Title
Water Permeability and Elastic Properties of an Archaea Inspired Lipid Synthesized by Click Chemistry

Permalink
https://escholarship.org/uc/item/17x690gb

Journal
CHEMISTRY OF MATERIALS, 30(11)

ISSN
0897-4756

Authors
Leriche, Geoffray
Manafirad, Arash
Nguyen, Steven
et al.

Publication Date
2018-06-12

DOI
10.1021/acs.chemmater.8b00992

License
https://creativecommons.org/licenses/by-nc/4.0/ 4.0

Peer reviewed
Water Permeability and Elastic Properties of an Archaea Inspired Lipid Synthesized by Click Chemistry

Geoffray Leriche,*† Arash Manafrad,*§,‖ Steven Nguyen,*† Nia Bell,* Joseph P. Patterson,*‡,⊥ S. Thayumanavan,*§∥ Jerry Yang,* Anthony D. Dinsmore,*§∥ and Nathan C. Gianneschi*§,‖

†Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093-0358, United States
‡Department of Physics, University of Massachusetts, Amherst, Massachusetts 01003, United States
§Department of Chemistry, University of California, San Diego, La Jolla, California 92093-0358, United States
‖Departments of Chemistry, Materials Science & Engineering and Biomedical Engineering, Northwestern University, Evanston, Illinois 60208, United States

Supporting Information

Archaea have evolved mechanically and chemically robust membranes composed of unique lipids to survive in extreme environments (i.e., high temperature, low pH, and high osmotic strength).1 At high temperatures, the polar membrane lipids are mainly bolaamphiphile tetraether scaffolds with branched phytanyl side chains attached via ether bonds to the glycerol carbons at the sn-2,3 positions.2 Archaeal lipids have garnered biological and technological interest as vaccine adjuvants and drug delivery systems because of their non-immunogenic properties and ability to form membranes with reduced permeability.3–5 To obtain lipids with robust and unique membrane properties and overcome the limitations associated with extracting lipids,6 synthetic chemistry has been commonly used.7 Several research groups have designed synthetic routes to access a hemicyclic tetraether scaffold that mimics structural features found in archael tetraether lipids.8–10 Synthetic archaea-inspired boalipids have been experimentally investigated for a long time, mainly for their leakage properties. Thus, compared to liposomes made from commercially available dialcyl lipids, liposomes comprised of pure tetraether lipids generally showed high stability in membrane disrupting conditions,11 and enhanced retention of both ions12 (up to 100-fold) and organic small molecules13,14 (up to 9-fold). However, little information is known regarding the relationship between lipid packing and permeability of membranes made of archael-inspired tetraether lipids.

Herein, we report the synthesis of a new archaea-inspired tetraether lipid and study the mechanical elastic properties of membranes made from this boalipid. To access structural diversity in the hydrophobic moiety of the lipids and to tune membrane properties, we used Huisgen 1,3-dipolar cycloaddition strategy (click chemistry) to design and synthesize a tetraether lipid incorporating phytanyl side chains and polar 1,4 triazole rings known to change lipid packing (Figure 1).15 Notably, the [3+2] azide-terminal alkene cycloaddition approach has been used for lipid functionalization16 and tetra-acyl phospholipid synthesis.17 However, in this work we demonstrate the first example of the synthesis of phytanyl side chain incorporated tetraether lipids with 1,4-triazole rings. We next confirm the ability of lipids incorporating 1,4-triazole rings to form stable small and giant vesicles and examined their physical and mechanical properties using a micropipette aspiration technique. To probe the effect of the 1,4 triazole rings on membrane properties, we also explore the mechanical properties of a previously reported tetraether phospholipid with phytanyl side chains, GMGTPC,12 which lacks 1,4-triazole ring (Figure 1). This work presents the first report of elasticity measurements for synthetic archael-inspired lipids and provides evidence that tuning the hydrophobic core of tetraether bolalipids result in dramatic change in membrane elasticity, which likely arises from changes in lipid packing.

While Egushi et al. previously reported 32 step total syntheses of archael 32- and 72-membered macrocyclic tetraether lipids,18,19 current synthetic strategies to synthesize hemicyclic tetraether bolaamphiphiles with phytanyl side chains involve either O-alkylation20 or dimeric metathesis21,22 of phytanylated glycerol units. Here, we directed our efforts to design a synthetic scheme that would allow for the facile modulation and tuning of the hydrophobic lipid core to the glycerol scaffold while reducing the number of synthetic steps to access new tetraether lipids.

With our design, we envisioned that using a bis-azide hydrophobic linker, we could tether two alkylene-bearing glycerol...
units using click chemistry to easily form the tetraether scaffold (Figure 2a). The length of the hydrophobic linker (20 carbons) between the two triazole rings has been chosen to maintain an adequate hydrophilic–lipophilic balance that is crucial for the formation of stable vesicles. The synthesis begins with the preparation of alkyne phytanylated glycerol 4. First, phytanyl iodide 2 was prepared by the iodination of phytol in the presence of triphenylphosphine and imidazole, and then reacted with 2-phenyl-5-hydroxy-1,3-dioxane to generate the ether derivative 3 in 98% yield. After hydrogenation of the phytanyl olefin using Wilkinson’s catalyst and tert-butanol as a solvent, a selective ring-opening of the dioxane moiety using diisobutylaluminum hydride (DIBAL-H) led to the formation of the benzyl protected glycerol backbone 3 (79% yield, 2 steps). The primary alcohol of 3 was finally reacted with hex-5-yn-1-yl methanesulfonate using KOH in DMSO to give glycerol 4 with 79% yield. Organic bisazide 5 was prepared from the corresponding dibromide. Bis-azole derivative 5 was then reacted with four equivalents of alkyne glycerol 4 using copper(II) sulfate (CuSO₄) and sodium ascorbate in a mixture of dimethylformamide/water/chloroform to ensure complete solubility of reagents and product. The resulting benzylated tetraether scaffold 6, obtained by click chemistry with good yield (87%), was hydrogenated in the presence of palladium on carbon to produce diol 7. The final phospholipid, termed the Archaea-type Lipid 1 (ATL1) was generated in 75% yield by the reaction of diol 7 with 2-bromoethyl dichlorophosphate, which was followed by a nucleophilic displacement of the bromine with trimethylamine, as described previously. Therefore, this synthetic strategy enables the facile synthesis of a tetraether phospholipid lipid incorporating phytanyl side chains in high yields and only in 8 steps starting from phytol 1.

The new lipid was first characterized by differential scanning calorimetry (DSC), which confirmed that ATL1 lipids do not undergo a phase transition above room temperature (Figure S1). The absence of phase transition temperature is in good agreement with reported literature that shows lipids incorporating phytanyl side chains remain in liquid phase at room temperature. We next explored if stable liposomes can form with pure tetraether lipids incorporating 1,4-triazole rings. Cryo-electron microscopy analysis revealed that ATL1 lipid could form ~3.7 ± 0.6 nm thick membrane and ~50 nm diameter small unilamellar vesicles (SUVs) using a standard thin-film hydration followed by extrusion method (Figure 2b and Figure S2).

We next examined whether small molecules could be encapsulated and retained by SUVs made from ATL1 lipids and different mole percentages of cholesterol (10–50%). Calcein was encapsulated in liposomes at a self-quenching concentration of 80 mM and relief of self-quenching due to dilution on leakage was measured by monitoring calcein fluorescence at 515 nm, with excitation at 495 nm. Results from leakage experiments revealed that the presence of cholesterol in liposomal membrane dramatically increases calcein retention whereas liposomes made from pure ATL1 lipids were not able to retain the small molecule (Figure S3). Of all liposomal formulations tested, the greatest retention over 12 h was found when cholesterol content was 30 mol % whereas high and low cholesterol content displayed rapid release of the dye (e.g., 80% of leakage within 2 h with 50 mol % of cholesterol). In contrast, tetraether phospholipids incorporating phytanyl side chains, such as GMGTPC lipids that lack 1,4-triazole rings (Figure 1), have shown high retention for small molecules without the need for cholesterol while having similar membrane thickness to ATL1 (Figure S2). The reduced capability of ATL1 liposomes to retain encapsulated small molecules suggests that 1,4-triazole rings may create kinks in the hydrophobic core, which results in looser lipid packing when compared with liposomes made of GMGTPC lipids but still allows liposome formation. To test this hypothesis further, we fabricated giant unilamellar vesicles (GUVs) of both ATL1 and GMGTPC lipids (see Supporting Information for more details). The giant size of these vesicles (usually 20–40 μm diameter, Figure S4) enabled us to measure the area compressibility modulus, the lysis tension, and the water permeability in a direct way, i.e., application of force on the membrane.

Figure 2. (a) Synthesis of a tetraether phospholipid incorporating phytanyl side chains (Archaea-type Lipid 1 - ATL1) and (b) Cryo-TEM image of extruded SUV prepared with ATL1.
We used the micropipette aspiration technique to assess the mechanical properties of these GUVs. In this technique, the suction pressure applied to a fluid membrane results in a uniform and isotropic membrane tension. The changes in the aspiration length inside the micropipette can then be related to mechanical and physical quantities such as stretching modulus and water permeation coefficient (see Supporting Information for more details). GUVs composed of GMGTPC lipids were used as a point of comparison to probe the effect of 1,4-triazole rings on membrane properties. GUVs of both ATL1 and GMGTPC tetraether phospholipids were formed by electroformation (Figure S4). Water permeability of lipid membranes of both ATL1 and GMGTPC GUVs were measured via micropipette aspiration setup by increasing the solute (that is glucose) concentration in the surrounding hypertonic solution and then monitoring the rate at which water permeated through the membrane (Figure 3a and Figure S5). The permeability coefficient from at least 15 vesicles for each type of tetraether lipid were obtained (Figure 3b). The measured water permeability was 54 ± 3 μm/s for ATL1 and was substantially greater than the value of 21 ± 0.2 μm/s for GMGTPC. GMGTPC tetraether lipids showed lower water permeability than typical phosphocholine based diether lipids (≤18 carbons) with the reported permeability of membranes within the range of 30 to 150 μm/s. Hence, the presence of triazoles makes ATL1 more than 2-fold permeable to water compared with GMGTPC; we return to this point below.

Higher water permeability could also be a direct effect of looser lipid packing. Therefore, we compared the elastic properties of both lipids ATL1 and GMGTPC to study the effect of 1,4 triazole rings on lipid packing. Figure 4a shows the membrane tension versus fractional area expansion, obtained from the aspiration of ATL1 and GMGTPC GUVs as described in the Supporting Information. The increase in membrane tension is directly proportional to the areal expansion with the area compressibility modulus (K_a) being the proportionality constant. Cumulative results for stretching modulus measurements revealed a K_a of 134 ± 3 mN/m for GUVs made from ATL1. For comparison, GUVs made from GMGTPC lipids K_a was 291 ± 3 mN/m, which is more than twice stiffer than ATL1 lipids (Figure 4 and Figure S6). For both ATL1 and GMGTPC, the distribution of K_a values followed a unimodal distribution (Figure S6). This result indicates that all of the vesicles were unilamellar, because the value of K_a is expected to be proportional to the number of lamellae. This assertion is consistent with cryo-TEM images obtained for ATL1 lipids (Figure 2b). For both ATL1 and GMGTPC lipids, K_a lies within the range reported for various lipid systems (K_a = 135–380 mN/m) in parallel comparison. Furthermore, compared to the crystalline or gel states of the reported lipid systems, the moduli of both ATL1 and GMGTPC lipids can be considered soft, representing a liquid-like chain disorder for both lipids.

We then measured the maximum tension that could be sustained by the GUVs without rupture (the lysis tension, *τ*). For ATL1 GUVs, the measured value was *τ* = 2.4 ± 0.1 mN/m (±SEM). For GMGTPC, the value was 5-fold larger, *τ* = 11.0 ± 0.3 mN/m (Figure 4b and Figure S7). Considering the values of K_a and *τ*, the area strain at the rupture point (τ*K_a/K_λ*) drops from ~0.038 for GMGTPC to ~0.018 for ATL1 (Figure 4b). The cohesive energy density (toughness of the membrane) were obtained by considering the area under the stress–strain plots. For ATL1 and GMGTPC, the measured values were 0.025 and 0.18 mJ/m², respectively (Figure 4b and Figure S7).

The micropipette measurements show that the 1,4 triazole moieties in ATL1 membranes increased the water permeability by a factor of 2.6 ± 0.2, and decreased the stretching modulus K_a by a factor of 2.2 ± 0.1 and the lysis tension by a factor of 4.6 ± 0.2 relative to GMGTPC. We now turn to a discussion of these results based on the packing of the lipids and the polarity of the 1,4 triazole moieties. In general, the permeability...
coefficient is directly proportional to the partition coefficient of water between the membrane phase and aqueous solution, and also to the diffusion constant of water within the membrane phase. Triazole group is known to interact with biological molecules through hydrogen bonding and dipole interactions, thus we propose that due to these capabilities, the 1,4 triazole moiety enhances the water partition coefficient relative to GMGTPC. We also propose that 1,4 triazole rings perturb lipid packing, thus decreasing the mechanical strength. Interestingly, when we doped the ATL1 membrane with 30 mol % GMGTPC, a 36% increase in stretching modulus was observed. The cohesive energy of the tetraether lipid incorporating the 1,4 triazole groups was 0.018 mJ/m², which is a factor of 7.0 ± 0.2 lower than GMGTPC and low compared to natural phospholipids (0.05 to 0.5 mJ/m²). On a per-molecule basis, these energy densities are very close to thermal energy. Thus, thermal fluctuations should cause significant variations in density and increase in local lateral compressibility, which all would enhance the diffusion of water through the membrane and lower the work necessary to form molecular-packing defects. Therefore, this potential of defect formation can manifest itself with a higher membrane permeability to water and to small solutes.

Further, we base our reasoning for the compromised stretching modulus observed for ATL1 on the to decrease in the interfacial tension. We rule out the effect of membrane thickness on stretching modulus, because both lipids show similar thicknesses (Figure S2). We may follow Flory’s model, in which the area compressibility modulus of a bilayer is derived to be \( K_a = 6\Pi \) (where \( \Pi \) being the surface pressure of the monolayer). In a flat, tension-free membrane, the surface pressure of a monolayer is a constant of interfacial energy, \( \gamma \) for the exposure of the hydrocarbon to water, thus \( \Pi = \gamma \).

In conclusion, we used click-chemistry to develop a hemicyclic tetraether scaffold incorporating phytanyl side chains and 1,4 triazole rings. A new tetraether phospholipid with 1,4 triazole rings in the hydrophobic core was readily prepared and successfully used for small and giant vesicle with 1,4 triazole rings and 1,4 triazole rings. A new tetraether phospholipid was readily incorporated into the relatively hydrophilic 1,4 triazole rings in the hydrophobic core of an amphiphilic molecule could alter the macroscopic properties of a membrane by increasing permeability by a factor of 2.6 ± 0.2, and decreasing the stretching modulus \( K_a \) by a factor of 2.2 ± 0.1 and the lysis tension by a factor of 4.6 ± 0.2. We postulate that the hydrophilic nature of the triazole moiety and having large dipole moments probably results in higher partitioning of water molecules, thus compromising the membrane integrity. This work introduces new design principles for producing lipid membranes with distinct properties in a synthetically tractable manner.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemmater.8b00992.

**REFERENCES**

(1) Woese, C. R.; Magrum, L. J.; Fox, G. E. Archaebacteria. J. Mol. Evol. 1978, 11 (3), 245–252.
(2) Chong, P. L. G.; Ayesa, U.; Prakash Daswani, V.; Hur, E. C. On Physical Properties of Tetraether Lipid Membranes: Effects of Cyclopetane Rings. *Archaea* 2012, 2012, 1–11.
(3) Patel, G. B.; Zhou, H.; KuoLee, R.; Chen, W. Archaeosomes as Adjuvants for Combination Vaccines. *J. Liposome Res.* 2004, 14 (3–4), 191–202.
(4) Garg, T.; Singh, S.; Goyal, A. Stimuli-Sensitive Hydrogels: An Excellent Carrier for Drug and Cell Delivery. *Crit. Rev. Ther. Drug Carrier Syst.* 2013, 30 (5), 369–409.
(5) Spott, G. D. Archael Membrane Lipids and Applications. In *cls*; John Wiley & Sons, Ltd: Chichester, U. K., 2011.
(6) Uda, I.; Sugai, A.; Itoh, Y. H.; Itoh, T. Variation in Molecular Species of Polar Lipids from Thermoplasma Acidiphilum Depends on Growth Temperature. *Lipids* 2001, 36 (1), 103–105.
(7) Mercer, J. A. M.; Cohen, C. M.; Shuken, S. R.; Wagner, A. M.; Smith, M. W.; Moss, F. R.; Smith, M. D.; Vahala, R.; Gonzalez-Martinez, A.; Boxer, S. G.; Burns, N. Z. Chemical Synthesis and Self-Assembly of a Ladderene Phospholipid. *J. Am. Chem. Soc.* 2016, 138 (49), 15845–15848.
(8) Koyanagi, T.; Cao, K. J.; Leriche, G.; Onofrei, D.; Holland, G. P.; Mayer, M.; Sept, D.; Yang, J. Hybrid Lipids Inspired by Extremophiles and Eukaryotes Afford Serum- Stable Membranes with Low Leakage. *Chem. - Eur. J.* 2017, 23 (28), 6757–6762.
(9) Patwardhan, A. P.; Thompson, D. H. Efficient Synthesis of 40- and 48-Membered Tetraether Macrocyclic Biphasocholines. *Org. Lett.* 1999, 1 (2), 241–244.
(10) Markowski, T.; Drescher, S.; Meister, A.; Blume, A.; Dobner, B. Structure-Property Relationships in a Series of Diglycerol Tetraether Model Lipids and Their Lyotropic Assemblies: The Effect of Branching Topology and Chirality. *Org. Biomol. Chem.* 2014, 12 (22), 3649–3662.
(11) Benevgnu, T.; Réthoré, G.; Brard, M.; Richter, W.; Plusquellec, D. Archaeosomes Based on Novel Synthetic Tetraether-Type Lipids for the Development of Oral Delivery Systems. *Chem. Commun.* 2005, 44, 5536–5538.
(12) Koyanagi, T.; Leriche, G.; Onofrei, D.; Holland, G. P.; Mayer, M.; Yang, J. Cyclohexane Rings Reduce Membrane Permeability to Small Ions in Archaea-Inspired Tetraether Lipids. *Angew. Chem., Int. Ed.* 2016, 55 (5), 1890–1893.

(13) Arakawa, K.; Eguchi, T.; Kakinuma, K. Highly Thermostable Liposome from 72-Membered Macroyclic Tetraether Lipid: Importance of 72-Membered Lipid for Archaea to Thrive under Hyperthermal Environments. *Chem. Lett.* 2001, 30, 440–441.

(14) Leriche, G.; Cifelli, J. L.; Sibucuo, K. C.; Patterson, J. P.; Koyanagi, T.; Gianneschi, N. C.; Yang, J. Characterization of Drug Encapsulation and Retention in Archaea-Inspired Tetraether Liposomes. *Org. Biomol. Chem.* 2017, 15 (10), 2157–2162.

(15) O’Neil, E. J.; DiVittorio, K. M.; Smith, B. D. Phosphatidylcholine-Derived Bolaamphiphiles via Click Chemistry. *Org. Lett.* 2007, 9 (2), 199–202.

(16) Frisch, B.; Hassane, F. S.; Schuber, F. Conjugation of Ligands to the Surface of Preformed Liposomes by Click Chemistry BT. In *Liposomes: Methods and Protocols, Vol. 1: Pharmaceutical Nanocarriers*; Weissig, V., Ed.; Humana Press: Totowa, NJ, 2010; pp 267–277.

(17) Mitchell, G. M.; Hesketh, A.; Lombardi, C.; Ho, C.; Fyles, T. M. A Membrane-Spanning Macroyclic Bolaamphiphile Lipid Mimic of Archaeal Lipids. *Can. J. Chem.* 2012, 90 (3), 253–262.

(18) Eguchi, T.; Ibaragi, K.; Kakinuma, K. Total Synthesis of Archaeal 72-Membered Macroyclic Tetraether Lipids. *J. Org. Chem.* 1998, 63 (8), 2689–2698.

(19) Eguchi, T.; Arakawa, K.; Terachi, T.; Kakinuma, K. Total Synthesis of Archaeal 36-Membered Macroyclic Diether Lipid. *J. Org. Chem.* 1997, 62 (7), 1924–1933.

(20) Brard, M.; Richter, W.; Benvegnu, T.; Plusquellec, D. Synthesis and Supramolecular Assemblies of Bipolar Archaeal Glycolipid Analogues Containing a Cis-1,3-Disubstituted Cyclopentane Ring. *J. Am. Chem. Soc.* 2004, 126 (32), 10003–10012.

(21) Arakawa, K.; Eguchi, T.; Kakinuma, K. An Olefin Metathesis Approach to 36- and 72-Membered Archaeal Macroyclic Membrane Lipids. *J. Org. Chem.* 1998, 63 (14), 4741–4745.

(22) Febo-Ayala, W.; Morera-Félix, S. L.; Hrycyna, C. A.; Thompson, D. H. Functional Reconstitution of the Integral Membrane Enzyme, Isoprenylcysteine Carboxyl Methyltransferase, in Synthetic Bolalipid Membranes. *Biochemistry* 2006, 45 (49), 14683–14694.

(23) Koga, Y. Thermal Adaptation of the Archaeal and Bacterial Lipid Membranes. *Archaea* 2012, 2012, 1–6.

(24) Weinstein, J.; Yoshikami, S.; Henkart, P.; Blumenthal, R.; Hagins, W. Liposome-Cell Interaction: Transfer and Intracellular Release of a Trapped Fluorescent Marker. *Science* 1977, 195 (4277), 489–492.

(25) Angelova, M. I.; Dimitrov, D. S. Liposome Electroformation. *Faraday Discuss. Chem. Soc.* 1986, 81, 303–311.

(26) Veatch, S. L. Electro-Formation and Fluorescence Microscopy of Giant Vesicles With Coexisting Liquid Phases. In *Lipid Rafts*; McIntosh, T. J., Ed.; Humana Press: Totowa, NJ, 2007; pp 59–72.

(27) Olbrich, K.; Rawicz, W.; Needham, D.; Evans, E. Water Permeability and Mechanical Strength of Polysaturated Lipid Bilayers. *Biophys. J.* 2000, 79 (1), 321–327.

(28) Israelachvili, J. N. *Intermolecular and Surface Forces*, 3rd ed; Academic Press: San Diego, 2011.

(29) Evans, E.; Needham, D. Physical Properties of Surfactant Bilayer Membranes: Thermal Transitions, Elasticity, Rigidity, Cohesion and Colloidal Interactions. *J. Phys. Chem.* 1987, 91 (16), 4219–4228.

(30) Finkelstein, A. Water Movement through Lipid Bilayers, Pores and Plasma Membranes. *John Wiley & Sons, Inc*: New York, 1987; Vol. 4.

(31) Kolb, H. C.; Sharpless, K. B. The Growing Impact of Click Chemistry on Drug Discovery. *Drug Discovery Today* 2003, 8 (24), 1128–1137.

(32) Needham, D.; Nunn, R. S. Elastic Deformation and Failure of Lipid Bilayer Membranes Containing Cholesterol. *Biophys. J.* 1990, 58 (4), 997–1009.

(33) Nagle, J. F.; Scott, H. L. Lateral Compressibility of Lipid Mono- and Bilayers. Theory of Membrane Permeability. *Biochim. Biophys. Acta, Biomembr.* 1978, 513 (2), 236–243.