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Archaeal Lipids: Innovative Materials for Biotechnological Applications

Thierry Benvegnu,*[a] Loïc Lemiègre,[a] and Sandrine Cammas-Marion[a]

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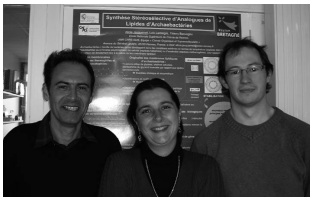
This microreview focuses on the development of diether-type and tetraether-type archaeal lipids as innovative materials for biotechnological applications, with special attention to synthetic analogues as well as to recent natural lipid structures.

1. Introduction

The Archaea domain is made up mainly of extremophile prokaryotic organisms and represents a third evolutionary line distinct from the well known and extensively studied Bacteria and Eukarya domains.[1–3] A variety of phylogenetic arguments has led several authors to suggest that archaebacteria may have played a key role in the early history of life.[4] Some archaea are thought to have survived until today because of their ability to retreat into harsh contemporary environments where otherwise successful competitors cannot follow them. These microorganisms colonize unusual living habitats and grow under extreme conditions including high salinity (halophiles), anaerobic atmosphere (methanogens), temperatures of 60 to 113 °C (thermophiles and hyperthermophiles) or 0 °C (psychrophiles), acidity around pH 0 (acidophiles and thermoacidophiles) or pressures around 400 terrestrial atmosphere. Research programs directed towards identification of the molecular adaptations responsible for their ability to survive and grow in such harsh environments have clearly emphasized the key role of membrane lipid components in overcoming the destabilizing conditions encountered in such extreme environments as hot acidic springs and submarine volcanic fields.[5–7] The archaea membrane core lipids are composed of saturated isoprenoid chains attached to glycerol through ether linkages with *sn*-2 stereochemistry, unlike that of conventional mesophilic lipids.[6] Common structures include the monomeric diphytanyldiglycerol diethers (Figure 1, a) and the dimeric macrocyclic dibiphytanyldiglycerol tetraethers and dibiphytanyl glycerol calditol tetraethers (Figure 1, b–h).

[a] UMR CNRS 6226 "Sciences Chimiques de Rennes", Equipe "Chimie Organique et Supramoléculaire", Ecole Nationale Supérieure de Chimie de Rennes, Université Européenne de Bretagne,
Av. Général Leclerc, 35700 Rennes, France
Fax: +33-2-23-23-80-46
E-mail: Thierry.Benvegnu@ensc-rennes.fr



Thierry Benvegnu (left) was born in Pau (France) in 1966. He graduated in chemistry and chemical engineering from the "Ecole Nationale Supérieure de Chimie de Rennes" (France, 1989) and obtained his Ph.D. at the University of Rennes (1993). After postdoctoral studies with Prof. P. A. Wender at the University of Stanford (USA, 1993–1994), he obtained an academic position as a lecturer at the ENSCR. He was appointed Professor in Organic Chemistry in Rennes (ENSCR, 2004). His research group is involved in the synthesis and the physicochemical evaluation of archaeal membrane lipids as innovative drug/gene delivery systems and in "green" synthesis of biocompatible and biodegradable surfactants from renewable raw materials.

Loïc Lemiègre (right) was born in Evreux (France) in 1976. He obtained his Ph.D. in organic chemistry from The University of Rouen (France, 2002) under the supervision of Dr. Jacques Maddaluno and Pr. Jean-Claude Combret. Then he moved successively to the laboratories of Pr. Eiichi Nakamura at The University of Tokyo (Japan, 2002–2004) and Pr. Jonathan Clayden at The University of Manchester (UK, 2004–2005) for postdoctoral studies. From September 2005, he has held a Maître de conférences (lecturer) position at the ENSCR (France). He is now interested in the synthesis and the self-organization of archaeal lipids and in the valorization of renewable raw materials.

Sandrine Cammas-Marion (middle) was born in Albi (France) in 1967. She graduated in chemistry and chemical engineering from the ENSCR (France, 1989) and obtained her Ph.D. in macromolecular science from the University of Paris VI (1993). After postdoctoral studies with Prof. K. Kataoka at the Science University of Tokyo (Japan, 1993–1995), she obtained an academic position as research assistant professor (Chargée de Recherche CNRS) with Prof. Ph. Guérin at the University of Paris XII (UMR 7581 CNRS, 1995–2000). She then moved to the team of Prof. G. Ponchel at the Faculty of Pharmacy of Châtenay-Malabry (UMR 8612 CNRS, 2000–2003). In July 2005, she joined the group of Prof. T. Benvegnu at the ENSCR, where her research interests are focused on the preparation of new degradable polymers of the poly(malic acid) family and on the study of degradable nanoparticle and archaeosome formulations of therapeutic interest.

The diether structures, termed archaeols, are found in almost all archaea, whereas the tetraether structures, termed caldarchaeols and calditoglycerocaldarchaeols, are found only in methanogenic, thermophilic and psychrophilic archaea.[8] More rarely, an acyclic structure (Figure 1, h) can be isolated from natural membranes, as in *Sulfolobus solfataricus* species.[9] In some thermoacidophilic and methanogenic species, the tetraether structures have been found to be mixtures of regioisomers with antiparallel (Figure 1, b) and parallel (Figure 1, c) arrangements of two glycerol moieties.[10,11] The tetraether backbone is thought to span the membrane from polar head groups on one side to head groups on the other side to form a monolayer membrane organization instead of the standard bilayer model (diether-type structures).[12] Covalent linkage is thus present in the middle of the lipid layer: each bipolar tetraether molecule is completely stretched and spans the entire membrane thickness. Figure 2 shows various forms and combinations that can be encountered in Archaea domain membranes. The left-hand structure has lipids that do not have bridging chains (bilayer model). The centre model shows a membrane in which all the lipids have bridging chains that cross the membrane (i.e., they are transmembrane: monolayer model). The last model structure shows a membrane characterized by a combination of bilayer/monolayer arrangements.

A striking feature of archaeal lipids is the occurrence of unusual carbohydrates, in the form of β-D-galactofuranosyl units.[13,14] The presence of these five-membered rings in such environments remains surprising, as glycofuranosides are much more rapidly hydrolyzed than their pyranosyl counterparts. Another particularly attractive point concerns the increasing proportion of cyclopentane rings (up to four five-membered rings in each of the biphytanyl chains) in thermoacidophilic lipids with increasing environmental temperatures.[15] The addition of cyclic structures in the transmembrane portions of the lipids appears to be a thermoadaptative response, resulting in enhanced membrane packing and reduced membrane fluidity. Surprisingly, the presence of these rings has also been observed in some psychrophilic membrane core lipids.[16] It should be noted that Montenegro et al. have shown by total synthesis and NMR comparison that the stereochemistry of the cyclopentanes in natural archaeal lipids is *trans*.[17]

Variants of the diphytanylglycerol diether and the dibiphytanyldiglycerol tetraether core lipid structures have also been identified in some archaea. The 36-membered macrocyclic diether core lipids, for example, occur in the thermophilic methanogen *Methanococcus jannaschii* (Figure 1, i),[18] whereas *Methanothermus fervidus* species contain tetraether lipids possessing a covalent cross-link at the centre of the isoprenoid chains (Figure 1, j).[19]

The existence of such a large variety of unusual lipid structures in Archaea raises the question of how these lipids function in the membranes of these organisms. Ether linkages are more stable than esters over a wide range of pH, and the branching methyl groups help to reduce both crystallization (membrane lipids in the liquid crystalline state at ambient temperatures) and membrane permeability (steric hindrance of the methyl side groups). The saturated alkyl chains would impart stability towards oxidative degradation, particularly in halophiles that are exposed to air and sunlight. The unnatural stereochemistry of the glycerol backbone would impart resistance to attack by phospholipases released by other organisms and would thus have a survival value for the extreme halophiles.[20]

The membranes of methanogens and thermoacidophiles essentially consist of bipolar monolayer structures. These molecular membrane-spanning components are thought to enhance membrane stability. Additionally, the presence of flexible cyclopentane units is believed to fine-tune the rigidity of the membrane in direct response to the growth temperature of the thermophiles (that is to say, it would keep the fluidity fairly constant as the temperature increases). The high proportions of glycosylated lipids present in membranes of both methanogens and thermoacidophiles may further stabilize their membrane structures through interglycosyl headgroup hydrogen bonding. The presence of large sugar heads towards the convex surface of the membrane may promote an asymmetric orientation, thus making the monolayer organization easier. Upon compression in film balance experiments, the bipolar lipids were shown to be able to adopt U-shaped configurations of the long branching chains tethering the two polar headgroups, at the air/water interface.[21,22] Furthermore, the permeation of small molecules and protons through archaeal bipolar tetraether lipid membranes is considerably reduced as a result of the particular physical structure of the lipid monolayer.[23] Finally, the presence of cyclic diether structures in species isolated from deep-sea hydrothermal vents may be related to the high pressures under which these archaebacteria live. Taken together, the higher rigidity and stability, lower pH sensitivity and higher pressure tolerance make archaeal lipid membranes better suited to extreme environments than the ester type of bilayer lipids found in Bacteria or Eukarya.

In order to study more deeply the relationship between the chemical structures of these unusual lipids and their physicochemical behaviour in membrane assemblies, significant amounts of a large variety of chemically pure lipids from natural sources are necessary. In spite of the recent improvements in the extraction and isolation of high quality archaeal lipids,[24] additional information relating to the functional roles of these lipids remains difficult to assess from natural lipid structures. In this context, the development of novel archaeal lipids – namely through the synthesis of several diether and bipolar tetraether structures that retain some of the essential structural features of archaeal membrane lipids – represents an interesting alternative. Approaches to synthetic acyclic or cyclic diethers and tetraethers related to archaebacterial membranes have been reported by several groups during the two last decades.[25–29] Most synthetic efforts have been directed toward the preparation of archaeal lipid analogues bearing phosphate-type polar heads and linear or branched aliphatic chains ether-linked to glycerol moieties. It seems clear that the rather

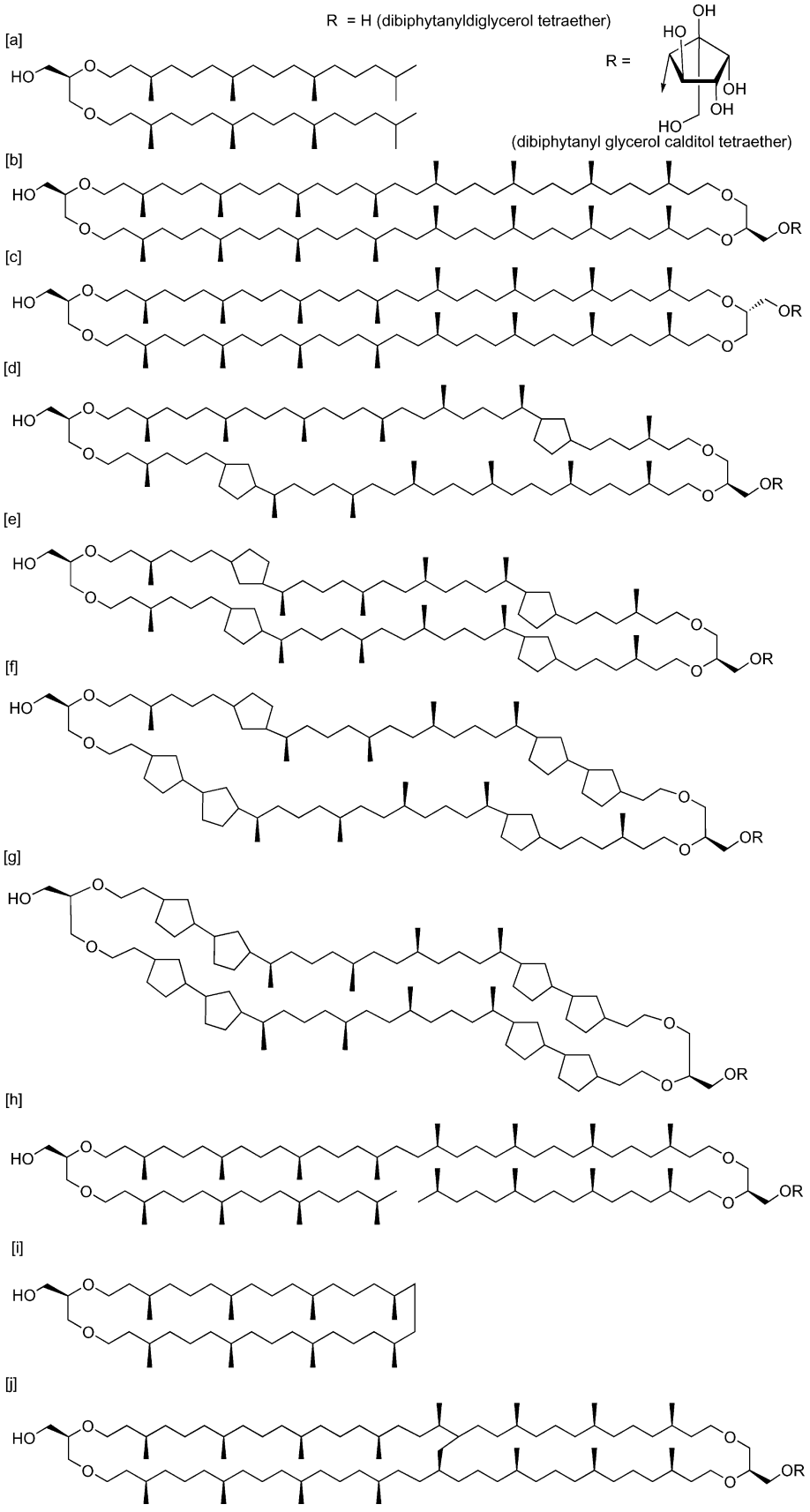


Figure 1. Archaeal lipid architectures: a) diphytanyl glycerol diethers, b–c) dibiphytanyl diglycerol (or glycerol calditol) tetraethers, d–g) internal cyclization in dibiphytanyl diglycerol (or glycerol calditol) tetraethers, h) acyclic diphytanyl diglycerol (or glycerol calditol) tetraethers, i) macrocyclic diphytanyl glycerol diethers, and j) internal covalent cross-linking in dibiphytanyl diglycerol (or glycerol calditol) tetraethers.

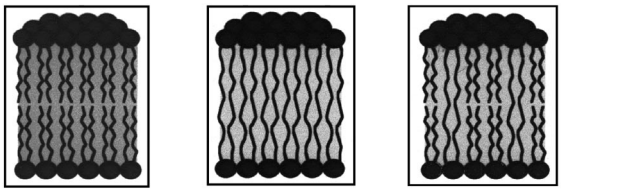


Figure 2. Models of membrane organizations.

intense development of these synthetic lipid analogues has significantly increased our understanding of this new class of amphiphiles in terms of membrane permeability, fluidity and packing properties. These novel molecules were found to represent materials with potential for the construction of stable liposomes and artificial membranes of technological interest.[13] Nevertheless, much remains to be learned of the functions of these atypical lipid structures with respect to the remarkable physical properties of their supramolecular assemblies. To evaluate the impact of additional molecular parameters on the membrane organizations and properties, we launched a program of synthesis and physicochemical evaluations of new diether-type and tetraether-type lipids. In particular, our attention was directed toward the functional roles of: 1) diether and tetraether lipid structures as molecular modulators of membrane fluidity and rigidity, 2) furanosyl units as original carbohydrate polar heads, 3) chirality of the monomers, 4) symmetrical or unsymmetrical structures of the bipolar tetraethers, and 5) cycle-containing bridging chains. Our major goals were both to improve our knowledge of these strange organisms and to propose original biotechnological applications from synthetic lipid analogues. Outlined below is a summary of our efforts in this field, together with a preliminary survey of the recent isolation and identification of novel archaeal membrane lipids from natural sources. In this review article we cover the synthesis and the self-assembling properties of our diether and tetraether lipid structures with a special focus on their potential applications in biotechnology. Additionally, we have selected some recently (2000–2008) published research work by several authors in the area of synthetic archaeolipids

2. Novel Archaeal Ether Polar Lipids

Besides the well known archaeal core membrane lipids shown in Figure 1, a great number of unusual archaeal polar lipids and archaeal neutral lipids have been found in various archaea, mainly as an adaptative response to modification of their living conditions. These recently elucidated structures of new core lipids and polar lipids from identified, unidentified or ancient archaea were reviewed in 2005 by Koga et al.[30] Among the structures described in that review, some are quite original and interesting. For example, macrocyclic isoprenoidal glycerol diethers containing one or two cyclopentane rings in their biphytanyl chain (Figure 3, a) were detected in carbonate crust from the Black Sea.[30,31] Stadnitskaia et al. identified such novel lipids in a new group of archaea able to oxidize methane anaerobically. A macrocyclic archaeal core membrane diether lipid of such a type was first isolated in *Methanococcus jannaschii*, a deep-sea hydrothermal vent methanogen. The work of Stadnitskaia et al. demonstrated for the first time that internal cyclopentane rings can be contained in macrocyclic diethers. However, it is still unclear why such archaea species should biosynthesize internal cyclopentane-containing diphytanyl glycerol diethers instead of internal cyclopentane-containing diphytanyl glycerol tetraethers. The authors hypothesized that this was probably related to an adaptation of physical properties of the membranes of archaea performing anaerobic methanotrophy in cold water environments.

Another interesting lipid has been named crenarchaeol. This cyclohexane-containing lipid (Figure 3, b) was isolated in non-thermophilic group I crenarchaeota, a subgroup of archaea present in seawater and lakes.[30,32–35] This lipid was also shown to be the most abundant lipid in *Crenarchaeum symbiosum*. Studies demonstrated that the internal cyclohexane ring formation was an adaptative response of crenarchaeota to the relative low-temperature environment in which this group has thrived, since this internal cyclohexane moiety is not present in (hyper)thermophilic archaea. Crenarchaeol can be found in oceans, lakes, river waters, soils and peats.

While H-shaped caldarchaeol had already been isolated from *Methanothermus fervidus* and related hyperthermophilic archaeal, hyperthermophilic euryarchaeota growing at neutral pH with optimal temperatures above 80 °C,[30] H-shaped caldarchaeols containing one, two, three or four cyclopentane rings (Figure 3c–f) have recently been found in specimens of "*Aciduliprofundum boonei*", a cultivated thermoacidophilic euryarchaeota from deep-sea hydrothermal vents growing at a lower temperature (70 °C) but under more acidic conditions (pH 4.5).[36] The positions of the covalent bonds in all these lipids have not been yet elucidated. Furthermore, the authors showed that these cyclopentane-containing H-shaped caldarchaeols in the membrane of *Aciduliprofundum boonei* mainly occurred with a phosphoglycerol head group. The functional role of such lipids is still not clear, but the presence of a covalent bond between the alkyl chains might protect the cells against membrane lysis at high temperature by reinforcing the strength of the monolayer membrane.

As described above, archaea are capable of adjusting their core lipid structures in order to be able to survive when their growing conditions are changing. Another important factor is the nature of the polar head groups. Tenchov et al., for example, have shown that membrane stability in hypersaline environments is influenced not only by the structures of the membrane lipids but also by the natures of the polar head groups.[37] These authors demonstrated that archaetidylglycerol methylphosphate (PGP-Me; Figure 3g) was an important archaeal lipid contributing in

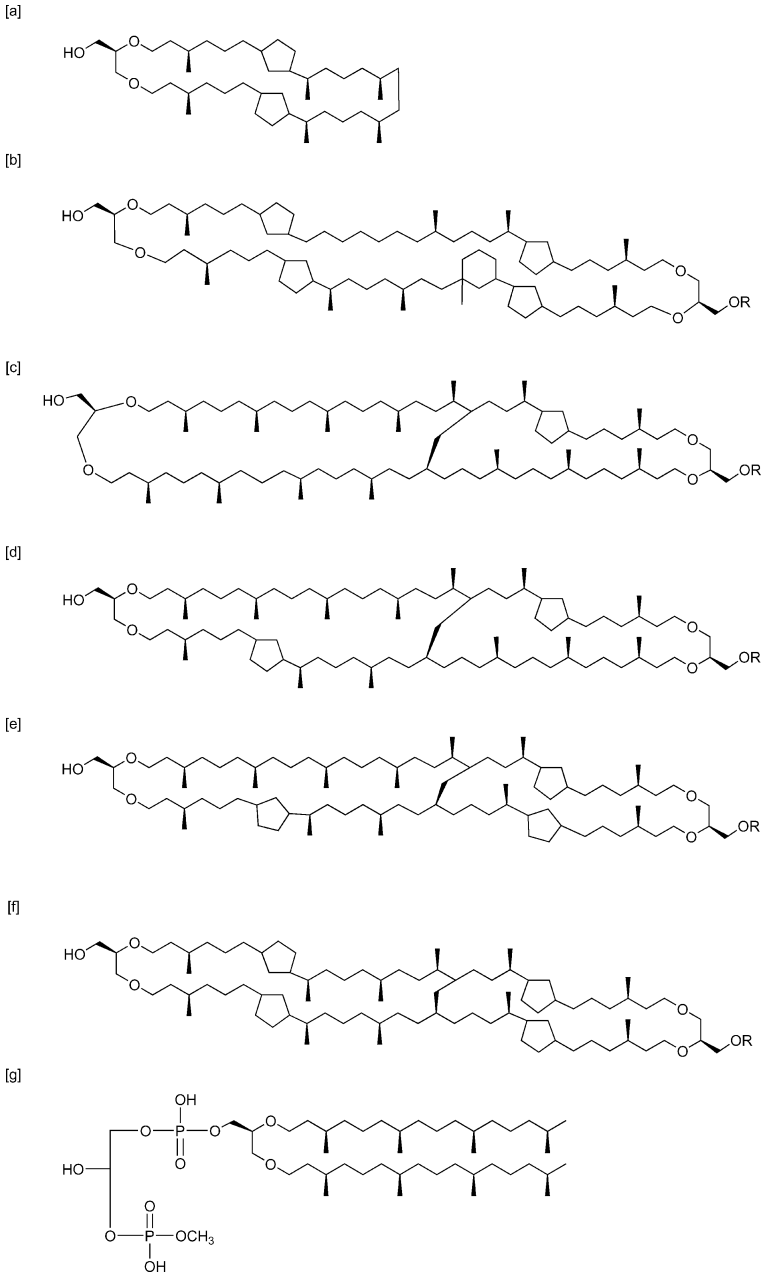


Figure 3. Unusual archaeal lipid structures: a) diphytanyl glycerol diether with two cyclopentane rings, b) crenarchaeol, c) H-shaped caldarchaeol containing one cyclopentane ring, d) H-shaped caldarchaeol containing two cyclopentane rings, e) H-shaped caldarchaeol containing three cyclopentane rings, f) H-shaped caldarchaeol containing four cyclopentane rings, and g) PGP-Me.

an essential way to membrane stability in hypersaline environments. From X-ray diffraction studies, they concluded that the forces preventing membrane aggregation in halophilic archaea appeared to be steric repulsion resulting from the large head group of PGP-Me.

Archaea are organisms that are able to adjust the biosynthesis of their core lipids as a function of their growth conditions. With improvements in analysis techniques, more and more archaeal lipids are being identified and isolated. However, a lot of work still has to be done in order to determine the exact roles played by such unusual lipids in the stabilities of the corresponding archaea.

3. Diether-type Archaeal Lipids: Synthesis and Supramolecular Assemblies

Lipids from methanogenic and halophilic archaebacteria are characterized at the molecular level by high proportions of diether-type components with 2,3-diphytanyl-*sn*-glycerol backbones.[38] Polar groups at the *sn*-1 positions in these lipids include various phosphate derivatives (phosphoserine, phosphoethanolamine and phosphoinositol) and glycosyl units (glucose, galactose).[39] In particular, glycosyl head groups in *Methanospirillum hungatei*, a methanogenic species, consist of repeating moieties in which the first sugar

attached to glycerol is β-D-galactofuranoside (β-Gal*f*) and the second either β-Gal*f* or α-D-glucopyranoside.[40] The presence of β-D-galactofuranose is a striking feature, because in mammalian glycolipids and glycoproteins D-galactose, as well as other hexoses, appears only in its pyranose cyclic form.[41] Some model amphiphiles have been synthesized in order to investigate the abilities of diether-type lipids to provide stable vesicles or planar membranes in water.[42] The synthetic molecules contain either linear alkyl chains or phytanyl residues linked to glycerophosphate derivatives. Upon sonication, these compounds furnish well defined liposomes that are particularly useful for the long-term storage of inorganic salts and for protein stability, both long-term stability and thermostability.[43,44] No study, however, had addressed the precise role or roles of furanosyl sugars in archaeal membranes. In this context, we focused our attention on the synthesis of some synthetic diether-type glycolipids possessing i) monosaccharide or disaccharide polar head groups selected from β-D-galactofuranose, β-D-glucofuranose, and α-D-mannofuranose and β-D-glucuronamide derived from β-D-glucofuranosidurono-6,3-lactone ("D-glucurone"), ii) optically pure (*R* or *S*) or racemic glycerol isomers, and iii) phytanyl, dihydrocitronellyl and/or straight alkyl chains, the phytanyl group being used to mimic the isoprene chains found in the lipids of archaeal membranes (Figure 4).[45–47]

The general synthetic pathways to the diethers involved the preparation of suitable diether-type alcohols from optically pure or racemic glycerol or glycidol, followed by their glycosylation with *n*-pentenyl furanosyl donors[48] derived from D-galactose (D-Gal), D-glucose (D-Glc) or D-mannose (D-Man) or direct reaction with D-glucurone (D-Glur). The key glycofuranosyl donors (Scheme 1, a) were prepared, in one-pot reactions, by ferric chloride-promoted glycosylations of pent-4-en-1-ol with D-glucose, D-mannose or D-galactose, followed by in situ acetylation. Subsequent introduction of the furanosyl units into diethers was achieved by use of standard *n*-pentenyl glycoside (NPG) conditions (NIS, 1.3 equiv., TESOTf, 0.3 equiv. with respect to donors) to afford the resulting glycosides. All reactions resulted in the specific formation of 1,2-*trans*-glycofuranosides, probably due to the C-2 ester neighbouring group participation and the anomeric configurations of the donors (Scheme 1, b). Conventional deacetylation with sodium methoxide resulted in effective deprotection of the hydroxy groups to yield the 1,2-*trans*-furanosides. The synthesis of the (1→6)-β-D-galactofuranoside dimer was achieved from the monogalactofuranoside diether, which was converted into the corresponding tri-*O*-benzyl-β-D-galactofuranoside by the known tritylation/benzylation sequence and was then coupled with a second *n*-pentenyl galactofuranosyl donor (Scheme 1, c).

After removal of the protective groups and purification, the disaccharide reproducing the structures of natural constituents of archaebacterial lipid membranes was isolated.[13,14] The glucurone-derived diether was prepared by treatment of commercially available D-glucurone with the glycerolipid and $BF_3{\cdot}OEt_2$ (2 equiv.) in THF at reflux to

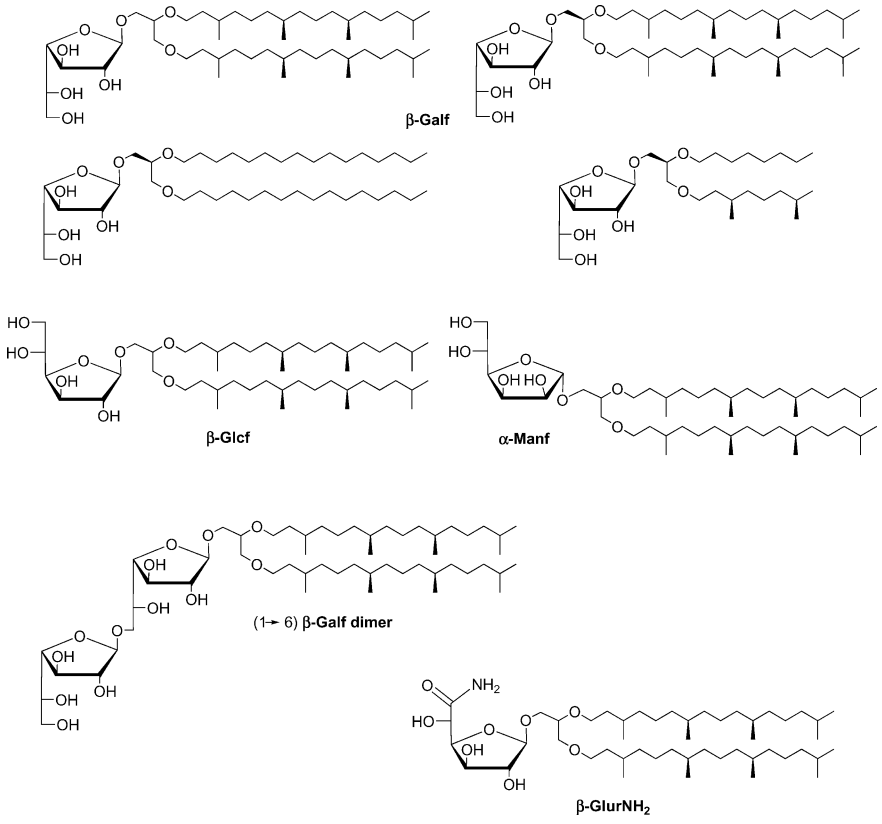
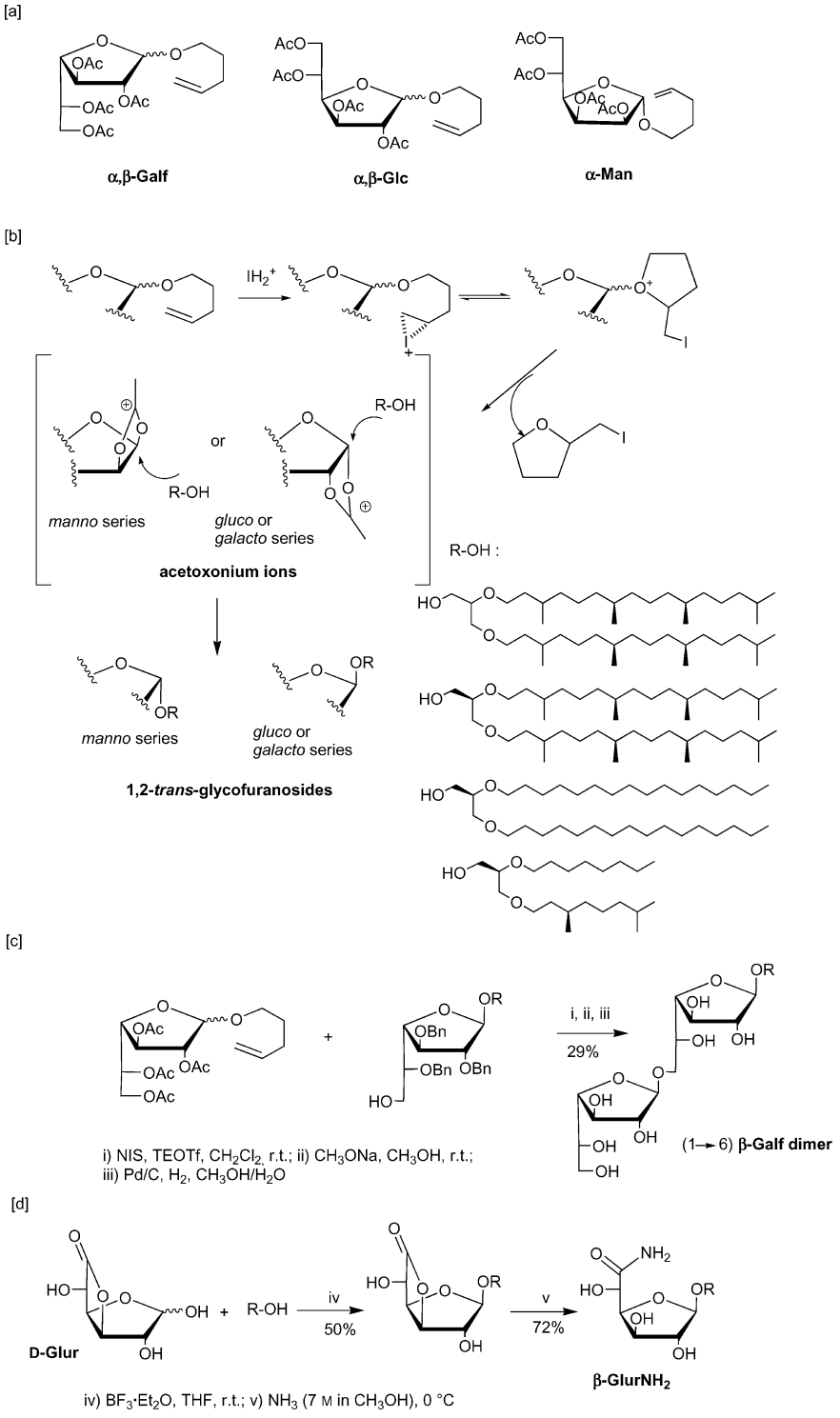


Figure 4. Synthetic diether-type glycofuranosyl lipids.



Scheme 1. Synthesis of glycofuranosyl diethers: a) glycofuranosyl donors, b) mechanistic pathway of the glycosylation reactions, c) synthesis of the (1→6)-β-D-Gal*f* dimer, d) synthesis of the glucuronamide derivative.

provide the expected furanoside lactone with high stereoselectivity (β:α = 9:1). The β-anomer was easily purified by column chromatography on silica gel and transformed into the corresponding amide (**Glur*NH*$_2$**) by lactone opening with ammonia (Scheme 1d).

Differential scanning calorimetry (DSC) measurements and observations by polarized transmitted-light thermal microscopy revealed that all of the Man-, Gal- or Glu-type glycosides possessing two methyl-branched aliphatic moieties exhibited no detectable melting points; instead they all formed glassy phases at very low temperatures. The lack of a melting point transition could be explained by the steric hindrance of the methyl groups to ordered packing of the hydrocarbon residues. These materials are thus in liquid

crystal phases at room temperature and can be cooled down to –50 °C without recrystallization occurring. On cooling, one compound possessing mixed linear and branched chains and with a *sn*-2 stereochemistry for the glycerol unit was found to give a glass transition point below –25 °C. It is interesting to note that only the glycolipid bearing two linear saturated chains exhibited a defined melting point in addition to the clearing point. In the presence of water, the monosaccharides (except for glucuronamide) exhibited hexagonal liquid crystalline mesophases probably resulting from their wedge-shaped structures. Disaccharide and glucuronamide lipids packed in lamellar arrays. These synthetic diethers were then sonicated in distilled water (1–5 mg mL^{-1}) at room temperature for 20 min. The resulting suspensions were centrifuged at 2000 *g* for 10 min to give the supernatants, which were then subjected to transmission electron microscopic observations in the presence of uranyl acetate as a staining agent. The glycolipids were found to self-assemble into various microstructures depending on the volumetric ratio of the hydrophilic to the hydrophobic parts. Compounds containing two identical C_{16} or C_{20} alkyl chains and a monosaccharide Gal, Man or Glu head group exclusively exhibited helical tubule-like structures of various sizes. These aggregates made by these wedge-shaped compounds can be seen as multiple bilayers in the tubular vesicular form.[49,50] Interestingly, the tubules formed appeared to have almost equidistantly separated parallel defect lines running across the long axes of the tubules (Figure 5, a). Lines of this nature are usually associated with chirality, and in particular with helicity, which arises from the way chiral molecules pack together. In the case of lipids possessing comparatively shorter hydrophobic aliphatic chains, myelin-type aggregates, which probably correspond to complex folding of rolled-up multilayers, were produced after sonication (Figure 5, b). In contrast, analysis of dispersions of the diether bearing a more voluminous disaccharidic head showed the presence of highly stable spherical vesicles of 80–500 nm diameters that preserved their morphologies for several months at ambient temperature (Figure 5, c). Bulkiness of the disaccharide polar head provides the phytanyl chains with the degree of freedom apparently necessary for the formation of vesicles. Similarly, sonicated aqueous dispersion of the glucuromamide lipid possessing a highly hydrated amide-type sugar moiety furnished stable vesicles of 200–700 nm diameters.[46]

In order to evaluate the biophysical significance of the macrocyclic ring structures in the deep-sea hydrothermal vent methanogen lipids (believed to decrease the fluidity of branched-chained lipid membranes), studies from synthetic cyclic lipids were envisaged by other research groups.[51] Notably, the 36-membered macrocyclic lipids **36MPC** and **36MGen** (Figure 6) bearing phosphocholine or gentiobiose polar heads were synthesized from the corresponding starting macrocyclic glyceryl alcohol **36MOH** (Scheme 2). More recently, the acyclic version of the gentiobiosyl diether was prepared through an in vitro biosynthetic pathway.[52] Fluorescence anisotropy measurements indicated that the macrocyclic structure produced a decrease in the fluidity

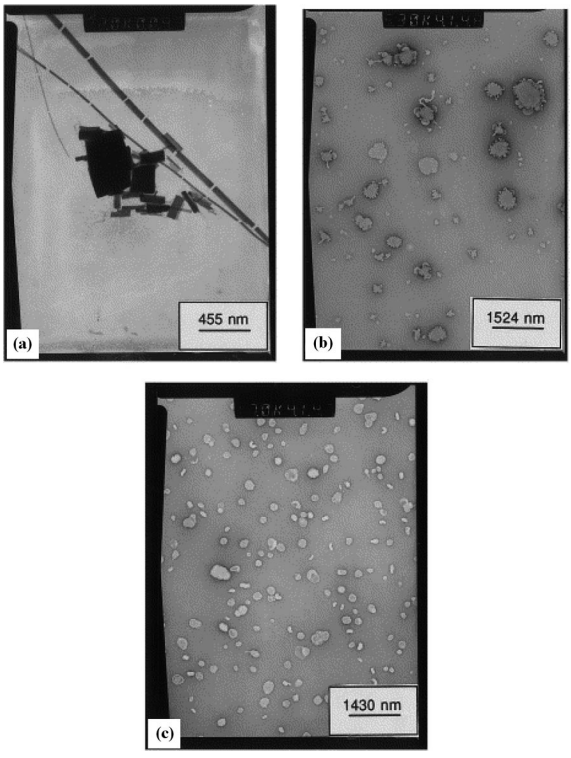


Figure 5. Electron micrographs of aqueous aggregates of glycofuranosyl diethers (stained with uranyl acetate): a) tubule-like assemblies obtained from monoglycofuranosides (except for glucuronamide), b) myelins from galactofuranoside possessing short-branched and linear C_8 alkyl chains, c) vesicles from galactofuranosyl dimer.

in the inner-membrane hydrophobic part more than in the membrane surface by limiting the motional freedom of the alkyl chains. The proton permeability was also significantly reduced through the introduction of a macrocyclic structure. Liposomal thermostability measurements suggested that the cyclic structure contributed to the formation of stable liposomes, especially at higher temperatures and for glycolipid derivatives. These findings clearly demonstrated that the 36-membered macrocyclic lipid membrane played

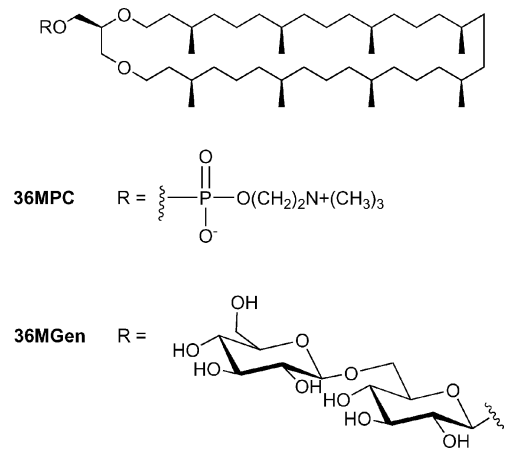
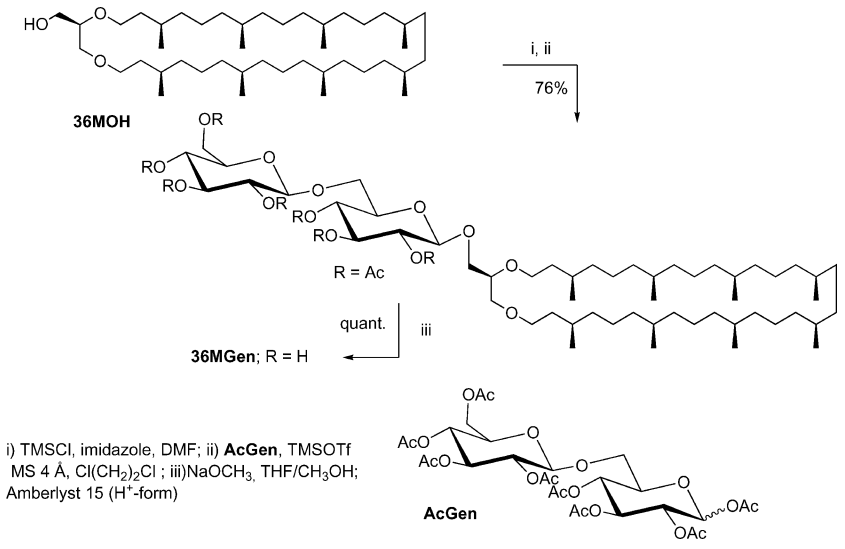


Figure 6. Structures of the synthetic macrocyclic diether phospholipid (**36MPC**) and glycolipid (**36MGen**).

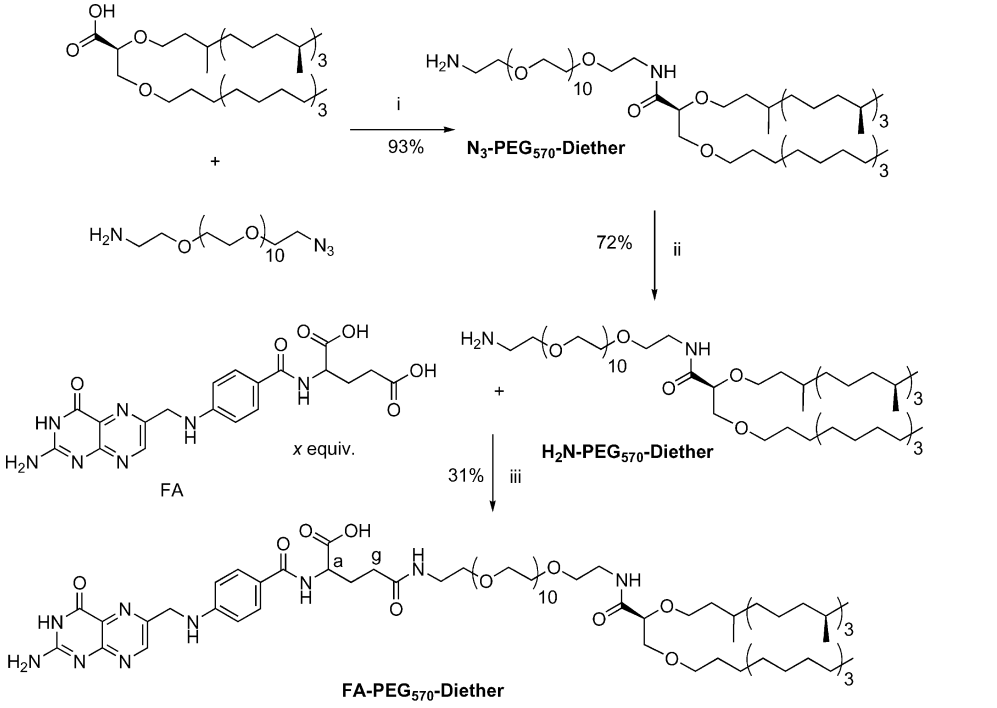


Scheme 2. Synthesis of diether glycolipid **36MGen**.

an important role in the adaption of thermophilic archaea to extreme environments.

In summary, the fluidity of lipid membranes based on diether structures can be readily controlled through the number of phytanyl chains (1 or 2) ether-linked to the glycerol unit, the macrocyclization of the lipid chains and the presence of glycosidic polar heads. In particular, the glycolipids interact intermolecularly with each other through strong hydrogen bonding, resulting in a decrease in the fluidity through the reduction of lateral lipid mobility.

With a view to developing liposomes composed of archaeal lipids as innovative gene/drug delivery systems, we recently envisaged the preparation of diether lipids bearing poly(ethylene glycol) (PEG) polymer moieties and their further functionalization with cell-targeting ligands (Scheme 3).[53] Coating of the liposome with the PEG



x / *y* / *z* = 3 : 3 : 3 **αFA-PEG$_{570}$-Diether / γFA-PEG$_{570}$-Diether / H$_2$N-PEG$_{570}$-Diether** = 40 : 48 : 12

i) TBTU (1.3 equiv.), DIEA (1.2 equiv.), CH_2Cl_2, r.t.; ii) PPh_3, THF/H_2O; iii) TBTU (*y* equiv.), DIEA (*z* equiv.), DMSO, r.t.

Scheme 3. Synthesis of pegylated diether (**H$_2$N-PEG$_{570}$-Diether**) and folate pegylated diether (**FA-PEG$_{570}$-Diether**).

makes it "invisible" to the recognition systems, and considerably increases its stability in the blood circulation system. The repulsive interaction between these PEG-liposomes – called "stealth" liposomes – and other particles, as well as their hydrophilicity, lead to a decrease in the rate of the absorption of the plasma proteins. A diether possessing both branched and linear alkyl chains was efficiently oxidized into the corresponding carboxylic acid by the TEMPO benzoate/$Ca(OCl)_2$ system. Introduction of a 10-unit PEG chain into the lipid structure was performed through a coupling reaction (TBTU, DIEA) between carboxylic acid derivative and commercially available dissymmetrical H_2N-PEG_{570}-N_3 (Scheme 3). After reduction of the azido function, the H_2N-PEGylated diether was isolated, and its functionalization with a folic acid (FA) moiety was successfully achieved under optimized coupling conditions (TBTU/FA/DIEA, 3 equiv.). The PEGylated and FA-PEGylated diethers (**H_2N-PEG_{570}-Diether** and **FA-PEG_{570}-Diether**) were mixed with a cationic lipid and were evaluated for in vitro gene delivery. These co-lipids have significantly increased transfection efficiencies from a level comparable to those seen with standard cationic lipid-based formulations (Lipofectamine®) to a higher level. In particular, folate diether demonstrated that in neutral complexes it could induce folate-/receptor-mediated internalization. This targeting co-lipid therefore permitted neutral lipoplexes to be used while still ensuring high transfection efficiencies and low cytotoxicity impacts.

4. Tetraether-Type Archaeal Lipids: Synthesis, Self-Assembly and Biotechnological Applications

Among the distinctive features of the thermophiles and thermoacidophiles are their unique membrane-spanning bipolar lipids. Indeed, these organisms respond to changes in ambient temperature through adaptations of the cytoplasmic membrane lipid composition, especially through a transition from diether to tetraether lipids. In addition to imparting the necessary mechanical and thermal stability to the membranes of their native organisms, the tetraether lipids are also capable of stabilizing vesicles formed by conventional bilayer-forming phospholipids, which opens up the possibility of biotechnological applications.[13] Several general surveys of the structural features of tetraether lipids have reviewed the properties and potential applications of novel liposomes or synthetic membranes made from natural/synthetic archaeal bipolar lipids.[54,55] During the past two decades, we[56–58] and other groups[27,59–61] have reported syntheses of tetraethers related to archaea membranes. In particular, several series of acyclic molecules in which the alkyl chain lengths ranged from 16 to 32 in carbon number – which represent shorter or similar lipid length than natural membrane components – were described. The goal of this section is to summarize recent research in this field, with the major focus on the four years from the end of 2004, as older work is covered by our previous overview.[26] Synthetic analogues either including an alkyl chain length of less than 30 carbon atoms or possessing a lipid domain comparable with the natural tetraether backbones ($C_{30–33}$ alkyl chains) are reported here.

4.1. Synthetic Tetraethers with Short Alkyl-Chain Structures

In order to study structure/property relationships of bipolar lipids in depth, simplified artificial structures that mimic archaeal membranes have been designed and synthesized. Attention was initially focused on analogous mole-

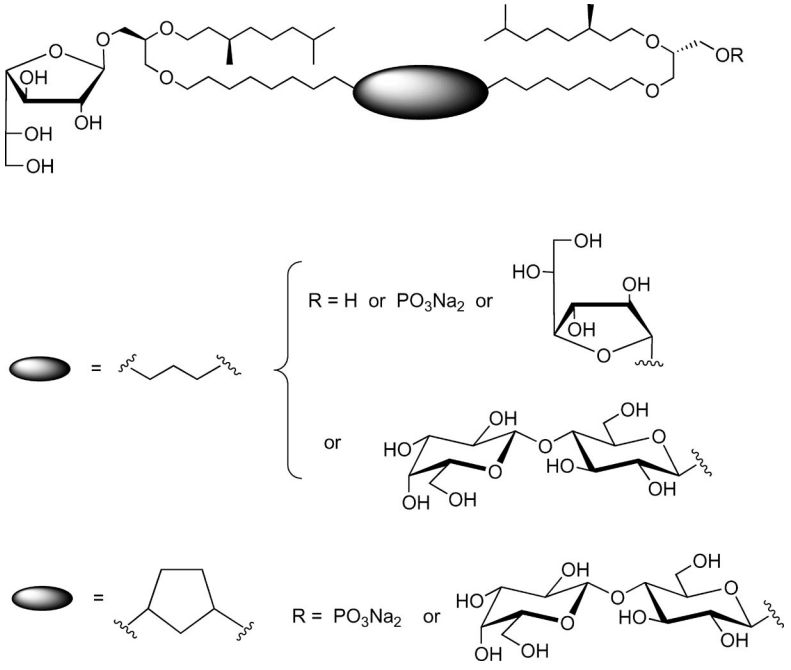


Figure 7. Structures of synthetic acyclic short tetraethers.

cules with $C_{16–28}$ alkyl chains possessing acyclic or macrocyclic structures. In our group, we studied the synthesis and the self-assembling properties of tetraether glycolipid analogues characterized by the presence of: i) either a hexadecamethylene bridging chain or a spacer of similar length containing a 1,3-disubstituted cyclopentane ring ether-linked to two glycerol units at the *sn*-3 and *sn*-3′ positions, ii) two (*R*)-dihydrocitronellyl chains attached to glycerol moieties at the *sn*-2 and *sn*-2′ positions, and iii) phosphate and/or glycosidic polar headgroups derived either from lactose or from D-galactofuranose (Figure 7).[62] An evaluation of the liquid crystalline behaviour showed that these compounds displayed thermotropic columnar phases as well as several lyotropic mesophases, and in particular lamellar structures.[58] In addition, an investigation of their properties in dilute aqueous media clearly demonstrated their ability to form stable transmembrane vesicles. Interestingly, the insertion of a cyclopentane ring into the middle of the bridging chain of the neutral bisglycosylated compound appeared to play an important role in the organization of the bipolar lipids within the membranes of the vesicles. The presence of the cyclopentyl ring was shown to affect both the arrangement of the unsymmetrical lipids in the membranes as well as the dissymmetrical distribution of the polar heads on each side of the monolayers.[58]

Pursuing our efforts in understanding the relationship between 1,3-disubstituted cyclopentane rings and transmembrane organizing properties of the bipolar lipids, we recently prepared additional unsymmetrical tetraether glycolipid analogues in which the cyclopentane unit was introduced with a totally controlled *cis* configuration, either into the middle of the aliphatic chain or at three methylene groups from the glycerol unit linked to the bulkier disaccharide residue (Figure 8).[63]

The construction of the symmetrical bridging chain (**symBC**) was performed through a double Wittig reaction between 6-benzyloxyhexanal and the bisphosphonium salt obtained from cyclopentane-*cis*-1,3-dicarbaldehyde,[57] followed by hydrogenation of the double bonds and removal of the benzyl groups (Scheme 4). Then, alkylation of the (*S*)-glycerol derivative[57] with the ditriflate of the aliphatic chain, subsequent hydrogenolysis of the benzyloxy groups and monobenzylation of the corresponding diol provided the monoprotected *O*-benzylated tetraether.

Our attention was next directed toward the synthesis of the lipophilic backbone possessing an unsymmetrical spacer (**unsymBC**) (Scheme 4). The additional difficulties in preparing such a structure lay in the dissymmetrization of the molecule due to the position of the cyclopentane ring in the spacer, which required the introduction of orthogonal protective groups. Our strategy for the synthesis of the monoprotected bridging chain was based upon two sequential Wittig reactions involving 10-(benzyloxy)decanal and 2-(tetrahydropyranyloxy)-ethanal as the aldehydes and final removal of the tetrahydropyranyl group under acidic conditions. The construction of the monobenzylated hemimacrocyclic structure involved two consecutive *O*-alkylations with triflates formed from glyceryl derivatives possessing different allyloxy or benzyloxy groups at the *sn*-1 position, followed by removal of the allyl group with Wilkinson catalyst. With the two tetraether-type diols to hand, the last crucial step consisted of the introduction of lactosyl and galactofuranosyl polar heads under standard coupling conditions.[57]

The supramolecular assemblies formed by these original amphiphiles with a totally controlled *cis* configuration in the 1,3-disubstituted cyclopentane moiety were then investigated in dilute aqueous media to evaluate the influence of the cyclopentyl ring position on lyotropic properties. With the compound incorporating a cycle into the middle of the main chain, freeze-fracture and cryotransmission electron microscopy experiments demonstrated unprecedented glycolipid supramolecular organizations involving two-by-two monolayer associations coupled with interconnection and fusion phenomena (Figure 9). These unusual bilamellar vesicles might result from unsymmetrical interfacial properties of the lipid layer induced by the presence of the cyclopentane ring. They may be the consequence of asymmetry of the membrane characterized by two distinct polar interfaces.

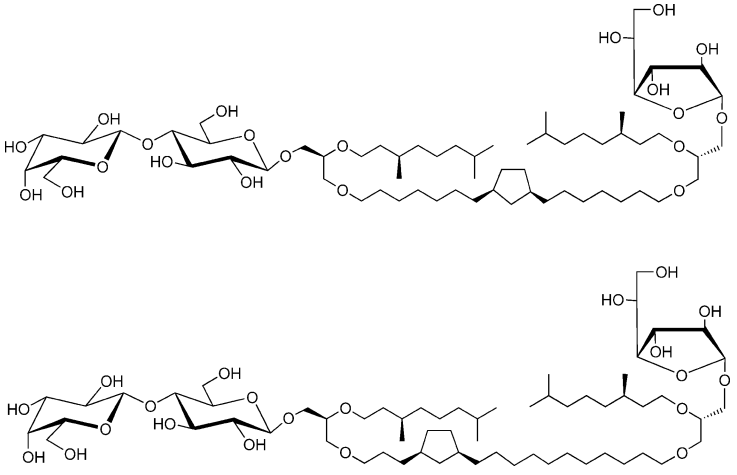
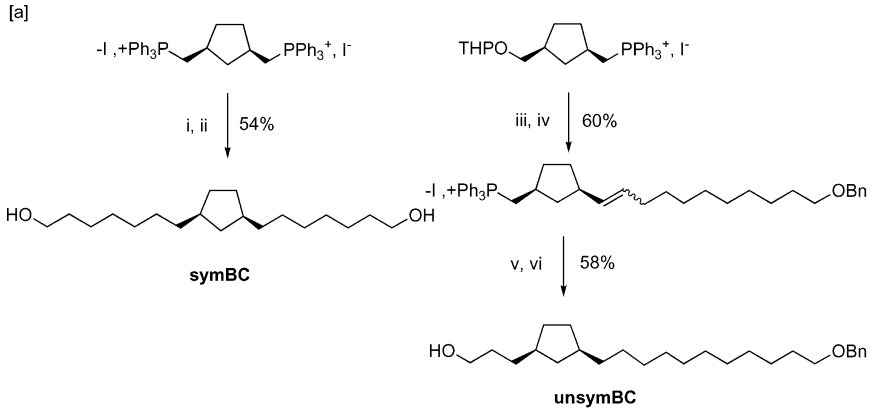
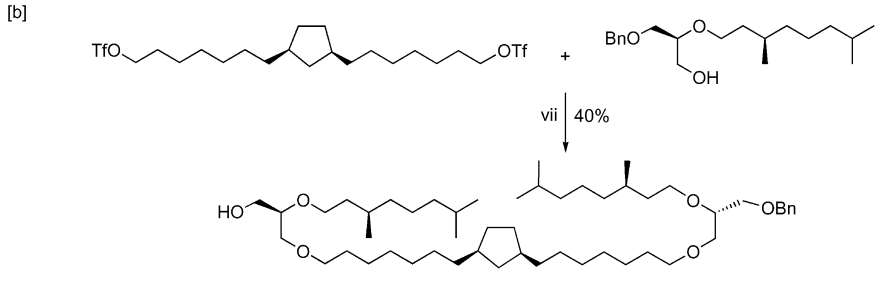


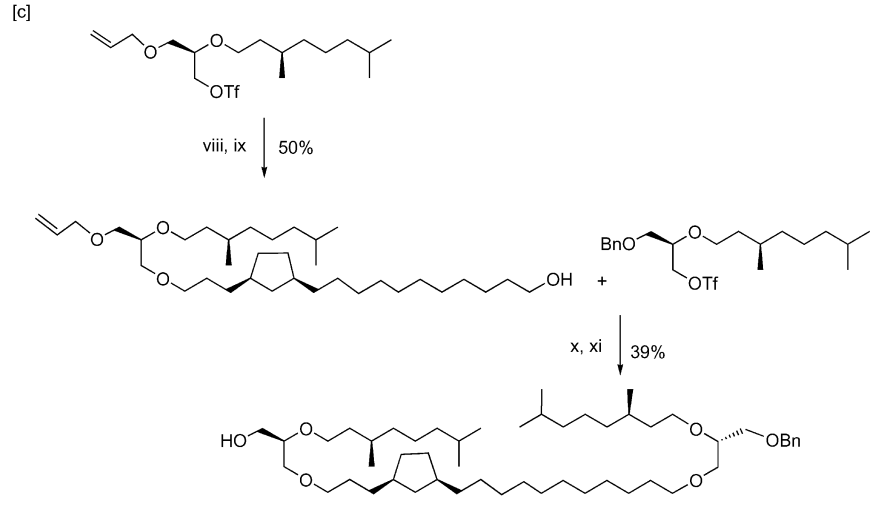
Figure 8. Structures of synthetic *cis*-1,3-disubtituted cyclopentane-containing glycolipid analogues.



i) *n*BuLi, THF, 0 °C, then BnO-$(CH_2)_5$-CHO; ii) H_2, Pd/C (10%), EtOH; iii) (a) *n*BuLi, THF, 0 °C, then BnO-$(CH_2)_9$-CHO. (b) *p*TsA, CH_3OH; iv) (a) I_2, imidazole, PPh_3, CH_2Cl_2. (b) PPh_3, CH_3CN; v) (a) *n*BuLi, THF, 0 °C, then THPO-CH_2-CHO. (b) H_2, Pd/C (10%), Et_3N, AcOEt; vi) *p*TsA, CH_3OH



vii) (a) KH, THF. (b) H_2, Pd/C (10%), AcOEt. (c) NaH, 15-5 crownether, BnBr, THF



vii) **unsymBC**, KH, THF, 0°C; ix) DDQ, CH_2Cl_2/H_2O (18:1 v/v); x) KH, THF, 0°C; xi) $(Ph_3P)_3RhCl$, toluene/EtOH/H_2O (1.25:2.65:0.4 v/v/v)

Scheme 4. Synthesis of the monoprotected *cis*-1,3-disubstituted cyclopentane-containing lipids: a) preparation of the symmetrical (**symBC**) and unsymmetrical (**unsymBC**) bridging chains, b) synthesis of monobenzylated symmetrical lipid, and c) synthesis of monobenzylated unsymmetrical lipid.

The corresponding glycolipid with a cyclopentane unit located at three methylene groups from the glycerol could not be dispersed in water to any great degree and it exhibited multilamellar onion-like vesicles and cubic mesophase in dilute media (Figure 10). As already shown by molecular modelling,[64,65] one may assume that the orientation of the lactose residue was affected by the position of the cyclopentane unit within the bridging chain. The decreased hydration may be associated with a less favourable arrangement of the bulkier polar headgroup relative to the surface membrane for hydration. In order to gain further insights into the structure/property relationships of these lipids,

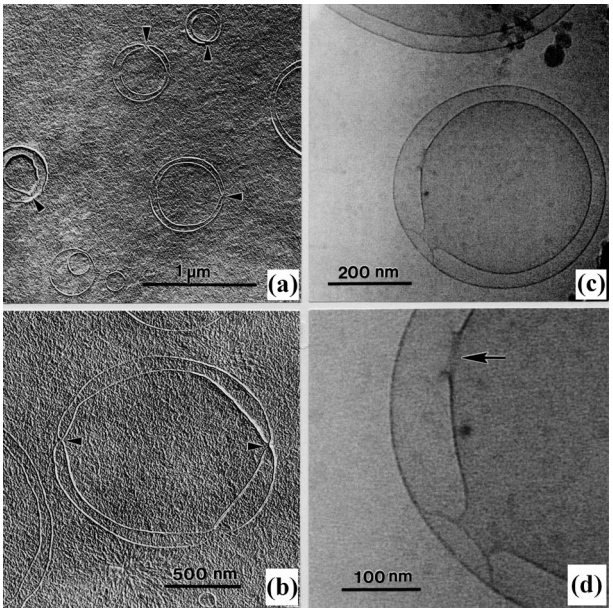


Figure 9. Freeze-fracture electron microscopy [FFEM (a–b)] and Cryo-TEM (c–d) of glycolipid possessing a cyclopentane moiety in the middle of the alipharic chain, after hydration by intensive stirring in excess water (99 wt.-%). a) Vesicles with two-by-two lamellar arrangements showing interconnections (arrowheads) between the two envelopes. b) Vesicle with two-by-two lamellar arrangement showing two tunnel-like interconnections (arrowheads). c) Cryo-TEM of vesicular two-by-two lamellar arrangements. The inside vesicle looks like an invagination of the outer envelope. d) At higher magnification, a second interconnection of the two membranes is clearly visible (arrow).

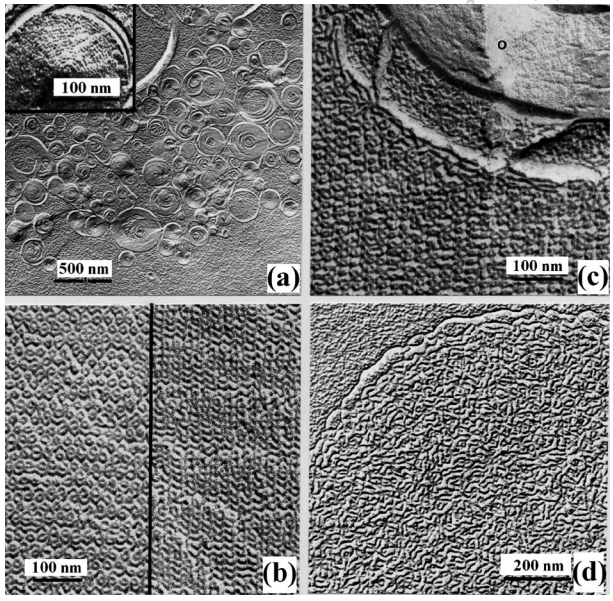


Figure 10. FFEM of glycolipid possessing a cyclopentane unit located at three methylene groups from the glyceryl unit, after hydration by intensive stirring in excess water (a, d) and gentle hydration at 95 wt.-% water content (b–c). a) Multilamellar onion-like vesicles are predominant. At larger magnification (inset), the multilamellar arrangement with a repeat distance of about 5 nm is visible. b) Two projections of a cubic 3D-structure with a repeat distance of the hexagonally arranged elements (tunnels, left part) of about 23 nm. c) Coexistence of a cubic phase structure and a multilamellar vesicle; sponge-like structured particles have not been found. d) Rarely detectable sponge-like structure after ultrasonication in excess water.

studies that are now in progress are directed towards more precise examination, with the aid of additional synthetic analogues, of the influence on the supramolecular assemblies of the stereochemistry and the number of 1,3-disubstitued cyclopentane units.

In parallel, a glycolipid analogue characterized by the presence of an aromatic ring (Figure 11) instead of the cyclopentane unit was synthesized (unpublished results). The presence of a 1,2-disubstituted phenyl ring in the middle of the bridging chain should mimic the constraint of a *cis*-double bond without the problems of the chemical reactivity of a related alkene. The mesomorphic properties of this amphiphile and its supramolecular assemblies formed in dilute aqueous media were also investigated to allow direct comparison with the related cyclopentane-containing bis-glycosylated lipids. On cooling of the isotropic liquid, observation of the glycolipid under a polarized optical microscope (POM) revealed the formation of needles that coalesced to give a texture characteristic of a hexagonal columnar phase (Figure 12).[66–71]

When water was added to the columnar mesophase, two different lyotropic phases were observed between crossed polarizers: an isotropic phase (possibly a cubic phase) and a birefringent texture at higher water concentrations (Figure 12b). The identification of the latter proved to be difficult, but the absence of myelin figures at the interface made the existence of a lamellar phase very unlikely. With 60% added water, six weak and broad small angle X-ray scattering (SAXS) reflections extended from s = 11 to 26×10^{-3} Å^{-1}; they could not be indexed easily to give a uniquely identified phase; however it is thought that they might correspond to a mixture of tridimensional phases. At

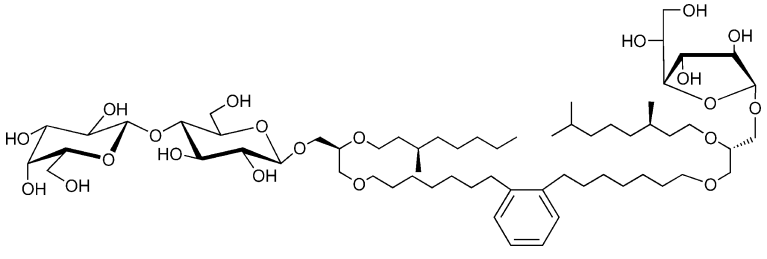


Figure 11. Structure of a glycolipid tetraether incorporating an aromatic ring into the bridging chain.

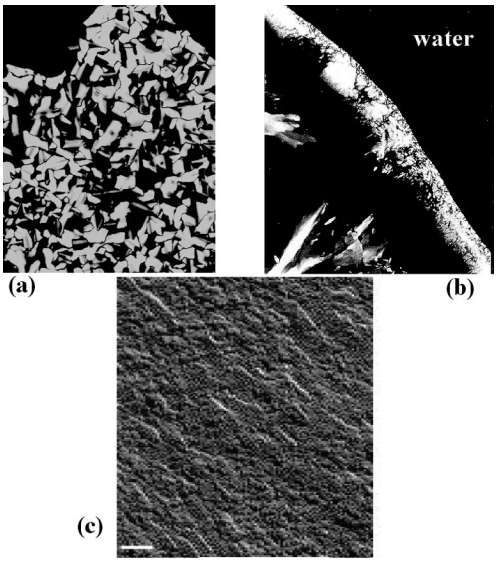


Figure 12. a) Optical fan defect texture associated with the thermotropic hexagonal columnar mesophase (crossed polarizers: enlargement 100×). b) Contact obtained between phenyl-containing glycolipid and water (crossed polarizers: enlargement 100×); top: birefringent mesophase; middle: isotropic cubic-type mesophase; bottom: thermotropic columnar mesophase. c) Freeze fracture electron microscopy; the bar corresponds to 100 nm.

higher water content the sample was isotropic and the SAXS was rather poor. The fractured surfaces obtained with freeze fracture electron microscopy displayed limited 2D-ordered domains (Figure 12, c), that resemble the 2D-ordered fracture planes of cubic phases.[72] Unlike with the analogues possessing a cyclopentane ring, it was not possible to determine clearly the type of supramolecular assemblies in a highly dispersed aqueous medium. The aromatic ring seems to confer specific properties on the novel bisglycosylated lipid, whereas the other bis-glycosylated derivatives were able to form vesicular assemblies. The presence of bulky aromatic rings probably hinders the parallel arrangement of the molecules, hence inhibiting the formation of a lamellar phase.

Very recently, the synthesis of diacetylenic cyclic tetraethers with 24-, 28- and 32-carbon chains with a view to constructing high-ordered thick monolayer membranes was reported (Figure 13).[73] Microscopic observations revealed that these molecules self-assembled into multilamellar vesicles in aqueous solution and into monolayer sheets on solid supports. In particular, fluorescence polarization measurements suggested that the molecular order of the 24-carbon alkyl chain lipid was higher than that of bilayer membranes in the liquid-ordered phase.

4.2. Synthetic Tetraethers with Longer Alkyl Chains (31-, 32- and 33-Atom Chains)

When the targeted applications of synthetic tetraethers involve their incorporation into liposomes to increase membrane rigidity and stability, artificial lipids including a longer acyclic or cyclic framework are required. We have thus developed archaeal lipid analogues based on a quasi-macrocyclic backbone and including cyclopentane rings.[74–76] Two types of structures were synthesized: one based on a fully 33-carbon chain and one on a 31-atom chain with two oxygen atoms (Scheme 5). Their syntheses are based on similar strategies starting from *cis*-cyclopentane-1,3-dimethanol. Wittig olefination and standard benzylation and hydrogenation afforded the C_{33} alkanediol. A simpler strategy based on Williamson ether formation, therefore including the two oxygen atoms originating from the 1,3-cyclopentane dimethanol, provided the C_{31} alkanediols. Conversion of these two C_{33} or C_{31} alkanediols into the corresponding ditriflates, couplings with two equivalents of a phytanyl glycerol derivative (**Phytgly**) and hydrogenolysis provided the two tetraether diols.[75]

These two 31- and 33-atom-chain tetraether lipids were then functionalized to evaluate their biotechnological interest. A large range of polar heads were symmetrically or unsymmetrically introduced onto one or both ends of these tetraether lipids (Figure 14), leading to a wide range of bolaamphiphile tetraethers bearing cationic polar heads (glycine betaine derivatives), neutral polar heads (lactose derivatives) or zwitterionic polar heads (phosphatidyl choline derivatives).

From a physicochemical point of view, we studied the membrane organization of liposomes (archaeosomes) containing solely dicationic tetraether lipid or diPC tetraether lipid, together with a dicationic tetraether lipid/DOPE (dioleylphosphatidylethanolamine, 95:5 w/w) mixture by freeze fracture electron microscopy (FFEM), the samples being etched before shadowing in order better to visualize the fracture propagation path. Interestingly, dispersion of these bipolar lipids at room temperature yielded vesicles that were not fractured along the midplane of the membrane in the usual way (Figure 15). The absence of a fracture midplane clearly indicates that the bipolar lipids span the membrane forming a monolayer as with natural tetraethers (Figure 15, parts a and c–d). The addition of DOPE (5%) yielded a small number of vesicles with midplane-fractured membranes (Figure 15, b). Nevertheless, even in the presence of the bilayer-forming DOPE, the predominant structures observed were small cross-fractured vesicles, due to the membrane-spanning tetraether lipids.

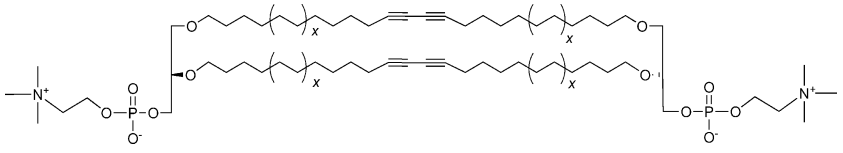
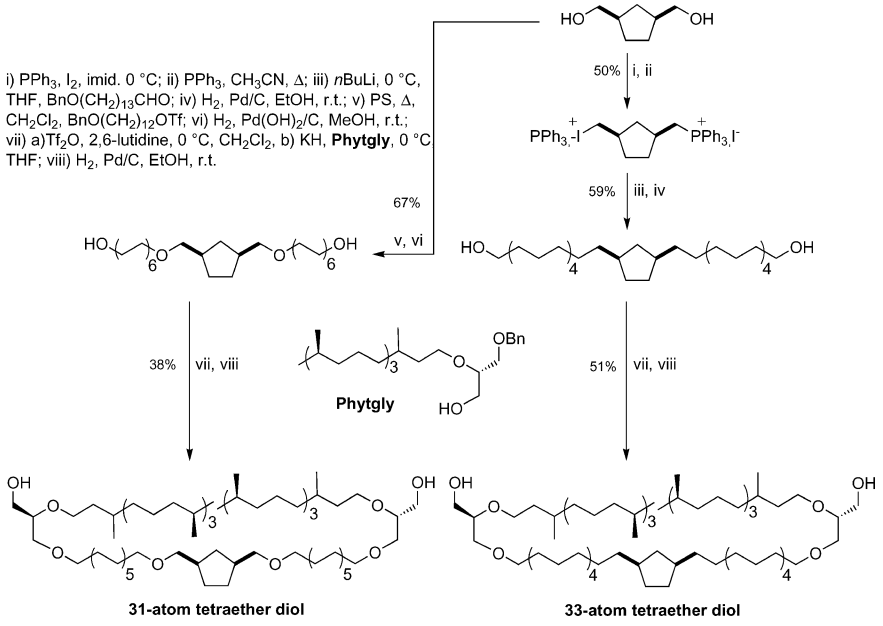


Figure 13. Cyclic lipids with C_{24} (x = 1), C_{28} (x = 2) and C_{32} (x = 3) alkyl chains.



Scheme 5. Synthesis of C_{31} and C_{33} alkyl chain tetraether lipids.

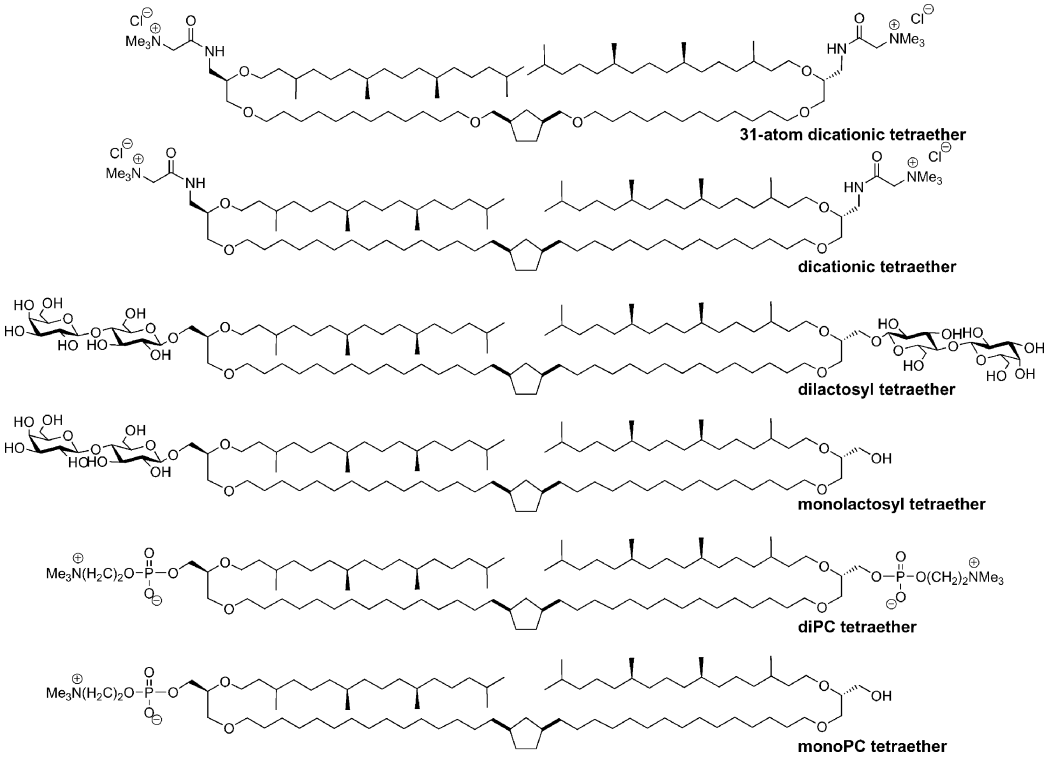


Figure 14. Functionalized tetraether analogues with various polar heads (cationic, neutral, zwitterionic).

The main applications so far envisaged with these synthetic cationic, neutral or zwitterionic tetraethers have been drug and gene delivery. The stabilities of the archaeosomes based on neutral and zwitterionic bolaamphiphiles even permit oral administrations to be envisaged. Indeed, we have evaluated their stabilities under conditions mimicking oral delivery; this study has shown that these synthetic analogues provide stable liposomes over wide temperature ranges, under acidic conditions (pH 2) and/or in the presence of fetal calf serum (FCS) or sodium cholate (reflecting bile salt-containing media).[76] In particular, incorporation of dilactosyl tetraether (30%) into egg PC liposome formulations significantly lowered the leakage of encapsulated 5(6)-carboxyfluorescein (CF) in sodium cholate (0.4%) and serum (FCS) media. Additionally, archaeosomes made exclusively from diPC tetraether have a pH stability that com-

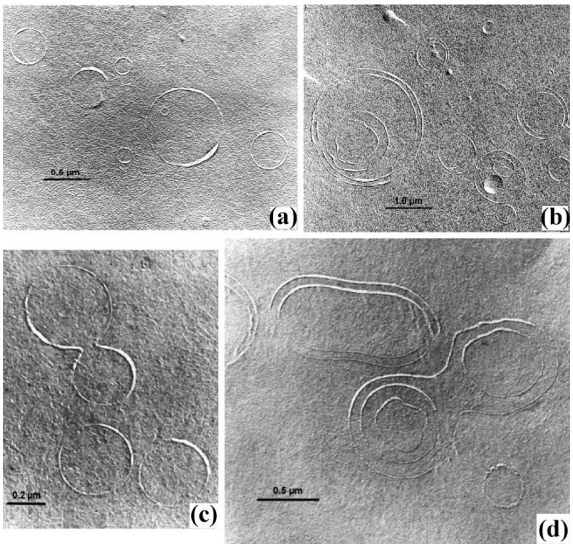
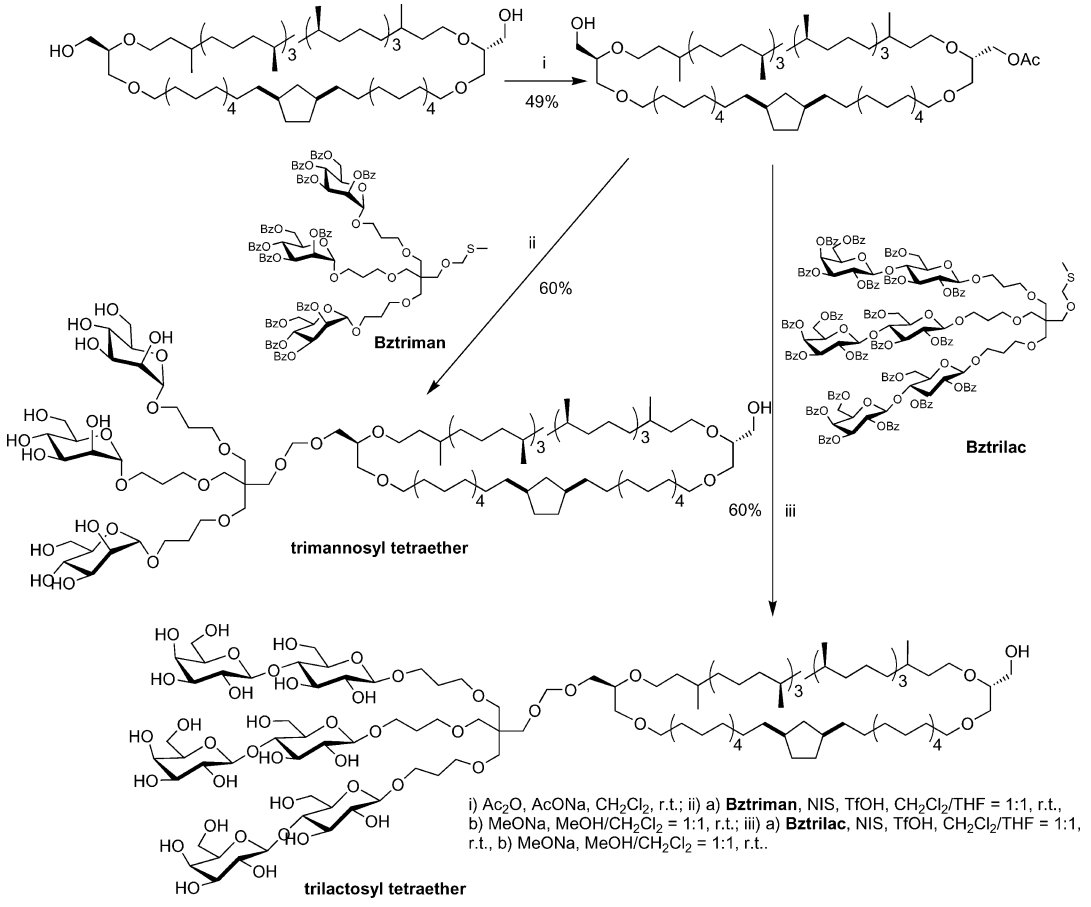


Figure 15. Freeze-fracture electron micrographs of cationic liposomes formed by dicationic tetraether lipid (a), by dicationic tetraether lipid/DOPE (95:5 w/w) (b), and by diPC tetraether lipid (c–d).

pares favourably to that of liposomes composed of natural *Thermoplasma acidophilium* tetraether lipids, which lost 20 to 30% of the marker after 10 min at pH 2.

Gene delivery systems based of the use of the cationic tetraether lipid or neutral tetraether diol (31-atom) were also envisaged.[74] The prerequisite for gene therapy are carrier systems that envelop the therapeutic nucleic acid and facilitate incorporation by the target cell. This special problem of drug delivery needs to take account of the special properties of negatively charged nucleic acids. Typically, cationic lipids can be used to complex nucleic acids, thereby forming so-called lipoplexes, and these complexes are then able to deliver genes into cells. With this in mind we envisaged two potential strategies: 1) the use of a cationic tetraether that plays both a complexation and a stabilization role, and 2) the use of a neutral tetraether diol that could form stabilized lipoplexes in association with cationic diester lipids.[74] In the first case, even though the complexation of the cationic tetraether was confirmed, no efficient transfections were achieved. The addition of DOPE (5%) increased the transfection efficiency and led us to think that the lipoplexes were too stable to permit the crucial escape of the DNA in the cytosol. In the second case, however, in which the tetraether diol was used as a neutral co-lipid (5–50%), high in vitro transfection efficiencies were achieved. In vivo assays to improve the potential of these tetraether lipids in gene delivery are now being conduced.

To improve our delivery systems based on archaeal lipid analogues we then introduced cell-targeting ligands (sugar



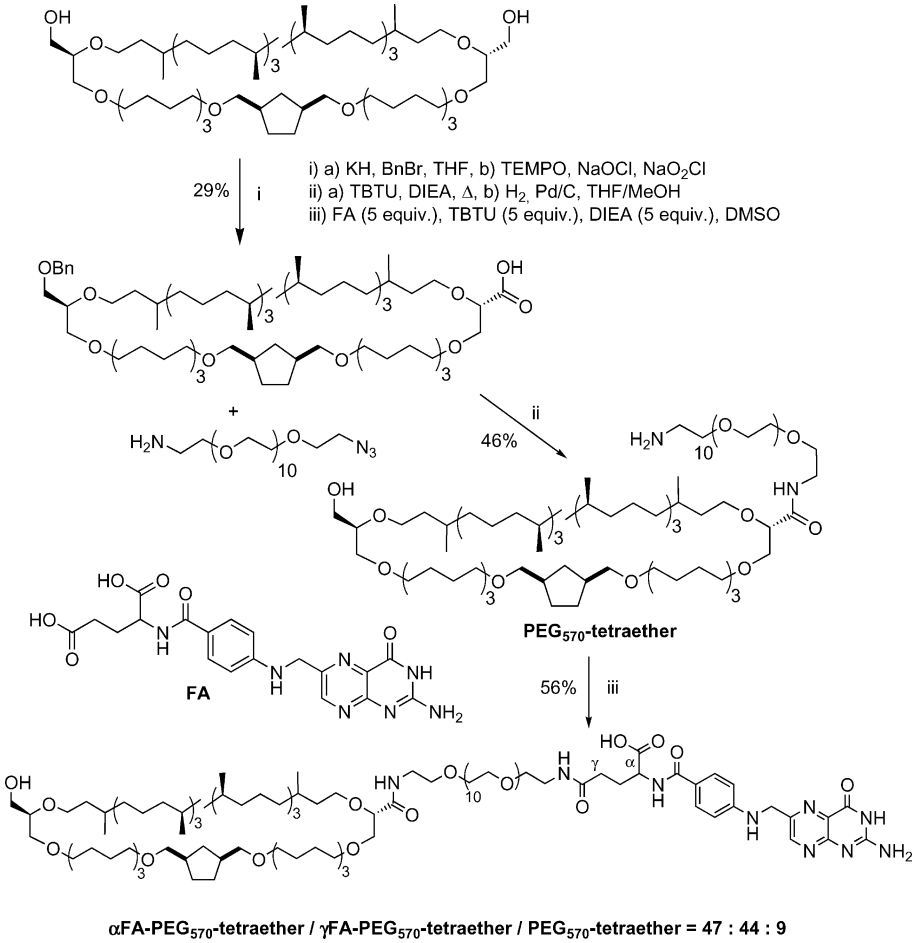
Scheme 6. Introduction of trimannosyl and trilactosyl clusters on tetraether lipids.

cluster[75] or folic acid derivatives[53]) at one end of the tetraether backbone (see Scheme 6 and 7). The dessymmetrization of the tetraether diol was achieved by monoacetylation (Ac_2O, AcONa) or monobenzylation (BnBr, NaH). In both cases statistical quantities of unreacted diol and diprotected derivative could be recovered in good yields. Trilactosyl (liver cell targeting) and trimannosyl (macrophage targeting) tetraether lipids were prepared through a glycosylation methodology on the monoacetylated tetraether and led to acetal functions, which can be regarded as labile linkages under acidic conditions (Scheme 6). A final deprotection step under Zemplèn conditions (MeONa, MeOH) provided trilactosyl and trimannosyl tetraethers. Note that sugar clusters are well known to enhance ligand/receptor interactions and thus to favour cell recognition.[77–79]

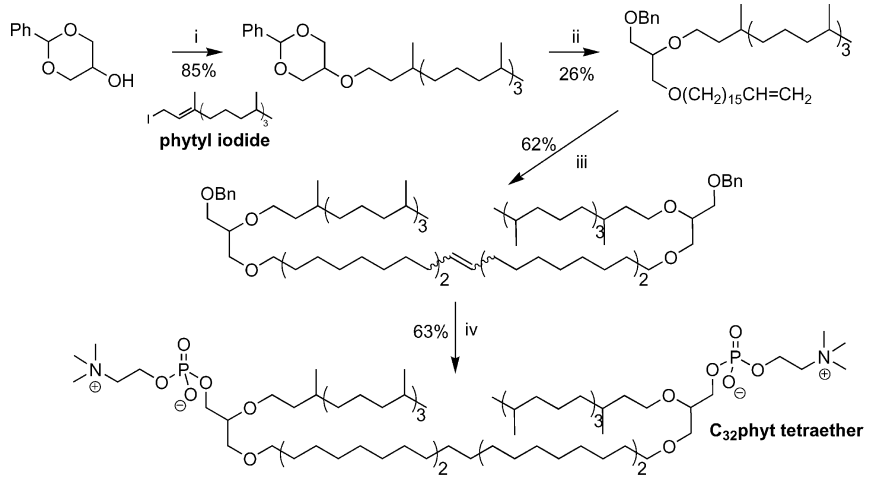
A folic acid moiety was introduced after preliminary functionalization of the monobenzylated tetraether with a 10-unit PEG chain. Oxidation of the alcohol function to the carboxylic acid and peptidic coupling with H_2N-PEG_{570}-N_3 provided the PEG_{570}-tetraether. Final coupling with folic acid (**FA**) in the presence of TBTU/DIEA led to a mixture of α and γ regioisomers (1:1) (Scheme 7).[53] The FA-PEG_{570}-tetraether was used in in vitro gene delivery assays, which showed good transfection efficiencies even with neutral lipoplexes, and clear folate-/receptor-mediated internalization was observed.

Additional synthetic tetraethers with acyclic backbones were recently evaluated by other research groups. Thompson et al. have prepared and studied the behaviour of quasi-macrocyclic tetraether lipids of various carbon chain lengths (20 to 32).[22,61,80] The synthetic scheme is based on olefin metathesis of two identical diethers (Scheme 8). A Williamson reaction between phytyl iodide and protected glycerol and hydrogenation provided the phytanyl derivative. Reductive opening of the cyclic acetal and the further introduction of the primary alcohol led to the ethylenic diether. The tetraether lipid was then formed through olefin metathesis between two equivalents of this diether induced by Grubbs' catalyst I. After the removal of the benzyl groups and the reduction of the double bond, the tetraether diol was functionalized by two phosphatidyl choline polar heads.

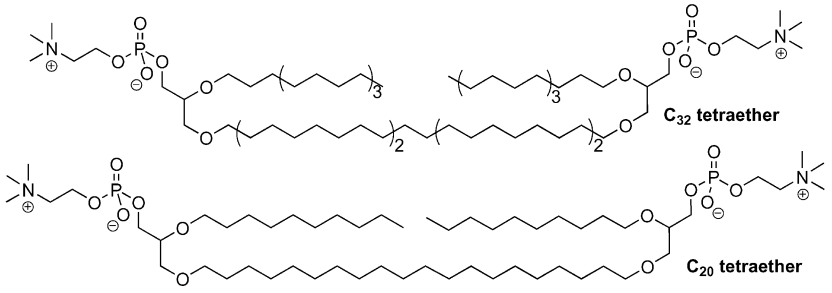
Thompson's research group has evaluated the functional reconstitution of the Ste14p membrane protein in C_{20}, C_{32} and C_{32}phyt tetraether lipids.[80] It appears that this protein recovers good activity when these types of tetraether lipids are present in the lipid membrane, and the thickness of the hydrophobic part has a dramatic influence on this activity as longer tetraether lipids induced a more active reconstitu-



Scheme 7. Preparation of highly functionalized tetraethers: **PEG_{570}-tetraether** and **FA-PEG_{570}-tetraether**.



i) a) NaH, **phytyl iodide**, Δ, THF, b) H_2, $Rh(PPh_3)_3Cl$, EtOH/THF, r.t.; ii) a) DIBAL-H, CH_2Cl_2, –78 °C to r.t., b) NaH, $CH_2=CH(CH_2)_{15}Br$, Δ, THF; iii) $(PCy_3)_2Ru=CHPh)Cl_2$, Δ, CH_2Cl_2; iv) a) H_2, $Pd(OH)_2$, THF/EtOH, r.t. b) $ClP=O(OCH_2CH_2O)$, Et_3N, THF, c) NMe_3, THF:DMF, 65 °C.



Scheme 8. Example of preparation of a tetraether lipid by olefin metathesis and structures of such tetraether lipids.

ted Ste14p. The same research group has also developed access to deuterated analogues by a similar strategy. The corresponding ^2H NMR spectroscopy study gave real insight into the lipid membrane structure.[81]

In summary, the presence of bipolar tetraether lipids in liposomes and artificial membranes may give rise to remarkable physical stability through the reduced degree of molecular translational freedom of their bipolar backbones and the adjustment of their chemical structures (cycle-containing bridging chains, polar headgroups, methyl branching…). Multifunctionality in the form of the ability to promote membrane stability, cell targeting, or "Stealth" properties can be efficiently obtained from original synthetic lipid analogues, which opens up the possibility of biotechnological applications.

5. Conclusions

In conclusion, archaeal lipids differ considerably from eubacterial and eukaryotic lipids in their structures and physical properties. In the last decade, more and more unique diether and tetraether lipids have been isolated and characterized from various archaea species, giving further insights into the physiological significance of ether lipids under extreme environmental conditions. This growing variety of archaeal lipid structures represents a rich source of inspiration for the conceptual development of innovative supramolecular assemblies and molecular materials. Of particular interest is the potential to control the fluidity/rigidity/permeability properties of liposomes and artificial membranes by modulating compositions in diether and tetraether lipids, as well as their chemical structures. As an example, a new generation of liposomes called Archaeosomes has been developed as innovative drug/gene delivery systems. In our research group, we have created an archaeal lipid toolbox (design, synthesis, physicochemical evaluations and biotechnological applications) allowing variability both in chemically pure structures (diether/tetraether backbones, carbohydrate, phosphorylated, glycine betaine neutral, anionic or cationic polar headgroups, functionalized bridging chains, stereochemistry, symmetrical or dissymmetrical structure of tetraethers, targeting ligand-containing PEGylated lipids) and in properties (membrane fluidity/rigidity/stability/permeability, cell/tissue targeting) to be achieved with a view to enlarging the scope of the use of these novel lipids (drug/gene delivery, Langmuir films, etc.).

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