An efficient synthesis of analogues of unsymmetrical archaeal tetraether glycolipids

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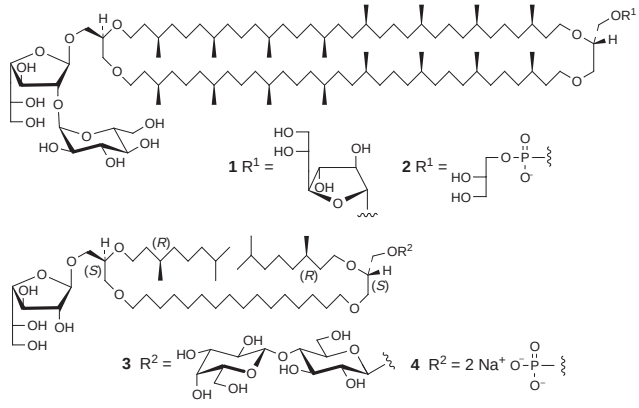
Unsymmetrical tetraether analogues of glycolipids found in archaebacteria, possessing either two different carbohydrate units or a saccharidic moiety and a phosphate group as polar heads and a quasi-macrocyclic lipid core, are efficiently synthesized from versatile chiral building blocks.

The Archaea domain, composed of a variety of extremophilic microorganisms, belongs to a third kingdom of life which is clearly distinct from the well-known Bacteria and Eucarya domains.[1–3] Preservation of the membrane function under extreme conditions such as high temperatures and strong acidity lies with the unique structure of archaebacterial lipids, which fundamentally differ from those of their bacterial and eucaryotic counterparts.[4] Of particular interest is the unusual molecular architecture of the macrocyclic tetraether lipids found in methanogenic and thermoacidophilic species.[5] These lipids are characterized by a bipolar structure with two head groups linked together by two C40 polyisoprenoid chains. Recently, Gräther and Arigoni demonstrated that several natural lipids were in fact nearly statistical mixtures of regioisomers differing in the relative orientation of their glycerol units.[6] The dimensions of the tetraether lipids are therefore sufficient to form monolayered membranes[5] and the presence of two head groups with different sizes at opposite sides of the monolayer is expected to induce membrane curvature.[4b] The presence of galactofuranose units in some glycolipids is puzzling since hexoses appear only in the pyranose form in mammalian glycoconjugates. The unique chemical design of these archaeal lipids represents an interesting opportunity to build exceptionally thermal stable vesicles which might be suitable for nano-scale technologies and for drug-delivery applications.[7]

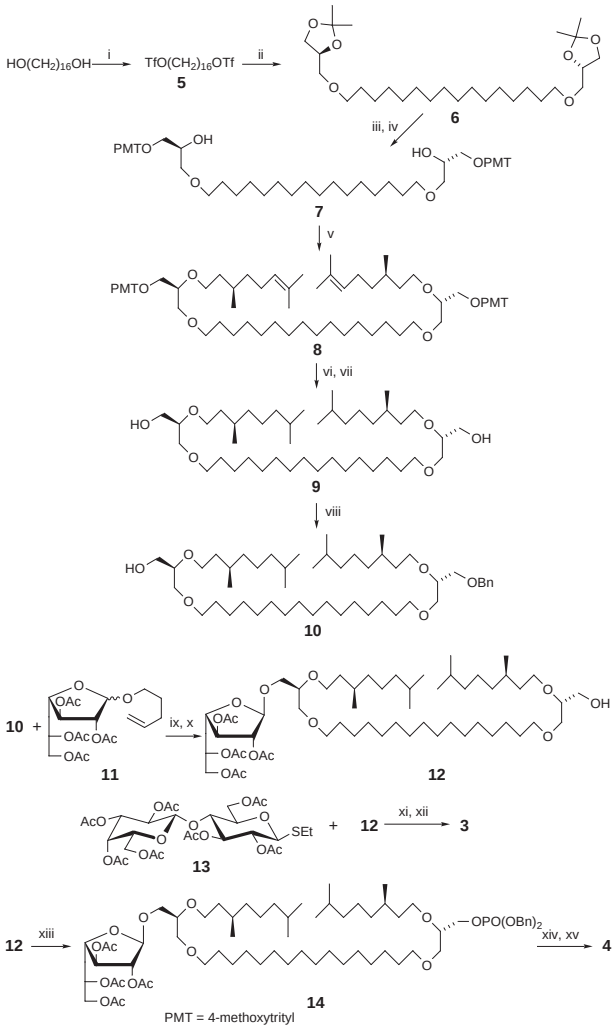
We report here the first synthesis of the chiral unsymmetrical quasi-macrocyclic tetraethers **3** and **4** as models of the natural



lipids **1** and **2** isolated from *Methanospirillum hungatei*, which is a methanogenic species.[5] The most characteristic structural features of the target molecules lies with (i) a lipid core consisting in a linear hexadecanemethylene spacer and two (*R*)-dihydrocitronellyl chains having a combined length equal to that of the bridging unit, attached to glycerol respectively at *sn*-3 and *sn*-2 positions, (ii) either a D-galactofuranose and a phosphate group or the former and a lactosyl unit as polar head groups at opposite ends of the lipid core, and (iii) an *S* stereogenic center at each glycerol backbone similar to that of the corresponding natural glycolipids **1** and **2**.[5] These synthetic bolaamphiphiles might show similar behaviour to cyclic lipids when aggregated or inserted into membranes.[8]

The general synthetic pathway for **3** and **4** (Scheme 1) involved the preparation of the key diol intermediate **9**, subsequent monoprotection of one of the hydroxy groups and sequential introduction of the polar heads. The crucial glycosylation or phosphorylation step of the monoglycosylated compound **12** should be performed under extremely mild conditions in order to preserve the highly hydrolysable β-D-galactofuranosidic linkage. Hemimacrocyclic diol **9** was obtained from (*S*)-(+)-2,2-dimethyl-1,3-dioxolan-4-ylmethanol [(*S*)-(+)-solketal],‡ hexadecane-1,16-diyl ditriflate **5**, and (*R*)-(−)-citronellyl bromide as the starting materials. After experimentation, we found that reaction of hexadecane-1,16-diol with Tf_2O in the presence of the hindered base 2,6-lutidine resulted in the efficient formation of the expected ditriflate **5**. Separation of the ditriflate from the base was easily accomplished by flash chromatography, thus affording compound **5** in 80% yield. Alkylation of two (*S*)-solketal units by **5** proceeded efficiently (83%) in refluxing CH_2Cl_2 for 24 h using 1,8-bis(dimethylamino)naphthalene (proton sponge: PS) as a base. Removal of the isopropylidene groups in the presence of a Dowex acidic resin in refluxing MeOH gave the tetraol (73%) which was selectively protected by *p*-methoxytritylation of the primary hydroxy groups. Williamson coupling of the remaining secondary hydroxy groups using commercially available (*R*)-(−)-citronellyl bromide provided the unsaturated tetraether **8** (80% yield). Palladium-catalyzed hydrogenation of **8** led to the formation of byproducts due to the unexpected cleavage of the ether linkages at the *sn*-2 glycerol sites. Addition of 1 equiv. of triethylamine in the hydrogenation media avoided this side reaction and furnished quasi-quantitatively the corresponding saturated lipid. Finally, the key diol **9** was readily prepared by removal of the trityl groups under acidic conditions.[9]

At this stage, the next challenging problem involved the selective protection of **9** so as to introduce two different polar head groups at opposite ends. After experimentation, we found that the protocol reported by Bouzide and Sauvé[10] was the most efficient methodology for the monoprotection of the diol. Treatment of **9** with Ag_2O and BnBr in CH_2Cl_2 led to the formation of the monobenzylated product **10** (50% yield) in addition to the dibenzylated derivative (10% yield) and the unreacted recyclable diol (36%). The introduction of the β-D-galactofuranoside unit was performed stereospecifically by way of *n*-pentenyl glycoside (NPG) glycosylation[11] from the key galactofuranosyl donor **11**.[12] After hydrogenolysis of the benzyloxy group, we envisaged either the glycosylation or the



Scheme 1 *Reagents and conditions*: i, Tf_2O, 2,6-lutidine, CH_2Cl_2, 0 °C, then room temp., 80%; ii, (*S*)-solketal, PS, CH_2Cl_2, reflux, 83%; iii, Dowex H^+ resin, MeOH, reflux; iv, 4-methoxytrityl chloride, pyridine, DMAP, THF, reflux, 60% over two steps; v, (*R*)-citronellyl bromide, NaH, 130 °C, 80%; vi, H_2, Pd/C, Et_3N, AcOEt; vii, HCO_2H, Et_2O, 70% over two steps; viii, Ag_2O, BnBr, CH_2Cl_2, 50%; ix, NIS, Et_3SiOTf, CH_2Cl_2; x, H_2, Pd/C, EtOH, 73% over two steps; xi, NIS, TESOTf, 4 Å molecular sieves, CH_2Cl_2; xii, MeONa, MeOH, 65% over two steps; xiii, 1*H*-tetrazole, $(BnO)_2PNPr^i_2$, CH_2Cl_2, then MCPBA, CH_2Cl_2, −40 to 0 °C, 80%; xiv, MeONa, MeOH; xv, H_2, Pd/C, MeOH, acetate buffer (pH 5) (3:1 v/v), then Amberlite® IR-120 (Na^+), MeOH, then gel-filtration on Sephadex LH-20 (1:2 CH_2Cl_2–MeOH), 85% over two steps

phosphorylation of the free alcohol under mild conditions in order to prevent any hydrolysis of the former β-D-galactofuranosidic bond. The glycosylation of **12** using lactosyl thioglycoside **13** as a donor[13] was carried out under NIS–Et_3SiOTf conditions and provided the desired β-D-glycoside in 70% yield. Deacetylation of the glycosyl hydroxy groups under standard conditions gave the totally deprotected bipolar lipid **3** in 93% yield.§

Having successfully prepared the bis-glycoside **3**, we turned our attention towards the introduction of a phosphate group onto intermediate **12**. Alkyl dibenzyl phosphate **14** was prepared by reacting alcohol **12** with dibenzyl *N*,*N*-diisopropylphosphoramidite and 1*H*-tetrazole followed by *in situ* mild oxidation of the resultant alkyl dibenzyl phosphite with MCPBA (80% yield).[14] The transformation of **14** into the phosphate salt **4** was performed by sequential deacetylation of the galactosyl unit, catalytic hydrogenolysis (Pd/C) in a buffered solvent mixture (MeOH–AcOH–NaOAc, pH 5) avoiding the glycoside hydrolysis and treatment with Amberlite® IR-120 (Na^+ form, water).

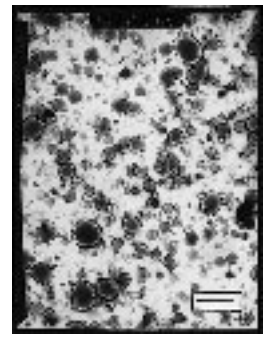


Fig. 1 Transmission electron micrograph of compound **4** vesicles negatively stained with uranyl acetate. The bar is 1500 nm.

Purification by gel filtration chromatography with Sephadex LH-20 furnished the targeted unsymmetrical glycolipid analogue **4** in 85% yield.

Aqueous dispersions of glycolipids **3** and **4** were sonicated at 60 °C for 20 min. Transmission electron microscopy revealed that phosphate **4** furnished spherical vesicles of 100–1000 nm diameter stable for several weeks at ambient temperature as shown in a representative micrograph (Fig. 1). Compound **3** provided myelin-type aggregates (not shown).

Further physicochemical measurements are under investigation.

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Notes and References

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‡ (*S*)-(+)-Solketal and its (*R*)-(−)-isomer are currently available on a kilogram scale. (*S*)-(+)-Solketal was obtained from Chemi S.p.A., 20092 Cinisello Balsamo, Italy.

§ All yields reported herein refer to isolated pure materials which have ^1H and ^{13}C NMR spectra, elemental analyses and high resolution mass spectral characteristics in accordance with the proposed structures. *Selected data* for **3**: $[\alpha]_D^{20}$ −26.7 (*c* 0.78 in MeOH); FABMS (*m*-nitrobenzyl alcohol matrix): Calc. for $[M + Na]^+$: 1195.7907. Found: 1195.7880. For **4**: $[\alpha]_D^{20}$ −26.4 (*c* 0.72 in MeOH); FABMS (*m*-nitrobenzyl alcohol matrix): Calc. for $[M + H]^+$: 973.6333. Found: 973.6331; Calc. for $[M - Na + 2H]^+$: 951.6514. Found: 951.6524.

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