Significance of the 72-Membered Macrocyclic Structure Found in Archaeal Membrane Lipids: Model Studies of the Macrocyclic Tetraether Diphospholipids by Calorimetric, ^{31}P NMR, and Electron Microscopic Analyses

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Described in this paper are the characterizations of the synthetic 72-membered macrocyclic tetraether phospholipids (**7**, **8**) and their acyclic congeners (**9**, **10**), the hydrocarbon chains of which are unbranched straight chains instead of isoprenoid chains such as found in those of archaea. Thermal analyses by differential scanning calorimetry (DSC) clearly showed that the phase transition temperature (T_c) of the cyclic tetraether lipids **7** and **8** was rather lower than those of the corresponding acyclic counterparts **9** and **10**. This trend was opposite to that of the diether lipids. The findings from ^{31}P NMR and electron microscopic analyses suggested that the lipid polymorphism of **7** and **8** was quite different from that of **9** and **10**. These results revealed that the cyclic structure in **7** and **8** contributed not only to the limitation of motional freedom at the hydrophobic region but also to the change of the lipid polymorphism in comparison with **9** and **10**. Furthermore, it was found that these physicochemical as well as polymorphic properties were indistinguishable between the regioisomers **7** and **8** (or **9** and **10**).

Living cells use a lipid membrane system as a barrier to separate the inside from the outside of a cell. Archaea (archaebacteria) including halophiles, methanogens, and thermoacidophiles possess unique membrane lipids distinct from those of bacteria and eucarya.[1] Archaeal lipids are composed of isoprenoid alkyl chains connected to *sn*-2 and -3 positions of glycerol with ether linkages, while bacterial and eucaryal lipids are mostly composed of straight-chain alkyls connected to *sn*-1 and -2 positions of glycerol with ester linkages. Some species of archaea possess 36- and/or 72-membered macrocyclic membrane lipids.[2] These features of the archaeal lipids appear to be favorable adaptation against extreme growth conditions (anaerobic, salt-rich, highly acidic, and/or high temperature). The unique structures of the archaeal lipids have attracted much attention in terms of biochemical and physicochemical aspects, and several model studies have been reported on the fluidity, permeability and thermostability of the membranes.[3] Menger et al. was the first to discuss the properties of the synthetic diether phospholipids with tethering of alkyl chains as a model of 36-membered lipid. They showed that, (i) the phase transition temperature T_c was raised by tethering, probably due to enlarged energy requirements to transit disordered conformation in the cyclic lipid, (ii) the phase transition enthalpy ΔH_c was lowered due to reducing the number of gauche C–C bonds, and (iii) the phase transition entropy ΔS_c was also reduced by preventing the motional freedom of the alkyl chain.[3i] Similar results were also obtained in our model studies with related phospholipids.[3l]

Some methanogens and thermoacidophiles possess tetraether bipolar membrane lipids of 72-membered macrocyclic structure. This unique membrane lipid is expected to form an unimolecular assembly, which seems similar to a covalently bound bilayer structure. It may be highly resistant to membrane fluidity, due to the limitation of lateral diffusion, which seems to play a significant role for some archaea to thrive under extreme environments.[4]

In order to investigate the polymorphism and physicochemical properties of the macrocyclic lipids, we have synthesized 72-membered macrocyclic tetraether lipids[5] and the corresponding acyclic tetraether lipids.[5a] Interestingly, Arigoni et al. found that the 72-membered lipids exist as an inseparable regioisomer mixture in terms of a glycerol arrangement such as **1** and **2** illustrated in Fig. 1,[6] which additionally prompted us to study the significance of the differences in physicochemical properties between these regioisomers. Previously, the properties of the archaeal tetraether lipids were mostly investigated by using the mixtures of numerous structures of natural origin.[7] Little was reported about the significance of macrocyclic structure and of the difference in features between the regioisomers in the tetraether lipids. In this paper, we describe the properties of the 72-membered macrocyclic model diphospholipids **7** and **8** characterized by

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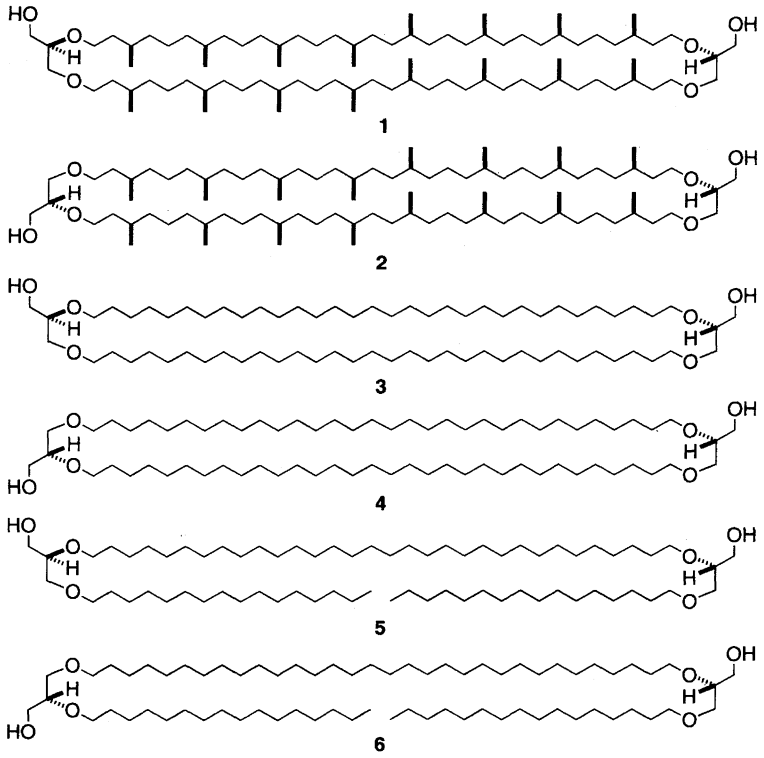


Fig. 1. Structures of tetraether core lipids.

calorimetric, ^{31}P NMR, and electron microscopic analyses, in comparison with the corresponding acyclic counterparts **9** and **10** (Fig. 2).

Results and Discussion

Recently, Menger et al. described a pioneering synthesis of a 72-membered bisphosphocholine derivative.[3k] But the lipid was reported to be an intractable solid due to poor solubility, which did not allow any further study of the physicochemical properties of the synthesized lipid. Instead of phosphocholine, we chose the well-studied phosphate structure as the polar head group.[3e,3f]

The synthetic routes to the lipids are illustrated in Scheme 1. In the presence of DMAP and pyridine, 72-membered diol **3**[5a] was treated with diphenylphosphoryl chloride[8] in benzene at 60 °C to give the corresponding bisphosphotriester **13** in an excellent yield. Then, the bisphosphotriester **13** was subjected to catalytic hydrogenation in the presence of platinum dioxide to afford for the first time, after recrystallization, the desired bisphosphoric acid derivative **7** in 40% yield. The regioisomer **8** was also synthesized similarly from diol **4**.

To figure out how the cyclic structure of tetraether lipids contributes to the physicochemical properties, comparative studies with the corresponding acyclic tetraether lipids are essential. Acyclic core lipids **5** and **6**[5a] were thus converted to the bisphosphoric acids **9** and **10**, respectively.

The physicochemical features of these diphospholipids were studied by differential scanning calorimetry (DSC), ^{31}P NMR, and electron microscopic analyses. First, we performed the DSC analyses of the lipids in ethylene glycol solvent.[9] The cyclic tetraether lipids appeared to show lower phase transition temperatures T_c (61.6 °C for **7**, 64.0 °C for **8**) than the corresponding acyclic counterparts (81.6 °C for **9**, 83.1 °C for **10**) (Table 1, Fig. 3). These diphospholipids are in solid state at ambient temperature. Interestingly, these trends were opposite to those of diether lipids. Both the phase transition enthalpies ΔH_c and the phase transition entropies ΔS_c of the cyclic lipids were also lower (ΔH_c = 1.7 kcal mol^{-1}, ΔS_c = 5.1 cal mol^{-1} deg^{-1} for **7**, ΔH_c = 2.9 kcal mol^{-1}, ΔS_c = 8.6 cal mol^{-1} deg^{-1} for **8**) than those of the acyclic lipids (ΔH_c = 13 kcal mol^{-1}, ΔS_c = 36 cal mol^{-1} deg^{-1} for **9**, ΔH_c = 14 kcal mol^{-1}, ΔS_c = 39 cal mol^{-1} deg^{-1} for **10**). Menger et al. reported that a cyclic diether lipid such as **11** possessed higher T_c and lower ΔH_c and ΔS_c than their acyclic counterpart **12**.[3i] A similar observation was also described by us.[3l] These results of diether models suggested that cyclization of the alkyl chains in the lipid molecules caused three significant effects: (i) T_c was raised due to an increased energy requirement for transition of the disordered

Table 1. Phase Transitions of the Tetraether Phospholipids **7**—**10**[a)]

Lipid	T_c (°C)	ΔH_c (kcal mol^{-1})	ΔS_c (cal mol^{-1} deg^{-1})
7	61.6	1.7	5.1
8	64.0	2.9	8.6
9	81.6	13	36
10	83.1	14	39

a) Thermograms were scanned at the temperature range from 50 to 130 °C at a rate of 2 °C min^{-1}. These lipids were dispersed in ethylene glycol.

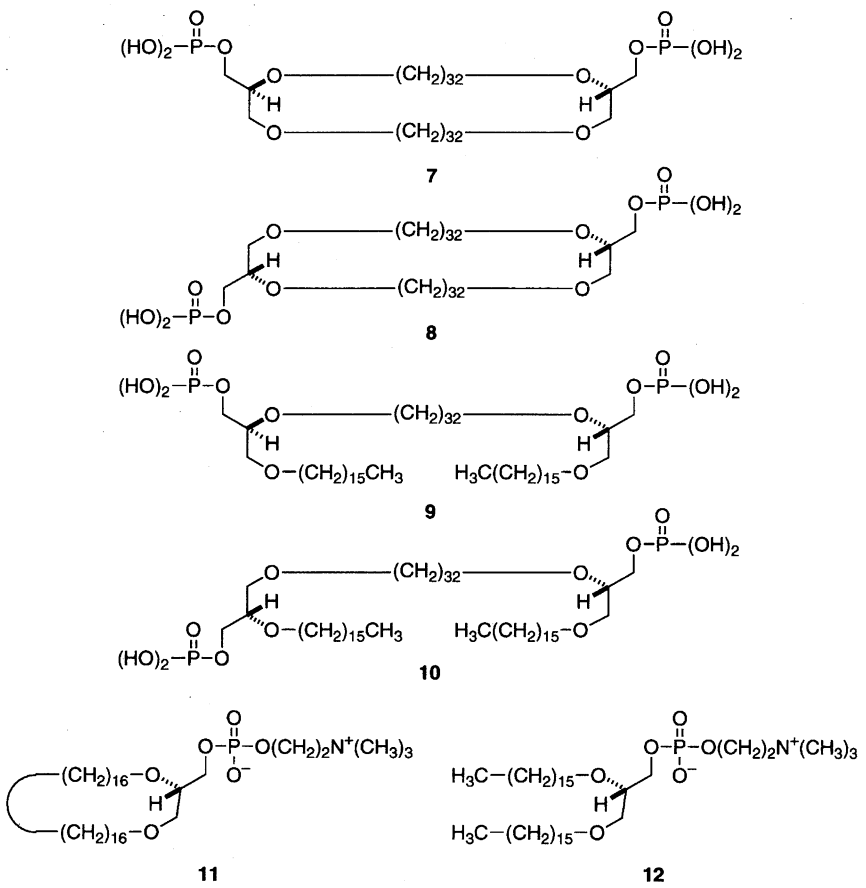
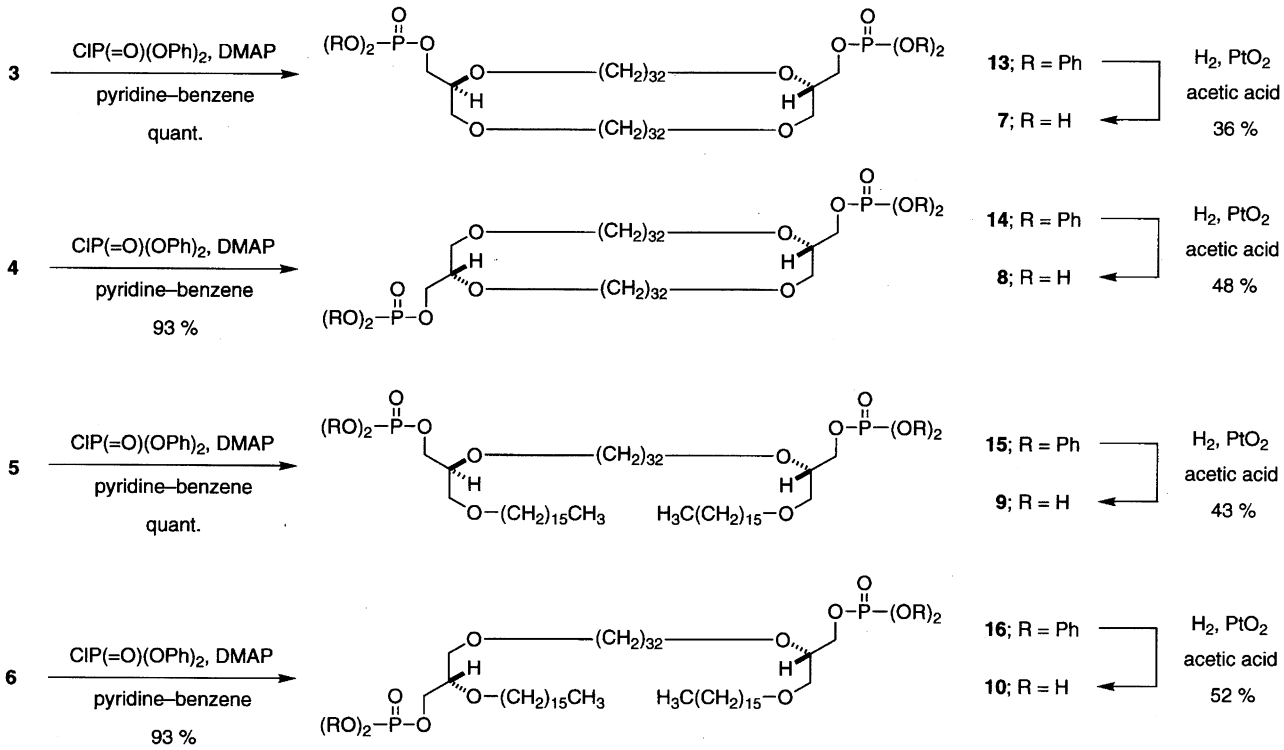


Fig. 2. Structures of tetraether model phospholipids **7**—**10**.



Scheme 1.

conformation, (ii) ΔH_c was lowered due to a reduced number of gauche C–C bonds, and (iii) ΔS_c was also lowered by preventing the motional freedom of the alkyl chains. Based on the observation that the values of T_c for **7** and **8** were

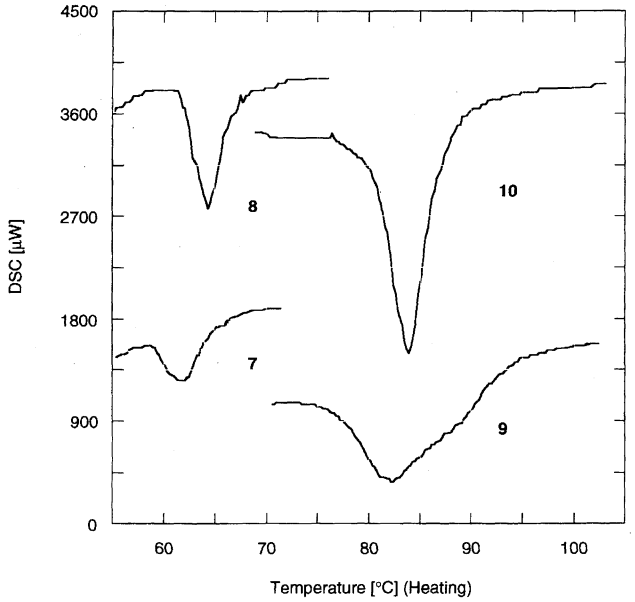


Fig. 3. DSC thermograms of tetraether phospholipids (cyclic; **7** and **8**, acyclic; **9** and **10**).

lower than those of **9** and **10**, it appears that the cyclic lipids require less energy for the phase transition than the acyclic counterparts. The polymorphic phase should thus be different between the cyclic and the acyclic tetraether lipids.

In addition, the values of T_c and ΔH_c were almost identical between the regioisomers of glycerol moiety such as **7** and **8**, or **9** and **10**, which suggests that the physicochemical and polymorphic features of regioisomers between **7** and **8** (or **9** and **10**) are indistinguishable. This may be a reason why archaea make no biosynthetic distinction of the regiochemistry on the glycerol arrangement of the 72-membered tetraether lipids.

^{31}P NMR spectroscopy of dispersed lipids is an effective method to get some insights into lipid polymorphism. The line shapes of ^{31}P NMR spectra indicate the polymorphic phase of phospholipids. For example, an axially symmetrical powder pattern indicates a lamellar polymorphic phase, while an isotropic signal indicates a vesicle and/or micellar structure.[10] The ^{31}P NMR spectra of the cyclic tetraether lipid **8** and the acyclic tetraether lipid **10** are shown in Fig. 4 (the spectra of **7** and **9** were identical to those of **8** and **10**, respectively). ^{31}P NMR spectra of **7** and **8** both only show an isotropic signal with a narrow half width (150 Hz) at the temperatures below and above T_c (Figs. 4a and 4c). For the acyclic lipids, axially symmetrical powder patterns were observed below T_c (20 °C), while sharp isotropic signals (half width; 500 Hz) were observed above T_c (100 °C) (Figs. 4b and 4d).

Next, the lipid polymorphism was directly observed with transmission electron microscopy (TEM). The micrographs are shown in Fig. 5. The cyclic lipids **7** and **8** (Figs. 5a and 5b, respectively) was mostly composed of large irregular fragments (diameter; 1—2 μm), and some small particles (diameter; 50—100 nm). The large irregular fragments were not derived from any obvious ordered structures. On the other hand, the polymorphism of the acyclic lipids **9** and **10** was composed mainly of small spheres (diameter; 50—100 nm) and a few larger spheres (diameter; 500 nm) (Figs. 5c and 5d, respectively).

The phosphocholine derivatives of **5** and **6** reported by Yamauchi's group[3a] could not form a vesicle structure, but rather showed a sheet-like polymorphism in distilled water. Moss et al. suggested that a mixture of acyclic tetraether lipid and diether lipid could form liposomes, but an acyclic

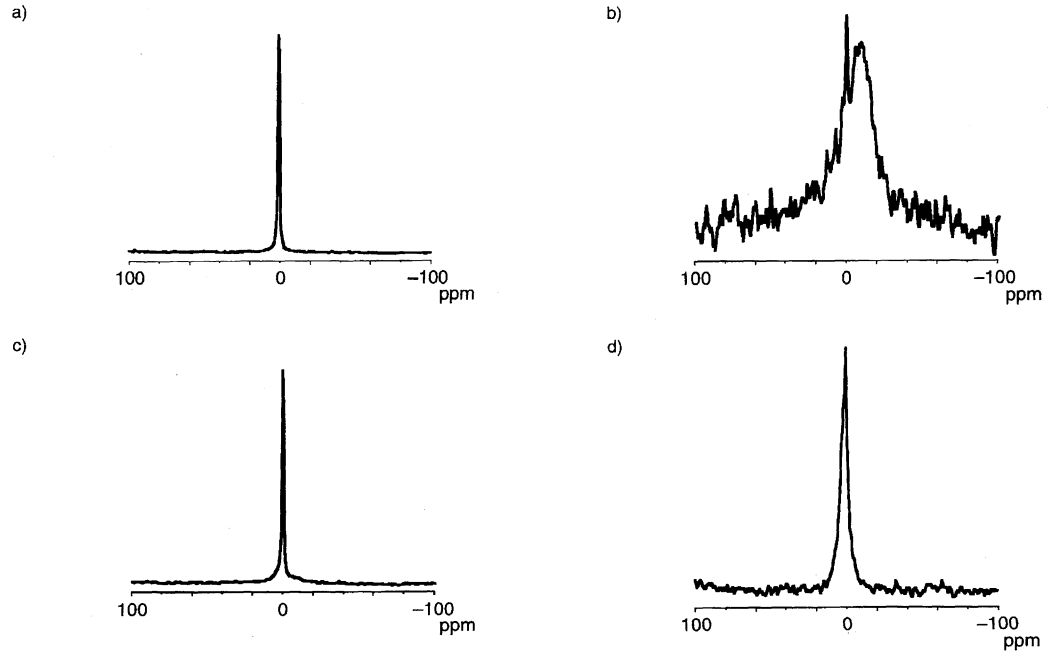


Fig. 4. ^{31}P NMR spectra of a) **8** at 20 °C, b) **10** at 20 °C, c) **8** at 75 °C, and d) **10** at 100 °C. Each lipid (10 mg) was dispersed in ethylene glycol (0.5 ml). Number of scan: ca. 20000.

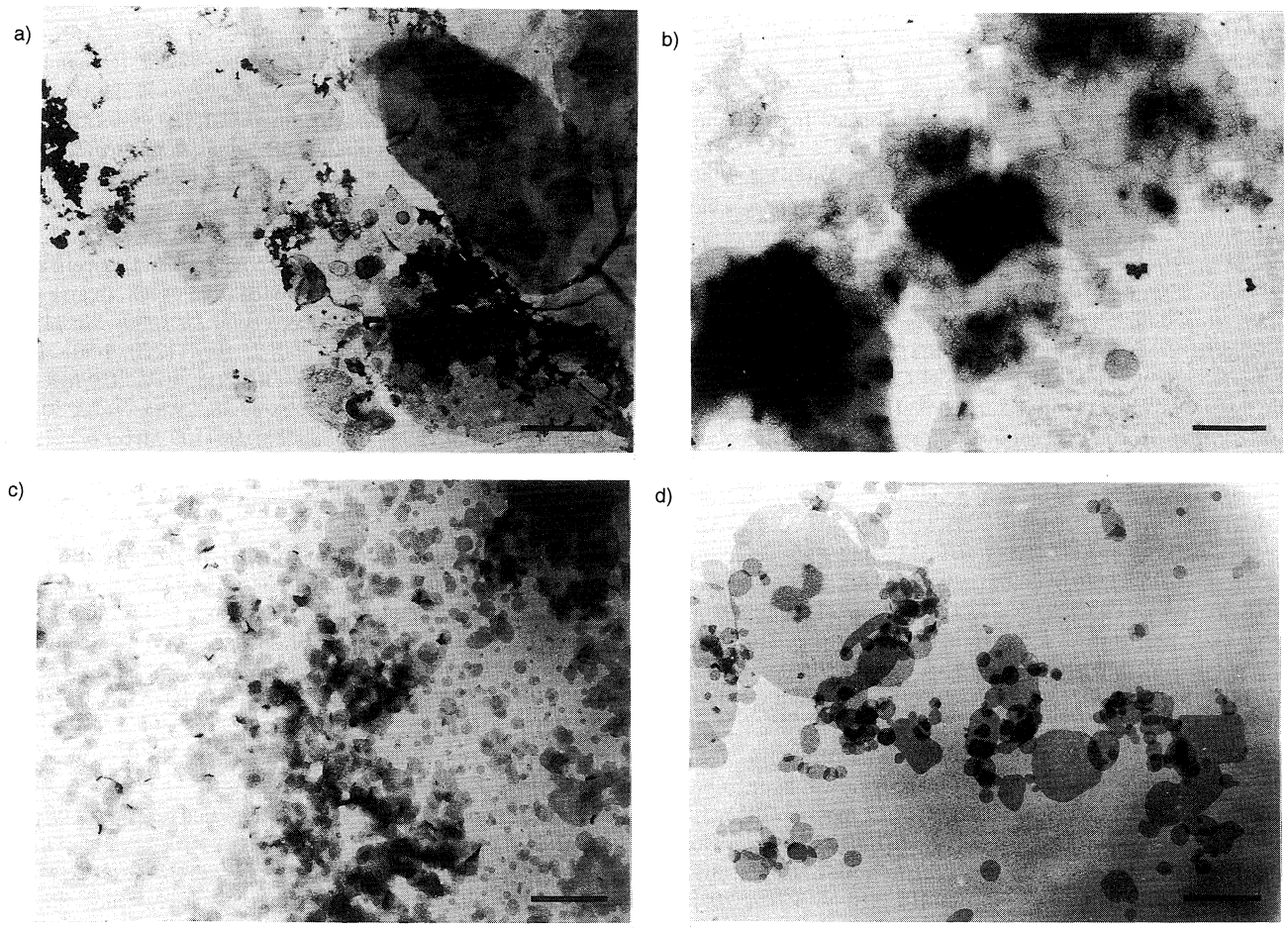


Fig. 5. Transmission electron micrographs of tetraether lipids. Scale bar = 1 μm. a) cyclic lipid **7**, b) cyclic lipid **8**, c) acyclic lipid **9**, and d) acyclic lipid **10**. These images were stained with 2% aqueous phosphotungstic acid (pH 7.0).

tetraether lipid assembled to form a U-shaped structure rather than a bilayer-bridged conformation.[3d,3g] The U-shaped structure was considered to be effective in space fillings at the curvature of small spheres.[3d,3g,7a] In fact, our synthetic acyclic tetraether lipid **9** and **10** aggregated in a Tris-HCl buffer as both small sphere and sheet-like structures, as observed in the TEM analyses. In contrast, the cyclic tetraether lipids **7** and **8** were aggregated mostly in irregular sheet-like structures, and some in small particles. This is consistent with the observation that ^{31}P NMR studies did not intrinsically give any information about irregular structure. The cyclic lipids **7** and **8** were unable to form particle structures like vesicles due to inferiority in the formation of U-shaped conformation. De Rosa et al. discussed the tetraether lipids with different polar head groups (PHGs) may be aggregated in the closed bilayer assemblies like vesicles,[7a,7c] where larger PHGs orient in the outer region of liposomes, while smaller PHGs orient in the inner region. The tetraether lipids mixed with the diester lipid might also form vesicles, since the diester lipid could act as the space filling at curvature.[7b] De Rosa et al. further investigated the polymorphic phase of the archaeal 72-membered tetraether core lipids (**1**, **2**, and the lipids in which each diphytanyl (phytanyl; 2,6,10,14-tetramethylhexadecyl) chain contains 0 to 4 cyclopentane rings) by DSC and X-ray scattering studies under dried conditions.[11] The tetraether core lipids were shown in the oriented lamellar phase ($L_{\beta'}$) at the temperature range from −19 to 19 °C; the lamellar structure melted above 19 °C due to the conformational changes in the alkyl chains.[11b] Similar phenomena may be the case in our synthetic model lipids **7** and **8**.

In conclusion, we were the first to synthesize and to characterize the 72-membered tetraether model phospholipids. The cyclic tetraether lipids were insufficient in forming closed structures like vesicles. The cyclic structure in **7** and **8** leads to a decrease in the mobility in the hydrophobic region, which causes apparent differences between the cyclic and acyclic lipids in terms of physicochemical and polymorphic properties.

Experimental

The 72-membered macrocyclic tetraether model compounds **3** and **4**, and their corresponding acyclic model compounds **5** and **6** were previously synthesized in our group.[5a] Melting points were measured with a Yanagimoto BY-1 melting point apparatus and are uncorrected. IR spectra were taken on a Horiba FT-710 Fourier transform infrared spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL LA-300 spectrometer. ^{31}P NMR spectra was recorded on a JEOL EX-270 spectrometer. Deuteriochloroform (99.8 atom% enriched, Merck) was used for the NMR solvent. ^{1}H NMR and ^{13}C NMR chemical shifts were reported in δ values based on internal TMS ($\delta_H = 0$), or solvent signal ($CDCl_3$ $\delta_C = 77.0$)

as reference. Phosphoric acid was used ($\delta_P = 0$) as an external standard for ^{31}P NMR. Column chromatography was carried out with a Kieselgel 60 (70—230 mesh, Merck). Pyridine was distilled from potassium hydroxide. Benzene was distilled from calcium hydride.

(2*S*,39*S*)-2,39-Bis(diphenylphosphoryloxymethyl)-1,4,37,40-tetraoxacyclodoheptacontane (13). Diphenylphosphoryl chloride (0.510 ml, 2.45 mmol) was added dropwise to a mixture of diol (**3**) (125 mg, 0.116 mmol) and 4-dimethylaminopyridine (DMAP) (67.2 mg, 0.550 mmol) in pyridine (12 ml) and benzene (24 ml) at room temperature, and the mixture was stirred at 60 °C for 80 h. Aqueous 2 M HCl (30 ml, 1 M = 1 mol dm^{-3}) was added and the mixture was extracted with CH_2Cl_2. The combined organic layer was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with benzene–EtOAc (10 : 1) to give bisphosphotriester (**13**) (156 mg, quant.) as a wax. ^{1}H NMR (300 MHz) δ = 1.25 (br, 112H), 1.50—1.61 (br, 8H), 3.37—3.67 (m, 14H), 4.21—4.39 (m, 4H), 7.16—7.36 (m, 20H). ^{13}C NMR (75 MHz) δ = 25.97, 26.05, 29.41, 29.57, 29.65, 29.90, 68.29 (d, J = 6.8 Hz), 69.62, 70.71, 71.73, 77.11 (d, J = 8.0 Hz), 120.07 (d, J = 5.0 Hz), 120.10 (d, J = 5.0 Hz), 125.31, 129.72, 150.54 (d, J = 7.5 Hz). ^{31}P NMR (109 MHz) δ = −12.00. IR (KBr pellet) 1292, 1464, 1473, 1489, 1591, 2848, 2918 cm^{-1}. Found: C, 73.16; H, 10.27%. Calcd for $C_{94}H_{158}O_{12}P_2$: C, 73.21; H, 10.33%.

(2*S*,38*S*)-2,38-Bis(diphenylphosphoryloxymethyl)-1,4,37,40-tetraoxacyclodoheptacontane (14). In the same way as described for the synthesis of **13**, the compound (**4**) (99.2 mg, 0.0920 mmol) was converted to bisphosphotriester (**14**) (132 mg, 93%) as a wax. ^{1}H NMR (300 MHz) δ = 1.25 (br, 112H), 1.50—1.61 (br, 8H), 3.37—3.66 (m, 14H), 4.21—4.39 (m, 4H), 7.16—7.36 (m, 20H). ^{13}C NMR (75 MHz) δ = 25.97, 26.06, 29.42, 29.58, 29.66, 29.92, 68.31 (d, J = 6.2 Hz), 69.66, 70.72, 71.74, 77.13 (d, J = 7.4 Hz), 120.08 (d, J = 5.0 Hz), 120.12 (d, J = 5.0 Hz), 125.30, 129.72, 150.57 (d, J = 7.5 Hz). ^{31}P NMR (109 MHz) δ = −12.03. IR (KBr pellet) 1292, 1464, 1473, 1489, 1591, 2848, 2918 cm^{-1}. Found: C, 72.92; H, 10.56%. Calcd for $C_{94}H_{158}O_{12}P_2$: C, 73.21; H, 10.33%.

2,2′-*O*-(1,32-Dotriacontanediyl)-3,3′-di-*O*-hexadecyl-1,1′-bis-*O*-(diphenylphosphoryl)-*sn*-diglycerol (15). In the same way as described for the synthesis of **13** and **14**, the compound (**5**) (226 mg, 0.209 mmol) was converted to bisphosphotriester (**15**) (325 mg, quant.) as a wax. ^{1}H NMR (300 MHz) δ = 0.88 (t, J = 6.8 Hz, 6H), 1.25 (br, 108H), 1.48—1.54 (m, 8H), 3.36—3.67 (m, 14H), 4.22—4.42 (m, 4H), 7.16—7.37 (m, 20H). ^{13}C NMR (75 MHz) δ = 14.12, 22.68, 25.99, 26.06, 29.35, 29.49, 29.58, 29.62, 29.65, 29.70, 29.72, 29.95, 31.91, 68.34 (d, J = 6.8 Hz), 69.36, 70.74, 71.79, 77.01 (d, J = 7.5 Hz), 120.07 (d, J = 5.0 Hz), 120.11 (d, J = 5.0 Hz), 125.27, 129.71, 150.55 (d, J = 6.8 Hz). ^{31}P NMR (109 MHz) δ = −12.06. IR (KBr pellet) 1294, 1458, 1469, 1489, 1591, 2848, 2914 cm^{-1}. Found: C, 72.88; H, 10.55%. Calcd for $C_{94}H_{160}O_{12}P_2$: C, 73.11; H, 10.44%.

2,3′-*O*-(1,32-Dotriacontanediyl)-2′,3-di-*O*-hexadecyl-1,1′-bis-*O*-(diphenylphosphoryl)-*sn*-diglycerol (16). In the same way as described for the synthesis of **13**, **14**, and **15**, the compound (**6**) (389 mg, 0.360 mmol) was converted to bisphosphotriester (**16**) (529 mg, 93%) as a wax. ^{1}H NMR (300 MHz) δ = 0.88 (t, J = 6.8 Hz, 6H), 1.26 (br, 108H), 1.48—1.54 (m, 8H), 3.36—3.67 (m, 14H), 4.23—4.43 (m, 4H), 7.14—7.36 (m, 20H). ^{13}C NMR (75 MHz) δ = 14.04, 22.60, 25.92, 25.99, 29.29, 29.40, 29.50, 29.53, 29.56, 29.64, 29.86, 31.84, 68.24 (d, J = 6.2 Hz), 69.25, 70.60, 71.66, 76.93 (d, J = 7.5 Hz), 119.98 (d, J = 5.0 Hz), 120.02 (d, J = 5.0 Hz), 125.18, 129.60, 150.47 (d, J = 6.8 Hz). ^{31}P NMR (109 MHz) δ = −11.92. IR (KBr pellet) 1294, 1469, 1489, 1591, 2850, 2918 cm^{-1}. Found: C, 73.04; H, 10.70%. Calcd for $C_{94}H_{160}O_{12}P_2$: C, 73.11; H, 10.44%.

(2*S*,39*S*)-2,39-Bisphosphoryloxymethyl-1,4,37,40-tetraoxacyclodoheptacontane (7). A mixture of bisphosphotriester (**13**) (158 mg, 0.102 mmol) and PtO_2 (204 mg) in glacial acetic acid (50 ml) was stirred at 100 °C under a hydrogen atmosphere for 60 h. The catalyst was filtered through a pad of Celite and washed with hot $CHCl_3$. The filtrate and washings were combined and concentrated to dryness. The residue was recrystallized from $CHCl_3$ to give bisphosphoric acid (**7**) (46 mg, 36%) as a colorless solid. Mp 113—116 °C. ^{1}H NMR (300 MHz) δ = 1.25 (br, 112H), 1.50—1.60 (br, 8H), 3.40—3.62 (m, 14H), 4.02—4.20 (m, 4H). ^{13}C NMR (75 MHz) δ = 26.06, 26.13, 29.44, 29.49, 29.58, 29.65, 29.72, 30.03, 64.16, 70.64, 70.68, 71.79, 76.75. ^{31}P NMR (109 MHz) δ = 1.76. IR (KBr pellet) 1055, 1246, 1464, 1473, 2848, 2918, 3465 cm^{-1}. Found: C, 67.89; H, 11.74%. Calcd for $C_{70}H_{142}O_{12}P_2$: C, 67.92; H, 11.56%.

(2*S*,38*S*)-2,38-Bisphosphoryloxymethyl-1,4,37,40-tetraoxacyclodoheptacontane (8). In the same way as described for the synthesis of **7**, the compound (**14**) (163 mg, 0.106 mmol) was converted to bisphosphoric acid (**8**) (63 mg, 48%) as a colorless solid. Mp 111—113 °C. ^{1}H NMR (300 MHz) δ = 1.26 (br, 112H), 1.51—1.57 (br, 8H), 3.39—3.65 (m, 14H), 4.03—4.37 (m, 4H). ^{13}C NMR (75 MHz) δ = 26.06, 26.13, 29.44, 29.58, 29.65, 29.71, 30.03, 64.16, 70.64, 70.67, 71.79, 76.74. ^{31}P NMR (109 MHz) δ = 1.93. IR (KBr pellet) 1051, 1244, 1464, 1473, 2848, 2918, 3452 cm^{-1}. Found: C, 68.06; H, 11.54%. Calcd for $C_{70}H_{142}O_{12}P_2$: C, 67.92; H, 11.56%.

2,2′-*O*-(1,32-Dotriacontanediyl)-3,3′-di-*O*-hexadecyl-*sn*-diglycero-1,1′-bisphosphoric Acid (9). In the same way as described for the synthesis of **7** and **8**, the compound (**15**) (325 mg, 0.210 mmol) was converted to bisphosphoric acid (**9**) (113 mg, 43%) as a colorless solid. Mp 93—95 °C. ^{1}H NMR (300 MHz) δ = 0.88 (t, J = 6.8 Hz, 6H), 1.26 (br, 108H), 1.56 (br, 8H), 3.40—3.75 (m, 14H), 3.95—4.15 (m, 4H). ^{13}C NMR (75 MHz) δ = 14.07, 22.71, 26.10, 26.19, 29.47, 29.53, 29.59, 29.73, 29.78, 31.99, 64.70, 70.49, 71.94, 72.05, 77.22. ^{31}P NMR (109 MHz) δ = 0.95. IR (KBr pellet) 1070, 1232, 1469, 2850, 2918, 3427 cm^{-1}. Found: C, 67.60; H, 11.87%. Calcd for $C_{70}H_{144}O_{12}P_2$: C, 67.81; H, 11.71%.

2,3′-*O*-(1,32-Dotriacontanediyl)-2′,3-di-*O*-hexadecyl-*sn*-diglycero-1,1′-bisphosphoric Acid (10). In the same way as described for the synthesis of **7**, **8** and **9**, the compound (**16**) (386 mg, 0.250 mmol) was converted to bisphosphoric acid (**10**) (160 mg, 52%) as a colorless solid. Mp 80—82 °C. ^{1}H NMR (300 MHz) δ = 0.88 (t, J = 6.8 Hz, 6H), 1.26 (br, 108H), 1.56 (br, 8H), 3.40—3.75 (m, 14H), 3.95—4.15 (m, 4H). ^{13}C NMR (75 MHz) δ = 14.04, 22.69, 26.07, 26.16, 29.36, 29.39, 29.61, 29.72, 29.78, 31.97, 66.13, 70.25, 71.07, 72.07, 77.98. ^{31}P NMR (109 MHz) δ = 1.37. IR (KBr pellet) 1061, 1242, 1471, 2850, 2918, 3421 cm^{-1}. Found: C, 67.56; H, 11.86%. Calcd for $C_{70}H_{144}O_{12}P_2$: C, 67.81; H, 11.71%.

Differential Scanning Calorimetry (DSC). Each diphospholipid (ca. 7 mg) dispersed in ethylene glycol (ca. 50 mg) was placed in an aluminum pan. Each dispersion was annealed once at a temperature above the T_c. Then, DSC thermogram was scanned at a heating rate of 2 °C min^{-1} (reference; same volume of ethylene glycol) from 50 to 130 °C. Phase transition point and phase transition enthalpy was measured with a Seiko SSC-5200 differential scanning calorimeter.

^{31}P NMR Spectra of Lipid Dispersion. Each lipid (ca. 10

mg) was dissolved in ethylene glycol (0.5 ml) while vortexing at a temperature below T_c (20 °C) or above T_c (75 °C for **7** and **8**; 100 °C for **9** and **10**) for 10 min. Phosphoric acid in D_2O was used as an external standard ($\delta_P = 0$). Measurements were performed both above and below T_c. Pulse width was 19 μs (90°), pulse delay 1 s, delay time 100 μs, number of scan ca. 20000.

Transmission Electron Microscopy (TEM). The negative staining method was used for examination of the fine structure of lipids. A mixture of lipid (1 mg) and a 50 mM Tris-HCl buffer (including 5 mM EDTA, pH = 7.5) (2 ml) was freezed, thawed and vortexed at 100 °C to afford a milky-white homogeneous suspension. Small amounts of the lipid dispersion and 2% aqueous phosphotungstic acid (pH 7.0) were both dropped onto formvar-coated grids. The prepared sample was observed with a Hitachi H-7000 transmission electron microscope at 75 kV. Specimens of the electron micrographs were displayed in Fig. 5 at a magnification of 20000.

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