Tetrahedron Vol. 41, No. 20, pp. 4567 to 4575, 1985
Printed in Great Britain.

0040-4020/85 $3.00 + .00
Pergamon Press Ltd.

SYNTHESIS OF STREPTOCOCCI PHOSPHATIDYL-α-DIGLUCOSYLDIGLYCERIDE AND RELATED GLYCOLIPIDS. APPLICATION OF THE TETRAISOPROPYLDISILOXANE-1,3-DIYL (TIPS) PROTECTING GROUP IN SUGAR CHEMISTRY. PART V*

C.A.A. van BOECKEL and J.H. van BOOM*

Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, the Netherlands

(*Received in UK* 18 *June* 1984)

Abstract - A short and convenient synthesis of phosphatidyl-α-diglucosyldiglyceride (i.e. compound 8d) and two related Streptococci glycolipids (i.e. compounds 6a and 6b) will be presented. 4',6'-Tetraisopropyl-disiloxane-1,3-diyl (TIPS) protected α-glucosyl diglyceride (i.e. compound 2) turned out to be a suitable protected precursor. Thus, compound 2 was selectively condensed with glucosyl bromide 3 to afford 4. Removal of the protecting groups from 4 gave glycolipid 6a. The "dynamic" properties of the TIPS protecting group were utilized to convert 4',6'-TIPS protected 4 into 3',4'-TIPS protected derivative 5a. Compound 5a could then be condensed with either a stearoyl fatty acid or a phosphatidyl moiety to give the fully protected derivatives 5c and 8a, respectively. Finally, removal of all the protecting groups from 5c and 8a afforded the glycolipid 6b and glycophospholipid 8d, respectively.

Introduction

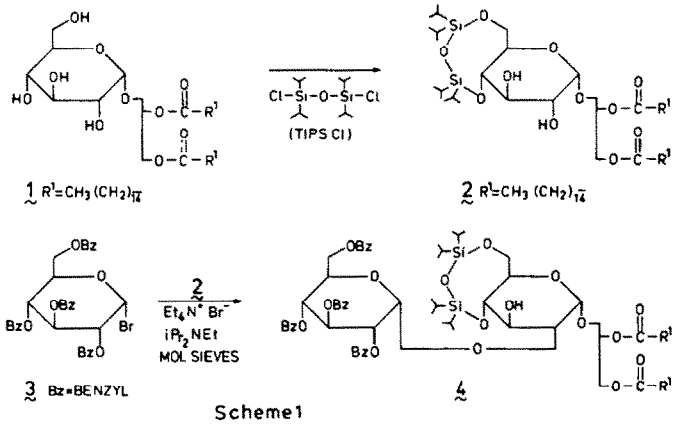
A special and well defined class of glyco(phospho)lipids occurs in Streptococci bacteria. The structure as well as the mutual relationship of many of these Streptococci glyco(phospho)lipids has been established[1-14]. Thus, the structure of practically all glyco(phospho)lipids found in Streptococci is characterized by the presence of a central α-glucosyldiglyceride moiety (i.e. fragment 1 in Scheme 1), which carries substituents on the 2'- and/or 6'-hydroxyl group. The substituent on the 6'-hydroxyl function may be a phosphatidyl or a fatty acid residue, whereas the substituent on the 2'-hydroxyl function proved to be an α-glucosyl moiety which, in turn, may be further functionalised.

Pieringer and Fischer[2,5,6,8,9] showed that the above mentioned Streptococci glyco(phospho)lipids have a metabolic relationship in which α-diglucosyldiglyceride (i.e. compound 6a in Scheme 2) turned out to be the most important precursor. The latter α-diglucosyldiglyceride (6a) is also the precursor of an acylated glycolipid[4,10,11] (i.e. compound 6b in Scheme 2), which was isolated from Streptococcus Lactis Kiel 42712. From the same species a lipoteichoic acid was isolated and it was established that glycolipid 6b functioned as its metabolic precursor[4,10,11]. In this paper[15] we wish to describe the synthesis of some major Streptococci glyco(phospho)lipids, i.e.: phosphatidyl-α-diglucosyldiglyceride (i.e. compound 8b in Scheme 3), α-diglucosyldiglyceride (i.e. compound 6a in Scheme 2) and also the 6'-acylated derivative of α-diglucosyldiglyceride (i.e. compound 6b in Scheme 2).

Synthesis of protected α-diglucosyldiglyceride 4 (Scheme 1)

In a previous report[16] we presented a convenient synthesis of α-glucosyldiglyceride (i.e. compound 1). In the same paper we also demonstrated that the α-glucosyldiglyceride 1, could be selectively

and simultaneously protected with the recently developed[17,18] tetraisopropyldisiloxane-1,3-diyl protective group to give 2 in 70% yield. At this stage we were anxious to find out if the 2'-hydroxyl function of compound 2 could be selectively condensed with a suitably protected α-glucopyranosyl moiety (i.e. compound 3 in Scheme 1). The feasibility of the selective glycosidation has been endorsed by several reports[19-21] which emphasized the enhanced reactivity of the 2-hydroxyl, in comparison with the 3-hydroxyl function, of α-glucopyranosyl derivatives. The difference in reactivity between the 2'- and 3'-hydroxyl group has been attributed[22] to the presence of an intramolecular hydrogen bridge between the 2'-hydroxyl function and the oxygen of the α-glycosidic bond. Furthermore, in our case we observed, by examining a molecular model of compound 2, that the 3'-hydroxyl function was shielded by an isopropyl substituent of the TIPS protecting group. The enhanced reactivity of the 2'-hydroxyl function of compound 2 was demonstrated by an acylation experiment in which compound 2 was treated with acetic anhydride in pyridine. After one hour at room temperature, TLC analysis indicated the formation of only one product. ^{1}H-NMR spectroscopy revealed the formation of a mono-acylated derivative, which turned out to be the expected 3-O-[2-O-acetyl-4,6-O-(tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranosyl]-1,2-di-O-palmitoyl-sn-glycerol.



Scheme1

We now, having established an enhanced reactivity of the 2'-hydroxyl function of compound 2, turned our attention to the selective introduction of a 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl moiety (i.e. formation of compound 4 in Scheme 1).

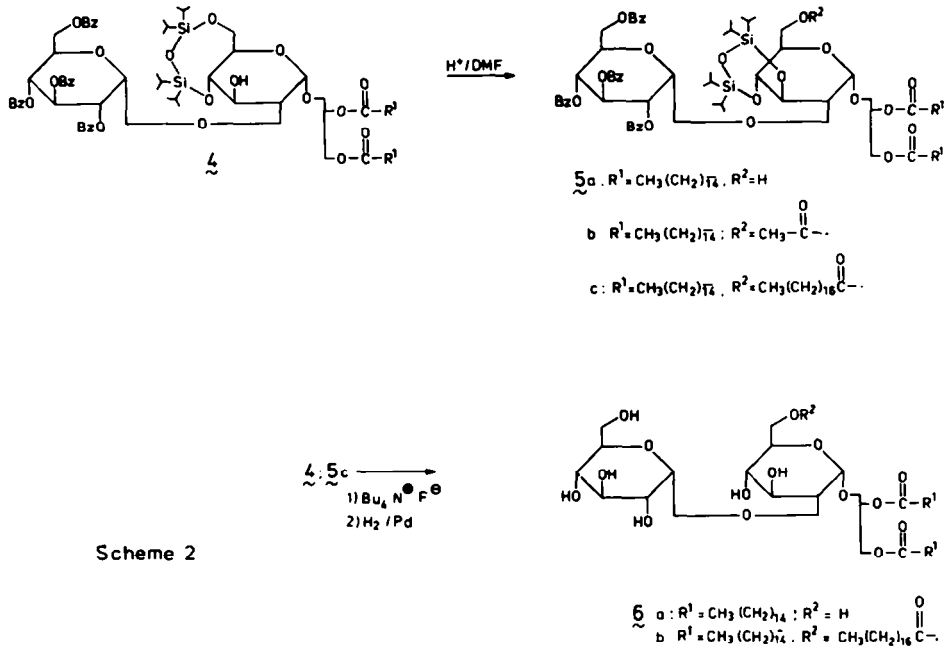
It should be noted that the silyl-primary oxygen bond of the 4',6'-TIPS protected derivative 2 is easily cleaved in an acid catalysed reaction[18,28]. Unfortunately, most of the α-glucosidations are performed under acidic conditions[23-27]. However, the α-glucosidation procedure developed by Lemieux et al.[23] may be conducted under essentially basic conditions.

Reacting together compound 2 and the bromide 3[29,30] under these reaction conditions afforded 4 in 45% yield. The structure of compound 4 was corroborated by ^{13}C-NMR spectroscopy.

Two resonances were observed in the anomeric region, the chemical shifts of which (i.e. 96.6 and 97.9 ppm) are characteristic for α-glucosidic bonds[31,32]. Further, the C_2, resonance at 80.4 ppm (7.8 ppm downfield in comparison with compound 2 confirmed[33] the selective glycosidation of the 2'-hydroxyl function of derivative 2.

Isomerization of 4',6'-TIPS protected derivative 4 into 3',4'-TIPS protected 5a and preparation of glycolipids 6a,b (Scheme 2)

At this stage of the synthesis we utilized the dynamic properties of the TIPS protecting group: i.e. conversion of the 4',6'-TIPS protected derivative 4 into the 3',4'-TIPS protected derivative 5a. Thus, compound 4 dissolved in dry DMF was treated with a catalytic amount of mesitylene sulfonic acid[18] to afford 5a in 84% yield. In order to locate the exact position of the TIPS protecting group we acylated derivative 5a, with acetic acid anhydride in pyridine and in the presence of 4-dimethylaminopyridine[34]. Analysis of the thus obtained acylated product by NMR spectroscopy, showed the formation of solely 5b. Thus, ^{1}H-NMR revealed the presence of one acetyl group, whereas ^{13}C-NMR spectroscopy indicated the acylation of the 6-hydroxyl function of 5a. The fully protected deriva-



Scheme 2

tive of glycolipid 6 (i.e. compound 5c) was obtained by reacting compound 5a with stearoyl chloride. The naturally occurring glycolipid 6b was obtained by complete removal of the benzyl and TIPS protecting groups from 5c. Deblocking of the TIPS group was effected by treating 5c with excess tetra-n-butylammonium fluoride (TBAF) in dry THF[35]. Hydrogenolysis of the above intermediate with hydrogen in the presence of palladium on charcoal afforded 6b in 74% yield. In the same way compound 4 was deblocked to afford α-diglucosyldiglyceride 6a as a white solid in 74% yield.

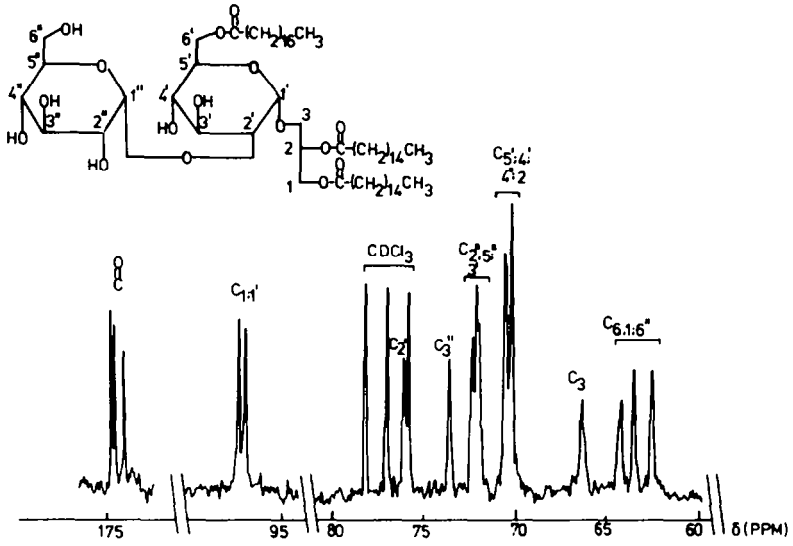
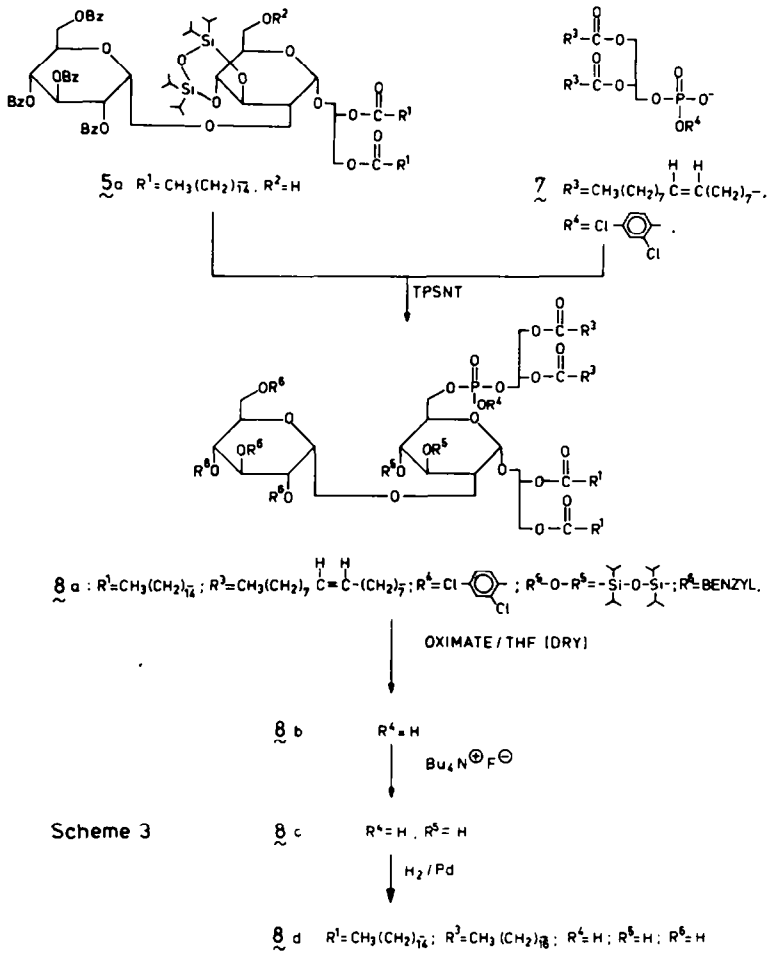


Figure 1. Part of the ^{13}C-NMR spectrum of compound 6b

The ^{13}C-NMR spectrum of glycolipid 6b is depicted in Figure 1[36]. Important resonances are: (i) three carbonyl resonances (at 173.8, 174.4 and 174.6 ppm); (ii) two α-anomeric resonances (at 97.6 and 97.1 ppm); (iii) the C_2'-carbon resonance (at 76.8 ppm), which due, to the glycosidation of the 2'-hydroxyl function, has been shifted downfield; (iv) the C_6'-carbon resonance (at 63.9 ppm), which is deshielded due to the presence of the O-stearoyl function (compare C_6" at 62.0 ppm).

Preparation of fully protected glycophospholipid 8a and removal of the protecting groups to afford glycophospholipid 8d (Scheme 3)

Coupling of the alcohol 5 with an excess of the triethylammonium salt of phosphatidyl part[16] 7 in the presence of activating agent 2,4,6-triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazole (TPSNT)[37].



Scheme 3

^{31}P-NMR spectroscopy of compound 8a showed in its proton decoupled spectrum two resonances, which are due to the presence of two diastereomers. Complete deblocking of the fully protected glycophospholipid 8a was accomplished in three distinct stages. Firstly, the 2,4-dichlorophenyl protecting group was deblocked quantitatively by the action of N^1,N^1,N^3,N^3-tetramethylguanidinium-syn-4-nitrobenzaldoximate[38] in dry THF[16] to give pure 8b in a quantitative yield. Secondly, the TIPS protecting group was removed from 8b by treatment with excess tetra-n-butylammonium fluoride in the presence of pyridinium chloride[16]. After work-up compound 8c was obtained as its triethylammonium salt in 95% yield. Finally, the triethylammonium salt of 8c was converted into the sodium salt of 8c, which was then submitted to catalytic hydrogenolysis (10% Pd/C). Under these conditions the benzyl ethers were cleaved and the oleoyl fatty moieties converted into stearoyl groups. Pure glycophospholipid 8d was isolated as its triethylammonium salt in 75% yield. The homogeneity and identity of phosphatidyl-α-diglucosyldiglyceride 8d were established by TLC and GLC analysis, as well as by ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectroscopy. TLC analysis of compound 8d revealed a single spot. Further, the chromatographical behaviour of 8d on TLC was compared with other synthetic Streptococci glyco(phospho)lipids: i.e. α-monoglucosyldiglyceride 1, α-diglycosyldiglyceride 6a, its acylated derivative 6b and phosphatidyl-α-monoglycosyldiglyceride[16]. The Rf-values of the synthesis glyco-(phospho)lipids were identical with the corresponding naturally occurring derivatives[5,9,39]. Compound 8b was also subjected to a sodium methanolate treatment in dry methanol/ether. GLC analysis

of the resulting fatty acid methyl esters indicated the presence of methyl palmitate and methyl stearate in equimolar amounts. ^{13}C-NMR spectroscopy of compound 8d (see Fig. 2) gave spectral data, which were in complete accordance with the structure of glycophospholipid 8d. For instance, (i) four low field carbonyl resonances; (ii) two coinciding resonances (at 97.1 ppm) of the α-anomeric carbon atoms C_1' and C_1"; (iii) one deshielded C_2'-carbon resonance (at 77.0 ppm); (iv) one deshielded C_6'-carbon resonance with a small phosphorus coupling (at 63.8 ppm). ^{31}P-NMR spectroscopy of compound 8d showed one resonance in the proton decoupled spectrum, which is additional prove for the homogeneity of glycophospholipid 8d. In conclusion, the data presented in this paper demonstrate that 4,6-TIPS protected α-glucopyranosyl derivatives (e.g. compound 2 in Scheme 1) are versatile and easily accessible building units for the synthesis of 1,2,6-tri-substituted glucose molecules (e.g. compounds 6b and 8d). As such, they may present a convenient alternative for differently protected, and far from easily accessible, glucose derivatives. For instance, Eby et al.[40] described

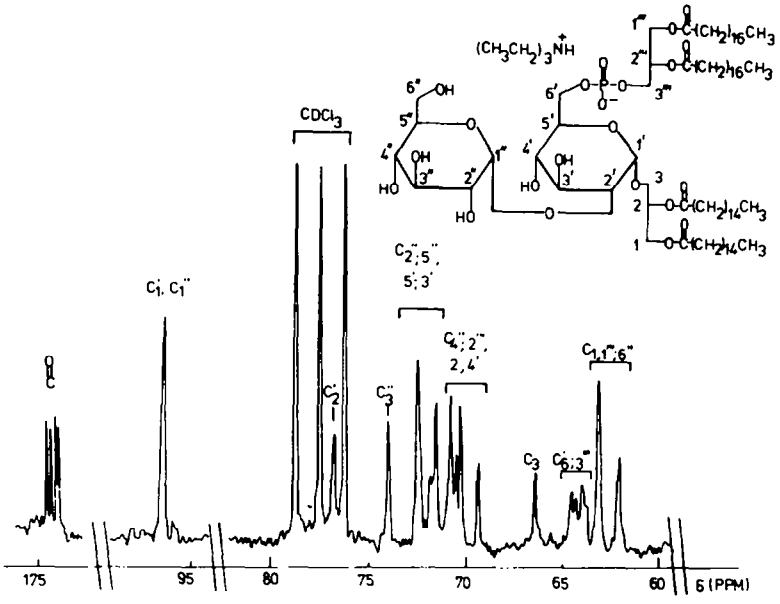


Figure 2. Part of the ^{13}C-NMR spectrum of compound 8d.

the synthesis of 2-O-allyl-3,4-di-O-benzyl-1,6-di-O-(N-phenylcarbamoyl)-β-D-glucopyranose, which derivative appeared to be a suitable precursor for the synthesis of 1,2,6-tri-O-substituted glucose derivatives, required for the preparation of dextran fragments. Furthermore, Gigg et al.[41,42] prepared, in a twenty-step synthesis, 3-O-(3,4-di-O-benzyl-α-D-glucopyranosyl)-1,2-O-isopropylidene-sn-glycerol and postulated that this glucosyl glycerol derivative would be a suitable precursor for the synthesis of Streptococci glycophospholipids.

Experimental*

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

p-Nitrobenzoyl-2,3,4,6-tetra-O-benzyl-α-D-glucopyranose (3.1 g, 4.5 mmol) was treated with a saturated solution of hydrogen bromide in dichloromethane (150 ml) for 10 min. The p-nitrobenzoic acid was removed by filtration and the solvent was evaporated under reduced pressure to give glucosyl bromide 3. Compound 3 was redissolved in a mixture of methylene chloride (30 ml) and N,N-dimethylformamide (0.7 ml), containing tetraethylammonium bromide (975 mg) and activated molecular sieves (4Å, 8 g). After 2 hr in the dark, the mixture was filtered off under dry nitrogen and the filtrate was added to a mixture of the 4',6'-TIPS protected derivative 2 (1.3 g, 1.34 mmol), diisopropylethylamine (0.65 ml) and activated molecular sieves 4Å (6 g). The reaction mixture was stirred for

* For general methods and materials see part III.

5 days under nitrogen at 20°C in the dark. TLC analysis (ether/petroleum ether, 4:1, v/v) indicated the formation of a more lipophilic product (Rf 0.80), a trace of p-nitrobenzoyl-2,3,4,6-tetra-O-benzyl-D-glucopyranose (Rf 0.72), decomposition products of glucosyl bromide 3 and non reacted alcohol 2 (Rf 0.3-0.5). The reaction mixture was filtered off and the filtrate was diluted with chloroform (100 ml) and washed with 10% aqueous $NaHCO_3$ (2x50 ml) and water (50 ml). The dried ($MgSO_4$) organic layer was evaporated to dryness and the resulting oil was redissolved in ether/petroleum ether (1:1, v/v) and applied to a column of Kieselgel H (120 g) suspended in ether/petroleum ether (2:5). Elution of the column with the same solvent mixture and evaporation of the appropriate fractions afforded compound 4 as a syrup. Yield 902 mg (45%). Rf 0.75 (ether/petroleum ether, 7:2). $[\alpha]_D^{25}$ +50.0 (c 1, chloroform).

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

To a solution of 4',6'-TIPS protected derivative 4 (400 mg, 0.267 mmol) in DMF (7 ml) was added a catalytic amount of mesitylene sulfonic acid (12 mg, 0.06 mmol). After 18 hr at room temperature, TLC analysis (chloroform/acetone, 97:3, v/v) indicated nearly complete conversion of derivative 4 (Rf 0.54) into the 3',4'-TIPS protected derivative 5a (Rf 0.22). The reaction was stopped by the addition of 10% $NaHCO_3$ (2 ml), and the reaction mixture was diluted with chloroform (150 ml) and washed with water (2x50 ml). The organic layer was dried ($MgSO_4$), concentrated to an oil and applied to a column of Kieselgel H (60 g) suspended in chloroform. Elution of the column with the same solvent and evaporation of the appropriate fractions afforded compound 5a as an oil. Yield 336 mg (84%). Rf 0.22 (chloroform/acetone, 97:3, v/v). ^{1}H-NMR ($CDCl_3$): δ values almost the same as for compound 4. ^{13}C-NMR ($CDCl_3$): δ 14.1, 22.7, 31.9 (s,2x$CH_2CH_2CH_3$); 11.7, 12.2, 12.7, 12.9 (s,4xSiC TIPS); 24.9, 34.0, 34.2 (s,2x$\underline{CH_2}\underline{CH_2}$COO); 17.4 (s,8x$CH_3$ TIPS); 62.4, 69.9, 66.1 (s,C1-C3 glycerol); 94.0, 75.6, 73.8, 72.5, 71.9, 62.0 (s,C1'-C6' glucose); 95.8, 79.5, 81.9, 77.5, 70.3, 68.3 (s,C1"-C6" glucose); 73.4, 74.5 (s,4xCH_2 benzyl); 137.9, 138.4, 138.7, 138.9 (s,4xC1 benzyl); 172.9, 173.1 (s,2xC=O).

Fully protected glycolipid 5c

To a solution of the 3',4'-TIPS protected derivative 5a (267 mg, 0.177 mmol) in dry pyridine/methylene chloride (3 ml, 1:2, v/v) was added dropwise, stearoyl chloride (66 mg, 2.2 mmol) dissolved in dry methylene chloride (1 ml). After 1 hr at 20°C, TLC analysis (chloroform/acetone, 96:4, v/v) indicated a complete acylation of the alcohol 5a (Rf 0.40) to give 5c (Rf 0.75). The reaction mixture was taken up in chloroform (100 ml) and washed with 10% aqueous $NaHCO_3$ (50 ml) and water (50 ml). The chloroform extract was dried ($MgSO_4$) and evaporated *in vacuo* to give the crude derivative 5c, which was chromatographed on a small column of Kieselgel H (15 g). Elution of the column with ether/petroleum ether (1:1, v/v) afforded pure protected glycolipid 5c. Yield 302 mg (96%). Rf 0.74 (ether/petroleum ether, 8:3, v/v). ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (c,119H,2x$(CH_2)_{13}CH_3$,$(CH_2)_{15}CH_3$,4xSiCH-$(CH_3)_2$); 2.2-2.4 (c,6H,3xCH_2COO); 2.9-5.4 (c,28H,2x glucose, glycerol,4xCH_2 benzyl); 7.0-7.3 (c,20H, 4xbenzyl-arom). ^{13}C-NMR ($CDCl_3$): δ 14.1, 22.7, 31.9 (s,3x$CH_2CH_2CH_3$); 11.8, 12.2, 12.7, 12.9 (s,4x SiC TIPS); 24.9, 34.0, 34.2 (s,3x$\underline{CH_2}\underline{CH_2}$COO); 17.3 (s,8x$CH_3$ TIPS); 62.5, 69.8, 66.0 (s,C1-C3 glycerol); 94.2, 75.7, 73.9, 72.6, 69.8, 63.1 (s,C1'-C6' glucose); 95.7, 79.4, 81.9, 77.5, 70.4, 68.3 (s,C1"-C6" glucose); 73.5, 74.5 (s,4xCH_2 benzyl); 137.9, 138.4, 138.7, 139.0 (s,4xC1 benzyl); 170.6, 172.8, 173.1 (s,3xC=O).

3-O-[2-O-(α-D-glucopyranosyl)-6-O-stearoyl-α-D-glucopyranosyl]-1,2-di-O-palmitoyl-sn-glycerol (i.e., glycolipid 6b)

Fully protected derivative 5c (230 mg, 0.12 mmol) was dissolved in dry THF (3 ml) and treated with tetra-n-butylammonium fluoride (0.5 ml of a 0.8M solution in THF). After 10 min at 20°C, when TLC analysis (chloroform/acetone, 96:4, v/v) revealed complete conversion of starting material 5c (Rf 0.75) into a desilylated intermediate (Rf 0.21). The reaction was stopped by the addition of acetic acid (0.1 ml) and the reaction mixture was concentrated under reduced pressure to give an oil, which was applied to a column of Kieselgel H (50 g) suspended in chloroform. Elution of the column with chloroform/acetone (97:3, v/v) and collection of the appropriate fractions afforded, after evaporation of the solvent, the desilylated intermediate as a white solid in quantitative yield. The latter intermediate was now dissolved in ethylacetate/ethanol (30 ml, 5:1) and treated with

hydrogen (3 atm.) in the presence of 10% palladium on charcoal (250 mg). After 16 hr, the catalyst was filtered off and washed with warm pyridine (50 ml, 40-60°C). The solvent was removed under reduced pressure and the crude material was chromatographed on a column of Kieselgel H (12 g) suspended in chloroform/methanol (92:8, v/v). The column was developed with the same solvent mixture and after collection of the appropriate fractions pure glycolipid 6b was obtained as a white waxy solid. Yield 112 mg (74%). Rf 0.33 (chloroform/methanol, 88:12, v/v). $[\alpha]_D^{25}$ +65.1 (c 1, chloroform). ^{1}H-NMR ($CDCl_3/CD_3OD$): δ 0.8-1.8 (c,91H,2x$(CH_2)_{13}CH_3$,$(CH_2)_{15}CH_3$); 2.2-2.4 (c,6H,3xCH_2COO); 2.9-4.5 (c,glucose and glycerol resonances except H2,H1',H1"); 4.88, 4.98 (2xd,2H,H1',H1" glucose, J1-2=3.2 Hz); 5.0-5.4 (c,1H,H2 glycerol). ^{13}C-NMR ($CDCl_3/CD_3OD$): δ 14.2, 23.0, 32.2 (s,3xCH_2CH_2-CH_3); 29.5, 29.7, 30.0 (m,$(CH_2)_n$); 25.2, 34.4, 34.6 (s,$\underline{CH_2}\underline{CH_2}COO$), 62.0 (s,C6"); 63.9 (s,C6'); 74.0 (s,C3"); 70.7, 70.3 (m,C2',C4',C5',C4"); 72.4, 72.7 (m,C3',C2",C5"); 63.1 (s,C1); 66.1 (s, C3); 76.8 (s,C2'); 97.1, 97.6 (s,C1',C1" glucose); 173.8, 174.4, 174.6 (s,3xC=O).

3-O-[2-O-(α-D-glucopyranosyl)-α-D-glucopyranosyl]-1,2-di-O-palmitoyl-sn-glycerol (i.e. glycolipid 6a)

Fully protected α-glucosyldiglyceride 4 (120 mg, 0.948 mmol) was deprotected as described for the preparation of 6b. The thus obtained crude glycolipid 6a was purified on a column of Kieselgel H (15 g). Elution of the column with chloroform/methanol (92:8 → 88:12, v/v) afforded pure glycolipid 6a as a white solid. Yield 64 mg (74%). Rf 0.38 (chloroform/acetone/acetic acid/methanol/water, 50:20:10:10:5, v/v). $[\alpha]_D^{25}$ +69.4 (c 1, chloroform). ^{1}H-NMR ($CDCl_3/CD_3OD$): δ 0.8-1.8 (c,58H,2x$(CH_2)_{13}(CH_3)$; 2.2-2.4 (c,4H,2xCH_2COO); 2.9-4.5 (c,glucose and glycerol resonances except H2,H1',H1"); 4.80, 4.90 (2xd,2H,H1',H1" glucose,J1-2=3.2 Hz); 5.0-5.4 (c,1H,H2 glycerol). ^{13}C-NMR ($CDCl_3/CD_3OD$): δ 14.1, 22.8, 32.0 (s,2x$CH_2CH_2CH_3$); 29.4, 29.6, 29.9 (m,$(CH_2)_n$); 25.1, 34.3, 34.4 (s,CH_2CH_2COO); 61.6 (s,C6'); 62.0 (s,C6"); 74.0 (s,C3"); 70.5, 70.3 (m,C2,C4',C4"); 72.4, 72.7, 72.8 (s,C3',C5', C5",C2"); 63.1 (s,C1); 66.1 (s,C3); 76.8 (s,C2'); 96.8, 97.0 (s,C1',C1" glucose); 173.6, 174.1 (s, 2xC=O).

Fully protected glycophospholipid 8a

A mixture of the alcohol 5a (330 mg, 0.218 mmol) and the triethylammonium salt of 7 (260 mg, 0.31 mmol) was dried by repeated coevaporation with anhydrous pyridine (3x10 ml) and TPSNT (110 mg, 0.29 mmol) was added to the resulting solution (ca 2 ml). After 1.5 hr at 20°C, TLC analysis (chloroform/acetone, 96:4, v/v) showed complete disappearance of the alcohol 5a (Rf 0.40) and the formation of the fully protected glycophospholipid 8a (Rf 0.68). The reaction mixture was diluted with chloroform, washed with 10% aqueous $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The crude material was purified on a column of Kieselgel H (40 g) suspended in petroleum ether/ether (1.5:1, v/v). Elution occurred with the same solvent mixture and after evaporation of the appropriate fractions pure glycophospholipid 8a was obtained as an oil. Yield 424 mg (85%). Rf 0.65 (ether/petroleum ether, 8:1, v/v). $[\alpha]_D^{25}$ +42.5° (c 1, chloroform). ^{13}C-NMR ($CDCl_3$): δ 14.1, 22.7, 31.9 (s,4x$CH_2CH_2CH_3$); 11.7, 12.2, 12.9 (s,4xSiC TIPS); 17.3 (s,8xCH_3 TIPS); 24.8, 34.0 (s,4x$\underline{CH_2}\underline{CH_2}COO$); 62.5, 69.7, 66.3 (s,C1-C3 glycerol); 61.6, 69.1 (^{3}Jc-p=6.8 Hz); 66.4 (broad,m,C1'",C2'",C3'" phosphatidyl); 94.1, 75.7, 73.5, 72.6, 70.3 (broad); 67.7 (broad,m,C1'-C6' glucose); 95.7, 79.4, 81.9, 77.5, 70.4, 68.3 (s,C1"-C6" glucose); 73.5, 74.3, 74.5 (s,4xCH_2 benzyl); 129.6, 130.0 (s,2xC=C cis); 139.0, 138.7, 138.4, 137.9 (s,4xC1 benzyl); 145.3 (^{2}Jc-p=5.4 Hz); 126.3 (^{3}Jc-p=6.9 Hz); 130.2, 130.8, 128.0, 122.3 (m,C1-C6 2,4-dichlorophenyl); 172.6, 172.8, 173.0 (s,4xC=O). ^{31}P-NMR ($CDCl_3$): δ -6.71, -7.01 (s, two diastereomers).

Preparation of partically protected derivative 8c

The fully protected glycophospholipid 8a (250 mg, 0.110 mmol) was dissolved in dry THF (2 ml). To this solution was added syn-4-nitrobenzaldoxime (130 mg, 0.62 mmol) and N^1,N^1,N^3,N^3-tetramethylguanidine (67 mg, 0.58 mmol). After 4.5 hr at 20°C, when TLC analysis (chloroform/acetone, 96:4, v/v) revealed quantitative removal of the 2,4-dichlorophenyl group, the solution was neutralized with acetic acid (33 µl) and the solvent was evaporated under reduced pressure. The thus obtained crude 8b was purified on a small column of Kieselgel 60 (7 g). Elution of the column with chloroform/methanol (100:0 → 90:10, v/v),afforded, after collection of the appropriate fractions and extraction of these fractions with triethylammonium bicarbonate (TEAB, 2x10 ml, 1M, pH 7.5), pure 8b (triethylammonium salt) in quantitative yield (244 mg). The thus obtained derivative 8b (0.110

mmol) was now dissolved in dry THF together with some pyridinium hydrochloride (28 mg, 0.24 mmol) and tetra-n-butylammonium fluoride was added (1 ml of 1 0.8M solution). After 3 hr at 20°C, TLC analysis (chloroform/acetone/methanol/acetic acid/water) indicated complete conversion of compound 8b (Rf 0.73) into 8c (Rf 0.65). Dowex 50W cation-exchange resin (100-200 mesh, 3 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 residue was applied to a column of Kieselgel 60 (8 g). Elution of the column with chloroform/methanol (100:0 → 85:15, v/v) followed by TEAB extraction of the appropriate fractions (2x10 ml) afforded pure compound 8c (triethylammonium salt) as an oil. Yield 202 mg (95%). ^{13}C-NMR ($CDCl_3$): δ 14.1, 22.7, 31.9 (s,CH_2CH_2-CH_3); 24.9, 34.1, 34.3 (s,4xCH_2CH_2COO); 29.3, 29.7 (m,$(CH_2)_n$); 27.2 ($\underline{CH_2}C$=C); 8.6, 45.7 (s,triethylammonium); 62.7, 69.8, 66.3 (s,C1-C3 glycerol); 62.7, 70.3 (broad); 64.0 (^{2}Jc-p=5 Hz,m,C1'"-C3'" phosphatidyl); 97.6, 80.6, 70.6, 68.9, 71.0 (broad); 63.7 (^{2}Jc-p=6 Hz,m,C1'-C6' glucose); 97.9, 79.6, 82.0, 77.6, 70.6, 68.4 (s,C1"-C2" glucose); 73.1, 73.4, 75.0 (s,4xCH_2 benzyl); 129.7, 129.9 (s,4xC=C); 137.9, 138.1, 138.3, 138.8 (s,4xC1 benzyl); 172.9, 173.2 (s,4xC=O). ^{31}P-NMR ($CDCl_3$): δ 1.53 (s).

Glycophospholipid 8d

Compound 8c was converted into the sodium-form by running a solution of the triethylammonium salt of 8c (120 mg, 0.065 mmol) dissolved in methanol/THF (2:1, v/v) through a column (12x2 cm^2) of Dowex 50W cation-exchange resin (100-200 mesh, sodium-form), suspended in the same solvent mixture. After concentration of the appropriate fractions, the sodium salt of 13c was dissolved in a mixture of isopropanol/ethylacetate/acetic acid (3:3:1, v/v, 20 ml) and hydrogenated over 10% palladium on charcoal (300 mg) at 4 atm for 18 hr. TLC analysis (chloroform/acetone/methanol/acetic acid/water, 50:20:10:10:5, v/v) indicated ca 90% conversion of the starting material (Rf 0.72) into the glycophospholipid 8d (Rf 0.30). The catalyst was filtered off and washed with warm pyridine/methanol (50 ml, 4:1, v/v, 40-60°C). The solvent was evaporated under reduced pressure and the residue was coevaporated twice with toluene (50 ml). The crude material was then chromatographed on a small column of Kieselgel 60 (10 g), suspended in chloroform/methanol (80:20, v/v). The column was eluted with chloroform/methanol (80:20 → 60:40) and the appropriate fractions were collected and concentrated in vacuo. The glycophospholipid 8d was redissolved in chloroform/methanol (100 ml, 3:1, v/v) and extracted with TEAB (2x20 ml, 1M, pH 7.5) to give the triethylammonium salt of 8d as a waxy solid. Yield 72 mg (75%). Rf 0.30 (chloroform/acetone/methanol/acetic acid/water, 50:20:10:10:5, v/v). $[\alpha]_D^{25}$ +36.1 (c 1, chloroform). ^{1}H-NMR ($CDCl_3/CD_3OD$): δ 0.8-1.8 (c,133H,2x$(CH_2)_{13}CH_3$,2x$(CH_2)_{15}CH_3$,3xCH_3 triethylammonium); 2.1-2.4 (c,8H,2xCH_2COO); 3.10 (q,6H,3xCH_2 triethylammonium); 3.3-4.5 (m,glucose and glycerol except H2',H2'" glycerol and H1',H1" glucose); 5.0 (dd,2H,H1',H1",J=3.2 Hz); 5.1-5.4 (c,2H,2xHCOOR). ^{13}C-NMR ($CDCl_3/CD_3OD$): δ 14.2, 23.0, 32.3 (s,4x$CH_2CH_2CH_3$); 29.7, 30.0 (m,$(CH_2)_n$); 25.3, 34.6, 34.4 (s,4x$\underline{CH_2}\underline{CH_2}COO$); 8.7, 46.3 (s,triethylammonium); 63.1, 70.3, 66.4 (s, C1-C3,glycerol); 97.1, 77.0, 71.6, 69.4, 71.9 (broad); 63.8 (m,C1'-C6' glucose); 97.1, 72.6, 74.2, 70.9, 72.6, 62.1 (s,C1"-C6" glucose); 63.1, 70.6 (^{3}Jc-p=7.5 Hz); 63.8 (broad,m,C1'"-C3'" phosphatidyl); 173.7, 173.9, 174.2, 174.4 (s,4xC=O). ^{31}P-NMR ($CDCl_3/CD_3OD$): δ 0.87 (s).

References and Notes

*. Part III and IV, see foregoing papers in this issue.
1. R.A. Pieringer, Biochem. and Biophys. Research Comm., 49, 502 (1972).
2. W. Fischer, H. Landgraf and J. Herrmann, Biochim. Biophys. Acta, 306, 353 (1973).
3. S.B. Feinman, B. Prescott and R.M. Cole, Infection and Immunity, 8, 752 (1973).
4. R.A. Laine and W. Fischer, Biochim. Biophys. Acta, 529, 250 (1978).
5. W. Fischer in Lipids, Vol I, p. 255, Eds. R. Paoletti and G. Jacini, New York.
6. W. Fischer, I. Ishizuka, H.R. Landgraf and J. Herrmann, Biochim. Biophys. Acta, 296, 527 (1973).
7. S.G. Batrakov, E.F. Il'ina, N.A. Ushakova and L.D. Bergel'son, Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, 12, 2796 (1974).
8. R.T. Ambron and R.A. Pieringer, J. Biol. Chem., 246, 4216 (1971).
9. W. Fischer and H. Landgraf, Biochim. Biophys. Acta, 380, 227 (1975).
10. H.U. Koch and W. Fischer, Biochemistry, 17, 5275 (1978).
11. W. Fischer, D. Schuster and R.A. Laine, Biochim. Biophys. Acta, 389 (1979).
12. W. Fischer, Biochim. Biophys. Acta, 487, 89 (1977).
13. W. Fischer, H.U. Koche, P. Rössel, F. Fiedler and L. Schmuck, J. Biol. Chem., 255, 4550 (1980).
14. W. Fischer. H.U. Koche P. Rössel and F. Fiedler J. Biol. Chem. 225 4557 (1980).
15. For a short communication see: C.A.A. van Boeckel and J.H. van Boom, Chemistry Letters, 581 (1981).

16. C.A.A. van Boeckel and J.H. van Boom: see foregoing paper part III.
17. W.T. Markiewicz, J. Chem. Research (S), 24 (1979); W.T. Markiewicz and M. Wiewiorowski, Nucl. Acids Res. s5, 185 (1978).
18. C.H.M. Verdegaal, P.L. Jansse, J.F.M. de Rooy and J.H. van Boom, Tetrahedron Letters, 157 (1980).
19. N.K. Kochetkov, A.Ya. Khorlin and A.F. Bochkov, Dokl. Akad. Nauk. SSSR, 162, 104 (1965).
20. N.K. Kochetkov, A.Ya. Khorlin and A.F. Bochkov, Tetrahedron, 23, 693 (1967).
21. D. Beck and K. Wallenfels, Liebigs Ann., 655, 173 (1962).
22. A.F. Bochkov and G.E. Zaikov, Chemistry of the O-glycosidic bond, p. 100, Pergamon Press, Oxford (1979).
23. R.U. Lemieux, K.B. Hendriks, R.V. Stick and K. James, J. Am. Chem. Soc., 97, 4056 (1975).
24. P.A. Gent and R. Gigg, J. Chem. Soc. Perkin Trans I, 1779 (1975).
25. H. Paulsen and O. Lockhoff, Chem. Berichte, 114, 3079 (1981).
26. J.R. Pougny, M.A.M. Nassr, N. Naulet and P. Sinaij, Nouv. J. Chim., 2, 389 (1978).
27. R.R. Schmidt and J. Michel, Angew. Chemie, 92, 183 (1980).
28. C.A.A. van Boeckel and J.H. van Boom, Tetrahedron Letters, 3705 (1980).
29. T. Ishikawa and H.G. Fletcher, Jr., J. Org. Chem., 34, 563 (1969).
30. C.A.A. van Boeckel, J.J. Oltvoort and J.H. van Boom, Tetrahedron, 37, 3751 (1981).
31. K. Bock and C. Pedersen, J. Chem. Soc. Perkin II, 293 (1974).
32. H. Friebolin, G. Keilich, N. Frank, U. Dabrowski and E. Siefert, Org. Magn. Resonance, 12, 216 (1979).
33. J.B. Stothers, 'Carbon-13 NMR Spectroscopy', Academic Press, New York, 1972.
34. H. Vorbrüggen, Angew. Chemie I.E., 569 (1978).
35. E.J. Corey and A. Venkateswarlu, J. Am. Chem. Soc., 94, 6190 (1972).
36. The ^{13}C-NMR spectroscopy data were compared with data of dextrans, containing 2-O-α-D-glucosylated-α-D-glucopyranosyl residues: F.R. Seymour, R.D. Knapp, E.C.M. Chen, A. Jeanes and S.H. Bishop, Carbohydrate Research, 71, 231 (1979).
37. J.F.M. de Rooy, G. Wille-Hazeleger, P.H. van Deursen, J. Serdijn and J.H. van Boom, Recl. Trav. Chim. (Pays-Bas), 98, 93 (1979).
38. C.B. Reese, R.C. Titmas and L. Yau, Tetrahedron Letters, 2727 (1978).
39. W. Fischer, Biochim. Biophys. Acta, 306, 368 (1973).
40. R. Eby and C. Schuerch, Carbohydrate Research, 79, 53 (1980).
41. P.A. Gent and R. Gigg, Carbohydrate Research, 49, 325 (1976).
42. P.A. Gent and R. Gigg, Chem. Phys. Lipids, 17, 111 (1976).