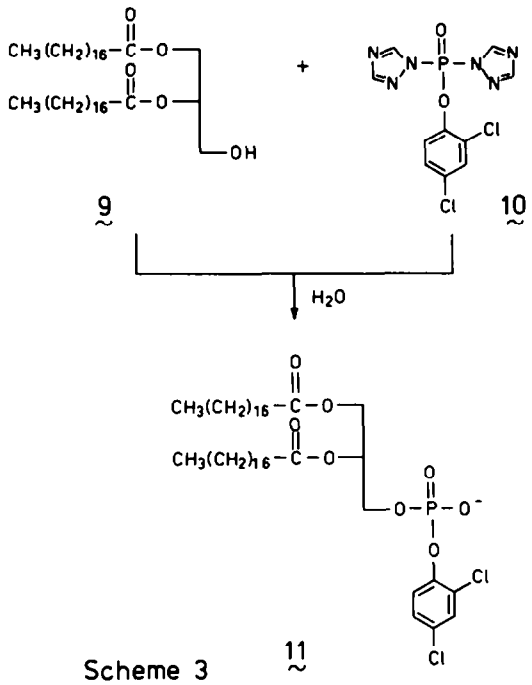
Scheme 2

glycerol (7a) in 89% overall yield. Compound 7a was then acylated with oleoyl chloride[3] to give 7b in 80% yield. The levulinoyl protective groups of derivative 7b were removed by treatment with hydrazine in pyridine-acetic acid. Purification of the crude material by column chromatography afforded pure 3-O-(β-D-glucopyranosyl)-1,2-di-O-oleoyl-sn-glycerol 7c as a waxy compound in 78% yield.

Preparation of the 3',4'-tetraisopropyldisiloxane-1,3-diyl protected derivative 8

In a previous paper[3] we demonstrated that we were able to perform a selective phosphorylation of the 6'-hydroxyl function of an α-glucosylglycerol derivative by utilizing the dynamic properties of the tetraisopropyldisiloxane-1,3-diyl (TIPS) protecting group[19,20].

The same approach could also be applied successfully for the preparation of phosphatidyl-β-glucosyldiglyceride 12c. Thus, compound 7c was dissolved in dry pyridine and treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPSCl)[21]. Work-up and purification by short column chromatography afforded 3-O-[4,6-O-(tetraisopropyldisiloxane-1,3-diyl), β-D-glucopyranosyl]-1,2-di-O-oleoyl-sn-glycerol in 91% yield. It is worthwhile mentioning that no disubstituted derivative of 7c could be detected by TLC analysis. In contrast, protection of an α-glucosyldiglyceride with TIPSCl led to the formation of nearly 20% disubstitution[3]. In this case the lack of selectivity may be due to the enhanced reactivity of the 2-hydroxyl function of an α-glucose derivative with regard to a β-analog[22]. In the next step, 3-O-[4,6-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-glucopyranosyl]-1,2-di-O-oleoyl-sn-glycerol was isomerized[3,20]. The isomerization reaction was performed in DMF with pyridinium hydrochloride as a mild acidic catalyst[23] to afford compound 8 in 57% yield. Recovered 4',6'-TIPS protected derivative of 7c was used again for the isomerization reaction to afford another portion of compound 8 (overall yield of 8 was 78%).

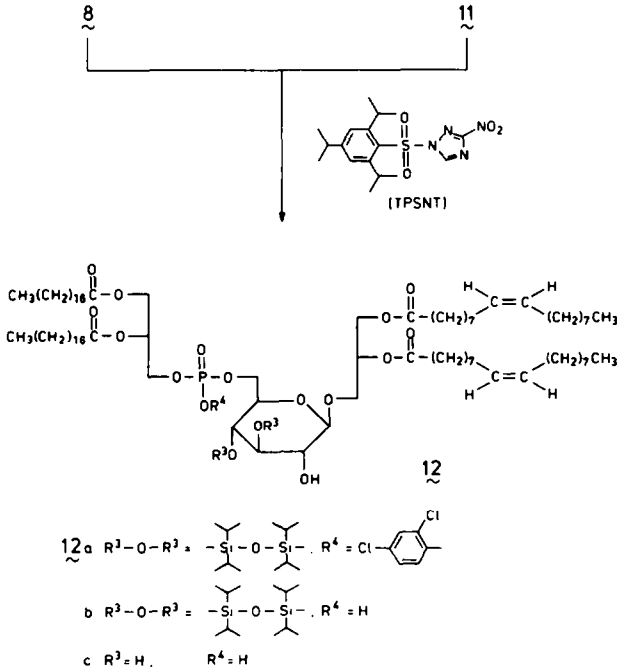


Scheme 3

Preparation of 1,2-di-O-stearoyl-sn-glycero-3-phospho-(2,4-dichlorophenol) (11)

The synthesis of the suitably protected phosphatidyl part 11 is outlined in Scheme 3 and consists of the phosphorylation of 1,2-di-O-stearoyl-sn-glycerol (9) by the phosphoditriazolide[24,25] method. Thus, a solution of 10 was added to optically pure 1,2-di-O-stearoyl-sn-glycerol 9[27] in pyridine. After two hours at room temperature, water was added to hydrolyze the phosphotriazolides. Crude compound 11 was purified by high speed column chromatography[28] to afford compound 11 in 92% yield.

Synthesis of protected glycophospholipid 12a and removal of the blocking groups to afford 12c



Scheme 4

Fully protected glycophospholipid 12a was prepared by condensing together the triethylammonium salt of phosphatidyl derivative 11 with 8 in the presence of the activating agent 2,4,6-triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazole (TPSNT)[29]. After one and a half hour at room temperature, TLC analysis indicated the formation of two diastereoisomers of 12a in a ratio of four to three. The crude reaction mixture was worked-up and chromatographed on Kieselgel to afford 12a in 86% yield. ^{31}P-NMR spectroscopy of purified 12a showed two resonances in the hydrogen decoupled spectrum. The intensities of the resonances were comparable with intensities estimated by TLC analysis of the diastereomeric mixture. Finally, the 2,4-dichlorophenyl and the TIPS protecting groups were removed from 12a in a two-step deblocking procedure. Firstly, compound 12a in dry THF[3,30] was treated with N^1,N^1,N^3,N^3-tetramethylguanidinium-4-nitro-benzaldoximate[31] to give 12b. Thereafter, compound 12b was treated with tetra-n-butylammonium fluoride (TBAF)[32] in dry THF in the presence of pyridinium hydrochloride[3]. Purification of the crude mixture by short column chromatography afforded 12c as a waxy solid in 85% yield.

The homogeneity and identity of compound 12c were corroborated by GLC and TLC analysis as well as by ^{1}H-NMR, ^{31}P-NMR and ^{13}C-NMR spectroscopy. GLC analysis of compound 12c, after sodium methoxide treatment, revealed the presence of equimolar amounts of methyl palmitate and methyl stearate. Hydrogen decoupled ^{31}P-NMR spectroscopy of 12c showed only one resonance due to the presence of one phosphodiester linkage. ^{13}C-NMR spectroscopy data of compound 12c were in complete accordance with its expected structure. Relevant resonances are: (i) the anomeric carbon atom at 103.7 ppm, which is characteristic[33] for a β-glucosidic bond; (ii) the cis substituted double bond at 129.7 and 130.0 ppm; (iii) the resonance of the 6 carbon atom at 63.4 ppm with a small phosphorus coupling.

Experimental*

3,4-O-isopropylidene-D-mannitol (2a)

1,2-3,4-5,6-Tri-O-isopropylidene-D-mannitol[11] (1) (20 g, 66.2 mmol) was dissolved in 70% aqueous acetic acid (400 ml) and kept at 40°C. After 1.5 hr the solvent was evaporated at reduced pressure and coevaporated twice with toluene. The light yellow oil was dissolved in acetone (600 ml) and shaked with K_2CO_3 to remove the last traces of acetic acid.

Excess K_2CO_3 and D-mannitol, which was formed during the acid hydrolysation step, were removed by filtration and the solvent was evaporated in vacuo to give an oil. The oil was dissolved in chloroform (100 ml) and some Kieselgel was added (5 g). After filtration of the chloroform to remove the Kieselgel, petroleum ether was added until compound 2a crystallized. Yield 12.0 g (82%); Rf 0.22 (chloroform/methanol, 92:8, v/v); m.p. 85°C.

1,2-Di-O-benzyl-sn-glycerol (3)

3,4-O-Isopropylidene-D-mannitol (2a) (10 g, 45 mmol) and benzyl chloride (32.8 g, 260 mmol) were dissolved in dry N,N-dimethylformamide (DMF, 100 ml). The mixture was cooled (ice-water bath) and NaH (6 g, 250 mmol) was added carefully. After the addition was finished the suspension was stirred for 1.6 hr at 60°C, when TLC analysis (toluene/acetone, 5:1, v/v) showed the formation of a single product (Rf 0.77), methanol was added (5 ml) to destroy excess NaH. Water was added (300 ml) and the diluted reaction mixture was extracted with ether (3x50 ml). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The thus obtained crude 1,2,5,6-tetra-O-benzyl-3,4-O-isopropylidene-D-mannitol (2b) was dissolved in a mixture of dioxan/methanol/1N aqueous HCl (200 ml; 3:6:1, v/v) and heated under reflux for 2 hr. TLC analysis (toluene/acetone, 5:1, v/v) showed complete conversion of compound 2b (Rf 0.77) into 1,2,5,6-tetra-O-benzyl-sn-glycerol (Rf 0.43). The reaction mixture was cooled and extracted with chloroform (3x75 ml). The combined organic layers were washed with 10% aqueous $NaHCO_3$ (2x50 ml) and water (50 ml) dried ($MgSO_4$) and concentrated to an oil. The oil was dissolved in methanol (900 ml) and an aqueous solution of $NaIO_4$ was added (15 g in 900 ml of water). The reaction mixture was stirred for 2 hr at 20°C, after which time TLC analysis (toluene/acetone, 5:1, v/v) revealed the oxidation to be complete (Rf 0.43 → 0.68). The reaction mixture was diluted with methanol (400 ml) and cooled. The precipitate was filtered off and to the filtrate was added $NaBH_4$ (16 g, 0.43 mmol). After 1 hr, TLC analysis indicated the reac-

* For general methods and materials see foregoing paper part III.

tion to be complete (Rf 0.68 → 0.50). The reaction mixture was neutralized with acetic acid and the solvent was evaporated to a small volume. The water layer was extracted with chloroform (3x50 ml), dried ($MgSO_4$) and the resulting oil was chromatographed on a column of Kieselgel 60 (300 g) suspended in chloroform. Elution of the column with chloroform/acetone (100:0 → 93:7, v/v) and collection of the appropriate fractions afforded 1,2-di-O-benzyl-sn-glycerol (3). Yield 10.8 g (88%); Rf 0.5 (toluene/acetone, 5:1, v/v). $[\alpha]_D^{25}$ -17.2 (c 1, chloroform). ^{1}H-NMR ($CDCl_3$): δ 2.6-2.8 (broad,1H,OH); 3.4-3.7 (c,5H,glycerol); 4.48, 4.60 (2xs,4H,OCH_2 benzyl); 7.2-7.4 (c,10H,benzyl-arom). ^{13}C-NMR: δ 62.7, 65.0, 78.1 (s,C1-C3 glycerol); 70.1, 72.1 (s,2xCH_2 benzyl); 127.6, 127.7, 128.3, 137.9, 138.2 (m,benzyl-arom).

3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-sn-glycerol (5b)

To a solution of 1,2-di-O-benzyl-sn-glycerol (3) (50 g, 18.4 mmol), $HgBr_2$ (3.65 g, 10 mmol) and $Hg(CN)_2$ (2.55 g, 10 mmol) in dry acetonitrile (50 ml) was added dropwise, over a period of 30 min at 20°C, a solution of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (4) (7.91 g, 20 mmol). After another 30 min, when TLC analysis (ether/petroleum ether, 4:1, v/v) showed the reaction to be complete (Rf product 5a 0.30), the reaction mixture was concentrated to an oil. The oil was redissolved in chloroform (300 ml) and washed with aqueous KBr (3x150 ml of a 1M solution) and water (150 ml). The dried ($MgSO_4$) organic layer was concentrated to dryness and redissolved in a small volume of ether and applied to a column of Kieselgel H (350 g). Pure product 5a was eluted from the column with ether/petroleum ether (5:1, v/v) and concentrated to an oil. Yield 9.7 g (88%).

Compound 5a (7.5 g, 12.5 mmol) was dissolved in ethanol (200 ml) and treated with hydrogen in the presence of palladium on charcoal (10%). After one day, the catalyst was filtered off and the ethanol was evaporated under reduced pressure to afford crude 5b. Crude 5b was crystallized from ether to give pure 5b. Yield 3.98 g (74%). Rf 0.34 (chloroform/methanol, 9:1, v/v). $[\alpha]_D^{25}$ -8.8 (c 0.62, chloroform). m.p. 107-109°C. ^{1}H-NMR ($CDCl_3$): δ 1.94-2.10 (4xs,4xCH_3); 3.5-3.8 (c,6H,glycerol and H5'); 4.0-4.35 (c,2H,2xH6'); 4.55 (d,1H,H1',J1'2'=8.0 Hz); 4.8-5.4 (m,3H,H2',H3',H4'). Anal. $C_{17}H_{26}O_{12}$ (422.2); calcd. C 48.32; H, 6.21, found C 48.55; H, 6.26.

3-O-(2,3,4,6-tetra-O-β-D-glucopyranosyl)-sn-glycerol-1,2-O-isopropylidene-sn-glycerol (6a)

To a solution of compound 5b (1.27 g, 3 mmol) in acetone (40 ml) was added 2,2-dimethoxypropane (5 ml) and para-toluenesulfonic acid (100 mg). After 1 hr at 20°C, the mixture was neutralized with triethylamine (0.15 ml) and evaporated to dryness. The crude material was redissolved in chloroform (100 ml) and washed successively with 10% aqueous $NaHCO_3$ (50 ml) and water (50 ml). The chloroform layer was dried ($MgSO_4$), evaporated to dryness and the residue was crystallized from diisopropyl ether to afford pure 6a. Yield 1.30 g (94%). $[\alpha]_D^{25}$ -11.0 (c 1, chloroform). Rf 0.68 (chloroform/methanol, 92:8, v/v); m.p. 110-111°C. Anal. $C_{20}H_{30}O_{12}$ (462.4); calcd. C 51.94; H, 6.54, found C 51.88; H, 6.65.

3-O-(2,3,4,6-tetra-O-levulinoyl-β-D-glucopyranosyl)-sn-glycerol (7a)

Compound 6a (0.97 g, 2.1 mmol) in dry methanol (10 ml) and a catalytic amount of sodium was left for 4 hr at 20°C, when TLC analysis (chloroform/methanol, 97:13, v/v) showed the reaction to be complete (Rf 0.78 → 0.07). The reaction was stopped by the addition of Dowex 50W (H^+) cation-exchange resin (5 g). The resin was filtered off, and the filtrate was evaporated to give a residue, which was dried by repeated coevaporation with pyridine (3x10 ml).

The thus obtained compound 6b (2.1 mmol) was dissolved in pyridine (10 ml) and levulinic acid anhydride (2.7 g, 12 mmol) was added together with a catalytic amount of 4-dimethylaminopyridine and the mixture was stirred at 20°C. After 45 min at 20°C, TLC analysis showed complete conversion of the starting material 6b into the product 6c. Water (1 ml) was added to destroy excess levulinic acid anhydride and the reaction mixture was diluted with chloroform (100 ml), washed with 10% aqueous $NaHCO_3$ (2x50 ml) and water (50 ml). The organic layer was dried ($MgSO_4$) and evaporated to dryness.

Crude 6c was now dissolved in 70% acetic acid (10 ml) and, after 3 hr at 50°C, TLC analysis (chloroform/methanol, 92:8, v/v) indicated the conversion of compound 6c (Rf 0.64) into the more polar compound 7c (Rf 0.36). The solvent was removed under reduced pressure and the resulting oil was coevaporated twice with toluene. The crude material was subjected to chromatographic purification on a column of Kieselgel H (60 g), which was eluted with chloroform/methanol (94:6, v/v). After col-

lection of the appropriate fractions, pure 3-O-(2,3,4,6-tetra-O-levulinoyl-β-D-glucopyranosyl)-sn-glycerol (7a) was obtained as an oil. Yield 1.21 g (89%). Rf 0.36 (chloroform/methanol, 92:8, v/v). $[\alpha]_D^{25}$ -12.2 (c 1, chloroform). ^{1}H-NMR ($CDCl_3$): δ 2.15 (4xs,12H,4xCH_3); 2.40-2.66 (c,16H,4xCH_2CH_2); 3.5-3.8 (c,6H,glycerol and H5'); 4.0-4.35 (c,2H,2xH6); 4.55 (d,1H,H1',J1'2'=8.0 Hz); 4.8-5.4 (m, 3H,H2',H3',H4').

3-O-(β-D-glucopyranosyl)-1,2-di-O-oleoyl-sn-glycerol (7c)

To compound 7a (1.21 g, 1.87 mmol), dissolved in pyridine (10 ml) was added dropwise a solution of oleoyl chloride (2.36 g, 6 mmol) in methylene chloride (5 ml). The reaction mixture was stirred overnight at 20°C. TLC analysis (toluene/acetone, 5:1, v/v) showed the presence of one major product together with some minor impurities. Water (0.5 ml) was added and the reaction mixture was kept at 20°C for 1 hr. Then the reaction mixture was dissolved in chloroform (100 ml) and washed with 10% aqueous $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried ($MgSO_4$) and concentrated to an oil, which was chromatographed on a column of Kieselgel H (80 g) suspended in chloroform. Elution with chloroform/acetone (96:4 → 92:8, v/v) gave, after concentration of the appropriate fractions pure 7b. Yield 1.77 g (80%).

Compound 7b (1.45 g, 1.23 mmol) was now dissolved in pyridine (5 ml) and a 1M solution of hydrazine hydrate in pyridine/acetic acid (25 ml, 3:2, v/v) was added. After 10 min at 20°C chloroform was added (150 ml) and the reaction mixture was washed successively with ice-cold water (4x100 ml), 10% aqueous $NaHCO_3$ (2x50 ml) and water (50 ml). The dried ($MgSO_4$) organic layer was concentrated to an oil and applied to a column of Kieselgel 60 (60 g). Elution of the column with chloroform/methanol (97:3 → 92:8, v/v) afforded 3-O-(β-D-glucopyranosyl)-1,2-di-O-oleoyl-sn-glycerol (7c) as a waxy solid. Yield 757 mg (78%). Rf 0.32 (chloroform/methanol, 92:8, v/v). $[\alpha]_D^{25}$ -10 (c 1, chloroform). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (c,50H,$(CH_2)_n$ oleoyl); 1.8-2.1 (c,8H,4xCH_2C=C); 2.2-2.4 (t,4H,2xCH_2COO, J=7.5 Hz); 3.0-4.0 (c,8H,2xH6',H2',H3',H4',H5',2xH3 glycerol); 4.0-4.7 (c,3H,2xH1 glycerol, H1'); 5.0-5.4 (c,5H,2xCH=CH,H2 glycerol). ^{13}C-NMR ($CDCl_3$): δ 14.1, 22.8, 31.9 (s,2x$CH_2CH_2CH_3$); 27.2 (s, 4xCH_2C=C); 29.2-29.8 (m,$(CH_2)_n$); 24.9, 34.0, 34.1 (s,CH_2CH_2CO); 63.0, 68.0, 69.4 (s,C1-C3 glycerol); 61.5, 73.4, 75.5, 70.2, 76.3 (s,C2'-C6' glucose); 103.6 (s,C1' glucose); 129.8, 130.0 (s,C=C cis); 173.1, 173.4 (s,2xC=O).

3-O-[3,4-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-glucopyranosyl]-1,2-di-O-oleoyl-sn-glycerol (8)

To a stirred solution of compound 7c (743 mg, 0.95 mmol) in pyridine (5 ml) was added dropwise at -15°C during 3 hr (ice-salt bath) 1,2-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPSCl) (0.38 ml, 1.2 mmol) in pyridine (5 ml). The reaction was stopped by the addition of water and the reaction mixture was dissolved in chloroform (200 ml), washed with 10% aqueous $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried ($MgSO_4$) and evaporated to dryness. A solution of this material in chloroform was applied to a column of Kieselgel H (60 g) suspended in chloroform. The column was eluted with chloroform/acetone (98:2, v/v) and the pure fractions were concentrated to afford pure 3-O-[4,6-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-glucopyranosyl]-1,2-di-O-oleoyl-sn-glycerol as an oil. Yield 881 mg (91%).

The latter compound (881 mg, 0.86 mmol) was dissolved in dry DMF (5 ml) together with pyridinium hydrochloride (200 mg, 1.74 mmol). The reaction mixture was stirred for 16 hr at 20°C, when TLC analysis (chloroform/methanol, 94:6, v/v) indicated nearly 60% conversion of the starting material (Rf 0.31) into product 8 (Rf 0.59). The reaction was stopped by the addition of a 2M solution of triethylammonium bicarbonate (0.5 ml). The reaction mixture was concentrated under reduced pressure, dissolved in chloroform and washed with water (2x50 ml). The organic phase was dried ($MgSO_4$) and concentrated to an oil, which was applied to a column of Kieselgel H (60 g). The column was developed with chloroform/acetone (98:2, v/v) and pure 8 was isolated (523 mg, 0.51 mmol) together with the starting material (369 mg, 0.36 mmol). The recycled starting material was subjected to the same procedure to afford an additional portion of compound 8 (192 mg, 0.19 mmol). Overall yield of compound 8: 715 mg (78%). Rf 0.59 (chloroform/methanol, 96:4, v/v). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (c,78H,2x$(CH_2)_6CH_3$,2x$(CH_2)_5$,4xCH$(CH_3)_2$ TIPS); 1.9-2.2 (c,8H,4xCH_2C=C); 2.2-2.5 (c,4H,2x CH_2COO); 3.2-4.6 (c,11H,H1'-H6' glucose,2xH1 and 2xH3 glycerol); 5.0-5.5 (c,5H,2xCH=CH,H2 glycerol). ^{13}C-NMR ($CDCl_3$): δ 14.1, 22.7, 31.9 (s,2x$CH_2CH_2CH_3$); 12.2, 12.3, 12.9 (s 4xSiCH TIPS); 17.3 (s,8xCH_3 TIPS); 27.2 (s,4xCH_2C=C); 24.9 (s,2xCH_2CH_2COO); 34.1, 34.3 (s,2xCH_2COO); 62.8, 70.3, 68.2

(s,C1-C3 glycerol); 74.0, 79.4, 72.8, 76.5, 62.2 (s,C2'-C6' glucose); 103.4 (s,C1' glucose); 129.7, 130.0 (s,C=C cis); 173.2, 173.4 (s,2xC=O).

1,2-di-O-stearoyl-sn-glycero-3-phospho-(2,4-dichlorophenol) (11)

To a solution of 1,2,4-triazole (207 mg, 3 mmol) in dry THF (5 ml) and triethylamine (0.42 ml, 3.1 mmol) was added dropwise a solution of 2,4-dichlorophenyl-phosphorodichloridate[26] (420 mg, 1.5 mmol) in THF (2.5 ml). After stirring for 30 min at 20°C, the reaction mixture was filtered to remove the triethylammonium hydrochloride. To the thus obtained ditriazolide 10 in THF was added dropwise 1,2-di-O-stearoyl-sn-glycerol (625 mg, 1 mmol) (9) in pyridine (6 ml). After 3 hr at room temperature, water (0.5 ml) was added to the reaction mixture and, after another 10 min, the reaction mixture was concentrated under reduced pressure. The residue was purified by high speed chromatography on a column of Kieselgel 60 suspended in chloroform/methanol (90:10, v/v). Elution of the column with the same solvent mixture gave pure phosphatidyl derivative 11. Compound 11 in chloroform (100 ml) was then extracted with aqueous triethylammonium bicarbonate (1M TEAB, pH 7.5, 50 ml) to afford 11 as the triethylammonium salt. Yield 868 mg (92%). Rf 0.48 (chloroform/methanol/25% ammonia, 70:20:3, v/v). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (c,75H,2x$(CH_2)_{15}CH_3$,3xCH_3 triethylammonium); 2.05-2.22 (c,4H,2xCH_2COO); 3.05 (q,6H,3xCH_2N,J=7.5 Hz); 4.06 (t,2H,CH_2OP,J=7.5 Hz); 4.08-4.5 (AB part ABX,2H,CH_2OOCR); 5.05-5.4 (c,1H,HCOOCR); 7.06-7.65 (m,3H 2,4-dichlorophenyl). ^{31}P-NMR ($CDCl_3$): δ -5.75 (s).

Fully protected glycophospholipid 12a

To an anhydrous solution of the triethylammonium salt of compound 11 (255 mg, 0.27 mmol) and the alcohol 8 (270 mg, 0.263 mmol) in pyridine (2 ml) was added 2,4,6-triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazole (0.3 mmol). After 1.5 hr at room temperature, TLC analysis (chloroform/acetone, 98:2, v/v) showed the presence of two diastereomers of compound 12a (Rf 0.35 and 0.44). The mixture was diluted with chloroform (100 ml) and washed with aqueous 10% $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure and chromatographed on Kieselgel H (15 g). Elution of the column with chloroform/acetone (100:0 → 98:2) afforded after evaporation of the appropriate fractions pure 12a. Yield 416 mg (86%). Rf 0.35 and 0.44 (ratio, 4:3) (chloroform/acetone, 98:2, v/v). $[\alpha]_D^{25}$ -15 (c 1, chloroform). ^{13}C-NMR ($CDCl_3$): δ 14.1, 22.7, 32.0 (s,4x$CH_2CH_2CH_3$); 12.2, 12.8, 13.0 (s,4xSiC TIPS); 17.0 (s,8xCH_3 TIPS); 24.9, 34.1, 34.8 (s,4xCH_2CH_2COO); 27.2 (s,4xCH_2C=C); 29.4, 29.8 (m,$(CH_2)_n$); 62.4, 70.0 (s,C1-C3 glycerol); 73.0, 79.1, 75.1 (^{3}Jc-p=6.8 Hz); 68.6 (broad,m,C2'-C6' glucose); 61.5, 69.2 (^{3}Jc-p=6.8 Hz); 67.8 (broad,m,C1''-C3'' glycerol); 103.1 (s,C1' glucose); 129.7, 129.9 (s,2xC=C cis); 145.3 (^{2}Jc-p=5.4 Hz); 126.3 (^{3}Jc-p=6.9 Hz); 130.3, 130.9, 128.0, 122.3 (m,C1-C6 2,4-dichlorophenyl); 172.6, 173.0, 173.3 (s,4xC=O). ^{31}P-NMR ($CDCl_3$): δ -6.92, -7.12 (two diastereomers).

Glycophospholipid 12c

To a solution of compound 12a (285 mg, 0.155 mmol) in dry THF (3 ml) was added syn-4-nitrobenzaldoxime (140 mg, 0.85 mmol) together with N^1,N^1,N^3,N^3-tetramethylguanidine (94 mg, 0.82 mmol). After 3 hr at 20°C, TLC analysis (chloroform/methanol/acetone/acetic acid/water, 60:5:15:5:0.5) indicated complete removal of the 2,4-dichlorophenyl group (Rf 0.91 → 0.61). Acetic acid was added to the reaction mixture (60 mg, 1 mmol) and the solvent was evaporated. The crude material (12b) was applied to a small column of Kieselgel H (6 g) which was eluted with chloroform/methanol (100:0 → 96:4, v/v). After evaporation of the solvent, pure 12b (284 mg) was obtained in quantitative yield. Compound 12b (284 mg, 0.155 mmol) was now dissolved in dry THF and a solution of tetra-n-butylammonium fluoride in THF (1 ml of a 0.8 M solution) was added together with pyridinium hydrochloride (46 mg, 0.4 mmol). After 3 hr at 20°C, TLC analysis (chloroform/acetone/methanol/acetic acid/water, 60:15:5:5:0.5, v/v) showed a complete removal of the TIPS protecting group (Rf 0.84 → 0.24). The reaction was stopped by the addition of Dowex 50W cation-exchange resin (100-200 mesh, ammonium-form, 2 g). The resin was removed by filtration and washed with THF/methanol (3:1, v/v). The crude reaction mixture was concentrated and applied to a column of Kieselgel H (10 g) suspended in chloroform/methanol (98:2, v/v). Elution of the column with chloroform/methanol (98:2 → 92:8, v/v) and collection of the appropriate fractions afforded, after evaporation of the solvent, pure 12c as an oil. The oil was dissolved in chloroform and extracted with triethylammonium bicarbonate (1M TEAB, pH 7.5, 50 ml) to afford, after removal of the solvent, the triethylammonium salt of 12c as a waxy

compound. Yield 200 mg (85%). $[\alpha]_D^{25}$ -12 (c 1, chloroform). Rf 0.24 (chloroform/acetone/methanol/acetic acid/water, 60:15:5:5:0.5, v/v). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (c,125H,2x$(CH_2)_{15}$,CH_3,$(CH_2)_n$ oleoyl,3xCH_3 triethylammonium); 1.8-2.1 (c,8H,4xCH_2C=C); 2.2-2.4 (c,8H,4xCH_2COO); 3.1 (q,6H,3xCH_2N,J=7.5 Hz); 3.0-4.5 (m,glucose and glycerol except H2 glycerol); 5.05-5.5 (c,6H,2xCH=CH,2xH2 glycerol). ^{13}C-NMR ($CDCl_3$/CD_3OD): δ 14.1, 22.8, 32.0 (s,4x$CH_2CH_2CH_3$); 25.0, 34.2, 34.3 (s,4x$\underline{CH_2}\underline{CH_2}$COO); 29.2-29.8 (m,$(CH_2)_n$); 27.3 (s,2x$\underline{CH_2}$C=C); 8.7, 45.7 (s,triethylammonium); 62.2, 70.0, 67.8 (s,C1-C3 glycerol); 62.6, 70.4, 63.8 (broad,s,C1"-C3" glycerol); 73.7, 78.4, 68.6, 76.0 (broad), 63.4 (broad) (m,C2'-C6' glucose); 103.7 (s,C1' glucose); 129.7, 130.0 (s,2xC=C); 173.3, 173.5, 173.8 (s,4xC=O). ^{31}P-NMR ($CDCl_3$): δ 1.19 (s).

References and Notes

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