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SYNTHESIS OF PHOSPHATIDYL-α-GLUCOSYL-DIACYLGLYCEROL CONTAINING PALMITIC AND OLEIC ACID ESTERS

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Summary: The recently introduced 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane protecting group enabled us to synthesize a naturally occurring phosphatidyl-α-glucosyl-diacylglycerol *via* a phosphotriester intermediate.

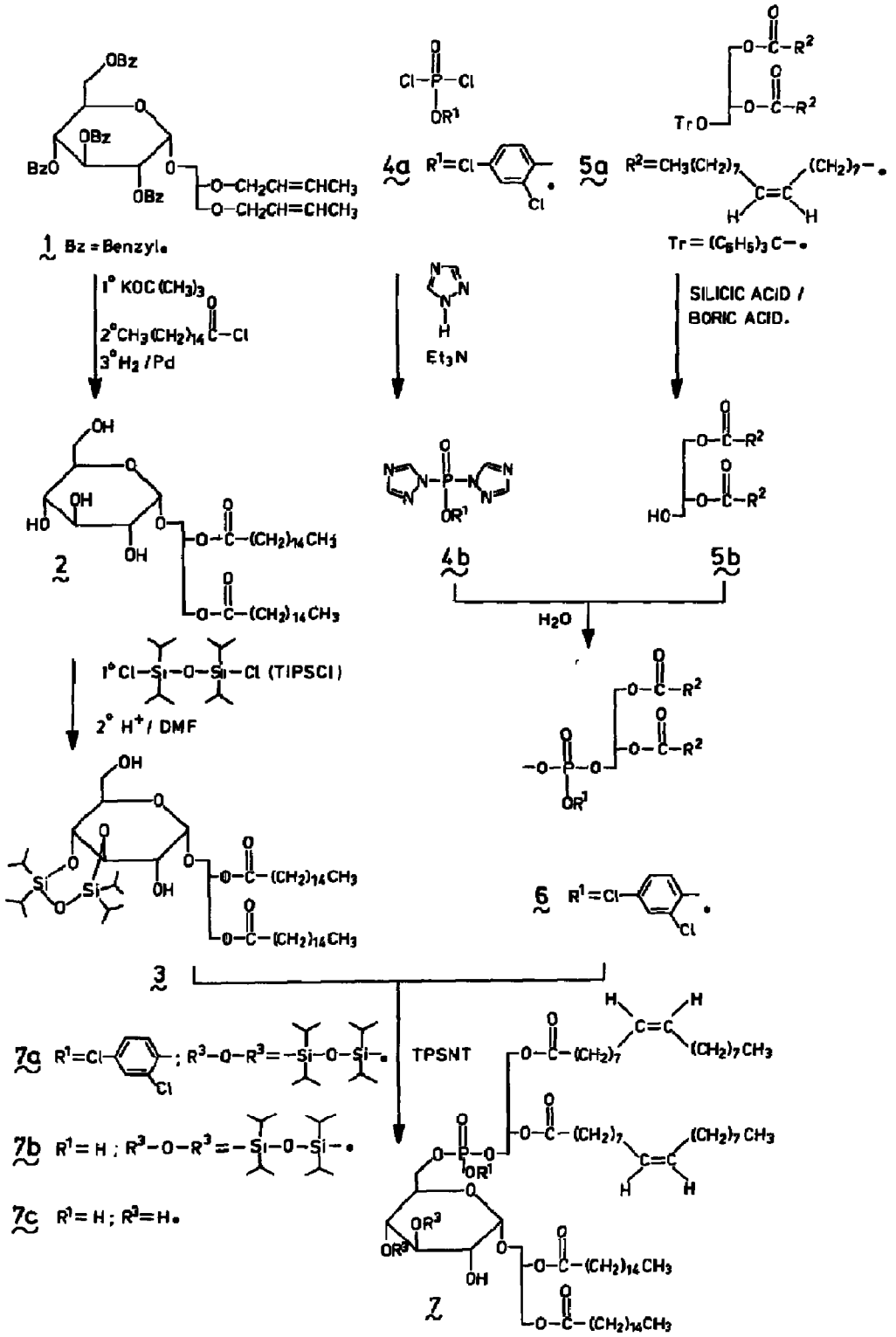
The phosphoglycolipid phosphatidyl-α-glucosyl-diacylglycerol (*i.e.*, compound 7c) has been isolated from the cell wall of Pseudomonas diminuta[1] and was subsequently recognised as a minor component of the polar lipid fraction located in the cytoplasmic membrane of group A,D,N Streptococci[2]. The fatty acid composition of this phosphoglycolipid has been established and showed to consist predominantly of oleic and palmitic acid[1,2].

In this paper we wish to report, for the first time, the synthesis of this naturally occurring phosphoglycolipid (*i.e.*, compound 7c) containing saturated as well as unsaturated fatty acids.

The compound to be synthesized contains four ester bonds, one α-glucosidic linkage and a phosphodiester linkage. The latter linkage connects the monoglucosyl diglyceride moiety 2 with the 1,2-di-oleoyl-sn-glycerol unit 5b (R^2=oleoyl).

The presence of the unsaturated fatty acid in the molecule to be synthesized excludes, at the final stage of the synthesis, the use of the commonly applied benzyl protecting group. Similarly, the base-labile ester bonds necessitate the application of protecting groups which can be removed only by acid or under neutral conditions. Apart from the limitations cited above in making use of well known protective groups, we were also confronted with the problem of introducing a phosphodiester function and an α-linkage between a glucose and a glycerol derivative. Nonetheless, we achieved our goal by the following strategy.

The α-linkage between D-glucose and sn-glycerol was introduced by condensing 2,3,4,6-tetra-O-benzyl-D-glucosyl bromide[3] (8.5 mmole) with the primary hydroxyl group of 1,2-di-O-(but-2-enyl)-sn-glycerol[4] (6.5 mmole), under the conditions of Lemieux[5], to afford compound 1 as a syrup (4.7 mmole). Removal of the but-2-enyl groups was performed by treating 1 (1.9 mmole) with potassium-t-butoxide (6 mmole) in DMSO[6] (20 ml) for 3 hr at 60°C. Work-up of the crude reaction mixture and purification by short column chromatography[7] gave 3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-sn-glycerol (1.5 mmole) as a homogeneous viscous oil. The diol function present in the latter intermediate was acylated with palmitoyl chloride (4 mmole) in methylene chloride-pyridine for 4 hr at 20°C to give a benzyl protected monoglucosyl-diglyceride as a waxy compound (1.3 mmole). In the next step the benzyl groups were removed by hydrogenolysis over palladium on charcoal to afford the monoglucosyldiglyceride 2 which, after purification by column chromatography, was isolated as a


1 Bz = Benzyl.
1° KOC(CH3)3
2° CH3(CH2)14C(=O)Cl
3° H2 / Pd
2
1° Cl-Si-O-Si-Cl (TIPSCl)
2° H+ / DMF
3
4a R1=Cl
Et3N
4b
5a R2=CH3(CH2)7-CH=CH-(CH2)7-.
Tr = (C6H5)3C-.
SILICIC ACID / BORIC ACID.
5b
H2O
6 R1=Cl
7a R1=Cl ; R3-O-R3 = -Si-O-Si-.
7b R1 = H ; R3-O-R3 = -Si-O-Si-.
7c R1 = H ; R3 = H.
TPSNT
7

white solid (0.95 mmole): softening at 79°C, $[\alpha]_D^{25}$= + 44.0° (c=1, $CHCl_3$), ^{1}H-NMR, H_1' : δ= 4.84 ppm (d), J= 3.2 Hz[8].

A crucial step in the synthesis of 7a consisted of the selective protection[16] of the 3'- and 4'-hydroxyl functions of 2 by means of the recently developed tetraisopropyldisiloxane-1,3-diyl (TIPS) protecting group[9]. The latter protective group showed to be very convenient[10] to protect simultaneously the 4 and 6 hydroxyl functions (4-6-TIPS) of methyl-α-D-glucopyranoside. Furthermore, it was also demonstrated[10] that a 4-6-TIPS protected methyl-α-D-glucopyranoside could easily be converted by acid into a 3-4-TIPS protected derivative. The same reaction sequence was now applied for the synthesis of the suitably protected derivative 3. Thus reacting together 2 (0.8 mmole) with the bifunctional silylating agent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPSCl; 1.0 mmole) at -15°C in pyridine afforded the 4',6'-TIPS derivative of 2 (0.56 mmole). The latter was readily isomerized by mesitylenesulphonic acid (0.1 mmole) in dry DMF. After 8 h at 20°C, when TLC-analysis revealed no further progress of the isomerization, the reaction mixture was worked-up and purified by column chromatography to give pure 3[8] (0.39 mmole), $[\alpha]_D^{25}$= +62.8° (c=1, $CHCl_3$) as a waxy compound.

The final step in the synthesis of the fully protected glycophospholipid 7a consisted of the regiospecific introduction of a phosphotriester linkage between the phosphatidyl component 6 and the primary hydroxyl function of the glucose derivative 3. The synthesis of 6 was accomplished by phosphorylation of 1,2-dioleoyl-sn-glycerol, which was obtained after mild removal of the trityl protecting group from 5a[11] by silicic-boric acid column chromatography[12]. Thus addition of the phosphorylating agent 4b[13] (2 mmole) in dry THF to a stirred solution of 5b (1.35 mmole) in dry pyridine gave, after purification by short column chromatography and extraction with triethylammonium bicarbonate (TEAB, 2M, pH 7.5), 6[8] (1.22 mmole) as a homogeneous oil (^{31}P-NMR: δ= -5.94 ppm). Coupling of 6 (Et_3NH^+-salt; 0.41 mmole) and partially protected 3 (0.39 mmole) to give 7a, was effected in dry pyridine under the influence of the coupling agent 2,4,6-triisopropylbenzenesulphonyl-3-nitro-1,2,4-triazolide (TPSNT; 0.43 mmole)[14]. After 1.5 h at 20°C, the crude reaction mixture was worked-up and purified by short column chromatography, to afford 7a[8] (0.28 mmole) as a homogeneous oil (^{31}P-NMR: δ= -6.85 and -7.03 ppm: two diastereomers). In the above phosphorylation reaction we observed the formation of a product which contained, on the basis of ^{31}P-NMR spectroscopy, two phosphotriester functions. The latter compound (yield 5% based on 3), which was formed by diphosphorylation of 3 with 6, could easily be removed by short column chromatography.

In order to obtain the phosphoglycolipid 7c (R^1=R^3=H) the TIPS and the 2,4-dichlorophenyl protecting groups had to be cleaved from 7a. Firstly, the 2,4-dichlorophenyl group was deblocked selectively and quantitatively by the action of N^1, N^1, N^2, N^2-tetramethylguanidinium *syn*-4-nitro-benzaldoximate (1 mmole) in dry THF[15]. After 3 hr at 20°C, the crude product 7b was worked-up and purified over a small bed of silicagel. In the second step, the removal of the TIPS group was achieved by treating 7b (0.2 mmole) with tetrabutylammonium fluoride (1 mmole) in THF containing pyridine-HCl salt (0.45 mmole) during 1.5 hr at 20°C. Work-up of the reaction mixture, and purification by short column chromatography, followed by conversion of the compound into the triethylammonium salt afforded pure glycophospholipid 7c (R^1=R^3=H) as a colourless waxy solid (0.175 mmole). The homogeneity and identity of 7c was ascertained by TLC-analysis, ^{1}H-NMR (anomeric proton: δ= 4.85 ppm, d, J= 3.4 Hz), ^{31}P-

NMR (δ=1.74 ppm) and ^{13}C-NMR spectroscopy (anomeric carbon: δ=99.6 ppm; double bond (cis) carbon atoms: δ=129.7 and 130.0 ppm). Further, treatment of 7c (R^1=R^3=H) with sodium methoxide in dry methanol followed by GLC-analysis showed the presence of methyl palmitate and methyl oleate in equimolar amounts.

In conclusion, the synthetic route described in this paper presents an elegant method to the synthesis of phosphatidyl-α-glucosyl-diacylglycerol and other naturally occurring (phospho)glycolipids. For instance, compound 3 glucosylated on the 2-hydroxy function of the glucose moiety promises[17] to be a very convenient and general intermediate for the synthesis of several (phospho)glycolipids which occur in gram-positive bacteria[2].

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16. Direct phosphorylation of 2 with the phosphatidyl derivative 6 gave an intractable mixture of compounds. C.A.A. van Boeckel, unpublished results.
17. Thus, the 4-6 TIPS-protected derivative of 2 could be glucosylated selectively at the 2-hydroxy function. Acidic isomerization of the disiloxane group afforded a 3-4 TIPS derivative having a free 6-hydroxy group;
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