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SYNTHESIS OF 2,3-DI-O-PHYTANYL-1-O-[GLUCOSYL(GALACTOSYL)-β(1→6)-MANNOSYL-α(1→2)-GLUCOSYL-α(1→1)]-SN-GLYCEROL. PURPLE MEMBRANE GLYCOLIPIDS

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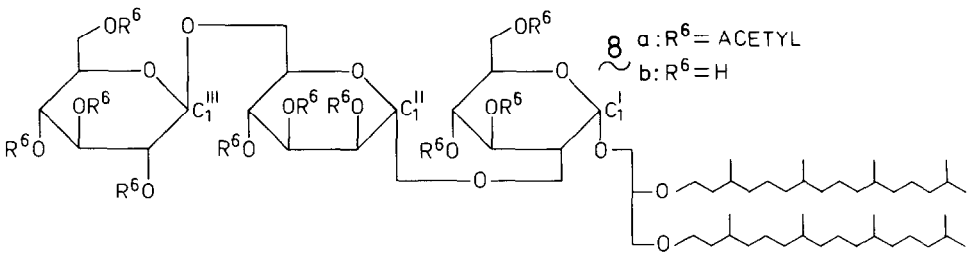
Summary: A convenient approach to the synthesis of triglycosyl-2,3-di-O-phytanyl-sn-glycerols will be presented. Special attention will be paid to the use of the following protective groups in carbohydrate chemistry: the 2,2,2-trichloroethoxycarbonyl, the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl and the o-dibromomethyl benzoyl group.

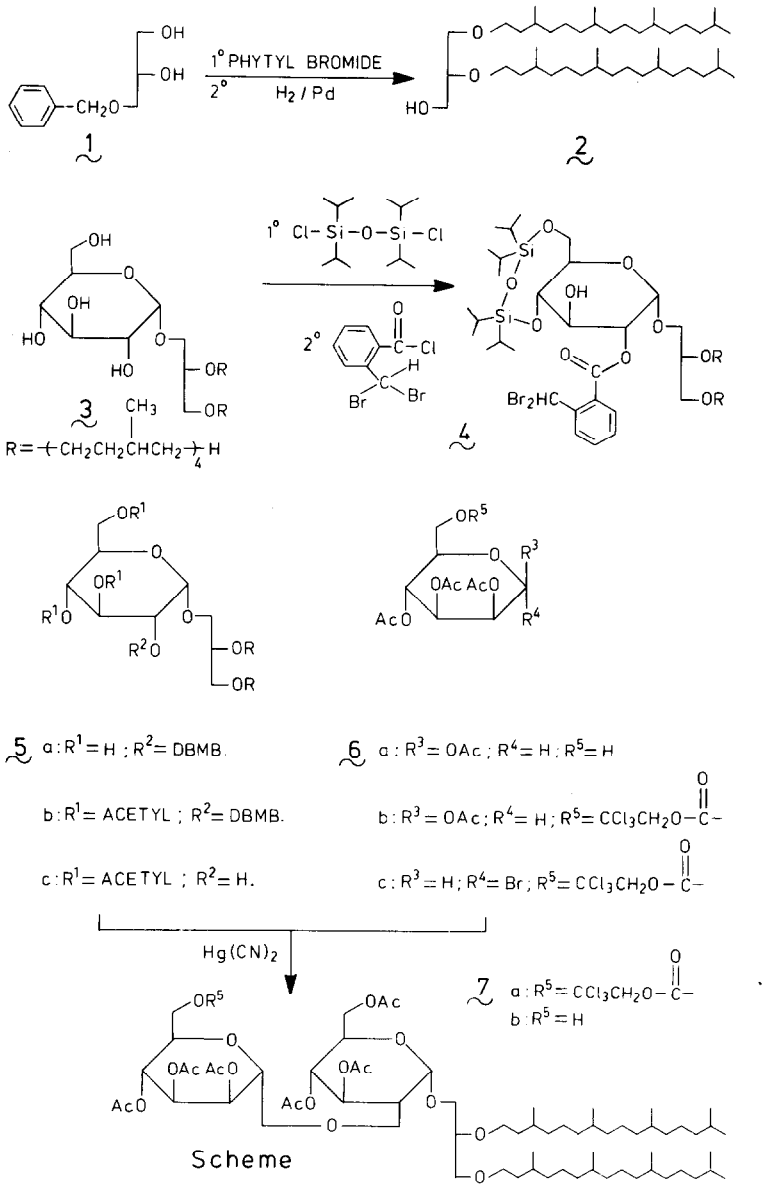
The lipid composition of the purple membrane of extremely halophilic bacteria has been discussed in detail[1,2,3] and shown to be of considerable interest in connection with the well known[4] function of this membrane as a light-driven proton pump. Some of the major polar lipids which are exclusively present in the purple membrane, have been identified as triglycosyl-2,3-di-O-phytanyl-sn-glycerol derivatives[1,2,3,5].

As part of our programme[6] to synthesize naturally occurring glyco(phospho)lipids we present, for the first time, a convenient route to the preparation of purple membrane constituents e.g. the triglycosyl-diphytanyl-glycerol (compound 8b)[5] of Halobacterium marismortui.

The molecule to be synthesized contains three sugar units and one di-phytanyl-sn-glycerol moiety which is α-linked to the glucose at the reducing end of the trisaccharide. The glucosyl glycerol part of the molecule is α-linked via the 2-hydroxyl group of the glucose with mannose. The mannose-glucose-di-phytanyl-glycerol moiety is an invariable part of a class of glycolipids present in the purple membrane. For this reason we synthesized first the properly protected diglycosyl derivative 7b (see Scheme) which has a free hydroxyl group at the 6-position of the mannose residue. The latter alcoholic function can now be β-linked to different sugars (e.g. glucose or galactose) which are naturally components of the triglycosyl-diphytanyl-glycerol of the purple membrane.



The strategy we followed for the synthesis of the diglycosyl derivative 7a(b) is illustrated in the Scheme. The first step involves the preparation of 2,3-di-O-phytanyl-sn-glycerol(2). Treatment of 1-O-benzyl-sn-glycerol (1;14.8 mmol)[7] with phytyl bromide[8] (40 mmol) in dry DMF with sodium hydride (60 mmol) for 20 h at 20°, afforded, after work-up and purification by short column chromatography, 1-O-benzyl-2,3-di-O-phytyl-sn-glycerol (10.4 mmol) as a colourless oil. The removal of the benzyl group and the reduction of the double bond in the phytyl



moiety was simultaneously effected by catalytic hydrogenolysis (10% Pd/C). Purification of the crude product afforded 2,3-di-O-phytanyl-sn-glycerol 2 [8.7 mmol; $[\alpha]_D^{25}$+7.1 (c 3 $CHCl_3$)] as a homogeneous oil. The introduction of the required α-linkage between glycerol unit 2 and a properly-protected glucose derivative was accomplished by using the conditions of Lemieux[9]. Thus, tetraethylammonium bromide was added to a solution of 2,3,4,6-tetra-O-benzyl-D-glucosyl bromide[10] and 2 in the presence of molecular sieves (4Å). Work-up of the reaction mixture afforded the benzylated derivative as an oil in 70% yield (^{13}C NMR $\delta C_{1'}$=97.1 ppm). Removal of the benzyl groups by catalytic hydrogenolysis and purification of the product afforded pure 3 as a waxy compound (^1H NMR;$\delta H_{1'}$=4.82 ppm, (d),J 3.3 Hz).

We now turned our attention to the introduction of the α-glucosydic linkage between mannose and the 2-hydroxyl group of the glucose derivative 3. Previously[6d], we showed that it was possible to introduce selectively an interglucosidic linkage between the 2-hydroxyl group of a 4-6 tetraisopropyl-1,3-diyl (TIPS) protected α-glucosyl-glycerol derivative and a suitable protected glucose derivative. In this particular case the interglucosidic linkage was introduced under essential basic conditions, which do not lead to isomerization of the TIPS function. However, the formation of the required α-linkage had to be performed under the acidic conditions of Helferich[11], which may induce[13] isomerization of the TIPS group. Nonetheless, we attained key-intermediate 5c(R^1=Ac;R^2=H) by the following five-step procedure. The TIPS-group was used for the temporarely blocking of the 4'-6'-hydroxyl groups of 3. The o-di-bromomethyl-benzoyl (DBMB)[12] group could be applied for the selective protection of the 2'-hydroxyl group of the 4'-6'-TIPS protected derivative of 3 to give 4. Removal of the TIPS group from 4 with fluoride-ions gave compound 5a (R^1=H;R^2=DBMB) which, after acylation followed by removal of the DBMB group, afforded the required compound 5c. Thus 4'-6'-TIPS protected[6c,13] 3 (1.2 mmol) was treated with o-dibromomethyl benzoyl chloride[12] (1.4 mmol) at 0° to give, after work-up, compound 4 [1.1 mmol; ^{1}H NMR: $H_{1'}$=5.30 (d), $J_{1',-2'}$ 3.7Hz; $H_{2'}$=5.0(dd), $J_{1',-2'}$ 3.7 and $J_{2',-3'}$ 9.7Hz]. The TIPS group was removed from 4 (1.0 mmol) with fluoride-ions[13] to afford, after column chromatography, pure 5a [0.6 mmol; ^{1}H NMR: $H_{1'}$=5.30 (d), $H_{2'}$=5.00(dd)]. Compound 5a was quantitatively converted into the 3', 4', 6'-triacetylated derivative 5b by standing overnight in a mixture of Ac_2O and pyridine. The DBMB group was now removed by adding silver triflate to a stirred solution of 5b (0.5 mmol) in acetone/water/lutidine (98:2:3). After 2 h at 20°, the silver salts were removed and morpholine[12] (15 mmol) was added to the filtrate. After 5 min, the reaction mixture was worked-up and purified by short colum chromatography, to give 5c (0.48 mmol) as a homogeneous oil [$[\alpha]_D^{25}$+49.0 (c=1 $CHCl_3$); ^{1}H NMR (δppm): $H_{1'}$=4.94(d); $H_{3'}$=5.00 (t); $H_{4'}$=5.30(t)]. Derivative 5c can now be coupled with a properly protected mannose unit. For the protection[14] of the hydroxyl groups of mannose (e.g. 6b) we used the acetyl and the 2,2,2-trichloroethoxycarbonyl (TCEC)[15] protective groups. The 6-hydroxyl group was protected with the acid-stable TCEC group, and the other alcoholic groups with acetyl groups. This choice had the following advantages. The acid-stable TCEC group survived the acid conditions necessary to convert the acetoxy group at the anomeric center into the bromide 6c and also the subsequent Helferich coupling condition. Furthermore, the TCEC group could be removed selectively and without migration of the remaining acetyl groups. Thus, mannose derivative 6a (5 mmol)[16] and 2,2,2-trichloroethyl chloroformate (5.5 mmol) were treated together in THF/pyridine at 0°. Work-up of the mixture after 1 h gave fully protected 6b (4.9 mmol) as a white foam (^{1}H NMR: H_1=5.94 ppm). Compound 6b was converted into the required bromo derivative 6c by adding water to a stirred solution of 6b in phosphorus tribromide and acetic anhydride[17]. After 1 h at 0°, work-up of the reaction mixture afforded 6c (^{1}H NMR: H_1=6.25 ppm) in 95% yield. The synthesis of 7a was now undertaken. Bromide 6c (2.4 mmol) was added dropwise to alcohol 5c (0.8 mmol) in acetonitrile/nitromethane (25:1,v/v) and in the presence of $Hg(CN)_2$[11] (2 mmol). After 4 h at 20°, the reaction mixture was worked-up and purified by column chromatography, to give 7a (0.68 mmol) as a colourless oil [^{13}C NMR(δppm); $C_{1'}$=94.8, $C_{1''}$=95.5, J_{C-H} 175Hz]. Quantitative removal of the TCEC group was performed by treating 7a (0.34 mmol) in THF/AcOH with activated zinc-dust[18] for 20 min at 20°. Work-up of the reaction mixture afforded 7b in a quantitative yield. ^{13}C NMR data of 7b indicated that no acetyl migration had occurred (^{13}C NMR(δppm):

$C_{6'}$=61.8;$C_{6''}$=61.0). The free 6''-hydroxyl function of 7b was now condensed with a glucose molecule to afford the required β-glucosidic bond. Thus, 7b (0.32 mmol) and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (0.7 mmol) in dry acetonitril were reacted together in the presence of $HgBr_2$ (0.35 mmol) and $Hg(CN)_2$ (0.35 mmol)[11]. After 1 h, the reaction mixture was worked-up and purified by short colum chromatography, to afford pure 8a 0.26 mmol: [^{13}C NMR(δppm); $C_{1'}$=94.7;$C_{1''}$=95.4;$C_{1'''}$=100.7]. The desired glycolipid 8b was obtained quantitavely after complete deprotection of 8a with base (NaOMe in MeOH/Ether). The identity and homogeneity of 8b was unambiguously affirmed by ^{1}H NMR, ^{13}C NMR(δ$C_{1'}$=97.1;$C_{1''}$=98.9 and $C_{1'''}$=103.9 ppm), MS, TLC analysis[5] and optical rotation $[\alpha]_D^{25}$+35.0[5]; c=1 $CHCl_3$. In the same way, starting from 7b, we prepared the glycolipid from Halobacterium cutirubrum[2] by introducing a β-galactose[19] instead of a β-glucose terminal unit.

In conclusion, the methodology described in this paper presents an elegant route to the synthesis of purple membrane glycolipids. For instance, key-intermediate 7b is also a convenient precursor for the synthesis of the glycolipid sulfate of Halobacterium salinarium[3] and Halobacterium cutirubrum[2].

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