Supramolecular Self-Assembling Properties of Membrane-Spanning Archaeal Tetraether Glycolipid Analogues

Grégory Lecollinet,[a] Annette Gulik,[b] Grahame Mackenzie,[c] John W. Goodby,[c] Thierry Benvegnu,*[a] and Daniel Plusquellec[a]

Abstract: The self-assembling properties of a new series of archaeal tetraether glycolipid analogues **1**–**6** that are characterized by a bipolar architecture with two similar or different glycosidic and/or phosphate polar heads and a lipid core possessing a cyclopentane unit and/or branched chains were studied by means of differential scanning calorimetry, optical microscopy, X-ray scattering, freeze-fracture electron microscopy and dynamic light scattering. Unsymmetrical phosphate derivatives **1** and **2** spontaneously formed thermostable multilamellar and unilamellar vesicles in which most of the bipolar lipids adopted a trans-membrane conformation, as revealed by freeze-fracture electron microscopy. Supramolecular aggregates of neutral glycolipids **3**–**6** were found to depend on both the saccharidic polar heads and the chain composition. The presence of one glycosidic residue with rather marked hydrophilic properties, such as the lactosyl moiety, was required to allow the formation of multilamellar vesicles. Surprisingly, the introduction of a cyclopentane unit in the bridging chain was able to induce an apparent two-by-two membrane association: this unusual behaviour might be the result of unsymmetrical interfacial properties of the lipid layer caused by the presence of the cyclopentane unit.

Keywords: amphiphiles · archaea · monolayers · self-assembly · vesicles

Introduction

Archaeal membranes are composed of a variety of unusual lipids that include phospholipids and glycolipids derived from diphytanylglycerol diether or dibiphytanyldiglycerol tetraethers, which are well-adapted to the extreme environmental conditions of archaebacteria, for example, halophiles, methanogens and thermoacidophiles (Figure 1).[1] The ether linkages are more stable than esters over a wide range of pH, and the branching methyl groups in the isoprenoid chains help to reduce crystallisation. Furthermore, tetraether-based lipids found in methanogenic and thermophilic species possess aliphatic chains that form bipolar monolayers in which the lipids span the membrane.[2] Another important point concerns the presence of cyclopentane rings in the isoprenoid chains of thermoacidophilic lipids that may correspond to an adaptive response to the change in temperature of the local environment.[3–4] The relationship between the chemical structure and the physicochemical behaviour has been established for several natural lipids derived from extremophilic thermoacidophiles.[5] However, given the difficulty in obtaining significant amounts of a large variety of chemically pure lipids from natural sources, the functional role of

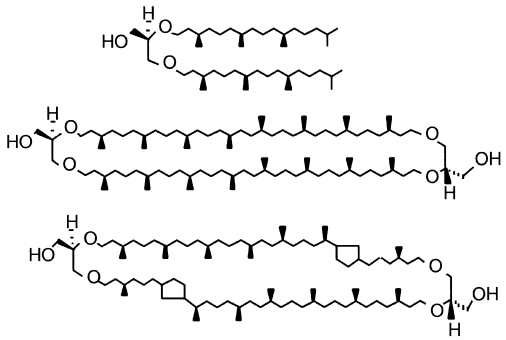


Figure 1. Typical basic structures of methanogenic and thermoacidophilic archaeal membrane lipids.

[a] Dr. T. Benvegnu, Dr. G. Lecollinet, Prof. D. Plusquellec
Ecole Nationale Supérieure de Chimie de Rennes
Synthèses et Activations de Biomolécules
CNRS UMR 6052, Institut de Chimie de Rennes
Avenue du Général Leclerc, 35700 Rennes (France)
Fax: (+33) 2-2323-8046
E-mail: thierry.benvegnu@ensc-rennes.fr
daniel.plusquellec@ensc-rennes.fr

[b] Dr. A. Gulik
Centre de Génétique Moléculaire, CNRS
91198 Gif-sur-Yvette Cedex (France)
E-mail: agulik@cgm.cnrs-gif.fr

[c] Dr. G. Mackenzie, Prof. J. W. Goodby
The Department of Chemistry
Faculty of Science and the Environment
The University of Hull, Hull HU6 7RX (UK)
E-mail: g.mackenzie@chem.hull.ac.uk
j.w.goodby@chem.hull.ac.uk

  0947-6539/02/0803-0585 $ 17.50+.50/0

complex bipolar amphiphiles is difficult to assess. The evaluation of well-defined synthetic analogues capable of reproducing the self-organization of archaeal bolaamphiphiles represents an interesting alternative, since it allows for the establishment of relationships between the molecular structures of the lipids and the architectures and thermal stabilities of their self-organising supramolecular assemblies.

Although some investigations pertaining to the self-assembling properties of synthetic phosphate-type tetraethers have been undertaken,[6] studies on the effects of 1) the symmetrical or unsymmetrical structure of the lipids, 2) the presence of anionic phosphate and/or neutral saccharidic moieties and 3) the structure and composition of the bolaphilic bridging chain are less common.

Within this context, we have developed and optimised the synthesis of novel symmetrical and unsymmetrical hemi-macrocyclic bipolar glycolipids **1**–**6** (Figure 2), characterized by the presence of: 1) a hexadecamethylene spacer or a spacer of similar length that also contains a 1,3-disubstituted cyclopentane ring, linked as an ether to two glycerol units at the *sn*-3 and *sn*-3′ positions, 2) two branched (*R*)-dihydrocitronellyl chains attached to glycerol moieties at the *sn*-2 and *sn*-2′ positions and 3) phosphate and/or glycosidic polar headgroups derived from either lactose or D-galactose (in a furanoid cyclic form, as found in some natural methanogenic lipids).[1b] For example, compounds **2** and **6** are 4:1 diastereomeric mixtures of *cis*–*trans* isomers of the 1,3-disubstituted cyclopentane ring. The presence of this diastereoisomeric mixture results from the synthetic pathway used from the preparation of the aliphatic linker.[7c] Therefore, our efforts have been directed towards evaluating the influence of the precise molecular architecture, such as the nature of the two polar moieties and the incorporation of a cyclopentane ring into the bridging chain, on the self-organising properties, namely, on the formation of liquid-crystalline thermotropic and lyotropic phases.

The results on the preparation of tetraethers **1**, **4** and **5**, which possess a linear aliphatic bridging group, were recently published.[7a–c] We now report on the synthesis of glycolipids **2** and **6**, which have a cyclopentane ring incorporated into the middle of the bridging chain, and also a monoglycosylated analogue **3**. The physicochemical behaviour of all the glycolipids **1**–**6** are presented. These studies clearly reveal striking differences in the supramolecular self-organising properties of the materials depending on the hydrophilicity of the headgroups and the introduction of a cyclopentyl moiety into the aliphatic bridging unit.

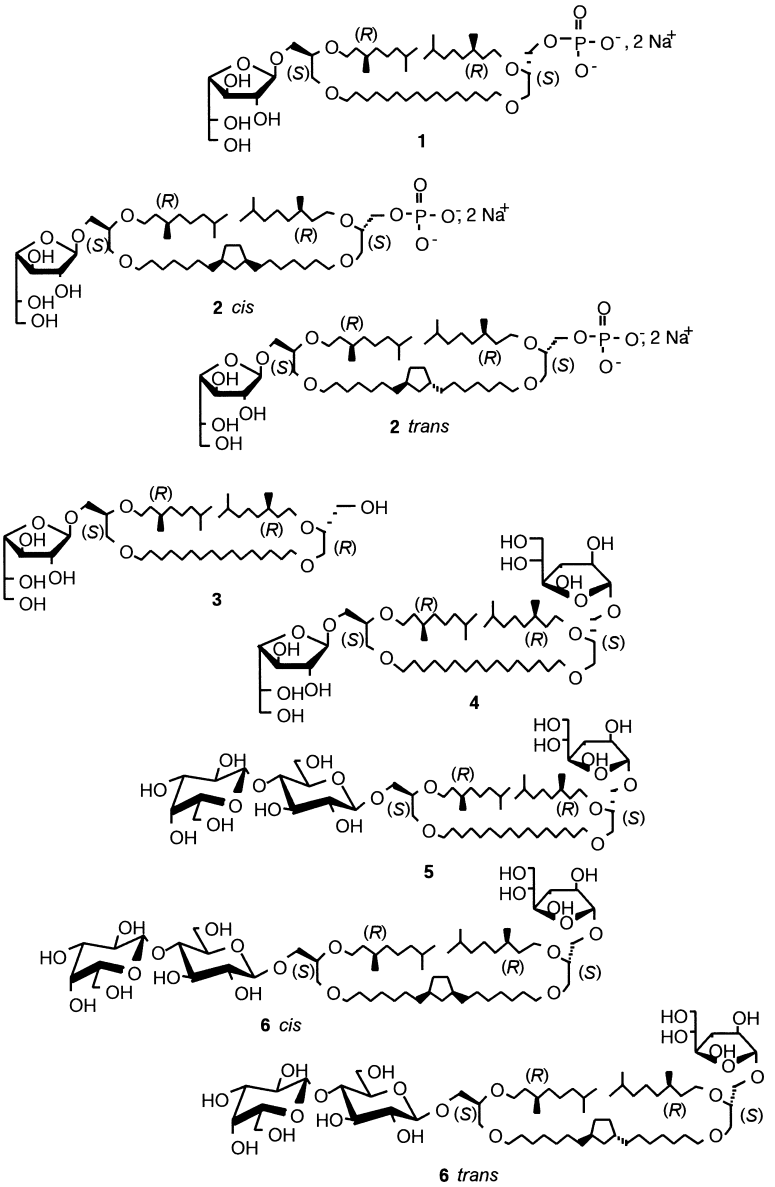
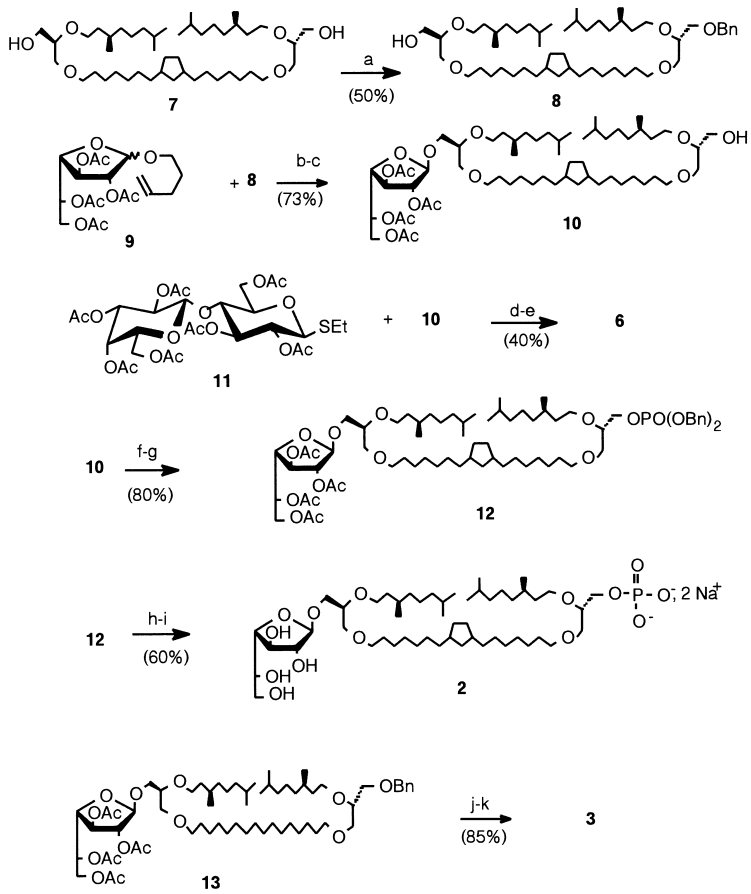


Figure 2. Synthetic symmetrical and unsymmetrical hemi-macrocyclic lipid analogues **1**–**6** of natural methanogenic or thermoacidophilic lipids. Compounds **2** and **6** are a 4:1 diastereomeric mixture of *cis*–*trans* isomers of the 1,3-disubstituted cyclopentane ring.

Results

Syntheses: The novel amphiphiles **2**, **3** and **6** were synthesized according to the strategy described for the preparation of compounds **1**, **4** and **5**[7c] (Scheme 1). The synthesis of the unsymmetrical glycolipids **2** and **6** involved the monobenzylation of the quasi-macrocyclic diol **7**[7c] followed by the introduction of the two different polar groups at opposite ends of the lipophilic core. Glycosylation of the monoprotect-

0947-6539/02/0803-0586 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 3



Scheme 1. Reagents: a) Ag_2O, BnBr, CH_2Cl_2; b) NIS, Et_3SiOTf, CH_2Cl_2; c) H_2,Pd/C,EtOH; d) NIS, Et_3SiOTf, CH_2Cl_2; e) CH_3ONa, CH_3OH; f) 1*H*-tetrazole, $(BnO)_2PN(iPr)_2$, CH_2Cl_2; g) *m*-CPBA, CH_2Cl_2, $-40\,^{\circ}C$ to $0\,^{\circ}C$; h) CH_3ONa, CH_3OH; i) 1) H_2, Pd/C, CH_3OH, acetate buffer (pH 5) (3:1 v/v), 2) Amberlite IR-120 (Na^+), CH_3OH, 3) gel-filtration on Sephadex LH-20; j) CH_3ONa, CH_3OH; k) H_2, Pd/C, EtOH.

ed diol **8** with *n*-pentenyl D-galactofuranoside **9**,[7c] by means of standard *n*-pentenyl glycoside chemistry (NIS-Et_3SiOTf), stereospecifically provided the corresponding β-glycoside. Removal of the benzyl group afforded the monoglycosylated lipid **10** in 95% yield. The phosphate or the β-lactosyl moieties were then introduced, as described for the preparation of derivatives **1** and **5**.[7c] Monoglycosylated lipid **3**, which possesses a free hydroxyl group, was obtained by the removal of the acetate and benzyl groups of intermediate **13**, which was employed in the preparation of bolaamphiphiles **1** and **5**.

Thermotropic and lyotropic properties: The thermotropic- and lyotropic-phase behaviour of anionic and neutral tetraether glycolipids **1**–**6**, as well as the characterization of their supramolecular aggregates in dilute aqueous media, were studied by means of differential scanning calorimetry (DSC), polarized optical microscopy (POM), X-ray scattering (XRS), freeze-fracture electron microscopy (FFEM) and dynamic light scattering (DLS). It is important to note that for all of the materials, a diffuse band characteristic of fluid chains was observed in the wide-angle X-ray scattering (WAXS) region; the band was centred around 4.8 $Å^{-1}$, a value slightly higher than the usual value of 4.5 $Å^{-1}$ found for aliphatic chains, as already observed for isoprenoid lipids obtained from *Sulfolobus solfataricus*.[5a, b] In some cases, an additional band was also observed around 8 $Å^{-1}$.

*Tetraether glycolipids that possess a phosphate head-group (**1** and **2**)*: The determination of the thermotropic liquid-crystalline behaviour of the phosphorylated compounds **1** and **2** by POM or DSC was not possible because of their strong hydroscopic properties. However, without added water, compounds **1** and **2** appeared to exhibit columnar mesophases. Small-angle X-ray scattering (SAXS) for compound **1** gave six reflections at $s = 20.0$, 35.1 (strong), 40.3, 53.3, 60.4, 71.1 $\times$ 10^{-3} $Å^{-1}$ that were compatible with a *P*-centred rectangular structure ($hk = 11$, 20, 02, 22, 13, 31, 04, 40, 33, 24, 06 reflections) of lattice dimensions 28.5×49.8 Å. In the case of tetraether **2**, two small-angle X-ray reflections were observed with a spacing ratio $1:\sqrt{3}$ that corresponded most likely to the first two reflections ($h = 10$, 11) of a hexagonal phase with a lattice dimension of 34.2 Å.

As soon as water comes into contact with the columnar phases of **1** and **2**, fluid rod myelin-type structures were observed between crossed polarizers. The structures produced typical irregular foldings, and helical and coiled forms (Figure 3a,b). The observation of Maltese crosses (Figure 3b) was also indicative of the presence of large multilamellar vesicles.[8] The contact preparations between compounds **1** and **2** and water revealed similar bands of lamellar mesophase and also an optically isotropic (possibly cubic) phase was present (Figure 3c). The X-ray scattering of hydrated glycolipid **1** was rather complex and depended on the amount of water present. Compound **1** tended to exhibit a lamellar phase as the amount of water was increased; for 80% added water, three reflections ($h = 1, 2, 3$) were observed, with a repeat dimension of 102 Å. The X-ray diffraction pattern of hydrated compound **2** consisted of four reflections ($h = 1, 2, 3, 4$) corresponding to a lamellar $L\alpha$ phase: with 50% added water, the lattice dimension was 58 Å.

In more dilute aqueous media, dispersions of **1** and **2** produced vesicles without sonication, as shown by freeze-fracture electron microscopy (Figure 4a,b); the vesicles were cross-fractured indicating the absence of a fracture plane along the mid-plane of the membrane. This result suggests that the lipid molecules do not have a U-shaped conformation but instead are trans-membrane. Therefore, the membrane is composed of a lipid monolayer. It is interesting to note that the vesicles were still stable at 60 °C, as shown by FFEM and

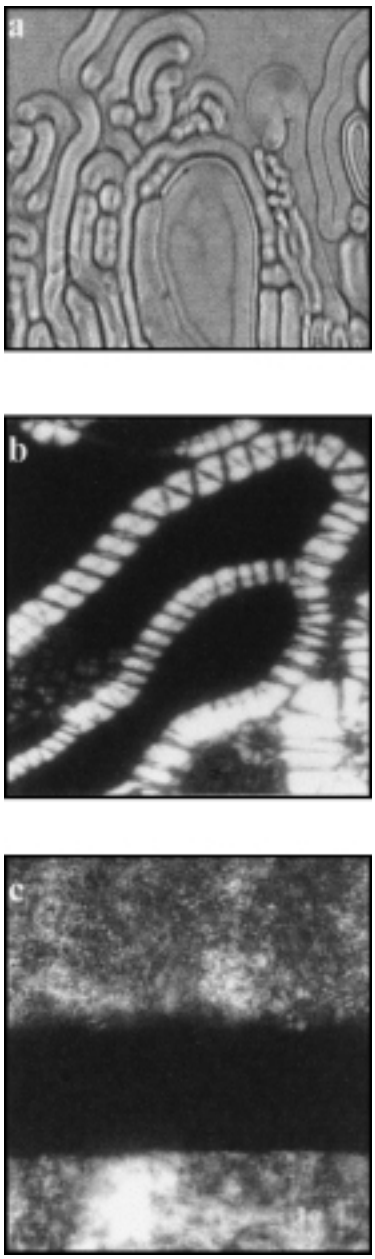


Figure 3. Optical textures of phosphorylated compounds **1** and **2** in the presence of water (crossed polarizers). a,b) Myelins and Maltese crosses (enlargement 200 ×). c) Contact obtained between compound **2** and water (enlargement 100 ×); top: $L\alpha$ mesophase; middle: isotropic cubic-type mesophase; bottom: thermotropic columnar mesophase.

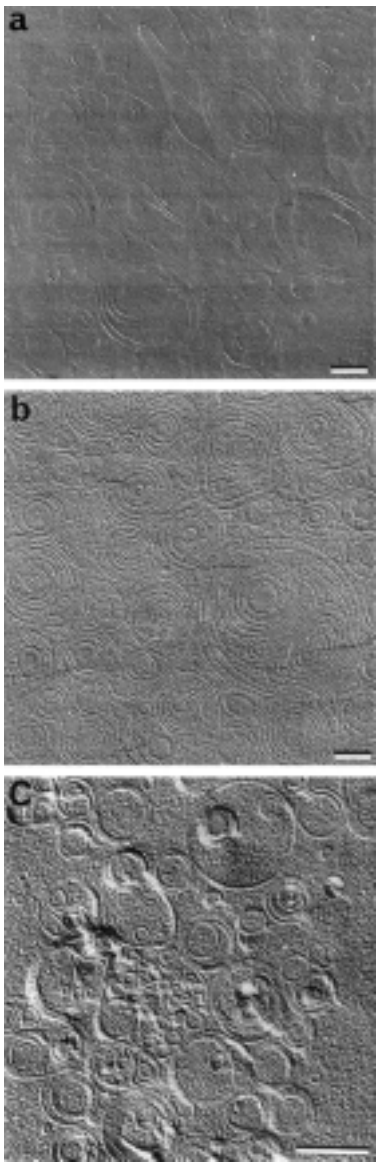


Figure 4. Freeze-fracture electron microscopy (FFEM) of glycolipids **1**, **2** and **5**. a) Compound **1**. b) Compound **2**. c) Compound **5**. In b and c, the fractured samples were etched before shadowing in order to visualize the propagation path much more clearly. In c, the vesicles consisted of very closely packed multilayers. In all cases, the vesicles were cross-fractured: the lipid molecules spanning the membrane and forming a monolayer prevent the fracture as a result of propagation along the mid-plane of the membrane. The size of the bar is 250 nm.

by the size distribution obtained from dynamic light scattering measurements.

*Tetraether glycolipids possessing one or two saccharidic moieties (**3**–**6**):* Self-assembling properties of neutral tetraether glycolipids **3**–**6** were examined in order to establish the influence of the presence of one or two sugar residues at opposite ends of the bridging group, as well as to investigate the effects of the incorporation of a cyclopentane unit into the lipophilic spacer with respect to the trans-membrane organization of the lipids. Compound **3**, which possesses a galactofuranosyl unit at one end and a free hydroxyl group at the other end of the bridging chain, was liquid at room temperature and did not display any amphiphilic properties: it behaved as a non-miscible oil with respect to water, even at 0 °C.

The presence of a sugar unit at each end of the bridging group engendered the materials with liquid-crystalline properties. The symmetrical tetraether **4**, which contains two similar galactofuranosyl residues, was transparent and optically isotropic at 20 °C in the absence of water. Upon heating, a birefringent texture slowly appeared at ≈70 °C. Identification of the resulting mesophase by POM proved to be impossible because of the formation of numerous paramorphotic defects leading to a highly mosaic texture with small grain sizes. However, on cooling from the isotropic liquid, the

  0947-6539/02/0803-0588 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 3

material produced a fanlike defect texture that resulted from the formation of a columnar phase (Figure 5a).[7b, 9a–f] At room temperature, X-ray scattering gave six reflections with the spacing ratios $\sqrt{6}:\sqrt{8}:\sqrt{14}:\sqrt{16}:\sqrt{20}:\sqrt{22}$. This corresponds to the reflections $hkl = 211$, 220, 321, 400, 420, 332 of a cubic phase, space group no. 230 (Q230) with a lattice dimension of 73.8 Å. The cubic phase Q230 has been often observed in lipid systems; it consists of two three-dimensional networks of one polarity separated by a continuous medium of opposite polarity. In the wide-angle region, a broad band characteristic of fluid chains was observed at 4.8 $Å^{-1}$, as well as an additional band centred around 8 $Å^{-1}$. A thermotropic and reversible transition took place at 70 °C. The corresponding XRS displayed only one slightly broad scattering reflection at 30 $Å^{-1}$. No detectable band was present in the WAXS.

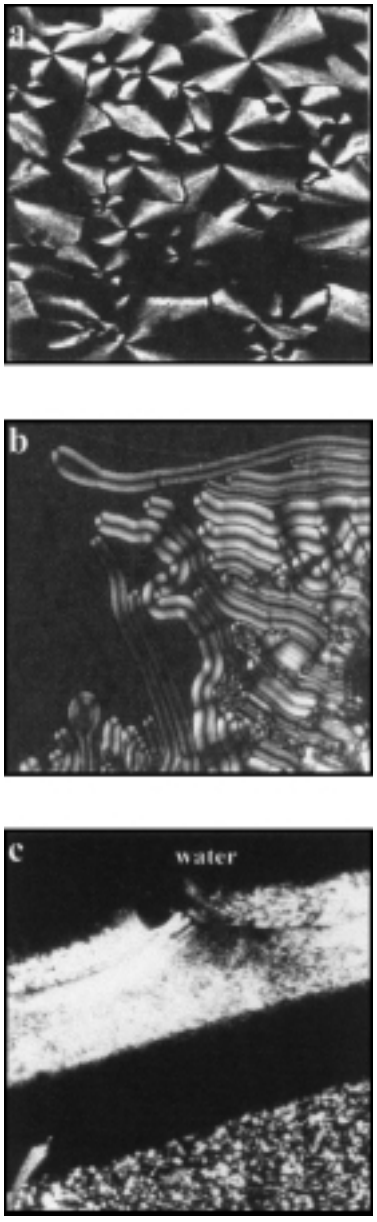


Figure 5. Optical textures of bis-glycosylated compounds **5** and **6** (crossed polarizers). a) Fan defect texture associated with the thermotropic columnar mesophase (enlargement 100×). b) Myelins formed after addition of water (enlargement 200×). c) Contact obtained between compound **5** and water (enlargement 100×); top: birefringent $L\alpha$ mesophase; middle: isotropic cubic-type mesophase; bottom: thermotropic columnar mesophase.

In the presence of water and at room temperature, three SAXS reflections were observed ($h = 1, 2, 3$) corresponding to a lamellar $L\alpha$ phase. However, glycolipid **4** did not take up much water and the lamellar phase coexisted with an excess water; the maximum dimension was 39.8 Å. Consequently, this compound, which possesses two similar monosaccharidic moieties, did not disperse easily in water; the amount of water within the phase was so small that the sample was very viscous, almost solid, and required a spatula to be spread onto the freezing planchett. During this preparation, the water partly evaporated, and some rarely observed vesicles and lamellae were identified by FFEM (not shown).

The physicochemical behaviour of the unsymmetrical bis-glycosylated lipids **5** and **6** was examined; these lipids contain polar sugar head-groups of different sizes, namely lactosyl and galactofuranosyl units, along with different lipophilic linkers. In the absence of water, tetraether **5**, which contains a hexadecamethylene spacer, appeared to be optically isotropic. On cooling the sample from its clearing point, the material gave a fanlike defect texture associated with a disordered hexagonal columnar phase (Figure 5a).[9a–f] When water was added to this hexagonal mesophase, two lyotropic mesophases were formed: an isotropic phase (possibly a cubic phase) at low concentrations of water, and a birefringent lamellar phase at higher concentrations of water (Figure 5c). At the interface between the lamellar phase and water, myelin structures were formed; these are indicative of the possible formation of vesicles in dilute solutions (Figure 5b).

Prior to heating, and in the absence of water, the SAXS spectrum of compound **5** gave six reflections in the spacing ratio $\sqrt{6}:\sqrt{8}:\sqrt{14}:\sqrt{16}:\sqrt{20}:\sqrt{22}$, which corresponds to a cubic phase Q230 with a lattice parameter of 90 Å. An irreversible transition took place on heating to the clearing point: SAXS displayed three reflections ($hk = 10$, 11, 20) which correspond to the formation of a hexagonal phase with a lattice parameter of 44 Å.

After mixing **5** with water and without preheating, the diffraction pattern displayed two SAXS reflections ($h = 1,2$) that correspond to the formation of a lamellar $L\alpha$ phase. The lamellar spacing was found to be 45.8 Å with 20% added water; in the presence 40% added water, the dimension was slightly larger, 47.6 Å. This means that the maximal amount of water within the lamellar phase is much lower than 40%. In the presence of 50% water/glycerol (2:1), the lamellar dimension was slightly larger, namely 50.2 Å. Therefore, tetraether **5** could not be homogeneously dispersed in water, even under dilute conditions. However, by hydrating the lipid film,[10a–b] it was possible to obtain multilamellar vesicles which were cross-fractured (Figure 4c).

The thermotropic behaviour as well as the lyotropic liquid crystal properties in concentrated media of glycolipid **6**, which possesses a cyclopentane unit, were found to be rather similar to those observed for the bis-glycosylated analogue **5**. In the absence of water, the tetraether **6** was found to exhibit an optically isotropic phase. SAXS showed two reflections at $s =$ 25.6, 29.6 $Å^{-1}$ in the spacing ratio $\sqrt{6}:\sqrt{8}$ which probably correspond to the first two reflections of a Q230 cubic

structure with a lattice parameter of 96 Å. In the contact preparation between compound **6** and water, isotropic and birefringent phases were observed. A lamellar $L\alpha$ phase was found to be present and was characterized by the presence of three reflections in SAXS and by a lattice dimension that did not exceed 48.7 Å with less than 40% water added. This parameter was slightly higher (53 Å) in the presence of the water/glycerol (2:1) system. In dilute conditions, FFEM revealed a rather complex behaviour: multilayer onionlike vesicles, dispersed multilamellar or bilamellar vesicles and also highly curved unilamellae were observed (Figure 6a,c). No cross-fractures along the mid-planes of the membranes were found, and only fractures across the membranes were observed. A particularly striking feature appeared in the vesicle organization displaying a two-by-two membrane association. We checked that in the absence of glycerol, which was used as a cryoprotectant, the same association of two layers could be observed. Furthermore, many curved structures seemed to stick and fuse together. Consequently, the introduction of a cyclopentane unit into the hydrocarbon chain appears to play an important role in the organization of the molecules within the membranes.

Discussion

The main objective of this study was to relate the molecular architectures of the various archaeal hemi-macrocyclic analogues to their influence on the nature and the properties of their self-organised supramolecular assemblies. All of the compounds investigated, except for the monosaccharidic derivative **3**, formed lyotropic lamellar phases. Tetraethers **1** and **2**, which contain one highly hydrophilic anionic phosphate group, were able to spontaneously give rise to swelled multilamellar and unilamellar vesicles in dilute systems. Most likely, the phosphate groups are distributed on both sides of the membrane. It is noteworthy that the presence of the cyclopentane ring within the bridging chain did not modify the behaviour in dilute conditions. FFEM did not reveal the presence of convex and concave surfaces characteristic of bilayered vesicular aggregates. In all cases, the vesicles were cross-fractured. Therefore, the lipid molecules span the membrane and consequently they prevent the fracture from propagating along the mid-plane of the monolayer.

Self-organizing properties of neutral glycolipids **3**–**6** were found to depend on both the polar and the hydrophobic parts of the molecules. Compound **3** behaved as an oil; the two glycosylated and unsubstituted glycerol head-groups are not able to segregate from the hydrocarbon region. Such a situation has been already observed for archaeal tetraether lipids above the transition temperature (*T*c) of the hydrocarbon chain.[5] Glycerol dialkyl-glycerol tetraether (GDGT), which contains two unsubstituted glycerol units, can form a lamellar phase below its *T*c; however, it behaves as an oil above *T*c. In glycerol dialkyl-nonitol tetraether (GDNT), which contains one nonitol and one glycerol unit, and in other tetraether lipids containing one polar head-group and one unsubstituted glycerol, the glycerol end tends to segregate from the polar interface and to mix within the disordered hydrocarbon chains.[5a, b] Here, we were dealing with lipids with liquid chains, as shown by WAXS. Because of the weak hydrophilicity of the galactofuranosyl moiety, the presence of such a sugar residue at both terminal ends was required to bring amphiphilic character to the molecule. However, compound **4** could not be dispersed in aqueous medium. The replacement of one galactofuranosyl residue by one lactosyl unit in **5** and **6** provided the molecules with a higher affinity to water and allowed the formation of multilamellar vesicles. Glycolipid **5** exhibited multilamellar onions with closely packed lamellae which resulted from the limited amount of water it could incorporate, as shown by SAXS. The presence of a cyclopentane unit

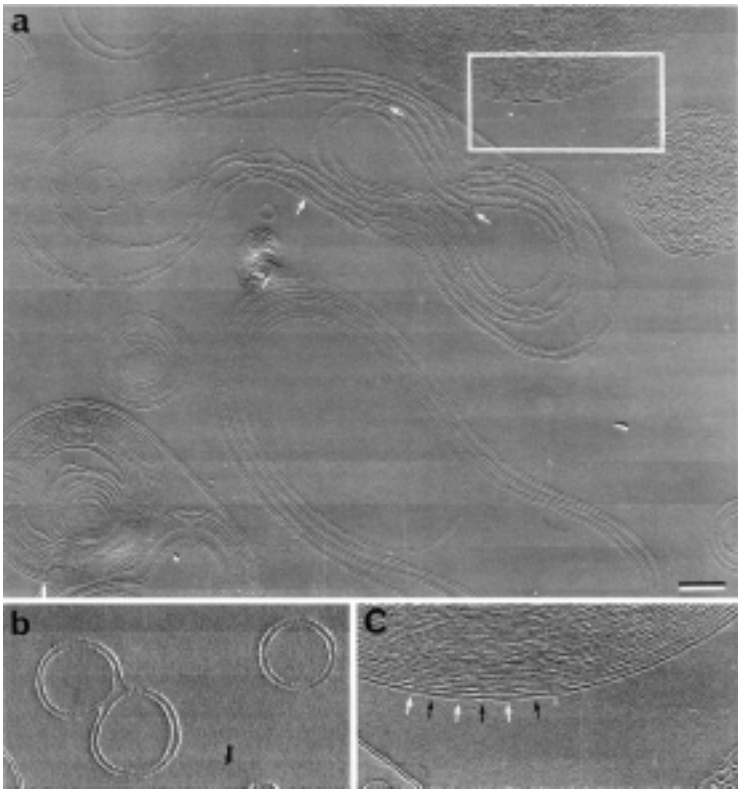


Figure 6. FFEM of compound **6**. Vesicles and lamellae were cross-fractured. For a better visualization, the fractured samples were etched prior to shadowing. Remarkable and unusual features were observed: association of two membranes, highly curved membranes and fused membranes (see arrows). In b, vesicles with two membrane layers; on the left, the morphology seems to result from a fusion process ($M = 48000$). In c ($M = 45000$), enlargement of the selected area in a. The size of the bar is 500 nm.

0947-6539/02/0803-0590 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 3

in the hydrocarbon chain of **6** revealed some significant differences in dilute conditions, although SAXS provided the same type of results as for compound **5**. Here again, the vesicles and lamellae were cross-fractured. The origin of the unusual association of two layers as well the presence of highly curved lamellae and fused lamellae, as shown by FFEM, should result from the presence of both *cis* and *trans* isomers of the 1,3-disubstituted cyclopentane. According to molecular modeling,[11] the cyclopentane unit may play a role in the orientation of the polar moiety of GDNT, a neutral tetraether lipid molecule derived from extremely thermoacidophilic lipids. Consequently, one might expect distinct interfacial properties, depending on the stereochemistry of the 1,3-disubstituted cyclopentane. The unusual association of two layers for compound **6** as well as the presence of highly curved lamellae may be the consequence of several features: 1) a dissymetry of the membrane characterized by two distinct polar interfaces, 2) a non-homogenous distribution of the two *cis* and *trans* isomers, either within the same type or in different types of supramolecular assemblies (vesicles and/or highly curved lamellae) and 3) a possible folding of the non-symmetrical membrane with distinct polar interfaces, and some sticking and fusing of membrane domains.

Conclusion

The results obtained from this study clearly demonstrate that both head-groups and chain composition of tetraether-type lipids influence their ability to form trans-membrane systems. The introduction of one highly hydrophilic moiety, such as an anionic phosphate group, into the second terminal side of a monoglycosidic lipid leads to the spontaneous formation of stable monolayer vesicles. Conversely, the self-organization of less polar bis-glycosylated compounds was found to depend more strongly on the lipophilic core of the molecules and particularly the nature of the linker. Thus, we have observed, for the first time to our knowledge, that the presence of a cyclopentane ring in the middle of the bridging chain may affect both the arrangement of the unsymmetrical bis-glycosidic lipids in the membranes as well as the dissymmetric distribution of the polar heads on each side of the monolayers. Consequently, studies that are now in progress are aimed at examining more precisely, from additional synthetic analogues, the influence of the stereochemistry, the position and the number of 1,3-disubstituted cyclopentane on supramolecular properties.

Experimental Section

Materials: ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively. Fast-atom bombardment (FAB) mass spectra were acquired on a MS/MS ZabSpec TOF Micromass spectrometer with *m*-nitrobenzylic alcohol as the matrix. Merck 60 H (5–40 μm) silica gel was used for column chromatography. Analytical TLC was performed on Merck 60 F_{254} silica-gel non-activated plates. A solution of 5% H_2SO_4 in EtOH was used to develop the plates. Pent-4-enyl-2,3,5,6-tetra-*O*-α,β-D-galactofuranoside (**9**), diol **7**, monoglycosylated compound **13** and lipids **1**, **4** and **5** were prepared as previously described.[7c] Chemicals were purchased from Acros or Fluka Chemika Co. Solvents were of reagent grade and were distilled under N_2 before use: THF from sodium/benzophenone, CH_2Cl_2 from P_2O_5. Unless otherwise noted, non-aqueous reactions were carried out under a nitrogen atmosphere.

Methods: Phase identifications by optical microscopy were carried out with either a Zeiss Universal or a Leika DMLS polarizing transmitted light microscope equipped with a Mettler FP82 microfurnace in conjunction with a FP80 Central Processor. Photomicrographs were obtained from the Leika microscope equipped with a digital SSc-DC38P camera. Typically, investigations into the lyotropic phases were carried out by simply running a small amount of water onto a dry sample sandwiched between a cover slip and a slide. X-ray scattering experiments were performed with a focusing Guinier temperature-controlled camera with monochromatic $Cu_{K\alpha 1}$ radiation ($\lambda = 1.54$ Å) and linear collimation. The samples, prepared by mixing controlled amounts of lipid and water, were placed between two mica windows in vacuum-tight cells. Positions in reciprocal space are specified by the parameter $s = 2\sin\theta\lambda^{-1}$ Å^{-1}, where 2θ is the scattering angle and λ is the wavelength. In the small-angle scattering (SAXS) region, the position of the scattering reflections allows the determination of the type of organization at large distances. The wide-angle (WAXS) region provides information at small distances and particularly on the chain state. For freeze-fracture electron microscopy, a small drop of the preparation, containing glycerol as a cryoprotectant (30/70, glycerol/water), was deposited on a thin copper planchett, which was then rapidly frozen in liquid propane. The samples were frozen from various temperatures, according to specific requirements. Freeze fracture was performed with a Balzers 301 freeze etch unit. The samples were fractured at $-125\,^\circ$C in a vacuum of $< 10^{-6}$ Torr and subsequently shadowed with PtC; when necessary the samples were heated to $-105\,^\circ$C, etched for 2 min and cooled to $-125\,^\circ$C prior to shadowing. The replicas were washed with a sodium dodecyl sulfate (SDS) solution, rinsed with water and examined in a Philips 410 electron microscope. Particle size distributions were measured with a 3000 Zetasizer Malvern Instrument. Tensiometry measurements were performed by means of the ring method with a tensiometer Kruss K10T.

3,3′-*O*-[1,18-Octadecan-(8,11-methylidene)methylene]-2,2′-di-*O*-[(*R*)-3,7-dimethyloctyl]-1′-*O*-benzyl-*sn*-diglycerol (8): Ag_2O (539 mg, 2.33 mmol) and benzyl bromide (237 μL, 2.02 mmol) was added to a stirred solution of diol **7** (1.13 g, 1.55 mmol) in CH_2Cl_2 (30 mL). The reaction was refluxed for 7 h in the dark and filtered through a silica gel pad. Evaporation of the solvent followed by flash chromatography (eluted with a mixture of petroleum ether and EtOAc, the volume ratio was changed from 9:1 to 3:1) gave the monobenzylated product **8** as a colorless oil (760 mg, 50%), in addition to the starting material **7** (43%) and the diprotected diol (6%). $R_f = 0.54$ (petroleum ether/EtOAc 4:1); $[\alpha]_D^{20} = +7.9$ ($c = 1.04$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.61$ (m, 1H; cyclopentyl ring C*H*H), 0.79–0.90 (m, 18H; 6CH_3), 1.01–1.41 and 1.45–1.92 (2m, 51H; 12CH_2, cyclopentyl ring 2CH, 2CH_2, 1CH*H*, 2(CH_2CH(CH_2)$_3$CH)), 2.19 (m, 1H; OH), 3.38–3.76 (m, 18H; CH_2OH, CH_2OBn, 2CH_2OCH_2, 2C*H*OCH_2, 4OCH_2), 4.54 (s, 2H; CH_2Ph), 7.22–7.35 (m, 5H; Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 19.73$, 19.74, 22.68, 22.79, 22.80 (6CH_3), 24.74, 26.18, 26.21, 28.03, 28.75, 28.77, 29.58, 29.62, 29.70, 29.74, 29.85, 29.88, 29.95, 29.98, 31.70, 33.10, 36.77, 36.78, 36.85, 37.17, 37.19, 37.43, 37.46, 39.35, 39.38 (12CH_2, 1 cyclopentyl ring CH_2, 2CH_2CH(CH_2)$_3$CH), 38.84 (cyclopentyl ring 2CH, 1CH_2), 40.19 (cyclopentyl ring 2CH), 40.79 (cyclopentyl ring CH_2), 63.18 (CH_2OH), 68.71, 68.95, 70.37, 70.83, 71.02, 71.75, 71.94 (CH_2OBn, 2CH_2OCH_2, 4OCH_2), 73.43 (CH_2Ph), 78.00, 78.34 (2C*H*OCH_2), 127.57, 127.66, 128.38, 138.51 (Ph); elemental analysis calcd (%) for $C_{52}H_{96}O_6$ (817.2): C 76.42, H 11.84; found: C 76.69, H 11.86.

3,3′-*O*-[1,18-Octadecan-(8,11-methylidene)methylene]-2,2′-di-*O*-[(*R*)-3,7-dimethyloctyl]-1-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-*sn*-diglycerol (10): Compound **8** (620 mg, 0.76 mmol) and pent-4-enyl 2,3,5,6-tetra-*O*-acetyl-α,β-D-galactofuranoside **9**[7c] (505 mg, 1.21 mmol) were combined, rotoevaporated twice with toluene and then dried for 2 h under vacuum. A solution of this mixture in dry CH_2Cl_2 (30 mL) at RT was treated under nitrogen with *N*-iodosuccinimide (342 mg, 1.52 mmol) followed by dropwise addition of triethysilyl trifluoromethanesulfonate (83 μL, 0.342 mmol). The mixture was stirred until TLC analysis indicated complete disappearance of the starting acceptor **9** (10–15 min). Several drops of triethylamine were added to the reaction mixture until it turned into a yellow solution. The resulting solution was diluted with CH_2Cl_2,

washed successively with 10% aqueous sodium thiosulfate, 0.5% aqueous HCl and brine, dried over $MgSO_4$ and rotoevaporated. The crude product was purified by silica gel chromatography with petroleum ether/EtOAc (4:1) to yield 3,3′-*O*-[1,18-octadecan-(8,11-methylidene)methylene]-2,2′-di-*O*-[(*R*)-3,7-dimethyloctyl]-1-*O*-(2,3,5,6-tetra-*O*-acetyl-*β*-D-galactofuranosyl)-1′-*O*-benzyl-*sn*-diglycerol as a colorless oil (665 mg, 76%). $R_f = 0.54$ (petroleum ether/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.62$ (m, 1H; cyclopentyl ring C*H*H), 0.82–0.90 (m, 18H; 6CH_3), 1.02–1.41 and 1.44–1.93 (2m, 51H; 12CH_2, cyclopentyl ring 2CH, 2CH_2, CH*H*, 2($CH_2CH(CH_2)_3CH$), 2.02–2.14 (4s, 12H; 4CH_3CO), 3.37–3.66 (m, 17H; C*H*HOCH, CH_2OBn, 2CH_2OCH_2, 2C*H*OCH_2, 4OCH_2), 3.75 (dd, J = 4.58, 10.17 Hz, 1H; CH*H*OCH), 4.21 (dd, ^3J(H,H) = 7.63, 12.21 Hz, 1H; Gal*f* H6), 4.25 (dd, ^3J(H,H) = 4.07, 6.10 Hz, 1H; Gal*f* H4), 4.33 (dd, ^3J(H,H) = 4.07 Hz, 1H; Gal*f* H6′), 4.54 (s, 2H; CH_2Ph), 4.98 (dd, ^3J(H,H) = 1.35 Hz, 1H; Gal*f* H3), 5.04–5.09 (m, 2H; Gal*f* H1 and H2), 5.39 (m, 1H; Gal*f* H5), 7.22–7.35 (m, 5H; Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 19.68, 19.73, 19.74, 22.68, 22.78 (6CH_3), 20.75, 20.78, 20.83, 20.90 (4*C*H_3CO), 24.22, 24.73, 25.77, 26.20, 28.02, 28.48, 28.71, 29.19–29.97, 31.68, 32.82, 33.08, 36.77, 36.86, 37.14, 37.17, 37.43, 37.46, 39.36 (12CH_2, 1 cyclopentyl ring CH_2, 2CH_2CH(CH_2)$_3$CH), 38.83 (cyclopentyl ring 2CH, 1CH_2), 40.19 (cyclopentyl ring 2CH), 40.79 (cyclopentyl ring CH_2), 62.81 (Gal*f* C6), 67.51 (*C*H_2CHOCH), 69.02 (Gal*f* C5), 68.93, 69.36, 70.34, 70.49, 70.81, 71.73, 71.85 (*C*H_2OBn, 2*C*H_2OCH_2, 4OCH_2), 73.41 (*C*H_2Ph), 76.65 (Gal*f* C3), 77.66, 77.99 (2*C*HOCH_2), 79.94 (Gal*f* C4), 81.29 (Gal*f* C2), 105.81 (Gal*f* C1), 127.57, 127.65, 128.37, 138.48 (Ph), 169.62, 170.05, 170.13, 170.60 (4C=O); elemental analysis calcd (%) for $C_{66}H_{114}O_{15}$ (1146.6): C 69.07, H 10.01; found: C 69.16, H 9.84.

A solution of this compound (880 mg, 0.76 mmol) in EtOH (20 mL) was stirred in the presence of 10% palladium on activated charcoal (100 mg) and under an atmosphere of hydrogen gas at RT until TLC analysis indicated complete disappearance of the starting material. The catalyst was removed by filtration, and the filtrate was concentrated to dryness under vacuum to give the deprotected monoglycosylated lipid **10** as a colorless oil (783 mg, 95%). $R_f = 0.33$ (petroleum ether/EtOAc 7:3); $[\alpha]_D^{20} = -14.7$ (c = 2.9 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.63$ (m, 1H; cyclopentyl ring C*H*H), 0.82–0.90 (m, 18H; 6CH_3), 1.01–1.41 and 1.44–1.92 (2m, 51H; 12CH_2, cyclopentyl ring 2CH, 2CH_2, CH*H*, 2($CH_2CH(CH_2)_3CH$), 2.02–2.15 (4s, 12H; 4CH_3CO), 3.39–3.67 (m, 17H; C*H*HOCH, CH_2OBn, 2CH_2OCH_2, 2C*H*OCH_2, 4OCH_2), 3.73 (dd, J = 4.56, 10.17 Hz, 1H; CH*H*OCH), 4.22 (dd, ^3J(H,H) = 7.61, 12.21 Hz, 1H; Gal*f* H6), 4.25 (dd, ^3J(H,H) = 4.08, 6.10 Hz, 1H; Gal*f* H4), 4.33 (dd, ^3J(H,H) = 4.07 Hz, 1H; Gal*f* H6′), 4.97 (dd, ^3J(H,H) = 1.39 Hz, 1H; Gal*f* H3), 5.04–5.09 (m, 2H; Gal*f* H1, H2), 5.39 (m, 1H; Gal*f* H5); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 19.62, 19.65, 22.61, 22.71 (6CH_3), 20.68, 20.72, 20.77, 20.84 (4*C*H_3CO), 24.43, 24.66, 26.10, 26.14, 27.95, 28.68, 28.71, 29.50–29.91, 31.61, 33.02, 36.69, 36.77, 37.08, 37.35, 37.36, 39.26, 39.30 (12CH_2, 1 cyclopentyl ring CH_2, 2CH_2CH(CH_2)$_3$CH), 38.76 (cyclopentyl ring 2CH, 1CH_2), 40.11 (cyclopentyl ring 2CH), 40.72 (cyclopentyl ring CH_2), 62.74 (Gal*f* C6), 63.08 (CH_2OH), 67.44 (*C*H_2OCH), 69.28 (Gal*f* C5), 68.63, 68.95, 70.42, 70.93, 71.78, 71.86, (2*C*H_2OCH_2, 4OCH_2), 76.58 (Gal*f* C3), 77.59, 78.27 (2*C*HOCH_2), 79.87 (Gal*f* C4), 81.22 (Gal*f* C2), 105.75 (Gal*f* C1), 169.55, 169.98, 170.07, 170.53 (4C=O); elemental analysis calcd (%) for $C_{59}H_{108}O_{15}$ (1056.6): C 67.01, H 10.29; found: C 67.01, H 10.37.

3,3′-*O*-[1,18-Octadecan-(8,11-methylidene)methylene]-2,2′-di-*O*-[(*R*)-3,7-dimethyloctyl]-1-*O*-(*β*-D-galactofuranosyl)-1′-*O*-(*β*-lactosyl)-*sn*-diglycerol (6): Alcohol **10** (200 mg, 0.19 mmol) and lactosyl thioglycoside **11** (206 mg, 0.306 mmol) were dissolved in CH_2Cl_2 (2 mL) and 4 Å molecular sieves (150 mg) were added. The mixture was treated under nitrogen in the dark with *N*-iodosuccinimide (86 mg, 0.38 mmol) followed by dropwise addition of triethylsilyl trifluoromethanesulfonate (10 μL, 0.038 mmol). The reaction was quenched with a few drops of triethylamine after 5 min at RT. The resulting solution was diluted with CH_2Cl_2, washed successively with 10% aqueous sodium thiosulfate, water and brine, dried over $MgSO_4$ and rotoevaporated. The crude product was purified by silica gel chromatography with petroleum ether/EtOAc (11:9) to give the acetylated diglycosylated lipid as a colorless oil (178 mg, 56%). $R_f = 0.21$ (petroleum ether/EtOAc 1:1). A solution of sodium methoxide in CH_3OH (0.1M, 4 mL) was added to a solution of this compound (178 mg, 0.11 mmol) in CH_3OH (5 mL). The mixture was stirred for 10 h at RT, neutralized with an acidic resin (Amberlite IR 120), filtered and concentrated. The crude product was purified by silica gel chromatography (CH_2Cl_2/CH_3OH 4:1) to yield **6** as a pasty solid (90 mg, 67%). $R_f = 0.33$ (CH_2Cl_2/CH_3OH 4:1); $[\alpha]_D^{20} = -27.9$ (c = 1.2 in CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.59$ (m, 1H; cyclopentyl ring C*H*H), 0.75–0.87 (m, 18H; 6CH_3), 0.98–1.36, 1.40–1.58 and 1.62- 1.90 (3m, 51H; 12CH_2, cyclopentyl ring 2CH, 2CH_2, CH*H*, 2($CH_2CH(CH_2)_3CH$), 3.15–3.26 and 3.30–3.95 (2m, 36H; 4CH_2OCH, 2C*H*OCH_2, 4OCH_2, Gal*f* H2, Gal*f* H3, Gal*f* H4, Gal*f* H5, Gal*f* H6, Lac H2, Lac H3, Lac H4, Lac H4, Lac H5, Lac H6, Lac H2′, Lac H3′, Lac H4′, Lac H5′, Lac H6′), 4.22–4.31 (2d, ^3J (H,H) = 7.6 Hz, 2H; Lac H1, Lac H1′), 4.79 (m, 1H; Gal*f* H1); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 20.21, 20.24, 23.13, 23.23 (6CH_3), 25.86, 27.29, 29.14, 29.77, 29.83, 30.64, 30.78, 30.83, 30.86, 31.07, 32.68, 34.11, 37.91, 37.98, 38.21, 38.44, 40.51 (12CH_2, 1 cyclopentyl ring CH_2, 2CH_2CH(CH_2)$_3$CH), 39.87, 40.04 (cyclopentyl ring 2CH, 1CH_2), 41.39 (cyclopentyl ring 2CH), 41.89 (cyclopentyl ring CH_2), 61.87 (Lac C6), 62.44 (Lac C6′), 64.51 (Gal*f* C6), 68.38 (*C*H_2OCH), 69.58, 69.70 (2*C*H_2OCH_2), 70.23 (*C*H_2OCH, Lac C4′), 71.85, 72.02, 72.56 (4OCH_2), 72.38 (Gal*f* C5), 72.47 (Lac C2′), 74.60, 74.72 (Lac C2, Lac C3′), 76.24 (Lac C5), 76.41 (Lac C3), 77.00 (Lac C5′), 78.86, 79.16 (2*C*HOCH_2), 79.21 (Gal*f* C3), 80.54 (Lac C4), 83.10 (Gal*f* C2), 84.59 (Gal*f* C4), 104.55 (Lac C1′), 105.02 (Lac C1), 109.63 (Gal*f* C1); FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M+Na]^+$: 1236.6245; found: 1236.8254.

3,3′-*O*-[1,18-Octadecan-(8,11-methylidene)methylene]-2,2′-di-*O*-[(*R*)-3,7-dimethyloctyl]-1-*O*-(2,3,5,6-tetra-*O*-acetyl-*β*-D-galactofuranosyl)-1′-*O*-(dibenzylphosphono)-*sn*-diglycerol (12): Dibenzyl diisopropylphosphoramidite (370 μL, 1.11 mmol) and 1*H*-tetrazole (157 mg, 2.21 mmol) were added to a solution of alcohol **10** (780 mg, 0.738 mmol) in CH_2Cl_2 (20 mL). After 1 h under stirring at RT, the mixture was cooled to −40 °C and a solution of 3-chloroperoxybenzoic acid (ready for use, Acros, 70% purity with water, 364 mg, 1.47 mmol) in CH_2Cl_2 (6 mL) was added. The reaction mixture was heated to −10 °C and maintained at this temperature for 20 min. The solution was diluted with CH_2Cl_2, and then washed with 10% aqueous $Na_2S_2O_3$, 5% aqueous $NaHCO_3$, water and brine. The organic layer was dried ($MgSO_4$) and concentrated, and the residue was purified by flash silica gel chromatography (petroleum ether/EtOAc 7:3) to yield compound **12** as a colourless oil (778 mg, 80%). $R_f = 0.67$ (petroleum ether/EtOAc 7:3); $[\alpha]_D^{20} = -12.5$ (c = 1.2 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.62$ (m, 1H; cyclopentyl ring C*H*H), 0.81–0.89 (m, 18H; 6CH_3), 1.01–1.41 and 1.43–1.93 (2m, 51H; 12CH_2, cyclopentyl ring 2CH, 2CH_2, CH*H*, 2($CH_2CH(CH_2)_3CH$), 2.02–2.14 (4s, 12H; 4CH_3CO), 3.36–3.66 (m, 15H; C*H*HOCH, 2CH_2OCH_2, 2C*H*OCH_2, 4OCH_2), 3.75 (dd; J = 4.58, 10.17 Hz, 1H; CH*H*OCH), 3.98–4.16 (m, 2H; CH_2OPO(OBn)$_2$), 4.21 (dd, ^3J(H,H) = 7.12 Hz, ^2J(H,H) = 11.73 Hz, 1H; Gal*f* H6), 4.25 (dd, ^3J(H,H) = 6.10 Hz, 1H; Gal*f* H4), 4.33 (dd, ^3J(H,H) = 4.07 Hz, 1H; Gal*f* H6′), 4.99 (dd, 1H; Gal*f* H3), 5.02–5.10 (m, 6H; Gal*f* H1, Gal*f* H2, 2CH_2Ph), 5.39 (m, 1H; Gal*f* H5), 7.21–7.38 (m, 10H; Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 19.65, 19.66, 22.64, 22.65, 22.75 (6CH_3), 20.72, 20.76, 20.81, 20.88 (4*C*H_3CO), 24.70, 26.13, 26.18, 27.99, 28.70, 28.75, 29.58, 29.60, 29.66, 29.73, 29.77, 29.93, 29.95, 31.64, 36.74, 36.83, 37.05, 37.12, 37.36, 37.40, 39.31, 39.34 (12CH_2, 1 cyclopentyl ring CH_2, 2CH_2CH(CH_2)$_3$CH), 38.79 (cyclopentyl ring 2CH, 1CH_2), 40.15 (cyclopentyl ring 2CH), 40.77 (cyclopentyl ring CH_2), 62.78 (Gal*f* C6), 67.06 (*C*H_2OPO(OBn)$_2$), 67.48 (*C*H_2OCH), 69.20 (Gal*f* C5), 68.98, 69.26, 69.27, 69.32, 69.72, 70.46, 71.81(2CH_2Ph, 2CH_2OCH_2, 4OCH_2), 76.62 (Gal*f* C3), 77.21, 77.63 (2CHOCH_2), 79.92 (Gal*f* C4), 81.25 (Galf C2), 105.78 (Gal*f* C1), 127.57–135.92 (Ph), 169.58, 170.01, 170.09, 170.55 (4C=O); elemental analysis calcd (%) for $C_{73}H_{121}O_{18}P$ (1317.6): C 68.14, H 9.48; found: C 68.39, H 9.62.

3,3′-*O*-[1,18-Octadecan-(8,11-methylidene)methylene]-2,2′-di-*O*-[(R)-3,7-dimethyloctyl]-1-*O*-(*β*-D-galactofuranosyl)-*sn*-diglycerol-1′-*O*-phosphate, sodium salt (2). A 0.1M solution of sodium methoxide in CH_3OH (10 mL) was added dropwise to a solution of compound **12** (750 mg, 0.57 mmol) in CH_3OH (20 mL). The resulting mixture was stirred for 20 min at RT and then treated with a solution of acetic acid diluted in CH_3OH. The solvents were evaporated and the residue was diluted in a mixture of CH_3OH (15 mL) and an acetate buffer (pH 5, 5 mL). The mixture was stirred at RT in the presence of 10% palladium on charcoal (75 mg) under an atmosphere of hydrogen gas for 1 h. The catalyst was removed by filtration and the solvent partially evaporated. Resin Amberlite IR-120 (Na^+ form, 10 mL), pre-washed with water, was added to the mixture and the resulting solution was stirred for 12 h at RT. The residue was filtered, the filtrate was concentrated and the crude product was purified on a Sephadex LH-20

0947-6539/02/0803-0592 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 3

column (CH_2Cl_2/CH_3OH 1:2) to give the phosphate derivative **2** (370 mg, 60%). $R_f = 0.39$ ($CH_2Cl_2/CH_3OH/H_2O$ 7:3:0.5); $[\alpha]_D^{20} = -32.1$ ($c = 1.4$ in CH_3OH); ^1H NMR (400 MHz, [D$_5$]pyridine/D_2O, 25 °C): $\delta = 0.65$ (m, 1H; cyclopentyl ring C*H*H), 0.82–1.01 (m, 18H; 6CH_3), 1.05–1.92 (m, 51H; 12CH_2, cyclopentyl ring 2CH, 2CH_2, CH*H*, 2(CH_2CH(CH_2)$_3$CH), 3.41–4.00 (m, 15H; C*H*HOCH, 2C*H*$_2$OCH$_2$, 2C*H*OCH$_2$, 4OCH$_2$), 4.02–4.10 (m, 1H; CH*H*OCH), 4.15–4.40 (m, 1H; Gal*f* H6), 4.50 (m, 1H; Gal*f* H5), 4.68 (m, 1H; Gal*f* H4), 4.74 (m, 1H; Gal*f* H2), 4.92 (dd, 1H; Gal*f* H3), 5.58 (m, 1H; Gal*f* H1); ^{13}C NMR (100 MHz, CD_3OH, 25 °C): $\delta = 20.18$, 20.20, 23.10, 23.10, 23.19 (6CH_3), 25.88, 28.89, 27.30, 27.32, 29.17, 29.83, 29.84, 30.68, 30.79, 30.83, 30.86, 31.07, 31.08, 32.68, 34.11, 37.92, 37.99, 38.23, 38.27, 38.46, 38.49, 40.53 (12CH_2, 1 cyclopentyl ring CH_2, 2CH_2CH(CH_2)$_3$CH), 40.06 (cyclopentyl ring 2CH, 1CH_2), 41.41 (cyclopentyl ring 2CH), 41.89 (cyclopentyl ring CH_2), 64.54 ($CH_2OPO(ONa)_2$) 65.30 (Gal*f* C6), 68.42 (CH_2OCH), 69.69, 69.73 (2CH_2OCH_2), 71.99, 72.38, 72.54, 72.59 (Gal*f* C5, 4OCH_2), 78.91, 79.18 (2$CHOCH_2$), 79.55 (Gal*f* C3), 83.10 (Gal*f* C2), 84.69 (Gal*f* C4), 109.69 (Gal*f* C1); ^{31}P NMR (162 MHz, CD_3OD, 25 °C): $\delta = -1.54$ (s, 1P); FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M+H]^+$: 1013.6646; found: 1013.6609.

3,3′-*O*-(1,16-Hexadecamethylene)-2,2′-di-*O*-[(*R*)-3,7-dimethyloctyl]-1-*O*-(β-D-galactofuranosyl)-*sn*-diglycerol (3): A 0.1M solution of sodium methoxide in CH_3OH (3 mL) was added to a solution of peracetylated monoglycoside **13**[7c] (335 mg, 0.30 mmol) in CH_3OH (8 mL). The mixture was stirred for 30 min at RT and then neutralized by addition of several drops of dilute acetic acid solution in CH_3OH. The solvents were evaporated and the residue (300 mg) was diluted in EtOH (10 mL). The resulting solution was stirred in the presence of 10% palladium on activated charcoal (50 mg) under an atmosphere of hydrogen gas at RT for 2 h. The catalyst was removed by filtration and the filtrate was concentrated to dryness under vacuum to give the deprotected monoglycosylated lipid **3** as a colourless oil (250 mg, 83%). $R_f = 0.24$ (CH_2Cl_2/CH_3OH 92:8); $[\alpha]_D^{20} = -29.5$ ($c = 1.05$ in CH_3OH); ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.72–0.82$ (m, 18H; 6CH_3), 0.97–1.12, 1.13–1.33 and 1.37–1.55 (3m, 48H; 14CH_2, 2CH_2CH(CH_2)$_3$CH), 3.18–3.22, 3.31–3.69 and 3.81–3.91 (m, 24H, CH_2OCH, CH_2OH, 2$CHOCH_2$, 2CH_2OCH_2, 4OCH_2, Gal*f* H2, Gal*f* H3, Gal*f* H4, Gal*f* H5, Gal*f* H6); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 20.18$, 20.22, 23.09, 23.28 (6CH_3), 27.32, 29.19, 30.62, 30.64, 30.77–30.90, 38.24, 38.27, 38.45, 38.47, 40.54 (14CH_2, 2CH_2CH(CH_2)$_3$CH), 62.77 (CH_2OH), 64.53 (Gal*f* C6), 68.44 (*C*H_2OCH), 69.65, 69.74 (2*C*H_2OCH$_2$), 71.87, 72.00, 72.57, 72.61 (4OCH_2), 72.42 (Gal*f* C5), 78.89, 79.21 (2*C*HOCH$_2$), 80.83 (Gal*f* C3), 83.19 (Gal*f* C2), 84.56 (Gal*f* C4), 109.70 (Gal*f* C1); elemental analysis calcd (%) for $C_{48}H_{96}O_{15}$ (913.3): C 63.13, H 10.59; found: C 63.30, H 10.62.

[1] a) T. Langworthy in *The Bacteria*, *Vol. 8* (Eds.: C. R. Woese, R. S. Wolfe), Academic Press, New York, **1985**, pp. 459–497; b) M. De Rosa, A. Gambarcorta, A. Gliozzi, *Microbiol. Rev.* **1986**, *50*, 70–80; c) G. D. Sprott, *J. Bioenerg. Biomembr.* **1991**, *24*, 555–566.

[2] a) A. Gliozzi, R. Rolandi, M. De Rosa, A. Gambacorta, *J. Membr. Biol.* **1983**, *75*, 45–56; b) I. C. P. Smith in *Biomembranes-Membrane Fluidity*; *Vol. 12* (Eds.: M. Kates, L. A. Manson), Plenum, New York, **1984**, pp. 133–168.

[3] a) M. Tomoaia-Cotisel, E. Chifu, J. Zsako, A. Mocanu, P. J. Quinn, M. Kates, *Chem. Phys. Lipids* **1992**, *63*, 131–138; b) D. Blöcher, L. Six, R. Gutermann, B. Henkel, K. Ring, *Biochim. Biophys. Acta* **1985**, *818*, 333–342.

[4] a) M. De Rosa, E. Esposito, A. Gambacorta, B. Nicolous, J. D. Bu'Lock, *Phytochemistry* **1980**, *19*, 827–831; b) M. Kates in *The Archaebacteria: Biochemistry and Biotechnology* (Eds.: M. J. Danson, D. W. Hough, G. G. Lunt), Portland, London, **1992**, pp. 51–72.

[5] a) A. Gulik, V. Luzzati, M. De Rosa, A. Gambacorta, *J. Mol. Biol.* **1985**, *182*, 131–149; b) A. Gulik, V. Luzzati, M. De Rosa, A. Gambacorta, *J. Mol. Biol.* **1988**, *201*, 429–435; c) M. G. L. Elferink, J. G. de Wit, A. J. M. Driesen, W. N. Konings, *Biochim. Biophys. Acta* **1994**, *1193*, 247–254.

[6] a) J.-M. Kim, D. H. Thompson, *Langmuir* **1992**, *8*, 637–644; b) K. Yamauchi, M. Kinoshita, *Prog. Polym. Sci.* **1993**, *18*, 763–804; c) G. Wang, R. I. Hollingsworth, *J. Org. Chem.* **1999**, *64*, 4140–4147; d) T. Eguchi, K. Arakawa, K. Kakinuma, G. Rapp, S. Ghosh, Y. Nakatani, G. Ourisson, *Chem. Eur. J.* **2000**, *6*, 3351–3358; e) S. Takajo, H. Nagano, O. Dannenmuller, S. Ghosh, A.-M. Albrecht, Y. Nakatani, G. Ourisson, *New J. Chem.* **2001**, *25*, 917–929; f) L. A. Cuccia, F. Morin, A. Beck, N. Hébert, G. Just, R. B. Lennox, *Chem. Eur. J.* **2000**, *6*, 4379–4384

[7] a) G. Lecollinet, R. Auzély-Velty, T. Benvegnu, G. Mackenzie, J. W. Goodby, D. Plusquellec, *Chem. Commun.* **1998**, 1571–1572; b) R. Auzély-Velty, T. Benvegnu, D. Plusquellec, G. Mackenzie, J. A. Haley, J. W. Goodby, *Angew. Chem.* **1998**, *110*, 2665–2668; *Angew. Chem. Int. Ed.* **1998**, *37*, 2511–2515; c) G. Lecollinet, R. Auzély-Velty, M. Danel, T. Benvegnu, G. Mackenzie, J. Goodby, D. Plusquellec, *J. Org. Chem.* **1999**, *64*, 3139–3150.

[8] D. A. Van Dal, J. A. Bouwstra, A. Van Rensen, E. Jeremiasse, T. de Vringer, H. E. Junginger, *J. Colloid Interface Sci.* **1996**, *178*, 263–269.

[9] a) Y. Bouligand, *J. Phys. (Paris)* **1980**, *41*, 1307–1315; b) S. Chandrasekhar, G. S. Ranganath, *Rep. Prog. Phys.* **1990**, *53*, 57–84; c) M. Sugiura, M. Mimoda, T. Fukuda, T. Miyamoto, J. Watanabe, *Liq. Cryst.* **1992**, *12*, 603–611; d) G. Friedel, *Ann. Phys.* **1992**, *18*, 273–472; e) G. W. Gray, J. W. Goodby in *Smectic Liquid Crystals: Textures and Structures*, Leonard Hill, Philadelphia, **1984**; f) P. J. Collins, M. Hird in *Introduction to Liquid Crystals: Chemistry and Physics* (Eds.: G. W. Gray, J. W. Goodby, A. Fukuda), Taylor and Francis, London, **1997**.

[10] a) A. D. Bangham, M. M. Standish, J. C. Watkins, *J. Mol. Biol.* **1965**, *13*, 238–252; b) R. R. C. New in *Liposomes: A Practical Approach*, (Eds.: D. Rickwood, B. M. Hames), IRL, Oxford University Press, New York, **1990**.

[11] J. L. Gabriel, P. L. G. Chong, *Chem. Phys. Lipids* **2000**, *105*, 193–200.

Received: July 9, 2001 [F3400]