CHEMISTRY LETTERS, pp. 581-584, 1981.

SYNTHESIS OF MEMBRANE SUBSTANCES: PHOSPHATIDYL-α-DIGLUCOSYL DIGLYCERIDE AND RELATED GLYCOLIPIDS

Stan A.A.VAN BOECKEL and **Jacques H.VAN BOOM**

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

The recently developed tetraisopropyldisiloxane-1,3-diyl protecting group enabled us to synthesize a naturally occurring phosphatidyl-α-diglucosyl diglyceride as well as the glycolipid α-diglucosyl diglyceride and a derivative thereof which is acylated on the 6'-position by a fatty acid.

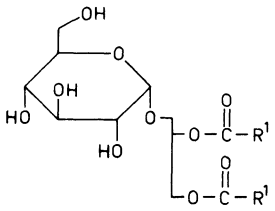
The phosphatidyl-α-diglucosyl diglyceride and its metabolic precursor α-diglucosyl diglyceride (i.e., compound 7d and 8a respectively) are major constituents of the polar lipid fraction of group A,D,N Streptococci[1,2] and have also been identified in Acholeplasma membranes.[3,4] An acylated derivative of α-diglucosyl diglyceride (i.e., compound 8b) proved to be the basic glycolipid of the corresponding lipoteichoic acid that has been isolated from Streptococcus Lactis Kiel 42712.[5,6] The phosphoglycolipid 7d as well as the glycolipid 8b are characterised by a terminal glucose unit that is joined via an α-linkage to the 2' position of the adjacent monoglucosyl diglyceride 1, which in turn is substituted on its 6' hydroxyl function. Up to now, only a few methods have been described for the synthesis of suitably protected glucopyranosyl[7] or 3-O-(α-glucopyranosyl)-sn-glycerol[8] derivatives, which may be converted into 1,2,6-tri-hydroxyl-substituted glucose molecules. However, the preparation of complex phosphoglycolipids by means of the above mentioned compounds will be rather laborious and time consuming.

We now wish to report for the first time a short and convenient synthesis, using tetraisopropyldisiloxane-1,3-diyl protected intermediates, of phosphatidyl-α-diglucosyl diglyceride 7d and also of two closely related glycolipids (i.e., 8a and 8b).

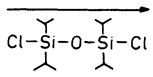
In an earlier report[9] we demonstrated that α-monoglucosyl diglyceride 1 could be selectively protected by the recently developed tetraisopropyldisiloxane-1,3-diyl (TIPS)[10,14] protecting group. Thus treatment of 1 with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPSCL) at -15°C in pyridine gave 2 (Yield 72%; $[\alpha]_{25}^{D}$ = +31.5° (c=1, $CHCl_3$); ^{13}C-NMR spectroscopy: δ C-1' = 99.1 ppm).

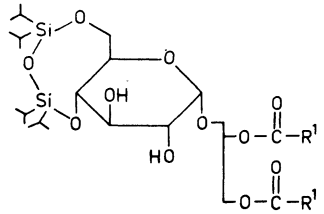
At this stage, we were anxious to find out if the more reactive[11] and less encumbered 2' hydroxyl function of 2 could be coupled via an α interglucosidic linkage with an activated 2,3,4,6-tetra-O-benzyl-glucopyranosyl derivative. In this respect, it should be noted that the silyl-primary oxygen bond of the 4'-6'-TIPS protected intermediate 2 is easily cleaved in an acid catalysed reaction.[14] As a consequence, the stereospecific condensation had to be performed under essentially basic conditions.[12] Thus a mixture of 2 (1.34 mmole) in dry methylene chloride (30 ml), DMF (0.5 ml), and diisopropylethylamine (0.65 ml) was treated with 3[13] (4.5 mmole) together with tetraethylammonium bromide (4.5 mmole) in the presence of powdered molecular sieves (4Å). The reaction mixture was stirred under an atmosphere of nitrogen for 5 days at 20°C and, after work-up, purified by short column chromatography[15] to afford the 4'-6'-TIPS protected derivative 4 as a homogeneous oil (0.60 mmole; $[\alpha]_{25}^{D}$ = +50.0°(c=1, $CHCl_3$); ^{13}C-NMR spectroscopy: anomeric carbons δ = 97.9; 96.6 ppm; δ C-2' = 80,4 ppm).

For the synthesis of the 6'-hydroxyl substituted (phospho)glycolipids 7d and 8b respectively the 4'-6'-TIPS protected derivative 4 was converted into the 3'-4'-TIPS protected glycolipid 5 by means of a recently developed acid catalysed isomerisation.[9,14] Thus treating 4 (0.52 mmole) in dry DMF (13 ml) with a catalytic amount of mesitylenesulphonic acid (0.1 mmole) and moni-

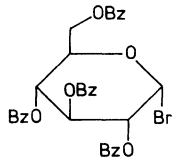


1 $R^1=CH_3(CH_2)_{\overline{14}}$.

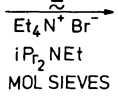


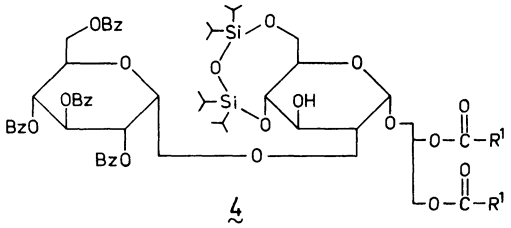


2 $R^1=CH_3(CH_2)_{\overline{14}}$.

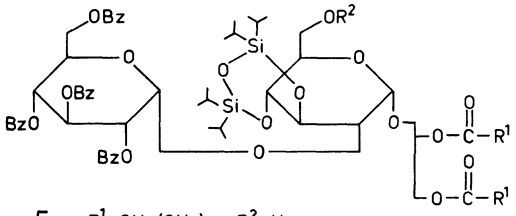


3 Bz=BENZYL





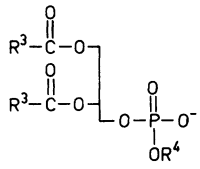
4



5 a : $R^1=CH_3(CH_2)_{\overline{14}}$; $R^2=H$.

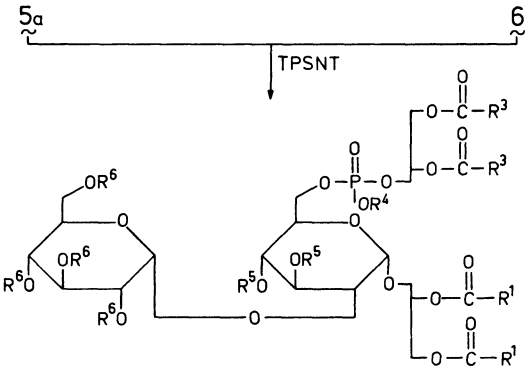
b : $R^1=CH_3(CH_2)_{\overline{14}}$; $R^2=CH_3-\overset{O}{\overset{\|}{C}}-$.

c : $R^1=CH_3(CH_2)_{\overline{14}}$; $R^2=CH_3(CH_2)_{16}\overset{O}{\overset{\|}{C}}-$.



6 $R^3=CH_3(CH_2)_7\overset{H}{C}=\overset{H}{C}(CH_2)_7-$.

$R^4=$ Cl



7 a : $R^1=CH_3(CH_2)_{\overline{14}}$; $R^3=CH_3(CH_2)_7\overset{H}{C}=\overset{H}{C}-(CH_2)_{\overline{7}}$; $R^4=$ Cl  ; $R^5-O-R^5=-\overset{}{Si}-O-\overset{}{Si}-$; $R^6=$BENZYL.

b : $R^1=CH_3(CH_2)_{\overline{14}}$; $R^3=CH_3(CH_2)_7\overset{H}{C}=\overset{H}{C}-(CH_2)_{\overline{7}}$; $R^4=H$; $R^5-O-R^5=-\overset{}{Si}-O-\overset{}{Si}-$; $R^6=$ BENZYL.

c : $R^1=CH_3(CH_2)_{\overline{14}}$; $R^3=CH_3(CH_2)_7\overset{H}{C}=\overset{H}{C}-(CH)_{\overline{7}}$; $R^4=H$; $R^5=H$; $R^6=$ BENZYL.

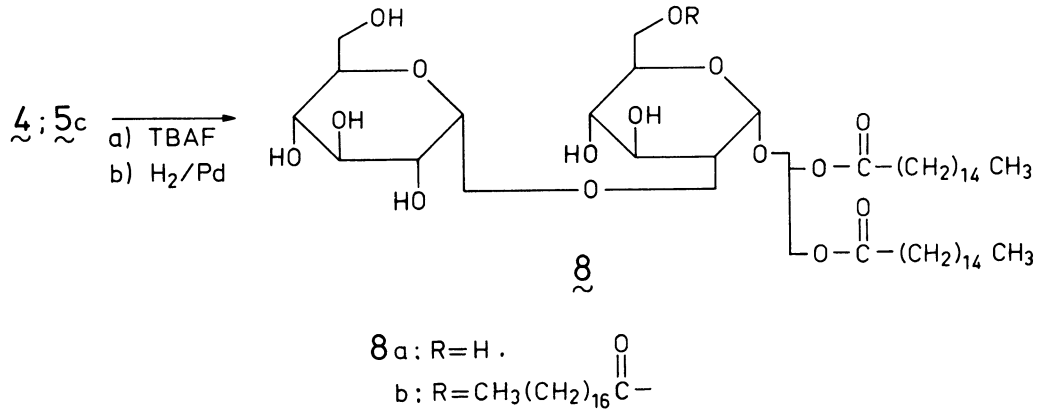
d : $R^1=CH_3(CH_2)_{\overline{14}}$; $R^3=CH_3(CH_2)_{\overline{16}}$; $R^4=H$; $R^5=H$; $R^6=H$.

toring of the reaction by TLC showed, after 18 hours, nearly complete conversion of 4 into a product (i.e., 5a, R^2 = H) with a lower Rf-value, Work-up and purification of the crude reaction product by short column chromatography afforded 5a (0.44 mmole) as a colourless oil. The identity of 5a (R^2 = H) was ascertained by ^{1}H-NMR and ^{13}C-NMR spectroscopy (anomeric carbons: δ = 95.8; 94.0 ppm; δ C-6' = 62.0 ppm; δ C-5' = 71,9 ppm) as well as by chemical means. Thus acetylation of 5a with acetic anhydride gave after work-up solely 5b as evidenced by ^{1}H and ^{13}C-NMR spectroscopy (δ C-6' = 63.0 ppm; δ C-5' ; 69.8 ppm). In the same way, using stearoyl chloride as the acylating agent in the solvent mixture pyridine/CH_2Cl_2, the fully-protected glycolipid 5c was isolated in a quantitative yield. The assemblage of the fully protected phosphatidyl-α-diglucosyl diglyceride 7a was now performed as follows. To a solution of 3'-4'-TIPS protected 5a (0.218 mmole) and the triethylammonium salt of 6[9] (0.31 mmole) in dry pyridine was added the activating agent 2,4,6-triisopropyl-benzenesulphonyl-3-nitro-1,2,4-triazolide[16] (TPSNT; 0.3 mmole). After 1.5 h. at 20°C, when TLC analysis revealed the formation of a single product, the reaction mixture was worked-up and purified by short column chromatography, to afford 7a as a colourless homogeneous oil (0.186 mmole). ^{13}C-NMR spectroscopy (anomeric carbons: δ = 95.7; 94.1); ^{31}P-NMR spectroscopy (δ = -6.71; -7.01 ppm: two diastereomers); $[\alpha]_{25}^{D}$ = +42.5° (c=1 $CHCl_3$).

Complete deblocking of the fully protected phosphoglycolipid 7a (0.11 mmole) 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^2,N^2-tetramethylguanidinium syn-4-nitro-benzaldoximate[17] (0.6 mmole) in dry THF[9] (2 ml). After 4.5 h. at 20°C, acetic acid (0.6 mmole) was added and the concentrated reaction mixture was purified over a small bed of silicagel, to give pure 7b (0.11 mmole). Secondly, the TIPS protecting group was now removed from 7b. Thus treating of 7b (0.11 mmole) with tetrabutylammonium fluoride (TBAF; 0.7 mmole) in dry THF (2.5 ml) containing pyridine -HCL salt (0.24 mmole) during 1.5 h. at 20°C afforded crude 7c. After work-up and short column chromatography, followed by its conversion into the triethylammonium salt, compound 7c was obtained as a colourless oil (0.105 mmole); ^{13}C-NMR spectroscopy (anomeric carbons δ = 97.9; 97.6 ppm). Finally, catalytic hydrogenolysis (10% Pd/C) of the benzyl protecting groups of 7c (sodium salt; 0.065 mmole) and simultaneously hydrogenation of the unsaturated fatty acids, afforded phosphatidyl-α-diglycosyl diglyceride 7d. The latter was purified by column chromatography and after extraction with triethylammonium bicarbonate (TEAB, 1M pH = 7.5) isolated as its triethylammonium salt (0.049 mmole) $[\alpha]_{25}^{D}$ = +36.1° (c=1 $CHCl_3$). The homogeneity and identity of 7d was ascertained by ^{13}C-NMR (two anomeric carbons δ = 97.1 ppm; δ C-2' = 77.0 ppm) ^{1}H-NMR, ^{31}P-NMR spectroscopy (δ = 0.87 ppm) and TLC analysis.[18]

The synthesis of the glycolipids 8a and 8b was accomplished by deblocking of the TIPS and benzyl protecting groups of the fully protected derivatives 4 and 5c, respectively. Thus, treatment of 4 (0.149 mmole) with excess TBAF in dry THF followed by hydrogenolysis (10% Pd/C) and purification of the reaction product by short column chromatography gave α-diglucosyl diglyceride 8a as a white solid[18] (0.105 mmole), $[\alpha]_{25}^{D}$ = +69.4° (c=1 $CHCl_3$); ^{13}C-NMR spectroscopy (anomeric carbons: δ = 97.0; 96.8 ppm; δ C-6' = 61.6 ppm). In the same way, starting from 5c, glycolipid 8b was obtained as a white solid[18] (70% yield), $[\alpha]_{25}^{D}$ = +65.1° (c=1 $CHCl_3$); ^{13}C-NMR spectroscopy (anomeric carbons δ = 97.1; 96.7 ppm; δ C-2' = 76.8 ppm; δ C-6' = 63.9 ppm).

In conclusion, the synthesis of the (phospho)glycolipids 7d, 8a, and 8b was performed by making use of the outstanding features of the TIPS protecting group. The latter turned out to be a dynamic protecting group for the following reasons: (a) the TIPS group could be introduced selectively to protect the 4'- and 6'-hydroxyl groups of a glucose derivative (i.e., com-



pound 1); (b) the 4'-6'-TIPS protected intermediate (i.e., compound 2) could be substituted selectively on its 2'-hydroxyl function (formation of 4); (c) the molecule (4) thus obtained could be isomerized, by an acid catalysed reaction, to afford the 3'-4'-TIPS protected compound (i.e., 5a); the latter process allowed further functionalisation of the 6'-hydroxyl group (i.e., conversion of 5a into 5c and 7a).

REFERENCES AND FOOTNOTES

1. W. Fischer, H.R. Landgraf and J. Herrmann, Biochim. Biophys. Acta, 306, 353 (1973).
2. W. Fischer in Lipids, Volume I. p. 255, Eds. R. Paoletti and G. Jacini, New York (1976).
3. P.F. Smith, J. of Bacteriology, 112, 554 (1972).
4. Å. Wieslander, A. Christiansson, L. Rilfors, and G. Lindblom, Biochemistry, 16, 3650 (1980).
5. W. Fischer, D. Schuster, and R.A. Laine, Biochim. Biophys. Acta, 575, 389 (1979).
6. W. Fischer et al., Chem. Phys. Lipids, 21, 103 (1978).
7. R. Eby and C. Schuerch, Carb. Res., 79, 53 (1980).
8. P.A. Gent and R. Gigg, Chem. Phys. Lipids, 17, 111 (1976).
9. C.A.A. van Boeckel and J.H. van Boom, Tetrahedron Letters, 3705 (1980).
10. W.T. Markiewicz, J. Chem. Research (S), 24 (1979); W.T. Markiewicz and M. Wiewiorowski, Nucl. Acids Res. (Special Report No. 5), 185 (1978)
11. A.F. Bochkov and G.E. Zaikov, Chemistry of the O-glycosidic bond, p. 100, Pergamon Press, Oxford (1979).
12. R.U. Lemieux, K.B. Hendriks, R.V. Stick, and K. James, J. Am. Chem. Soc., 97, 4056 (1975).
13. T. Ishikawa and H.G. Fletcher, Jr., J. Org. Chem., 34, 563 (1969).
14. C.H.M. Verdegaal, P.L. Jansse, J.F.M. de Rooij and J.H. van Boom, Tetrahedron Letters, 1571 (1980)
15. B.J. Hunt and W. Rigby, Chem. Ind. (London), 1868 (1967).
16. J.F.M. de Rooij, et al., Recl. Trav. Chim. (Pays-Bas), 98, 537 (1979).
17. C.B. Reese, R.C. Titmas, and L. Yau, Tetrahedron Letters, 2727 (1978).
18. TLC analyses of the (phospho)glycolipids 7d, 8a and 8b revealed single spots with identical Rf-values as the natural products. W. Fischer, Biochim. Biophys. Acta, 487, 74 (1977). See also references 1 and 2. The Rf-values were compared with α-monoglucosyl diglyceride 1 and phosphatidyl-α-glucosyl-diacylglycerol.[9]

(Received January 5, 1981)