Synthesis of Ether-Linkage-Based Cyclic Glycerophospholipid Carrying Methylene Moiety that Improves Membrane Fluidity

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Received 10 October 2008

Abstract: A cyclic glycerophospholipid, in which two glycerols are connected via alkyl chains to form a 64-membered macrocycle, was synthesized. The structural features that differentiate this from our analogous lipids previously reported are two methylene moieties in the alkyl chains, which were incorporated to improve membrane fluidity. Fluorescence recovery after photobleaching revealed that the membranes made from the methylene-containing cyclic lipid demonstrated fluidity at room temperature unlike those of a linear alkyl-chain lipid of equal ring size.

Key words: lipids, macrocycles, ether lipid, phospholipid, cyclic lipid, FRAP

Phospholipids are a major component of membranes in living cells. Some of these have been utilized to construct artificial membranes mimicking biomembranes and other micro/nanostructures that have beneficial functions.[1–5] Physical lability in the membranes sometimes arises as a problem in the design of these assemblies when their functions are utilized and examined.[6,7] We focused our attention on ether-linkage-based cyclic lipids as potent materials that could overcome this weakness. These are naturally found in some species of archaea distributed over extreme environments such as those with high temperatures.[8,9] Thus, a membrane that is composed of such lipids is expected that it would be much more stable than one composed of ester-based, two-tailed phospholipids. With this idea in mind, we are continuing to make progress with our research on ether lipids to establish membrane systems that work as a model of living cells or functional micro/nanostructures.

The feasibility of obtaining large quantities of ether cyclic lipids from natural sources presently appears dubious; only trace amounts can be obtained by standard culturing methods. Thus, we need to develop synthetic methods for the natural cyclic lipids or those mimics. We previously reported the preparation of ether lipids **1** (Figure 1), which mimic natural cyclic lipids.[10,11] These molecules consist of two glycerol components that are attached to a phosphorylcholine residue at each end and are connected via two long hydrocarbon chains to form a macrocycle (n = 8, 10, 12, and 14 where the total length of each chain is 20, 24, 28, and 32 carbons). Diyne moieties at the middle of the chains have been incorporated to make the molecule more rigid and keep the molecular conformation a straight I-shape. By doing so, self-assembly of the lipid molecules into monolayer membrane can be expected.[11,12] A series of lipids **1** with different chain lengths were prepared so that the thickness of the resulting vesicles would match a variety of functional membrane proteins that would be incorporated. We found that synthetic cyclic lipids were successfully formed into monolayer structures such as vesicles.[11,12] We also found that the membranes of lipid **1** (n = 8), which has a maximum thickness of 4.8 nm and a fluidic character at room temperature, reconstituted bacteriorhodopsin (a membrane protein found in the natural purple membrane of *Halobacterium salinarum* with a thickness of 4.9 nm).[13] However, we anticipated that ves-

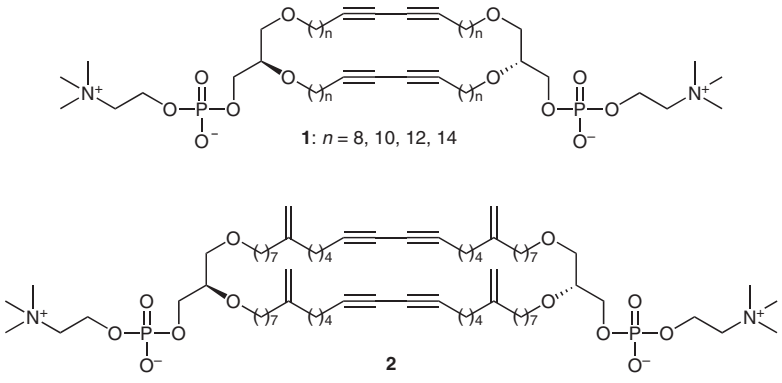
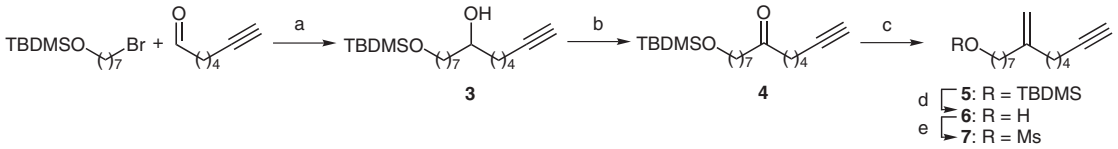


Figure 1

SYNLETT 2009, No. 5, pp 0759–0762
Advanced online publication: 24.02.2009
DOI: 10.1055/s-0028-1087935; Art ID: U11108ST

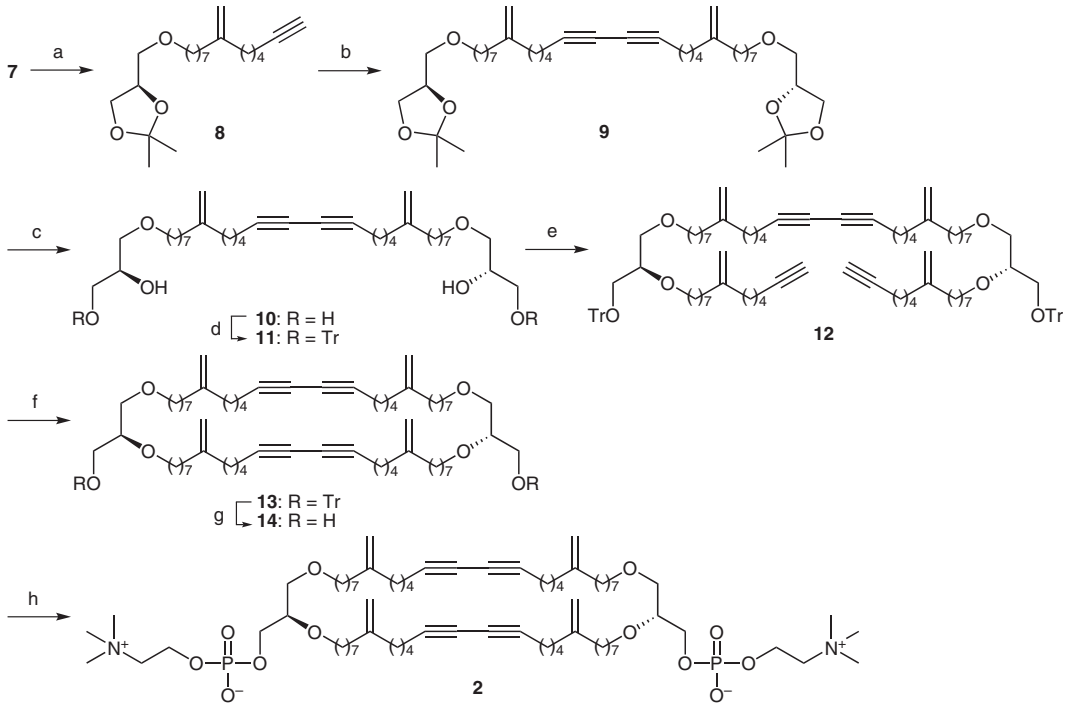


Scheme 1 *Reagents and conditions*: (a) Mg, THF, reflux, then hept-6-ynal, 0 °C, 91%; (b) cat. TPAP, NMO, CH_2Cl_2, r.t., 89%; (c) $MePh_3P^+I^-$, NaH, DMSO, r.t., 63%; (d) concd HCl, EtOH, r.t., 87%; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C, 91%.

icles made from lipids with longer chain lengths (**1**, n = 10, 12, 14) would be too rigid for reconstituting membrane proteins.[11] That is, the vesicles demonstrated gel-to-liquid-crystalline phase transition above room temperature (in water). This property is detrimental, because proteins exhibit functions in the liquid-crystalline phase but not in the gel phase, and they normally degenerate at higher temperatures.

To resolve this drawback with lipids **1** (n = 10), we introduced a 'horn' onto the chains connecting the glycerols. We expected that it would work as a steric hindrance to the aggregation of lipid molecules and weaken the interaction between molecules when the lipid membrane formed, so that the phase-transition temperature could be reduced. We chose a methylene group as the 'horn'. In natural membrane lipids incorporated olefin and isoprenoid moieties have been found to work as a functional group that drops the phase-transition temperature of a lipid membrane.[14,15] The alkylidene group we introduced this time is a novel type of such functionality that may have the ability to drop the phase-transition temperature, which gives high mobility to the membrane. Preparing and introducing a natural or related optically active isoprenoid moiety into an alkyl chain as its partial structure are tough works that require a complicated reaction sequence.[16] The alkylidene group can be introduced more easily (by using a practical method such as the Wittig reaction, for example) because it does not require a stereocontrolled or stereospecific reaction to introduce it. In other words, when the 'horn' moiety is introduced into the chain, a chemical conversion that leads to an additional asymmetric carbon into the molecule and makes it diastereomeric or racemic is detrimental (e.g., the Grignard reaction makes the carbonyl carbon racemic, yielding a mixture of stereoisomers). The structure of the ether lipid **2** synthesized in this work is outlined in Figure 1. We also report its membrane properties.

First, half the hydrocarbon chains that connected the two glycerols was synthesized. The procedure is outlined in Scheme 1. 7-Bromoheptan-1-ol with the hydroxy group being protected as TBDMS ether was joined with hept-6-ynal by using the Grignard reaction in THF. The alcohol **3** thus obtained was subjected to oxidation with TPAP/NMO to give ketone **4**. Then, the 'horn' moiety was introduced by using the Wittig reaction. Thus, the action of **4** with methylene triphenylphosphine generated from methyltriphenylphosphonium iodide and NaH in DMSO successfully gave olefin **5**. To perform further transfor-



Scheme 2 *Reagents and conditions*: (a) 1,2-isopropylidene-*sn*-glycerol, NaH, DMSO, r.t., 91%; (b) CuCl, TMEDA, O_2, acetone, reflux, 97%; (c) TsOH, MeOH, r.t., 71%; (d) TrCl, DMAP, Et_3N, CH_2Cl_2, r.t., 46%; (e) **7**, NaH, DMSO, 60 °C, 51%; (f) $Cu(OAc)_2$, pyridine, reflux, 61%; (g) TsOH, MeOH, r.t., 85%, (h) bromoethyl dichlorophosphate, Et_3N, benzene, r.t., then 30% aq Me_3N, *i*-PrOH–MeCN–$CHCl_3$, 60 °C, 50%.

mations, the TBDMS protecting group on **5** was replaced by the Ms group to yield compound **7**, which is the key counterpart in the preparation of the required cyclic lipids.

Next, by using the Williamson reaction, the half-chain part **7** was joined with a glycerol in which two of the three hydroxy groups were protected as acetonide (1,2-isopropylidene-*sn*-glycerol, Scheme 2). By using glycerol acetonide, the entire molecule became chiral. We consider that the lipid molecules needed to be chiral for the sake of mimicry with natural membrane lipids, mainly from the viewpoint of interaction with protein molecules. Then, terminal alkyne **8** was coupled to give diyne **9** by using a CuCl catalyst under Hay conditions in hot acetone.[17] Before the second chain moiety was introduced, the acetonide protective groups of **9** were replaced by Tr groups; the acetonides were hydrolyzed by using an acid catalyst, and the terminal primary hydroxy groups in **10**[18] in Scheme 2 were selectively masked by the Tr groups. The reaction of **11**[19] in Scheme 2 with NaH and then **7** in warm DMSO yielded **12**, which was cyclized to **13** under highly diluted Eglinton coupling conditions in the presence of excess $Cu(OAc)_2$ in refluxing pyridine.[20,21]

Finally, the Tr protecting groups on **13** were removed and the hydroxy groups were converted into phosphorylcholine residue by successive action with bromoethyl dichlorophosphate and trimethylamine. Isolation with gel permeation chromatography using $CHCl_3$–MeOH as an eluent gave the required 'horned' cyclic lipid **2** in pure form.[22]

The membrane fluidity composed of the 'horned' cyclic lipid **2** was proved by fluorescence recovery after photobleaching (FRAP). After bleaching with irradiation, the recovery of fluorescence was observed in the defined region of a monolayer membrane of **2** developed on a glass plate. This meant that the bleached molecules were exchanged with unbleached molecules on the membrane, thus revealing the mobility of lipid molecules that composed the membrane. However, the membranes of the previously synthesized straight chain analogue **1** (n = 10) demonstrated gel-like characteristics at room temperature.[11,23] Altogether, we think that the 'horn' worked well as an effective steric hindrance to improve membrane fluidity.

Acknowledgment

We are grateful to the AIST Upbringing of Talent in the Nanobiotechnology Course and the Promotion Budget for Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology for their support in this research.

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(17) **Selected Physical Data of 9**
^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30 (12 H, br s), 1.36 (6 H, s), 1.42 (6 H, s), 1.52 (8 H, quint, *J* = 5.4 Hz), 1.96–2.02 (8 H, m), 1.58 (8 H, br m), 2.26 (8 H, br m), 3.39–3.53 (8 H, m), 3.72 (2 H, dd, *J* = 8.2, 6.4 Hz), 4.05 (2 H, dd, *J* = 8.2, 6.4 Hz), 4.26 (2 H, quint, *J* = 6.0 Hz), 4.69 (4 H, br s). ESI-MS (TOF): *m/z* = 694 [M + Na^+], 672 [M + H^+].
(18) **Selected Physical Data of 10**
^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.32 (12 H, br s), 1.42 (4 H, quint, *J* = 7.2 Hz), 1.53 (8 H, quint, *J* = 3.4 Hz), 1.59 (4 H, quint, *J* = 7.0 Hz), 1.98–2.02 (8 H, br s), 2.28 (4 H, br m), 3.45–3.56 (8 H, m), 3.66 (2 H, dd, *J* = 11.4, 5.0 Hz), 3.73 (2 H, dd, *J* = 11.5, 4.1 Hz), 3.87 (2 H, quint, *J* = 4.7 Hz), 4.71 (4 H, br s). ESI-MS (TOF): *m/z* = 614 [M + Na^+], 591 [M + H^+].
(19) **Selected Physical Data of 11**
^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (12 H, br s), 1.40 (4 H, quint, *J* = 7.1 Hz), 1.51 (8 H, quint, *J* = 4.2 Hz), 1.96–1.99 (8 H, br m), 2.25 (4 H, br m), 2.42 (2 H, d, *J* = 4.6 Hz), 3.19 (4 H, qd, *J* = 7.6, 5.4 Hz), 3.40–3.54 (8 H, m), 3.94 (2 H, sext, *J* = 5.1 Hz), 4.69 (4 H, br m), 7.22 (6 H, t, *J* = 7.3 Hz), 7.29 (12 H, t, *J* = 7.3 Hz), 7.42 (12 H, d, *J* = 7.3 Hz). ESI-MS (TOF): *m/z* = 1098 [M + Na^+].
(20) **Procedure for the Preparation of 13**
To a solution of $Cu(OAc)_2$ (314 mg, 1.73 mmol) in refluxing pyridine (100 mL), a solution of **12** (257 mg, 0.173 mmol) in pyridine (10 mL) was added dropwise with a syringe pump over 5.5 h. The solution gradually turned from blue to dark green. After completion of the addition the solution was heated at the same temperature for 1 h. The mixture was cooled to r.t. and evaporated to give a residue, which was purified on a silica gel column chromatography eluted with hexane–EtOAc (10:1) to give **13** as a yellow oil; yield 157 mg (61%).
^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (24 H, br s), 1.39 (8 H, br m), 1.51–1.57 (16 H, br m), 1.96–2.01 (16 H, m), 2.28 (br s, 8 H), 3.13–3.20 (4 H, br m), 3.39 (4 H, br t, *J* = 6.9 Hz), 3.45–3.58 (10 H, m), 4.68 (8 H, br s), 7.20 (6 H, t, *J* = 7.1 Hz), 7.27 (12 H, t, *J* = 7.3 Hz), 7.45 (12 H, d, *J* = 7.8 Hz). ESI-MS (TOF): *m/z* = 1505 [M + Na^+].

(21) The sequence from **7** and glycerol moiety to **12** was altered from the one we previously reported for preparing a straight-chain analogue **1**.[10,11] At the step of the connection of chain moiety and protected glycerol in the previous study, we used 1-trityl-3-*p*-methoxybenzoyl-*sn*-glycerol, which was prepared by starting from 1,2-isopropylidene-*sn*-glycerol in three steps. Through this modification, several protection–deprotection steps could be omitted. The order in which the chains was introduced into the glycerol moiety was also changed. This change would cause an increase in the steric hindrance around the secondary hydroxy groups during the Williamson reaction sequence; however, the reaction proceeded without difficulties even though the yield of **12** was somewhat lower.

(22) Purification of **2** was conduced on an LC-908 instrument (Japan Analytical Industries) equipped with a JAIGEL-GS310 column [eluent, $CHCl_3$–MeOH = 1:2 (v/v)]. Colorless gummy oil. ^{1}H NMR (400 MHz, $CDCl_3$–CD_3OD = 2:1): δ = 1.30 (28 H, br s), 1.41 (12 H, br s), 1.52 (20 H, br s), 1.97–2.01 (16 H, t, *J* = 7.6 Hz), 2.27 (8 H, br m), 3.23 (18 H, s), 3.38–3.49 (6 H, m), 3.55–3.61 (12 H, m), 3.88 (4 H, br s), 4.25 (4 H, br s), 4.70 (8 H, br s). ESI-MS (TOF): *m/z* = 1350 [M + Na^+], 1328 [M + H^+].

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