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Synthesis of 2,3-di-*O*-phytanyl-1-*O*-(α-D-glucopyranosyl)-*sn*-glycerol derivatives, analogues of polar lipids isolated from a halophilic bacterial strain

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2,3-Di-*O*-phytanyl-1-*O*-glucopyranosylglycerol and polar derivatives of its 6′-glucose moiety have been synthesized. The target molecule contains the diphytanyl-*sn*-glycerol moiety which is α-linked to glucose. The key step in its synthesis involves the coupling of phytanyl bromide and isopropylidene threitol. We also demonstrated that the 6′-hydroxyl group of glycolipids can be functionalized without protection of the sugar moiety.

Keywords: sulfoglycolipid, phosphonoglycolipid, glycerol ether lipids, synthesis

Introduction

Halophilic bacteria, together with thermoacidophilic bacteria, are considered to represent a line of early divergence from the eukaryotic and eubacterial lines of evolutionary descent, and are designated as 'archaebacteria' [1]. From the halophilic bacterial strain R-4, Kates *et al.* [2] have isolated 2,3-di-*O*-phytanyl-1-*O*-[α-D-mannopyranosyl-6′-sulfate-(1′ → 2′)-*O*-α-D-glucopyranosyl]-*sn*-glycerol (**1**). This lipid is a glycerol ether with terpenoid hydrocarbons which contribute to the stability of the cell membrane, and it has a strong negative charge that enables it to exist in a high salt concentration environment. Our attention was directed towards the biological and physical properties of derivatives of glucosyl diphytanylglycerol ether. Here we report on the synthesis of these compounds.

Results and discussion

Boeckel and co-workers [3] synthesized the requisite diphytanyl glycerol ether (**8**) by treatment of 1-*O*-benzyl-*sn*-glycerol with phytanyl bromide. However, the etherification was extremely inefficient, even with long heating. To improve the overall yield, we opted to explore a novel L-tartaric acid based approach. 1,2-*O*-Isoproylidene-L-threitol (**4**) was prepared from L-tartaric acid by the method of Haines [4] in 61% overall yield. Etherification of **4** with phytanyl bromide under various conditions gave only low yields. This was circumvented by using 1-bromo-2-phytene (**5**) in the Williamson etherification step [5]. Thus, treatment of **5**, which was prepared from isophytol by PBr_3 and pyridine, with **4** and sodium hydride in THF in the presence of phase transfer catalyst (cetyltrimethylammonium bromide) gave the ether **6** in 74% yield. In the absence of the phase transfer catalyst, no etherification occurred. Catalytic reduction of **6** with Rh on an alumina catalyst followed by acid hydrolysis of the isopropylidene group yielded a diol (**7**) in 71% yield from **6**. Standard glycol cleavage [$Pb(OAc)_4$] followed by reduction with sodium borohydride gave the desired glycerol diphytanyl ether **8** in 74% yield. The total overall yield of **8** from L-tartaric acid was greatly improved to 26%.

The *O*-glucopyranosyl diphytanylglycerol ether was prepared in a number of ways, with Paulsen's procedure [6] working very well. Coupling 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl chloride with **8** in toluene using silver carbonate and silver perchlorate as promoter gave the corresponding glucosides in 88% yield (β:α ratio, 76:24). Both anomers were separated easily by flash chromatography. Debenzylation of the α-anomer by Birch reduction afforded 1-*O*-(α-D-glucopyranosyl)-2,3-*O*-diphytanyl-*sn*-glycerol (**10**) in quantitative yield.

The α-glucoside was converted into 6′-*O*-sulfate and 6′-*O*-(2″-*N*-methylamino)ethylphosphonate. In a previous study [7, 8], we demonstrated that derivatization of the 6′-hydroxyl group of glycolipids could be performed without the protection of other glycosidic hydroxyl groups. When **10** was treated with sulfurtrioxide/triethylamine complex in DMF [9, 10] followed by treatment on ion-exchange resin activated with sodium hydroxide, sodium sulfonate **2** was

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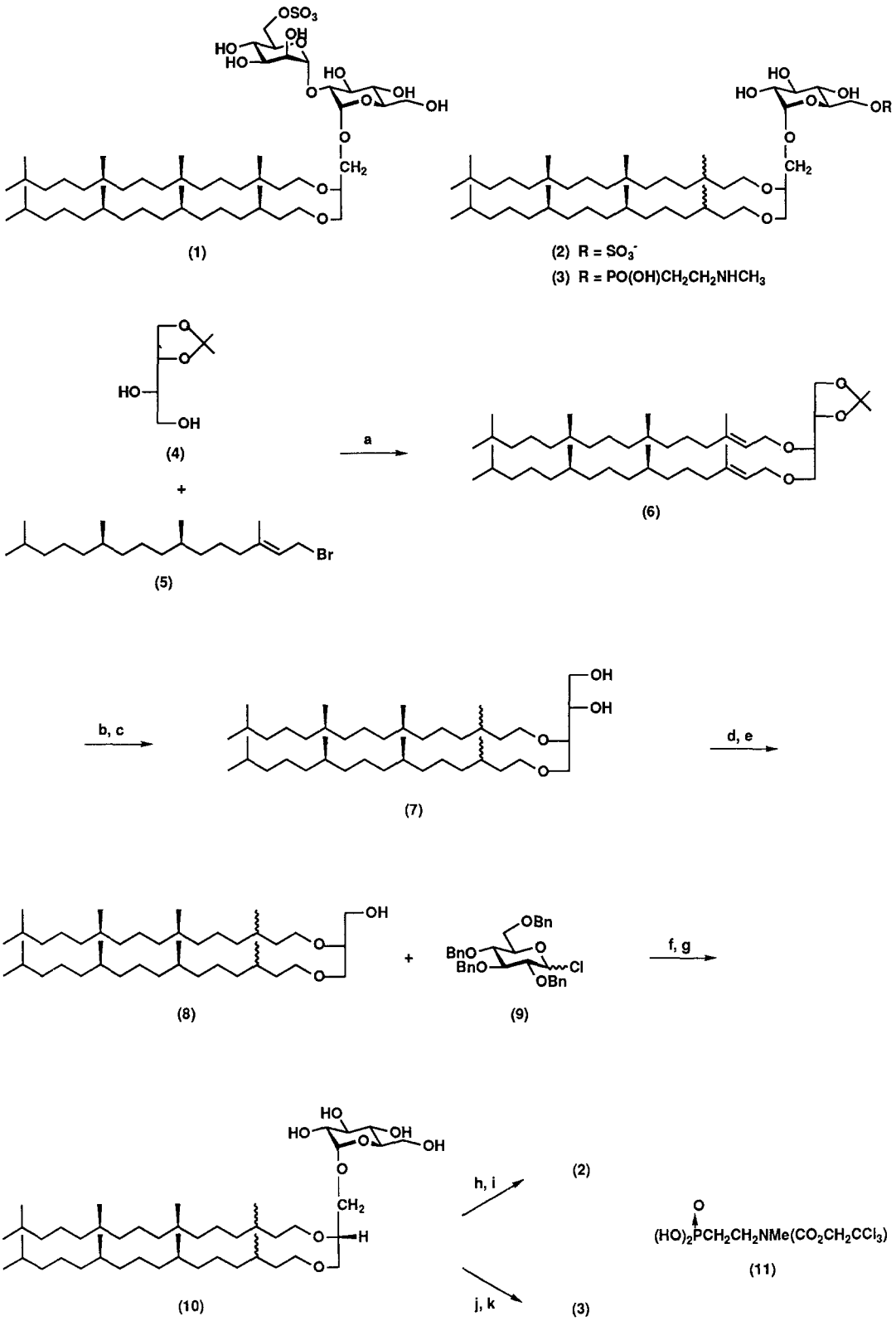

Scheme 1. Reagents and conditions: a, NaH, THF, $CH_3(CH_2)_{12}N(CH_3)_3Br$; b, H_2, Rh-Al_2O_3; c, EtOH, *p*-TsOH; d, $Pb(OAc)_4$; e, $NaBH_4$; f, Ag_2CO_3m $AgClO_4$, Et_4NBr; g, Li, liquid NH_3; h, SO_3-Et_3N; i, Amberlite CG-120 (Na^+ form); j, **11**, DEC; k, Zn, AcOH.

obtained in 47% yield. The molecular formula of **2**, $C_{49}H_{97}O_{11}SNa$, was determined by positive-ion mode FAB mass peak (*m/z* 939) [M + 2Na) and a negative-ion mode FAB mass peak (*m/z* 893) [M − 2H]. The position of the sulfate ester was determined by a conventional permethylation, hydrolysis, reduction, and acetylation sequence, which gave 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol. When **10** was allowed to react with 2-[*N*-methyl-*N*-(2′,2′,2′-trichloroethoxycarbonyl)-2-amino]ethylphosphonic acid [7, 8] and the coupling reagent 1-(3-diethylaminopropyl)-3-ethylcarbodiimide hydrochloride (DEC) followed by deprotection with zinc and acetic acid, phosphonate **3** was obtained in 21% yield. The molecular formula of **3**, $C_{52}H_{106}O_{10}NP$, was determined from a positive-ion mode FAB mass peak (*m/z* 936) [M + H] and a negative-ion mode FAB mass peak (*m/z* 934) [M − H]. The membrane, which is formed by sonication in water, showed a low gel-to-liquid crystalline phase temperature (T_m − 10 °C). Compounds **2** and **3** were both found to be devoid of anti-PAF activity against rabbit platelets. Further investigation of the biological activity of the new glycoconjugates is in progress.

In conclusion, we have demonstrated that it is possible to achieve the synthesis of glucopyranosyl diphytanyl glycerol ether in good yield and its 6′-functionalized glycolipids without protection of the other secondary hydroxyl groups of the sugar moiety.

Materials and methods

General methods

Melting points are uncorrected. THF, ether, benzene, and toluene were distilled from sodium benzophenone ketyl. Dichloromethane was distilled from phosphorus pentoxide. DMF was distilled from calcium hydride. Thin layer chromatography was performed on Merck silica gel (SiO_2) 60 analytical plates. Flash column chromatography was performed on Merck 230–400 mesh silica gel (SiO_2). Iatrobead column chromatography was performed using Iatrobeads® 6RS-8060.

Infrared spectra were recorded ona JASCO A 100 spectrometer. Electron impact and FAB mass spectra were recorded on a JEOL JMS-HX 100 spectrometer. Optical rotations were measured on a Horiba SEPA-200 spectrometer. Positive-ion mode FAB mass spectra were recorded with 3-nitrobenzyl alcohol as a matrix, and negative-ion mode FAB mass spectra were recorded with triethanolamine as a matrix. Proton nuclear magnetic resonance spectra were recorded at 60 MHz on a Hitachi R-600L or at 270 MHz on a JEOL GX 270 FT and at 500 MHz on a JEOL GX 500 FT spectrometer. Proton NMR data were recorded in ppm from TMS as an internal standard.

1,2-O-Isopropylidene-3,4-di-O-(2′-phytenyl)-L-threitol (**6**)

To a suspension of sodium hydride (4.68 g, 11.7 mmol) in THF (2 ml) was added a solution of **4** (0.277 g, 1.66 mmol) in THF (4 ml) at 0 °C. After the mixture had been stirred at room temperature for 2 h, a solution of 1-bromo-2-phytene (1.21 g, 3.42 mmol) and cetyltrimethylammonium bromide (59 mg, 0.34 mmol) in THF (1 ml) was added. The mixture was heated under reflux for 9 h. Upon completion of the reaction, ice was added, and the mixture was extracted with chloroform. The organic phase was washed successively with dilute hydrochloric acid and sodium hydrogen carbonate solution, then dried ($MgSO_4$), filtered and concentrated. The residue was purified by chromatography (SiO_2: eluted with benzene:ethyl acetate, 95:5) to give **6** (0.913 g, 74%) as a colourless oil. $[\alpha]_D$ +1.45° (c 2.06, $CHCl_3$). ^1H NMR data (C^2HCl_3, 270 MHz); δ 0.86 (t, *J* 6.0 Hz, 25H), 1.0–1.8 (m, 68H), 3.4–4.4 (m, 10H), 5.30 (t, *J* 6.5 Hz, 2H), 5.36 (t, *J* 6.5 Hz, 2H). HRMS data. Calculated for $C_{49}H_{90}O_4$, 718.6839; found, 718.6851.

1,2-Diphytanyl-L-threitol (**7**)

Compound **6** (2.259 g, 3.14 mmol) was dissolved in ethanol (50 ml), and Rh on alumina (5%, 0.20 g) was added. The mixture was hydrogenated at room temperature under atmospheric pressure. The mixture was filtered through a pad of Celite and concentrated. The crude hydrogenation product was dissolved in ethanol (50 ml), and *p*-toluenesulfonic acid (54 mg) was added. The mixture was heated under reflux for 2 h. Most of the solvent was evaporated and the resulting mixture was extracted with chloroform. The extract was washed with saturated sodium hydrogen carbonate and dried ($MgSO_4$), filtered and concentrated. The product was purified by chromatography (SiO_2: eluted with benzene:ethyl acetate 9:1) to give **7** (1.657 g, 77%) as a colourless oil. $[\alpha]_D$ +8.63° (c 1.07, $CHCl_3$). IR data (neat): 3400, 2950, 2920, 2860, 1460, 1380, 1110 cm^{-1}. ^1H NMR data (C^2HCl_3, 60 MHz): δ 0.8–2.0 (m, 78H), 3.4–4.0 (m, 10H). HRMS data. Calculated for $C_{44}H_{90}O_4$, 682.6816; found, 682.6816.

*1,2-Diphytanyl-*sn*-glycerol* (**8**)

Glycol **7** (1.42 g, 2.08 mmol) was dissolved in ethyl acetate (5 ml). Lead tetraacetate (1.00 g, 2.26 mmol) was added, and the mixture was stirred at room temperature under an argon atmosphere. When the starting material had been consumed (4 h), a small amount of ethyleneglycol was added. After the mixture had been filtered, the solvent was removed under reduced pressure. The residue was dissolved in ethanol (40 ml), sodium borohydride (1 g, 23.3 mmol) was added, and the mixture was stirred for 2 h at room temperature. After the solvent had been removed under reduced pressure, the residue was extracted with chloroform. The combined extracts were washed with 5% sodium hydroxide, dried ($MgSO_4$), and evaporated. The product was purified by

chromatography (SiO_2: eluted with benzene:ethyl acetate 95:5) to give **8** (1.01 g, 74%) as a colourless oil. $[\alpha]_D$ +7.63° (c 0.92, $CHCl_3$). IR data (neat): 3420, 2950, 2910, 2850, 1460, 1380, 1120, 1050 cm^{-1}. ^1H NMR data (C^2HCl_3, 270 MHz): δ 0.86 (t, *J* 6.0 Hz, 24H), 1.0–1.8 (m, 78H), 2.1 (br, 1H), 3.4–4.4 (m, 10H). FAB-MS data (positive-ion mode): *m/z* 653 $[M + 1]^+$, 373.

1-O-(α-D-*Glycopyranosyl*)-*2,3*-O-*diphytanyl*-sn-*glycerol* (**10**)

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranosyl chloride (**9**, 0.410 g, 0.758 mmol) was dissolved in toluene (10 ml). Molecular sieves 4 Å (3 g) and tetraethylammonium bromide (16 mg) were added and the mixture was stirred for 10 min. A solution of **8** (4.95 g, 0.758 mmol) in toluene (10 ml), silver carbonate (0.752 g, 2.73 mmol) and silver perchlorate (0.075 g, 0.37 mmol) were then added successively to the above solution. After 48 h the reaction mixture was filtered through Celite and concentrated under reduced pressure. The product was purified by chromatography (SiO_2: eluted with benzene:ethyl acetate 96:4) and further by flash chromatography (SiO_2: eluted with benzene:ethyl acetate 93:7) to give 1-*O*-(2′,3′,4′,6′-tetrabenzyl-α-D-glucopyranosyl)-2,3-*O*-diphytanyl-*sn*-glycerol (0.784 g, 88%) as a colourless oil. $[\alpha]_D$ +21.0° (c 1.54, $CHCl_3$), IR data (neat): 3020, 2950, 2920, 2850, 1500, 1450, 1380, 1360, 1210, 1100, 730, 700 cm^{-1}. ^1H NMR data (C^2HCl_3, 500 MHz): δ 0.8–1.7 (m, 20H), 3.9–4.0 (m, 12H), 4.5 (m, 8H), 7.0–7.4 (m, 20H).

A solution of the tetrabenzyl ether (0.215 g, 0.187 mmol) in THF (25 ml) was added to a stirred solution of lithium (104 mg, 0.0150 mol) in liquid NH_3 at −78 °C. The solution was stirred at −78 °C for 5 h, quenched with finely ground solid NH_4Cl, and allowed to warm to room temperature overnight. The residue was extracted with ethyl acetate, dried ($MgSO_4$) and concentrated. The product was purified by chromatography (SiO_2: eluted with chloroform:methanol 95:5) to give **10** (0.155 g, 100%) as a waxy solid. $[\alpha]_D$ +37.0° (c 3.12, $CHCl_3$). IR data ($CHCl_3$): 3400, 2950, 2850, 1460, 1380, 1150, 1000 cm^{-1}. ^1H NMR data (C^2HCl_3, 500 MHz): δ 0.8–1.7 (m, 78H), 3.0 (br, 4H), 3.4–4.0 (m, 15H), 4.85 (d, *J* 3.3 Hz, 1H). FAB-MS data (positive-ion mode), *m/z* 837 $[M + Na]^+$, 653, 373; (negative-ion mode), 813 $[M - 1]^-$.

1-O-(*6′*-O-*Sulfo*-α-*D*-*glucopyranosyl*)-*2.3*-O-*diphytanyl*-sn-*glycerol* (**2**)

To a solution of **10** (0.316 g, 0.387 mmol) in DMF was added a sulfur trioxide/triethylamine complex (0.13 g, 0.93 mmol). After the mixture had been stirred overnight at room temperature, the solvent was removed under reduced pressure. The product was purified by chromatography on Iatrobeads® (eluted with chloroform:methanol:water, 32:8:1 by vol) and then on Amberlite (CG-120, Na^+ form) to give **2** (0.168 g, 48%) as a waxy solid. IR data (neat): 3400, 2900, 1650, 1450, 1370, 1250, 1100, 930, 760 cm^{-1}. ^1H NMR data (C^2HCl_3, 500 MHz): δ 0.8–1.6 (m, 78H), 3.45–5.00 (m, 15H). FABMS data (positive-ion mode), *m/z* 939 $[M + 2Na]^+$, 923.837; (negative-ion mode) *m/z* 893 $[M - 2]^-$, 877.

1-O-{*6′*-O-[*2″*-(N-*Methylamino*)*ethylphosphonyl*]-α-D-*glucopyranosyl*}-*2,3*-O-*diphytanyl*-sn-*glycerol* (**3**)

To a solution of **10** (0.216 g, 0.268 mmol) in pyridine (4 ml) was added 2-[*N*-methyl-*N*-(2′,2′,2′-trichloroethoxycarbonyl)-2-amino]ethylphosphonic acid (0.100 g, 0.318 mmol) and DEC (0.100 g, 0.522 mmol). After the mixture had been sonicated for 2 h, the solvent was removed under reduced pressure. Purification by preparative layer chromatography (SiO_2: developed by chloroform:methanol:water, 56:36:10 by vol) gave a protected phosphonate (0.084 g, 30%) as a waxy solid. To a solution of the phosphonate derivative (80 mg, 0.072 mmol) in 90% acetic acid (1 ml) and THF (1 ml) was added an excess of freshly activated zinc powder. After 20 h stirring at room temperature, the reaction mixture was neutralized with saturated sodium bicarbonate solution and extracted with chloroform:methanol (87:13 by vol) and then with ethyl acetate. The combined extracts were dried ($MgSO_4$) and concentrated. Purification by preparative layer chromatography (SiO_2: developed by chloroform:methanol:water, 56:38:10 by vol) gave **3** (49 mg, 71%) as a waxy solid. IR data ($CHCl_3$): 3260, 2900, 1620, 1460, 1380, 1110, 1020 cm^{-1}. ^1H NMR data (C^2HCl_3, 500 MHz): δ 0.8–1.6 (m, 78H), 1.9–2.2 (m, 4H), 1.95 (s, 3H), 3.4–5.0 (m, 15H). FABMS data (positive-ion mode) *m/z* 936 $[M + 1]^+$; (negative-ion mode) *m/z* 934 $[M - 1]^-$, 140, 138.

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