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Synthesis and Supramolecular Assemblies of Bipolar Archaeal Glycolipid Analogues Containing a *cis*-1,3-Disubstituted Cyclopentane Ring

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Abstract: Unsymmetrical archaeal tetraether glycolipid analogues **1**−**2** incorporating a 1,3-disubstituted cyclopentane ring into the bridging chain have been synthesized. The cyclopentane has been 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. Freeze-fracture and cryotransmission electron microscopy experiments clearly demonstrated unprecedented glycolipid supramolecular organizations involving two-by-two monolayer associations coupled with interconnection and fusion phenomena. Furthermore, a significant difference in the hydration properties and in the lyotropic liquid crystalline behavior of bipolar lipids **1**−**2** was found depending on the position of the cyclopentane residue.

Introduction

Lipids of thermophilic and thermoacidophilic Archaebacteria are characterized by tetraether-type macrocyclic components possessing one or two polar headgroups derived from phosphate and/or sugar moieties at terminal ends of a lipidic backbone.[1] These tetraethers can be divided into two classes.[2] The first one is called glycerol-dialkyl-glycerol-tetraethers (GDGT, Figure 1; compounds **a**−**d**) and is formed by two biphytanyl ether chains linked at both ends to a glycerol unit. In the second class of molecules, glycerol-dialkyl-nonitol-tetraethers (GDNT, Figure 1; compounds **a′**−**d′**), a calditol group replaces one of the glycerol units. Recently, Sinaÿ et al.[3] demonstrated the polyhydroxylated cyclopentanic structure of this polyol. A particularly attractive point concerns the presence of 0−4 pentacyclic rings into each biphytanyl chain;[4] the number and position of the cyclopentanes were found to depend on the nature of the lipids and vary with the growing temperature of microorganisms. In fact, it has been shown that the extent of cyclization increases when the species are grown at increasing temperatures. The regular disposition of these cycles at two or six methylene groups starting from the glycerol or calditol units could result

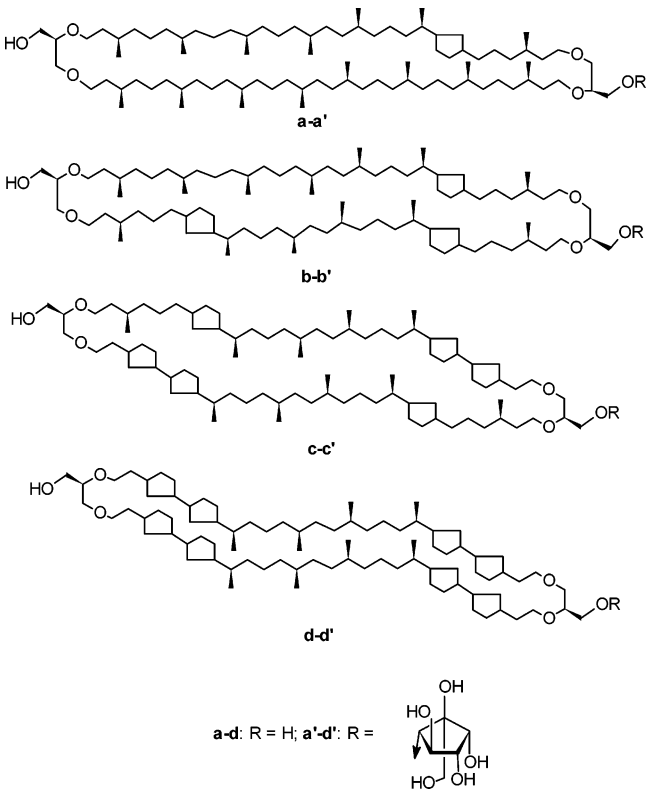

Figure 1. Typical basic structures of GDGT-type (**a**−**d**) or GDNT-type (**a′**−**d′**) tetraethers found in methanogenic and thermoacidophilic archaeal membrane lipids.

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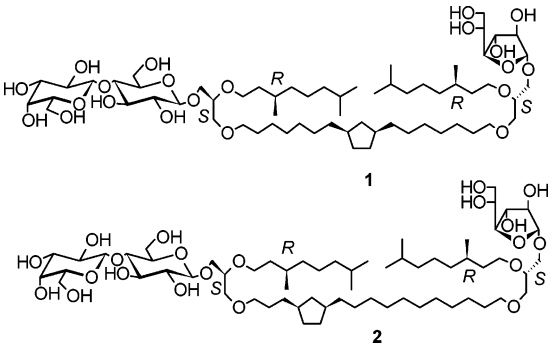

Figure 2. Synthetic unsymmetrical neutral archaeal glycolipid analogues **1**−**2**.

from the mechanism of biosynthesis that operates on alkyl chains starting from the middle of the isoprenoid system toward ether bonds.[2] Very little is known concerning the functional role of cyclopentanes within the membrane apart from the fact that they probably reduce the rotational freedom of the hydrocarbon chains. It is not clear, however, whether their presence corresponds to an adaptative response to environmental high temperatures or whether it results from a particular biosynthetic pathway. Seemingly, there does not appear to be any specific structural feature of the polar lipids of thermophiles since cyclopentyl rings were also found in lipids isolated from psychrophilic archaebacteria living at low temperatures.[5] These points may be of fundamental importance in understanding the structure−function relationship of natural archaeal membranes and may help the development of bipolar tetraether liposomes for biotechnological applications such as sterilization,[6] drug or antigen delivery,[7] and membrane protein/peptide reconstitutions.[8]

To provide further evidence for the crucial role of cyclopentanes on the membrane properties, we launched a program of synthesis and physicochemical evaluations of archaeal tetraether glycolipid analogues. Preliminary freeze-fracture electron microscopy experiments were carried out from synthetic acyclic tetraether-type glycolipids that incorporated a 1,3-disubstituted cyclopentane ring at midpoint of their bridging chain, as a diastereoisomeric mixture of cis−trans isomers.[9c] Interestingly, cyclopentane ring was found to play an important role in the organization of the bipolar lipids within the vesicle membranes. Pursuing our efforts on understanding the relationships between 1,3-disubstituted cyclopentane rings and transmembrane organizing properties of the bipolar lipids, we now report on a novel approach for the synthesis of the unsymmetrical tetraether-type glycolipids **1**−**2** (Figure 2) that possess lactosyl and galactofuranosyl units as polar headgroups and an acyclic lipophilic backbone characterized by the presence of (1) a bridging chain attached to two glycerols at *sn*-3 and *sn*-3′ positions and containing a *cis*-1,3-cyclopentane, either into the middle of the chain or located at three methylene groups from the *sn*-3 position of the glycerol unit linked to the lactosyl residue, and (2) two dihydrocitronellyl chains linked to glycerol at *sn*-2 and *sn*-2′ positions. The supramolecular assemblies formed by these original amphiphiles with a totally controlled cis configuration for the 1,3-disubstituted cyclopentane moiety were then investigated in dilute aqueous media to give further insights into the mechanisms involved in the bilamellar vesicle formation and to evaluate the influence of the cyclopentyl ring position on lyotropic properties.

Results and Discussion

(I) Synthesis of Bipolar Lipids 1−2. Bisglycosides **1** and **2** were synthesized according to a strategy that initially involved constructing the lipophilic monoprotected diols **17** or **24** (Scheme 1) followed by the sequential introduction of the saccharidic moieties. This novel synthetic pathway warrants the cis-configuration of the 1,3-disubstituted cyclopentane as well as modulates the position of the carbocycle within the bridging chain. Spacer **7** was prepared via a double Wittig reaction between 6-benzyloxy-hexan-1-al **5** and the bisphosphonium salt **4** obtained from *cis*-1,3-diformylcyclopentane[9b,c] followed by hydrogenation of the double bonds and removal of the benzyl groups. Then, alkylation of the (*S*)-glycerol derivative **16**[9b] with ditriflate **15**, subsequent hydrogenolysis of the benzyloxy groups, and monobenzylation of the corresponding diol provided the monoprotected tetraether **17**.

Our attention was next directed toward the synthesis of the lipophilic backbone **24** possessing a nonsymmetrical spacer. 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 that required the introduction of orthogonal protective groups. Our strategy for the synthesis of the monoprotected spacer **14** was based upon two sequential Wittig reactions involving phosphonium salts **9** and **12**. Compound **9** could be readily prepared by monoprotection of the *cis*-1,3-bis-(hydroxymethyl) cyclopentane **3** and conversion of the free alcohol unit to the corresponding phosphonium iodide. Phosphonium salt **12** was prepared by connection of the 10-benzyloxy-decan-1-al **10** with the cyclopentyl derivative **9** via standard Wittig reaction conditions followed by removal of the tetrahydropyranyl group and transformation of the hydroxyl unit into phosphonium salt. Formation of spacer **14** was finally accomplished by a second Wittig reaction between phosphonium salt **12** and 2-*O*-THP-ethan-1-al **13**[10] and subsequent reduction of the double bonds and removal of the tetrahydropyranyl group under acidic conditions.

Having the free alcohol **14** in hand, the last crucial step involved the construction of the hemimacrocyclic backbone **24** via two consecutive alkylations with triflates **20** and **23** formed from glyceryl derivatives **19** and **16**, respectively, possessing different allyloxy or benzyloxy groups at the *sn*-1 position (Scheme 1). The major unexpected difficulty in this sequence was the selective removal of the benzyl group from intermediate

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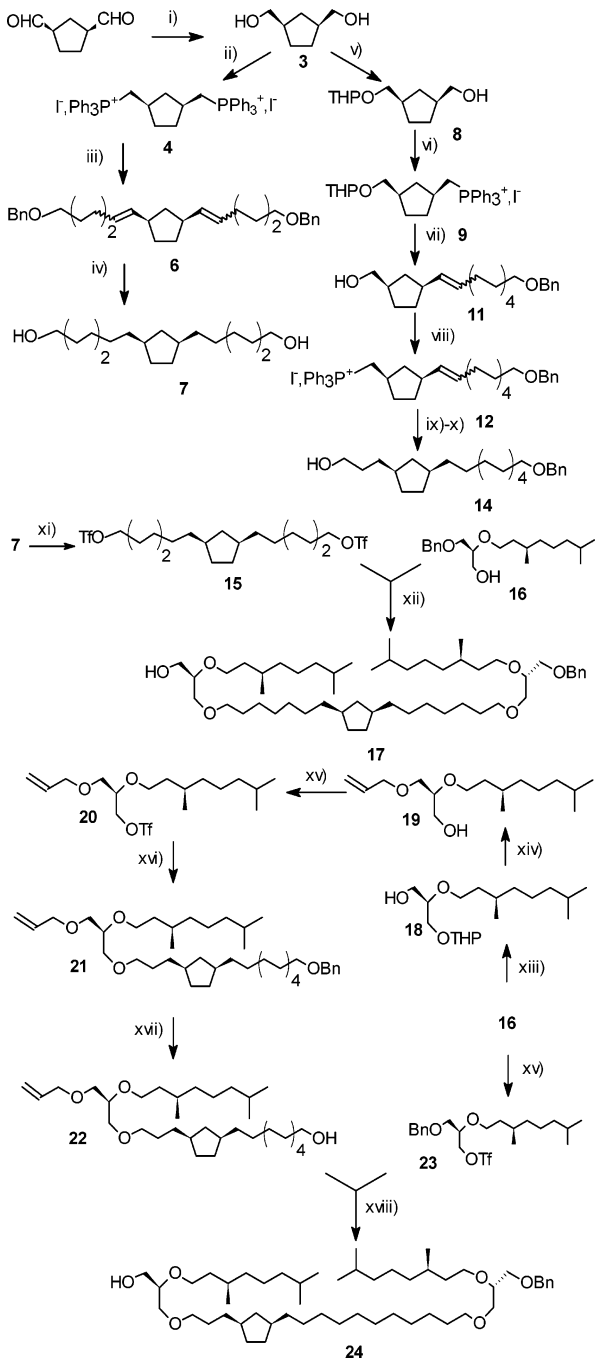
Scheme 1. Synthesis of the Monoprotected Alcohols **17** and **24**[a]



[a] (i) $NaBH_4$, CH_3OH, 0 °C, 80%. (ii) (a) I_2, imidazole, PPh_3, CH_3CN/Et_2O (1:3 v/v). (b) PPh_3, CH_3CN, reflux, 50% over two steps. (iii) *n*-BuLi, THF, 0 °C, then BnO−$(CH_2)_5$−CHO **5**, 55%. (iv) H_2, Pd/C (10%), EtOH, 98%. (v) DHP, Dowex 50WX2, toluene, 81%. (vi) (a) I_2, imidazole, PPh_3, CH_2Cl_2; (b) PPh_3, K_2CO_3, CH_3CN, 70% over two steps. (vii) (a) *n*-BuLi, THF, 0 °C, then BnO−$(CH_2)_9$−CHO **10**. (b) PTSA, CH_3OH, 75% over two steps. (viii) (a) See (vi) (a). (b) PPh_3, CH_3CN, 80% over two steps. (ix) (a) *n*-BuLi, THF, 0 °C, then THPO−CH_2−CHO **13**. (b) H_2, Pd/C (10%), Et_3N, AcOEt, 75% over two steps. (x) See (vii) (b), 77%. (xi) Tf_2O, 2,6-lutidine, CH_2Cl_2, 95%. (xii) (a) **16**, KH, THF, then **15**. (b) H_2, Pd/C (10%), AcOEt, 40% over two steps. (c) NaH, 15−5 crown-ether, BnBr, THF, 60%. (xiii) (a) DHP, PTSA, CH_2Cl_2. (b) See (iv), 65% over two steps. (xiv) (a) NaH, allylbromide, $(Bu)_4N^+Br^-$, THF. (b) PTSA, CH_3OH, 75% over two steps. (xv) (see xi), 0 °C, 95%. (xvi) **14**, KH, THF, 0 °C, then **20**, 70%. (xvii) DDQ, CH_2Cl_2/H_2O (18:1 v/v), 70%. (xviii) (a) **22**, KH, THF, 0 °C, then **23**, 50%. (b) $(Ph_3P)_3RhCl$, toluene/EtOH/H_2O (1.25:2.65:0.4 v/v/v) 78%. Bn = benzyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DHP = 2,3-dihydro-4H-pyran, THP = tetrahydropyranyl, PTSA = *p*-toluenesulfonic acid.

21 in the presence of an allyl ether unit. Deprotection by using selective standard conditions (Me_2S-BCl_3, CH_2Cl_2)[11] was ineffective. Surprisingly, oxidative deprotection with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) under conditions[12] ($CH_2Cl_2-H_2O$) specifically used for the cleavage of mono- or dimethoxylbenzyl ethers efficiently provided monoalcohol **22**. Compound **24** was next obtained in a two-step procedure by introduction of the second glyceryl moiety and selective removal of the allyl group.

The cis configuration of the 1,3-disubstituted cyclopentane in compounds **17** and **24** was clearly demonstrated by the presence of two distinct signals in ^1H NMR for the protons of the methylene group $CHCH_2CH$ for the cyclopentyl moiety, whereas a single signal should have appeared for the trans isomer.[9b] Monoprotected diols **17** and **24** were both obtained as a mixture of two diastereoisomers because of the presence of the (±)-*cis*-disubstituted cyclopentyl ring. It is noteworthy that (±)-*cis*-cyclopentanes were characterized by unique signals in NMR. At this stage, the sequential introduction of the β-D-galactofuranosyl and lactosyl units was performed in the same manner as previously described from lactosyl thioglycoside **25** and pent-4-enyl 2,3,5,6-tetra-*O*-α,β-D-galactofuranoside **26**[9b] (Scheme 2) and permitted us to isolate the corresponding glycolipids **1** and **2**.

(II) Transmission Electron Microscopy Studies. The supramolecular aggregates formed by these synthetic bipolar lipids in distilled water were examined by transmission electron microscopy (TEM). The membrane-spanning organization of the lipids was shown by freeze-fracturing from the observation of circles instead of convex and concave fracture faces visualized in the case of bilayered systems.[13] The absence of a fracturable midplane in the membranes indicates that the bipolar lipids do not adopt a U-bent shape but span the membrane to form a monolayer. The cross-fracture behavior, which is the absence of a preferential fracture plane, has further been shown to be indicative for monomolecular vesicle membranes and membrane spanning lipid molecules[14] and also in recent investigations.[15]

Additional confirmation of the aggregate morphologies assumed by these glycolipids was obtained by cryo-TEM experiments that allowed the direct visualization of the lamellar arrangements without any alteration. For glycolipid **1**, we have determined a membrane thickness of 3.0−3.5 nm in bent vesicular membranes (Figure 3a). This is in good agreement with the molecular length of the bolaamphiphiles **1**−**2** in a stretched conformation estimated to be about 2.8−3.0 nm.

Compound **1**, which possesses a cyclopentane in the middle of the main chain, gave rise to oligolamellar or bilamellar vesicles, but no multilamellar onionlike vesicles were observed as in the case of a mixture of cis−trans isomers.[9c] A two-by-two monolayer association frequently appeared from dispersed systems prepared either in the presence (Figure 3a) or the absence (see Supporting Information) of glycerol added as a cryoprotectant for FFEM. We also checked that after storage

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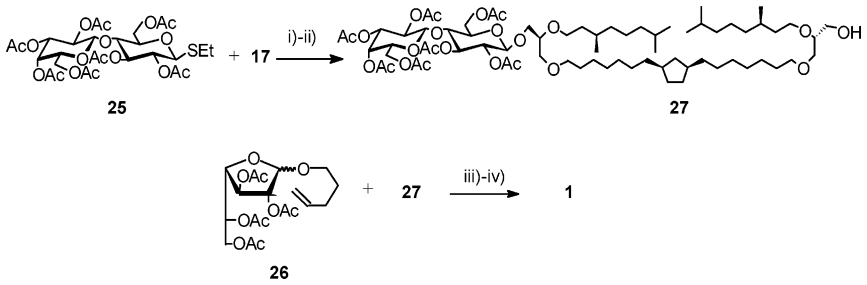
Scheme 2. Introduction of the Glycosidic Polar Head Groups in Compound **1**[a]



[a] (i) NIS, Et_3SiOTf, CH_2Cl_2, 48%. (ii) H_2, Pd/C, EtOH, >99%. (iii) NIS, Et_3SiOTf, CH_2Cl_2, 35%. (iv) MeONa, MeOH, >99%. NIS = *N*-iodosuccinimide. [b] Under the same conditions, **25** + **24** (then **26**) → → **2**.

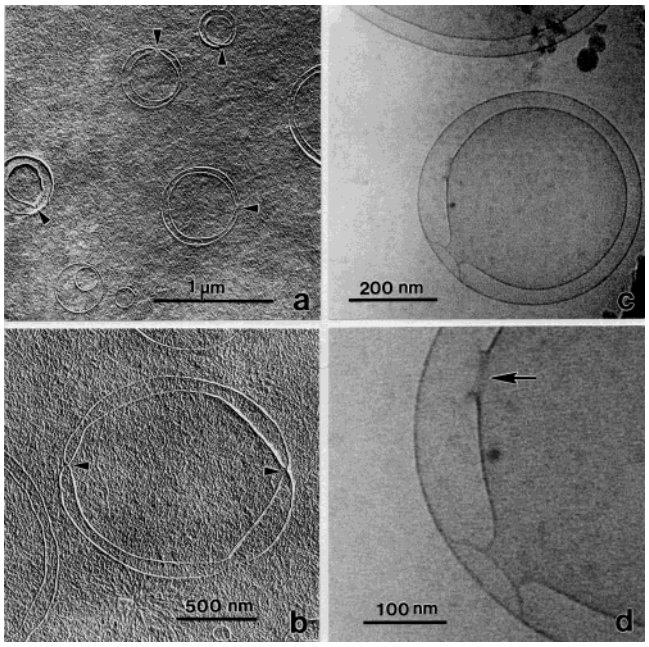


Figure 3. Freeze-fracture electron microscopy (FFEM; a, b) and Cryo-TEM (c, d) of glycolipid **1** after hydration by intensive stirring in excess water (99 wt %). (a) Vesicles with two-by-two lamellar arrangement showing an interconnection (arrowheads) between the two envelopes. (b) Vesicle with two-by-two 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).

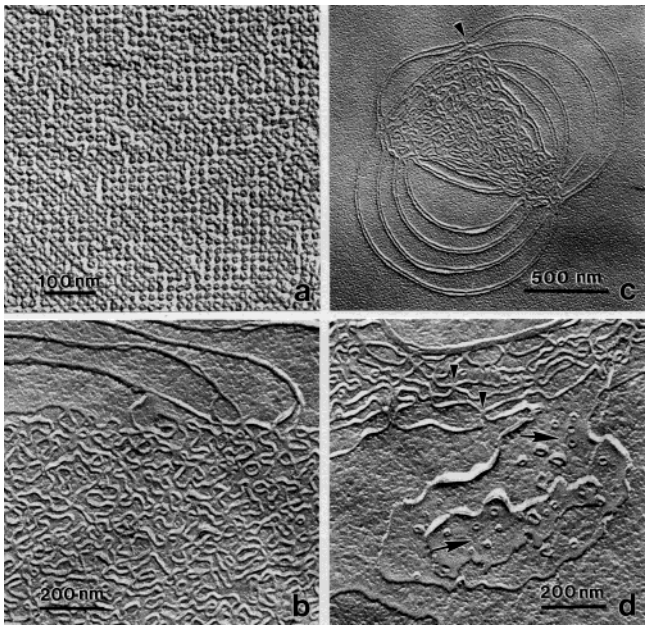


Figure 4. FFEM of compound **1** after gentle hydration at 94 wt % (a) and 97 wt % (b−d) water content. (a) Projection with a regular pattern of a cubic 3D structure. Repeat distance of about 18 nm of the orthogonally arranged elements (tunnels). (b) Projection of a spongelike 3D structure after storage over 10 days at room temperature. A transition to tightly coupled lamellae is visible. (c) Structural transition from a spongelike nucleus to peripheral two-by-two arranged lamellae. Interconnection at the end of two lamellar loops (arrowhead). (d) Tangentially running fracture plane near the surface of lamellar faces with a number of tunnel-like structures (arrows). They may be descended from interconnections of the sponge structure (arrowheads).

of the sample at room temperature and ultrasonication the same phenomenon could be obtained. Moreover, a particularly striking feature happened in several bilamellar systems. Surprisingly, the outer envelope of many two-by-two arranged lamellae shows openings such as invaginations favoring their connection with the inner vesicle structure as illustrated by FFEM (Figure 3a,b) and confirmed by cryo-TEM (Figure 3c,d). In many cases, several (two or even more) interconnections were revealed by TEM pictures (Figure 3b,d). The structures mainly appeared as an "X" intersection in the two dimensions of the FFEM image because the freeze fracture plane ran at the border of the opening and not across its center. Nevertheless, the opening of these bilamellar vesicle systems was shown at the upper part of Figure 3a and more clearly in the Supporting Information where the magnification is higher. These unexpected contacts may involve original fusion mechanisms that operate between two matched monolayers through the possible existence of specific interactions between sugar residues. A strong affinity between these headgroups could exhibit a cooperative orientation of the monomers within the lamellae, allowing a stronger coupling of two close monolayers; this original phenomenon may appear very early during the hydration process. To elucidate the mechanism of invaginated vesicle formation, additional electron microscopic experiments were then performed at lower hydration conditions.

Fresh dispersion of compound **1** with a 94 wt % water content showed after gentle hydration a regular structure pattern[16,17] (Figure 4a) resulting from a cubic 3D structure.[9c] After further dilution (97 wt % water content) and prolonged storage at room temperature (10 days), it exhibited a spongelike structure[13,16] (Figure 4b) that was transforming into bilamellar arrangements

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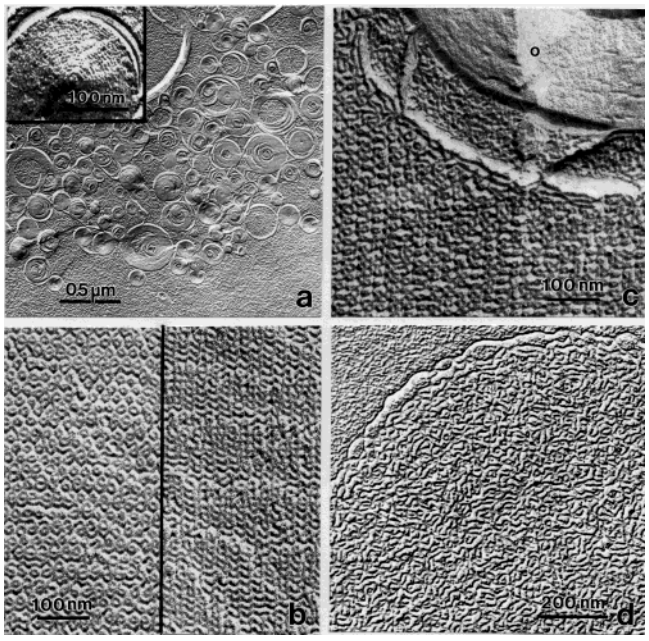

Figure 5. FFEM of glycolipid **2** after hydration by intensive stirring in excess water (a, d) and gentle hydration at 95 wt % water content (b, c). (a) Multilamellar onionlike vesicles are predominant. At larger magnification (inset), the multilamellar arrangement with a repeat distance of about 5 nm is visible. (b) Two projections of a cubic 3D structure with a repeat distance of about 23 nm of the hexagonally arranged elements (tunnels, left part). (c) Coexistence of a cubic phase structure and a multilamellar vesicle. Contrary to compound **1**, spongelike structured particles have not been found. (d) Rarely detectable spongelike structure after ultrasonication in excess water.

at its periphery (Figure 4b,d). These two-by-two arranged lamellae generally showed tunnel-like interconnections (Figure 4d). We assume that the interconnections are primary structures of the 3D sponge arrangement that are preserved during the transition to the vesicular lamellae. Because of a structural genesis from cubic to sponge and curved lamellar (vesicles), the frequent two-by-two arrangements of lamellae could be favored by these primary connections initially observed in the sponge-type aggregates (Figure 4d).

A great difference in the hydration properties of compounds **1** and **2** was observed depending on the position of the cyclopentyl moiety on the bridging chain. Only a part of glycolipid **2** could be dispersed in water, whereas compound **1** exhibited a complete dispersion under the same conditions. Compound **2** did not nearly form oligolamellar or two-by-two lamellar vesicles. The typical features were multilamellar onionlike vesicles with tightly packed lamellae (Figure 5a). After gentle hydration at 90 wt % water content, compound **2** gave a regular cubic 3D structure (Figure 5b,c) as bisglycoside **1**; however, no spongelike aggregates were found, even after a prolonged storage (14 days) and further dilution. A sponge structure with narrow meshes could be induced by ultrasonication in excess water (Figure 5d), but it was found extremely seldom. These results suggested that bipolar lipid **2** could adopt the same transmembrane conformation as in compound **1**, but the swelling of the lipid by incorporation of a large amount of water appeared to be much more restricted. Consequently, the position of the (±)-*cis*-1,3-disubstituted cyclopentane moiety within the main chain seems to play a major role on the lyotropic behavior of the tetraether-type lipids. The different dispersion morphologies cannot simply be a consequence of the (±)-*cis*-cyclopentyl residue mixture used, since the same behavior would have been observed from glycolipids **1** and **2**. Indeed, the presence of an identical (±)-cis isomeric system in both structures clearly demonstrates the profound positional effect of the cyclopentane on the self-assembling properties of these neutral archaeal glycolipid analogues. The presence of cyclopentanes in precise positions within the bridging chains may influence the orientation of the glycosidic polar headgroups attached to the tetraether backbone, as recently shown by molecular modeling.[18] When containing eight cyclopentane rings, the polar disaccharidic moiety of natural tetraether-type lipids was shown to run parallel to the membrane surface, whereas the headgroup is oriented perpendicularly in the absence of cyclopentanes.[18b] Therefore, one may assume that the orientation of the lactose residue of our synthetic glycolipids is affected by the cyclopentane position. The increased hydration for compound **1** in comparison with that of glycolipid **2** may be associated with a more favorable arrangement of the bulkier polar headgroup relative to the surface membrane for hydration.

Conclusions

The results obtained from this study clearly reveal the importance of the cyclopentane incorporation into bridging chains on the self-assembling behavior of neutral tetraether-type glycolipids. Utilizing novel synthetic neutral bipolar lipids possessing a 1,3-disubstituted cyclopentane with a controlled cis configuration, we have demonstrated, for the first time to our knowledge, the profound impact of the cycle position on the physicochemical properties of the monolayer membranes. Another major finding of this work concerns the formation of the two-by-two lamellar arrangements via the existence of primary tunnel-like connections between two matched monolayers. Consequently, the cyclopentane ring position within the bridging chains may modulate interactions between neutral tetraether-type membranes and the aqueous environment and between two monolayers next to each other. Insights gained into the structure−property relationships of these bolaamphiphiles can help explain the unusual organization and functionality of archaeal lipids in natural membranes as well as allow the design of new materials based upon cyclopentane ring-containing lipids capable of inducing original supramolecular systems. Applications of these monolayer systems for the encapsulation and the delivery of biomaterials are under investigation. Indeed, the positional effect of cyclopentanes may represent a new method for the control of membrane permeability and drug releasing.

Experimental Section

General. All commercially available chemicals were used without further purification, and solvents were carefully dried and distilled prior to use. Unless otherwise noted, nonaqueous reactions were carried out under a nitrogen atmosphere. Analytical TLC was performed on Merck 60 F_{254} silica gel nonactivated plates. A solution of 5% H_2SO_4 in EtOH was used to develop the plates. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Fast-atom bombardement (FAB)

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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. For freeze-fracturing, lipid dispersions (94−97 wt % and 99 wt % water content) were prepared by gentle stirring (low water content) or intensive stirring overnight at room temperature (high water content). Small amounts of the dispersions were sandwiched, after addition of glycerol (2.3:10, v/v, glycerol/water) as cryoprotectant, between copper profiles and were then rapidly frozen in a liquid 1:1 ethane/propane mixture cooled by liquid nitrogen. Fracturing and replication were carried out at −120 °C in a freeze-fracture apparatus BAF 400T (BAL-TEC, Liechtenstein) equipped with electron beam evaporators. All fracture samples were etched before Pt/C-shadowing to visualize the propagation patch much more clearly. Freeze-etching was repeated after storage of the samples at room temperature over 4 days (or more) and ultrasonication for 10 min in a cleaning batch immediately before freezing. Electron micrographs were prepared with a CEM 902A electron microscope (Zeiss, Germany). For cryo-TEM experiments, the CM 120 electron microscope (FEI Co., The Netherlands) equipped with cryo-Transfer (Gatan, USA) was used. Pent-4-enyl galactofuranoside **26**, lactosyl thioglycoside **25** and 1-*O*-benzyl-2-*O*-(*R*)-3,7-dimethyl-octyl-*sn*-glycerol **16** were prepared as previously described.[9b] The synthesis of 2-*O*-THP-ethan-1-al **13** has already been described.[10]

***cis*-1,3-Bis-(hydroxymethyl)cyclopentane (3).** Sodium borohydride (4.52 g, 120 mmol, 2 equiv) was added slowly at 0 °C to a stirred solution of *cis*-1,3-diformylcyclopentane (7.5 g, 59.8 mmol) in methanol (65 mL). After being stirred overnight at room temperature under N_2 atmosphere, the reaction mixture was quenched with water, extracted 3 times with the ethyl acetate/butanol (9:1 v/v) mixture. The combined extracts were dried ($MgSO_4$) and concentrated under vacuum. The residue was purified by flash column chromatography upon elution with EtOAc to afford **3** as a colorless oil (4.4 g, 56%); R_f = 0.3 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90−0.98 (dt, 1H, J = 9.1, 12.7 Hz), 1.33−1.41 (m, 2H), 1.72−1.79 (m, 2H), 1.95 (s, 1H), 1.94−2.00 (m, 1H), 2.13−2.20 (m, 2H, J = 6.6 Hz), 3.50−3.59 (m, 4H, J = 3.6 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 28.3, 32.8, 42.2, 67.3.

[(*cis*-2,5-Methylidene)-hexyl]-1,6-di-triphenylphosphonium Bis-iodide (4). To a solution of triphenylphosphine (19.4 g, 74 mmol, 2.2 equiv) and imidazole (5 g, 74 mmol, 2.2 equiv) in a mixture of acetonitrile/ether (1/3 (40 mL:120 mL) v/v), diiode (18.8 g, 74 mmol, 2.2 equiv) was added at 0 °C. After stirring of the mixture at room temperature in the dark for 10 min, we added a solution of diol **3** (4.38 g, 33.6 mmol) in ether (20 mL). The reaction mixture was stirred for 3 days at room temperature in the dark. The solution was washed with 10% aqueous sodium thiosulfate and extracted with diethyl ether. The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and concentrated to dryness. The crystallized triphenylphosphine oxide was filtered on silica gel with petroleum ether/EtOAc (8:2 v/v). The crude product (11.8 g, 33.6 mmol) was dissolved in acetonitrile (90 mL), and triphenylphosphine (26 g, 100 mmol, 3 eq) was added. The reaction mixture was refluxed for 2 days. Dilution with toluene (50 mL) gave the desired salt **4** as a white solid (14.3 g, 50%), which was recrystallized from a mixture of methanol and diethyl ether; *Rf* = 0.2 (CH_2Cl_2/MeOH 96/4 v/v); mp > 260 °C (MeOH/Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47−1.53 (m, 2H), 1.57−1.65 (m, 2H), 1.88−1.96 (m, 1H), 2.07−2.14 (m, 1H), 2.36−2.43 (m, 2H), 3.59−3.69 (m, 2H), 3.78−3.88 (m, 2H), 7.72−7.85 (m, 30H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 28.2, 28.7, 32.66, 32.74 (d, J = 8 Hz), 33.4, 41.8, 117.5−135.0; FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M^{2+},I^-]^+$, 747.1807; found, 747.1809.

1,18-Dibenzyloxy-(*cis*-8,11-methylidene)-octadec-6,12-diene (6). To a suspension of phosphonium diiodide **4** (500 mg, 0.57 mmol, 1 equiv) in dry THF (13 mL), butyllithium (0.8 mL, 1.2 mmol, 2.1 equiv) was added at 0 °C and under N_2 atmosphere. The reaction mixture was stirred for 20 min at 0 °C, and 6-benzyloxy-hexan-1-al **5** (250 mg, 1.3 mmol, 2.3 equiv) dissolved in dry THF (13 mL) was added. The solution was subjected to an extractive workup (quenching with water and extraction with petroleum ether/EtOAc (2:8 v/v)), and the crude product was purified by silica gel column column chromatography. Elution with a mixture of petroleum ether and ethyl acetate (99/1 v/v) yielded compound **6** as a colorless oil (150 mg, 55%); R_f = 0.8 (EP/EtOAc 8:2 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95−1.04 (m, 1H), 1.21−1.37 (m, 14H), 1.53−1.56 (m, 4H), 1.69−1.81 (m, 2H), 1.89−1.90 (m, 1H), 2.65−2.80 (m, 2H), 3.36−3.39 (t, 4H, J = 6.6 Hz), 5.12 (s, 4H), 5.18−5.28 (m, 4H), 7.15−7.21 (m, 10H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 26.3−32.4, 32.8, 38.3, 42.2, 70.6, 72.9, 128−134.5, 127.5, 127.7, 128.4, 138.6; elemental analysis calcd (%) for $C_{33}H_{46}O_2$: C, 83.49; H, 9.77. Found: C, 83.91; H, 10.20.

(*cis*-8,11-Methylidene)-octadecane-1,18-diol (7). A solution of **6** (383 mg, 0.81 mmol, 1 equiv) in ethanol (50 mL) was stirred in the presence of 10% palladium on activated charcoal (153 mg) under an atmosphere of hydrogen gas at room temperature for 24 h. The catalyst was removed by filtration after the solvent was heated to 40 °C, and the filtrate was concentrated to dryness under vacuum. The residue was purified by flash silica gel column chromatography (petroleum ether/EtOAc 7:3) to afford compound **7** as a colorless oil (232 mg, 97%); R_f = 0.2 (EP/EtOAc 8:2 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.51−0.56 (m, 1H), 1.31−1.75 (m, 30H), 1.79−1.84 (m, 1H), 3.64−3.68 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 26.0−30.0, 31.9, 33.0, 36.9, 40.4, 63.2; elemental analysis calcd (%) for $C_{19}H_{38}O_2$: C, 76.45; H, 12.83. Found: C, 76.04; H, 12.96.

***cis*-(1-Tetrahydropyranyloxymethyl-3-hydroxymethyl)-cyclopentane (8).** To 26.9 mmol (3.5 g) of compound **3** in a mixture of toluene (150 mL) and dihydropyran (7.5 mL), resin Dowex 50X ×2 (6 g) prewashed with methanol was added, and the reaction mixture was heated at 40 °C for 2 h. The residue was filtered and concentrated under vacuum. The crude product was chromatographed over silica gel with petroleum ether/EtOAc (8:2 then 6:4) to give the monoprotected diol **8** as a colorless oil (4.66 g, 81%); R_f = 0.3 (EP/EtOAc 8/2 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88−0.99 (m, 1H), 1.32−1.84 (m, 10H), 1.94−2.04 (m, 1H, J = 7.8 Hz), 2.11−2.19 (q, 1H, J = 7.8 Hz), 2.19−2.28 (q, 1H, J = 7.8 Hz), 3.27−3.34 (dt, 1H, J = 6.6, 9.4 Hz), 3.48−3.54 (m, 3H), 3.61−3.81 (dt, 1H, J = 7.6, 9.4 Hz), 3.84−3.88 (dt, 1H, J = 3.6, 7.6 Hz), 4.58 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.6, 25.5, 28.3, 28.8, 28.9, 30.7, 33.3, 33.4, 39.7, 42.3, 62.3, 67.4, 72.0, 72.1, 98.9, 99.0; elemental analysis calcd (%) for $C_{12}H_{22}O_3$: 0.1 H_2O: C, 66.69; H, 10.35. Found: C, 66.45; H, 10.47.

[(*cis*-2,5-Methylidene)-6-tetrahydropyranyloxy]-hexyl-1-triphenylphosphonium Iodide (9). To a solution of triphenylphosphine (27.5 g, 105 mmol, 3 equiv) and imidazole (14.3 g, 210 mmol, 6 equiv) in CH_2Cl_2 (80 mL), diiode (27.2 g, 105 mmol, 3 equiv) was added at 0 °C. The reaction mixture was stirred in the dark at room temperature for 10 min. Alcohol **8** (7.5 g, 35 mmol) dissolved in CH_2Cl_2 was then added at 0 °C, and the reaction mixture was stirred for 3 h. The solution was washed with 10% aqueous $Na_2S_2O_3$ and extracted with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated. The crystallized triphenylphosphine oxide was removed by filtration on silica gel with petroleum ether/EtOAc (8:2 v/v). To a stirred solution of the resulting crude product (10.9 g, 33.6 mmol) in acetonitrile (250 mL), triphenylphosphine (10.6 g, 40 mmol, 1.2 equiv) and potassium carbonate (5.6 g, 40 mmol, 1.2 equiv) were added. The reaction mixture was refluxed for 2 days. A column chromatography over silica gel with CH_2Cl_2 and CH_2Cl_2/MeOH (95/5) gave the phosphonium salt **9** as a white solid (13.5 g, 66%); R_f = 0.4 (CH_2Cl_2/MeOH 94/6 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02−1.14 (m, 1H, J = 10.9 Hz), 1.38−1.69 (m, 10H), 1.71−1.80 (m, 1H), 1.97−2.05 (m, 1H), 2.10−2.18 (m, 1H), 3.12−3.18 (dt, 1H, J = 6.4, 9.2 Hz), 3.37−3.43 (m, 1H), 3.45−3.53 (m, 1H), 3.55−3.80 (m, 3H), 4.42 (s, 1H), 7.64−7.80 (m, 15H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.6, 25.2, 27.7, 27.8, 28.8, 30.4, 33.2, 33.3, 34.4, 38.2, 38.3, 38.6, 38.7, 62.0, 62.4, 71.0, 71.1, 98.6, 98.9, 117.8, 118.6, 130.3, 130.4, 133.4,

133.5, 135.0; FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M - I^-]^+$ 459.2453; found: 459.2451.

1-Benzyloxy-10-en-(*cis*-12,15-methylidene)-hexadecan-16-ol (11). To a suspension of phosphonium salt **9** (1 g, 1.71 mmol) in dry THF (12 mL), butyllithium in hexane (2 M) (940 μL, 1.88 mmol, 1.1 equiv) was added at 0 °C and under a N_2 atmosphere. The reaction mixture was stirred for 15 min at 0 °C, and 10-benzyloxy-decan-1-al **10** (492 mg, 1.88 mmol, 1.1 equiv) dissolved in dry THF (13 mL) was added. The mixture was decolorated, and a solid appeared. The solution was subjected to an extractive workup (quenching with water and extraction with petroleum ether/EtOAc (6:4 v/v)), and the crude product was purified by silica gel column chromatography. Elution with a mixture of petroleum ether and ethyl acetate (98:2 v/v) yielded the desired compound as a colorless oil (600 mg, 80%); $R_f = 0.6$ (EP/EtOAc 8/2 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90−1.00 (m, 1H, $J = 6.4$, 11.4 Hz), 1.27−1.28 (m, 14H), 1.49−1.83 (m, 10H), 1.91−1.99 (m, 1H), 2.00−2.03 (m, 2H), 2.21−2.28 (m, 1H), 2.74−2.81 (m, 1H), 3.25−3.31 (dt, 1H, $J = 6.8$, 9.1 Hz), 3.44−3.51 (m, 3H), 3.60−3.64 (m, 1H, $J = 4.8$, 6.8 Hz), 3.87 (m, 1H), 4.50 (s, 2H), 4.58 (s, 1H), 5.22−5.38 (m, 2H), 7.25−7.34 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.6, 25.5, 26.2−32.8, 27.6, 30.8, 38.0, 38.3, 39.6, 62.2, 62.3, 70.5, 72.2−72.3, 72.9, 98.9, 128.9−134.8, 127.5, 127.8, 128.4, 138.7; elemental analysis calcd (%) for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47. Found: C, 79.67; H, 10.35. To a solution of this protected alcohol (540 mg, 1.22 mmol) in methanol (20 mL), *p*-toluenesulfonic acid (11.6 mg, 0.06 mmol, 0.05 equiv) was added. After being stirred for 2 h at room temperature, the solution was concentrated under vacuum, and the residue was then purified by column chromatography on silica gel (petroleum ether/EtOAc (98:2)) to afford compound **11** as a colorless oil (410 mg, 94%); $R_f = 0.2$ (EP/EtOAc 8/2 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87−0.99 (m, 1H, $J = 10.1$ Hz), 1.23−1.44 (m, 14H), 1.57−1.66 (m, 2H), 1.72−1.79 (m, 2H), 1.88−1.95 (m, 1H), 2.00−2.03 (m, 2H), 2.10−2.20 (m, 1H), 2.42−2.46 (m, 1H), 2.70−2.74 (m, 1H), 3.44−3.51 (m, 4H), 4.50 (s, 2H), 5.25−5.37 (m, 2H), 7.26−7.32 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 27.5, 26.2−32.8, 37.5, 38.3, 42.1, 67.5, 70.5, 72.9, 129.1, 134.6, 127.5, 127.6, 128.3, 138.6.

1-Benzyloxy-10-en-(*cis*-12,15-methylidene)-hexadecyl-16-triphenylphosphonium iodide (12). To a solution of alcohol **11** (1.240 g, 3.46 mmol) in CH_2Cl_2 (25 mL), imidazole (500 mg, 7.37 mmol, 2.1 equiv) and diiode (1.27 g, 4.91 mmol, 1.4 equiv) were added at 0 °C, and the resulting reaction mixture took a red coloration. Triphenylphosphine (1.09 g, 4.2 mmol, 1.2 equiv) in CH_2Cl_2 (25 mL) was added slowly at 0 °C; the solution became yellow, and the formation of a solid was observed. After being stirred 5 h in the dark, the solution was quenched with saturated aqueous sodium hydrogenocarbonate and extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and concentrated to dryness. A column chromatography on silica gel (CH_2Cl_2) gave the desired product (1.39 g, 86%). This product (5.9 g, 12.7 mmol) and additional triphenylphosphine (4.0 g, 15.24 mmol, 1.2 equiv) were dissolved in acetonitrile (140 mL). The reaction mixture was refluxed for 2 days. A column chromatography over silica gel with CH_2Cl_2 and CH_2Cl_2/MeOH (98:2) gave the phosphonium salt **12** as a white solid (7.24 g, 78%); *Rf* = 0.5 (CH_2Cl_2/MeOH 95:5 v/v); mp = 139−140 °C (MeOH/Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19−1.42 (m, 15H), 1.52−1.69 (m, 4H), 1.74−1.83 (m, 1H, $J = 6.0$ Hz), 1.89−1.97 (m, 2H), 2.18−2.31 (m, 1H), 2.58−2.65 (m, 1H), 3.45−3.48 (t, 2H, $J = 9.6$ Hz), 3.74−3.88 (d, 2H), 4.50 (s, 2H), 5.21−5.29 (m, 2H), 7.26−7.34 (m, 5H), 7.70−7.88 (m, 15H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 26.1−29.7, 27.4, 29.2, 32.0, 33.2, 34.5, 37.5, 42.5, 70.4, 72.8, 129.4,133.7, 127.5, 127.6, 128.3, 138.6, 118.0, 118.8, 130.5, 130.6, 133.7, 133.8, 135.1, 135.2; FABMS (*m*-nitrobenzyl alcohol matrix) for $C_{42}H_{52}OP$: calcd for $[M^+]$, 603.3756; found, 603.3755.

(*cis*-4,7-Methylidene)-18-benzyloxy-octadecan-1-ol (14). To a suspension of phosphonium salt **12** (1 g, 1.37 mmol) in dry THF (15 mL), butyllithium in hexane (1.6 M) (943 μL, 1.51 mmol, 1.1 equiv) was added at 0 °C under a N_2 atmosphere. The reaction mixture was stirred for 15 min at 0 °C, and 2-*O*-THP-ethan-1-al **13** (217 mg, 1.51 mmol, 1.1 equiv) dissolved in dry THF (10 mL) was added. The resulting mixture was decolorated, and the formation of a solid was observed. The solution was subjected to an extractive workup (quenching with water and extraction with diethyl ether), and the product was purified by silica gel column chromatography. Elution with a mixture of petroleum ether and ethyl acetate (the volume ratio was changed from 98:2 to 8:2 v/v) yielded the unsaturated compound resulting from the Wittig reaction as a colorless oil (480 mg, 75%); $R_f = 0.5$ (EP/EtOAc 95:5 v/v). A solution of this product (1.87 g, 4 mmol) in ethyl acetate (19 mL) was stirred in the presence of triethylamine (667 μL, 4.8 mmol, 1.2 equiv) and 10% palladium on activated charcoal (190 mg) under an atmosphere of hydrogen gas at room temperature for 12 h. The catalyst was removed by filtration, and the filtrate was concentrated to dryness under vacuum to give the compound resulting from the reduction of the double bonds, as a colorless oil (1.9 g); $R_f = 0.5$ (EP/EtOAc 95:5 v/v),); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.66 (m, 1H), 1.16−1.85 (m, 36H), 1.89−1.93 (m, 1H), 3.37 (1H), 3.46 (t, 2H, $J = 6.6$ Hz), 3.48−3.52 (m, 1H), 3.72 (m, 1H), 3.86−3.91 (m, 1H), 4.50 (s, 2H), 4.57 (s, 1H), 7.20−7.35 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.6−40.6, 62.2, 67.8, 70.5, 72.8, 98.7, 127.4, 127.5, 128.3, 138.6; elemental analysis calcd (%) for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09. Found: C, 79.10; H, 11.09. To a solution of this protected alcohol (2.08 g, 4.4 mmol) in methanol (50 mL), *p*-toluenesulfonic acid (84 mg, 0.44 mmol, 0.1 equiv) was added. The reaction mixture was stirred overnight and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (petroleum ether/EtOAc (from 95:5 to 8:2)) to give compound **14** (1.32 g, 77%) as a white solid; $R_f = 0.3$ (EP/EtOAc 95:5 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.64 (m, 1H, $J = 11.4$ Hz), 1.16−1.82 (m, 30H), 1.89−1.93 (m, 1H, $J = 5.6$ Hz), 3.46 (t, 2H, $J = 6.6$ Hz), 3.61 (m, 2H, $J = 6.6$ Hz), 4.50 (s, 2H), 7.26−7.34 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 26.2−40.7, 63.3, 70.6, 72.9, 127.5, 127.7, 128.4, 138.7; elemental analysis calcd (%) for $C_{26}H_{44}O_2$: C, 80.35; H, 11.42. Found: C, 80.01; H, 11.30.

3,3′-*O*-[1,18-Octadecan-(*cis*-8,11-methylidene)-methylene]-2,2′-di-*O*-[3-(*R*)-7-dimethyloctyl]-1′-*O*-benzyl-*sn*-diglycerol] (17). A solution of 2,6-lutidine (150 μL, 1.29 mmol, 3.8 equiv) in 20 mL of dry CH_2Cl_2 was cooled to 0 °C under N_2. Triflic anhydride (217 μL, 1.29 mmol, 3.8 equiv) was slowly introduced, and after a few minutes diol **7** (100 mg, 0.34 mmol) was added. The mixture was stirred for 15 min before water was added. The layers were separated, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were washed with 5% aqueous HCl and 5% aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography, eluting with a mixture of petroleum ether and EtOAc (9:1) to afford ditriflate **15** as a colorless oil ($R_f = 0.8$ (petroleum ether/EtOAc 9:1). The crude product was dissolved in dry tetrahydrofuran (3 mL), and a suspension of alcohol **16** (471 mg, 1.02 mmol, 3 equiv) and potassium hydride (35% in oil) (156 mg, 1.36 mmol, 4 equiv) in 3 mL of dry THF was added. The suspension was stirred for a few minutes at room temperature before we added water. The resulting mixture was extracted with ether: the combined extracts were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with a mixture of petroleum ether and ethyl acetate (95:5) to yield the acyclic diol (125 mg, 42%) as a yellow oil ($R_f = 0.8$ (EP/EtOAc 9:1 v/v)). A solution of this diol (1.77 g, 1.2 mmol) in ethyl acetate (50 mL) was stirred overnight in the presence of 10% palladium on activated charcoal (500 mg) under an atmosphere of H_2 at room temperature. The catalyst was removed by filtration, and the filtrate was concentrated to dryness under vacuum to give the deprotected diol as a colorless oil (780 mg, 89%); $R_f = 0.3$ (EP/EtOAc 8:2 v/v); $[\alpha]^{20}_D$ +11.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.58−0.66 (m, 1H), 0.84−0.89 (m, 18H), 1.05−1.83 (m, 48H), 1.86−1.95 (m, 1H), 3.4−3.7 (m, 18H); ^{13}C NMR (400 MHz,

$CDCl_3$) δ 19.67, 19.74, 19.8, 22.7−22.8, 24.4−40.8, 39.4, 40.2, 63.1, 68.7, 71.0, 71.9, 78.3; elemental analysis calcd (%) for $C_{45}H_{90}O_6$: C, 74.32; H, 12.47. Found: C, 73.92; H, 12.36. A solution of 270 mg of this diol (0.37 mmol, 1 equiv) and 45 μL of crown ether 15−5 in 13 mL of tetrahydrofuran was stirred at room temperature for 10 min, and sodium hydride (9 mg, 0.37 mmol, 1 equiv) was added. The reaction mixture was stirred for 10 min followed by dropwise addition of benzyl bromide (53 μL, 0.45 mmol, 1.2 equiv). The solution was refluxed overnight and quenched with addition of water. The aqueous phase was extracted with diethyl ether and ethyl acetate. The organic layers were dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by column chromatography on silica gel eluting with CH_2Cl_2 and then CH_2Cl_2/MeOH (96:4) to give alcohol **17** (0.5 g, 59%) as a colorless oil; $R_f = 0.5$ (EP/EtOAc 9:1 v/v); $[\alpha]^{20}_D$ +4.5° (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.51−0.59 (m, 1H), 0.75−0.80 (m, 18H), 1.00−1.70 (m, 50H), 1.80−1.84 (m, 1H), 2.17 (s, 1H), 3.34−3.66 (m, 18H), 4.48 (s, 2H), 7.18−7.26 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.6, 19.8, 22.7, 22.8, 24.4−40.4, 40.2, 63.1, 68.7, 68.9, 70.3, 70.8, 71.0, 71.7, 71.9, 73.4, 78.0, 78.4, 127.5, 127.6, 128.4, 138.5; elemental analysis calcd (%) for $C_{52}H_{96}O_6$: C, 76.42; H, 11.84. Found: C, 76.74; H, 12.06.

2-*O*-(3-(*R*)-7-Dimethyloctyl)-3-*O*-tetrahydropyranyl-*sn*-glycerol (18). To a solution of alcohol **17** (2 g, 6.2 mmol) and *p*-toluenesulfonic acid (60 mg, 0.3 mmol, 0.5 equiv) in 32 mL of CH_2Cl_2, dihydropyran (876 μL, 9.6 mmol, 1.5 equiv) was added dropwise at 0 °C. The solution was stirred overnight at room temperature, and 88 μL of triethylamine (0.63 mmol, 0.1 equiv) was added. After being stirred for 5 min, norit was introduced, and the mixture was stirred for a few minutes. The reaction mixture was concentrated and filtered on silica gel (CH_2Cl_2). After removal of the solvent, 1.8 g (4.4 mmol) of the protected compound was isolated. To this product dissolved in ethanol (45 mL), 10% palladium on activated charcoal (180 mg) was added, and the solution was stirred under H_2 atmosphere overnight. The catalyst was removed by filtration, and the filtrate was concentrated to dryness to give alcohol **18** (1.26 g, 66%) as a colorless oil; $R_f = 0.3$ (EP/EtOAc 8:2 v/v); $[\alpha]^{20}_D$ +9.4° (*c* 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.76−1.04 (2s, 9H), 1.10−1.84 (m, 16H), 3.27−3.34 (dt, 1H, $J = 6.6$, 9.4 Hz), 3.50−3.80 (m, 7H), 3.84−3.88 (dt, 1H, $J = 3.6$, 7.6 Hz), 4.60 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.6, 19.7, 22.7, 22.8, 24.8, 25.5, 28.0, 29.9, 30.7, 37.2, 37.5, 39.4, 62.3, 63.0, 69.1, 72.0, 78.4, 99.0.

1-*O*-Allyl-2-*O*-(3-(*R*),7-dimethyloctyl)-*sn*-glycerol (19). To a solution of alcohol **18** (1.70 g, 5.4 mmol) in dry tetrahydrofuran (60 mL), sodium hydride (60% in oil) (430 mg, 10.8 mmol, 2 equiv) was added slowly. The solution was stirred for 15 min, and tetrabutylammonium bromide (174 mg, 0.54 mmol, 0.1 equiv) and allyl bromide (701 μL, 8.1 mmol, 1.5 equiv) were then introduced. After being stirred for 5 h, the mixture was diluted with ether, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to dryness under vacuum. To this residue dissolved in methanol (50 mL), *p*-toluenesulfonic acid (100 mg, 0.53 mmol, 0.1 equiv) was added, and the resulting solution was stirred overnight at room temperature. The reaction mixture was concentrated under vacuum, and the residue was purified by silica gel column chromatography elution with a mixture of petroleum ether/EtOAc (9:1 then 8:2 v/v) to give alcohol **19** as a colorless oil (1.08 g, 75%); $R_f = 0.2$ (EP/EtOAc 9/1 v/v); $[\alpha]^{20}_D$ −7.9° (*c* 1.2, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86−0.89 (2s, 9H), 1.12−1.63 (m, 10H), 2.28 (s, 1H), 3.50−3.73 (m, 7H), 4.00−4.01 (d, 2H, $J = 5.6$ Hz), 5.19 (dd, 1H, $J = 1$ Hz, 10.4 Hz), 5.27 (dd, 1H, $J = 1.6$, 17.3 Hz), 5.88 (m, 1H, $J = 6.9$ Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.7, 22.7, 22.8, 24.7, 28.0, 29.8, 37.1, 37.4, 39.3, 62.9, 68.7, 70.1, 72.5, 78.5, 117.2, 134.5; elemental analysis calcd (%) for $C_{16}H_{32}O_3$: C, 70.54; H, 11.84. Found: C, 70.32; H, 11.76.

3-*O*-[1-Octadecan-(*cis*-4,7-methylidene)-18-benzyloxy-methylene]-2-*O*-[3-(*R*),7-dimethyloctyl]-1-*O*-allyl-*sn*-glycerol (21). A solution of 2,6-lutidine (720 μL, 6.16 mmol, 2 equiv) in 40 mL of dry CH_2Cl_2 was cooled to 0 °C under N_2. Triflic anhydride (1.04 mL, 6.16 mmol, 2 equiv) was slowly introduced, and after a few minutes, diol **19** (840 mg, 3.08 mmol) dissolved in 16 mL of CH_2Cl_2 was added dropwise. The mixture was stirred for 15 min before water was added. The layers were separated, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were washed with 5% aqueous HCl, 5% aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography, eluting with a mixture of petroleum ether and EtOAc (9:1) to afford compound **20** (1.12 g, 90%) as a colorless oil ($R_f = 0.6$ (petroleum ether/EtOAc 9:1). To a solution of this crude product in dry tetrahydrofuran (10 mL), a suspension of alcohol **14** (811 mg, 2.09 mmol, 1 equiv) and potassium hydride (35% in oil) (311 mg, 2.7 mmol, 1.3 equiv) in 30 mL of dry THF was added at 0 °C. The suspension was stirred for a few minutes at room temperature before water was added, and the resulting mixture was extracted with ether. The combined extracts were dried over $MgSO_4$, and the solvent was removed at reduced pressure. The residue was purified by column chromatography on silica gel with a mixture of petroleum ether and ethyl acetate (95/5) to yield compound **21** (900 mg, 68%) as a colorless oil; $R_f = 0.6$ (EP/EtOAc 9/1 v/v); $[\alpha]^{20}_D$ +1.3° (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.63 (m, 1H), 0.86 (m, 9H), 1.06−1.75 (m, 40H), 1.89−1.93 (m, 1H), 3.41−3.66 (m, 11H), 4.03 (m, 2H), 4.50 (s, 2H), 5.17 (d, 1H, $J = 10.4$ Hz), 5.26 (d, 1H, $J = 17.3$ Hz), 5.89 (m, 1H, $J = 6.4$ Hz), 7.26−7.35 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.7, 22.7, 22.8, 24.7−40.7, 69.0, 70.3, 70.6, 70.8, 71.9, 72.4, 72.9, 77.9, 116.9, 127.5, 127.7, 128.4, 134.9, 138.7.

3-*O*-[1-Octadecan-(*cis*-4,7-methylidene)-(18-hydroxy)-methylene]-2-*O*-[3-(*R*)-7-dimethyloctyl]-1-*O*-allyl-*sn*-glycerol (22). To a solution of compound **21** (1.8 g, 1.24 mmol) in CH_2Cl_2 (60 mL) and water (3.2 mL), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.41 g, 6.2 mmol, 5 equiv) was added. The reaction mixture was stirred vigorously for 20 h and then quenched with 5% aqueous of sodium carbonate (40 mL). The aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to dryness under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc (the volume ratio was changed from 99:1 to 9:1)) to give deprotected alcohol **22** as a colorless oil (470 mg, 68%); $R_f = 0.5$ (EP/EtOAc 8/2 v/v); $[\alpha]^{20}_D$ +1.5° (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.56 (m, 1H, $J = 11.7$ Hz), 0.80 (m, 9H), 1.00−1.72 (m, 40H), 1.83 (m, 1H, $J = 5.6$ Hz), 3.34−3.60 (m, 11H), 3.94 (d, 2H, $J = 5.6$ Hz), 5.10 (dd, 1H, $J = 1.5$, 10.4 Hz), 5.20 (dd, 1H, $J = 1.8$, 17.3 Hz), 5.84 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.7, 22.7, 22.8, 24.7−40.7, 63.2, 69.0, 70.3, 70,8, 72.0, 72.4, 78.0, 116.9, 134.9; elemental analysis calcd (%) for $C_{35}H_{68}O_4$: C, 76.03; H, 12.40. Found: C, 76.49; H, 12.31.

1-*O*-Benzyl-2-*O*-(3-(*R*)-7-dimethyloctyl)-3-*O*-triflate-*sn*-glycerol (23). A solution of 2,6-lutidine (442 μL, 3.80 mmol, 2 equiv) in 40 mL of dry CH_2Cl_2 was cooled to 0 °C under N_2. Triflic anhydride (639 μL, 3.80 mmol, 2 equiv) was slowly introduced, and after a few minutes diol **16** (612.5 mg, 1.90 mmol) dissolved in 10 mL of CH_2Cl_2 was added dropwise. The mixture was stirred for 15 min before water was added. The layers were separated, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were washed with 5% aqueous HCl, 5% aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography, eluting with a mixture of petroleum ether and EtOAc (9:1) to afford compound **23** (691 mg, 80%) as a colorless oil; $R_f = 0.6$ (petroleum ether/EtOAc 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 (m, 9H), 1.00−1.56 (m, 10H), 3.41−3.52 (m, 4H), 3.65 (m, 1H), 4.46 (s, 2H), 4.48 (d, 1H, $J = 6.1$ Hz), 4.56 (dd, 1H, $J = 3.1$, 10.4 Hz), 7.26−7.35 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.5, 22.6, 22.7, 24.7, 28.0, 29.7, 36.8, 37.3, 39.3, 67.8, 69.3, 73.7, 75.9, 76.0, 118 (q, $J = 320$ Hz), 127.8, 128.0, 128.6, 137.5.

3,3′-*O*-[1,18-Octadecan-(*cis*-4,7-methylidene)-methylene]-2,2′-di-*O*-[3-(*R*)-7-dimethyloctyl]-1′-*O*-benzyl-*sn*-diglycerol (24). To a solu-

tion of triflate (691 mg, 1.52 mmol, 1.6 equiv) in dry tetrahydrofuran (2 mL), a suspension of alcohol **22** (500 mg, 0.96 mmol, 1 equiv) and potassium hydride (35% in oil) (150 mg, 1.15 mmol, 1.2 equiv) in 6 mL of dry THF was added at 0 °C. The suspension was stirred for a few minutes at room temperature before water was added, and the resulting mixture was extracted with ether. The combined extracts were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel with a mixture of petroleum ether and ethyl acetate (95/5) to yield the corresponding acyclic compound (411.5 mg, 50%) as a colorless oil; R_f = 0.6 (EP/EtOAc 8:2 v/v); $[\alpha]^{20}_D$ −4.0° (*c* 0.8. $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.64 (m, 1H, J = 10.7 Hz), 0.85 (m, 18H), 1.10−1.73 (m, 50H), 1.91 (m, 1H), 3.41−3.64 (m, 18H), 4.01 (d, 2H, J = 5.6 Hz), 4.55 (s, 2H), 5.17 (d, 1H, J = 10.4 Hz), 5.27 (d, 1H, J = 17.0 Hz), 5.90 (m, 1H, J = 5.6 Hz), 7.26−7.34 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.7, 22.7, 22.8, 24.7−40.2, 68.9, 69.0, 70.3, 70.8, 71.7, 71.9, 72.4, 73.4, 78.0, 116.9, 127.6, 127.7, 128.4, 138.5, 134.9. To a solution of this compound (50 mg, 0.077 mmol) in a mixture of toluene/EtOH/H_2O (1.25 mL:2.65 mL:400 μL), Wilkinson catalyst $(Ph_3P)_3RhCl$ (15 mg, 0.016 mmol, 0.2 equiv) was added. The reaction mixture was heated at 100 °C for 24 h and stirred 12 h at room temperature. After removal of solvent, the residue was dissolved in diethyl ether. The organic layer was washed with water and brine, dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography eluting with a mixture of petroleum ether/EtOAc (95:5 v/v) to yield compound **24** (47 mg, 78%) as a colorless oil; R_f = 0.3 (EP/EtOAc 8/2 v/v); $[\alpha]^{20}_D$ +12.2° (*c* 1.07, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.65 (m, 1H, J = 10.9 Hz), 0.87 (m, 18H), 1.00−1.73 (m, 50H), 1.90 (m, 1H), 2.22 (s, 1H), 3.41−3.71 (m, 18H), 4.56 (s, 2H), 7.26−7.34 (m, 5H);^{13}C NMR (400 MHz, $CDCl_3$) δ 19.7, 22.7, 22.8, 24.7−40.7, 63.2, 68.7, 68.9, 70.3, 70.8, 71.0, 71.7, 72.2, 73.4, 78.0, 78.3, 127.6, 127.7, 128.4, 138.4; elemental analysis calcd (%) for $C_{52}H_{96}O_6$: C, 76.42; H, 11.84. Found: C, 76.95; H, 11.98.

3,3′-*O*-[1,18-Octadecan-(*cis*-8,11-methylidene)-methylene]-2,2′-di-*O*-[3-(*R*)-7-dimethyloctyl]-1-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*β*-D-glucopyranosyl]-*sn*-diglycerol (27). Compound **17** (109 mg, 0.13 mmol) and lactosyl thioglycoside **25** (136 mg, 0.2 mmol, 1.5 equiv) were combined, rotoevaporated twice with toluene, and then dried for 2 h under vacuum. A solution of this mixture in dry CH_2Cl_2 (4.5 mL) was added to 4 Å molecular sieves. The mixture was treated at 0 °C under N_2 and in the dark with *N*-iodosuccinimide (60 mg, 0.27 mmol, 2 equiv) followed by dropwise addition of triethylsilyl trifluoromethanesulfonate (12 μL, 0.05 mmol, 0.4 equiv). The reaction was quenched with a few drops of triethylamine after 5 min at room temperature. 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 with silica gel column chromatography with petroleum ether/EtOAc 7:3 to give the acetylated lactosylated lipid (89 mg, 48%); R_f = 0.5 (EP/EtOAc 6:4 v/v); $[\alpha]^{20}_D$ −5.2° (*c* 1, $CHCl_3$) as a colorless oil. To a solution of this product (55 mg, 0.04 mmol) in ethanol (2 mL), 10% palladium on activated charcoal (10 mg) was added. The solution was stirred under H_2 atmosphere overnight. The catalyst was removed by filtration, and the filtrate was concentrated to dryness. A purification by column chromatography on silica gel (petroleum ether/EtOAc 7:3)) gave the deprotected monoglycosylated lipid **27** (51 mg, >99%) as a colorless oil; R_f = 0.4 (EP/EtOAc 6:4 v/v); $[\alpha]^{20}_D$ −3.2° (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.52−0.61 (m, 1H, J = 9.9 Hz), 0.79−0.81 (m, 18H), 1.08−1.67 (m, 50H), 1.82−1.85 (m, 1H), 1.91−2.10 (6s, 21H), 3.31−3.63 (m, 18H), 3.75 (t, 1H, J = 9.4 Hz), 3.82−3.86 (m, 2H), 4.00−4.10 (m, 3H), 4.40−4.45 (m, 2H), 4.49 (d, 1H, J = 7.7 Hz), 4.82−4.86 (t, 1H, J = 8.2 Hz), 4.89−4.92 (dd, 1H, J = 3.3, 10.4 Hz), 5.01−5.06 (dd, 1H, J = 8.1, 10.3 Hz), 5.13 (t, 1H, J = 9.4 Hz), 5.28 (d, 1H, J = 2.7 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.6, 20.4, 20.8, 22.5, 22.6, 24.6−39.2, 40.7, 60.8, 62.0, 62.6, 66.6, 68.7, 69.0, 69.1, 70.2, 70.3, 70.5, 70.7, 71.0, 71.6, 71.7, 71.8, 72.5, 72.9, 76.2, 77.7, 78.3, 100.7, 101.0, 169.3, 169.7, 170.0, 170.3, 170.4, 170.6, 170.7; elemental analysis calcd (%) for $C_{71}H_{124}O_{23}$: C, 63.37; H, 9.29. Found: C, 63.09; H, 9.21.

3,3′-*O*-[1,18-Octadecan-(*cis*-8,11-methylidene)-methylene]-2,2′-di-*O*-[3-(*R*)-7-dimethyloctyl]-1-*O*-[4-*O*-(*β*-D-galactopyranosyl)-*β*-D-glucopyranosyl]-1′-*O*-(*β*-D-galactofuranosyl)-*sn*-diglycerol (1). Compound **27** (51 mg, 0.04 mmol, 1 equiv) and pent-4-enyl 2,3,5,6-tetra-*O*-acetyl-α,*β*-D-galactofuranoside **26** (24 mg, 0.06 mmol, 1.5 equiv) were combined, rotoevaporated twice with toluene, and then dried for 2 h under vacuum. A solution of this mixture in dry CH_2Cl_2 (2 mL) was added to 4 Å molecular sieves. The mixture was treated at 0 °C under N_2 and in the dark with *N*-iodosuccinimide (34 mg, 0.16 mmol, 4 equiv) followed by dropwise addition of triethylsilyl trifluoromethanesulfonate (6.8 μL, 0.03 mmol, 0.8 equiv). The reaction was quenched with a few drops of triethylamine after 10 min at room temperature. 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 with silica gel column chromatography with petroleum ether/EtOAc (7:3) to give the acetylated bisglycosylated lipid as a colorless oil (22 mg, 35%); R_f = 0.3 (EP/EtOAc 6:4 v/v); $[\alpha]^{20}_D$ −11.5° (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.57 (m, 1H, J = 10.2 Hz), 0.78−0.80 (m, 18H), 0.97−1.73 (m, 50H), 1.78−1.85 (m, 1H), 1.90−2.09 (m, 33H), 3.31−3.55 (m, 17H), 3.69 (q, 1H, J = 4.0 Hz), 3.72 (t, 1H, J = 9.6 Hz), 3.79−3.84 (m, 2H), 3.99−4.09 (m, 3H), 4.15 (m, 1H), 4.18 (m, 1H), 4.26 (dd, 1H, J = 4.3, 11.9 Hz), 4.40 (m, 2H), 4.48 (d, 1H, J = 7.9 Hz), 4.82−4.93 (m, 3H), 5.00 (m, 2H), 5.05 (m, 1H), 5.12 (t, 1H, J = 9.2 Hz), 5.27 (d, 1H, J = 3.1 Hz), 5.32 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.7, 19.8, 20.6, 20.9, 22.7, 22.8, 24.7−39.3, 40.2, 40.8, 60.8, 62.1, 62.8, 66.6, 67.5, 69.0, 69.2, 69.1, 69.3, 70.5, 70.7, 71.0, 71.7, 71.8, 72.6, 72.9, 76.4, 76.6, 77.7, 77.9, 79.9, 81.3, 100.9, 101.2, 105.8, 169.2, 170.6; elemental analysis calcd (%) for $C_{85}H_{142}O_{32}$: C, 60.91; H, 8.54. Found: C, 61.24; H, 8.72. A solution of sodium methoxide in CH_3OH (0.1 M, 131 μL) was added to a solution of the acetylated bisglycosylated lipid (22 mg, 0.013 mmol) in 2 mL of CH_3OH. The mixture was stirred for 2 h at room temperature, neutralized with a solution of acetic acid in methanol and concentrated under vacuum. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH/H_2O 7:3:0.5) to yield **1** as a pasty solid (16 mg, >95%); R_f = 0.7 ($CHCl_3$/MeOH/H_2O 7:3:0.5 v/v); mp = 125−130 °C; $[\alpha]^{20}_D$ −16.4° (*c* 1, MeOH/$CHCl_3$ 1:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.59 (m, 1H), 0.80 (m, 18H), 0.95−1.68 (m, 51H), 3.14−3.91 (m, 36H), 4.24 (2d, 2H, J = 7.4 Hz), 4.80 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 20.19, 20.23, 23.1, 23.2, 25.9−40.5, 41.2, 41.4, 41.9, 61.9, 62.4, 64.5, 68.4, 69.6, 69.7, 70.2, 70.3, 70.0, 71.8, 72.4, 72.5, 72.6, 72.8, 74.7, 74.8, 76.3, 76.5, 77.1, 78.9, 79.2, 79.3, 80.5, 83.2, 84.6, 104.6, 105.1, 109.7; FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M + Na]^+$, 1235.8220; found, 1235.8217.

3,3′-*O*-[1,18-Octadecan-(*cis*-4,7-methylidene)-methylene]-2,2′-di-*O*-[3-(*R*)-7-dimethyloctyl]-1-*O*-[4-*O*-(*β*-D-galactopyranosyl)-*β*-D-glucopyranosyl]-1′-*O*-(*β*-D-galactofuranosyl)-*sn*-diglycerol (2). Following the same strategy as for compound **1** and starting from alcohol **25**, compound **2** was obtained (first glycosylation: 50% yield; deprotection: 98% yield; second glycosylation: 58% yield; last deprotection: 86% yield), compound **2**: R_f = 0.7 ($CHCl_3$/MeOH/H_2O 7:3:0.5 v/v); mp = 196−199 °C, $[\alpha]^{20}_D$ −9.8° (*c* 0.86, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.59 (m, 1H), 0.80 (m, 18H), 0.95−1.68 (m, 51H), 3.14−3.91 (m, 36H), 4.24 (2d, 2H, J = 7.4 Hz), 4.80 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 20.19, 20.23, 23.1, 23.2, 25.9−40.5, 41.2, 41.4, 41.9, 61.9, 62.4, 64.5, 68.4, 69.6, 69.7, 70.2, 70.3, 70.0, 71.8, 72.4, 72.5, 72.6, 72.8, 74.7, 74.8, 76.3, 76.5, 77.1, 78.9, 79.2, 79.3, 80.5, 83.2, 84.6, 104.6, 105.1, 109.7; FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M + Na]^+$, 1235.8220; found, 1235.8220.

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Supporting Information Available: FFEM of compound **1** in the absence of glycerol and FFEM of compound **1** showing two-by-two vesicle membranes connected by a tunnel-like opening. This material is available free of charge via the Internet at http://pubs.acs.org.

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