Giant Vesicles from 72-Membered Macrocyclic Archæal Phospholipid Analogues: Initiation of Vesicle Formation by Molecular Recognition between Membrane Components

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Abstract: Stereochemically pure archæal acyclic bola-amphiphilic diphosphates **4** and **5**, with the basic structure of the phospholipids found in *Sulfolobus*, have been synthesized for the first time. The self-assembly properties have been compared with those of the nearly identical 72-membered macrocyclic tetraether phosphates **3a** and **3b**, analogues of the major phospholipid components of *Sulfolobus*, *Thermoplasma*, and methanogenic Archæa, which were also synthesized. Phase contrast and fluorescence microscopies have shown that the dipolar lipids **1** and **2** spontaneously formed vesicles. Whereas the macrocyclic dipolar phosphates **3** spontaneously formed vesicles (phase contrast and fluorescence microscopies), the bolaform phosphate **4** gave only a lamellar structure (synchrotron diffraction pattern: repeat distance of about 4.25 nm but with only a few layers). However, upon addition of the unphosphorylated precursors phytanol, phytol, or geranylgeraniol to the acyclic lipids **4** and **5**, giant vesicles were rapidly formed. Addition of *n*-hexadecanol or cholesterol did not lead to vesicle formation. Therefore it was concluded that this vesicle formation occurs only when the added molecule is closely compatible with the constituents of the lipid layer and can be inserted into the double layer. A slight mismatch (cholesterol or *n*-hexadecanol/polyprenyl chains) is therefore enough to block the insertion process presumably required for vesicle formation.

Keywords: amphiphiles · Archæa · macrocycles · molecular recognition · vesicles

Introduction

Archæa, the third major kingdom of living organisms, possess membrane lipids structurally unique in that their polar headgroups are linked to polyprenyl chains (unsaturated or saturated) by ether bonds, in contrast to the ester bonds and *n*-acyl chains of Eucarya (Procarya often contain also branched lipids, but of the *iso*- and *anteiso*-acyl type). Another striking feature of some archæal lipids is the presence of 36- (**2**; $Y = PO_3(CH_2)_2NMe_3$) or 72-membered rings (**3a**, **3b**; $X = PO_3H_2$), or of bolaform lipids (**4**, **5**; $X = PO_3H_2$).[1]

We have undertaken to study systematically the effect of these structural peculiarities on the self-assembly properties of natural archæal lipids and analogues, such as the formation of vesicles and their permeability. We have previously shown that the archæal 36-membered *macrocyclic* diether phosphatidylcholine **2** ($Y = PO_3(CH_2)_2NMe_3$) gave vesicles which were much less water-permeable than those composed of acyclic *n*-diacyl or *n*-dialkyl phosphocholines.[2]

The 72-membered macrocyclic tetraether phosphocholine lipids are major membrane lipids of the highly thermophilic *Sulfolobus* and *Thermoplasma*, and of some methanogenic Archæa.[1a] They carry two nonequivalent polar heads, based on glycerol (or more complex polyols[3]); two of the carbon atoms of glycerol are linked by ether bonds to C_{40} octaprenyl chains, themselves linked distally to the second headgroup. The diversity of these phospholipids comes from the nature of the headgroups and from the presence of zero to four cyclopentane rings per polyprenyl chain. It was also shown recently that the 72-membered lipids are present as a mixture of regioisomers **3a** and **3b** (with various complex polar

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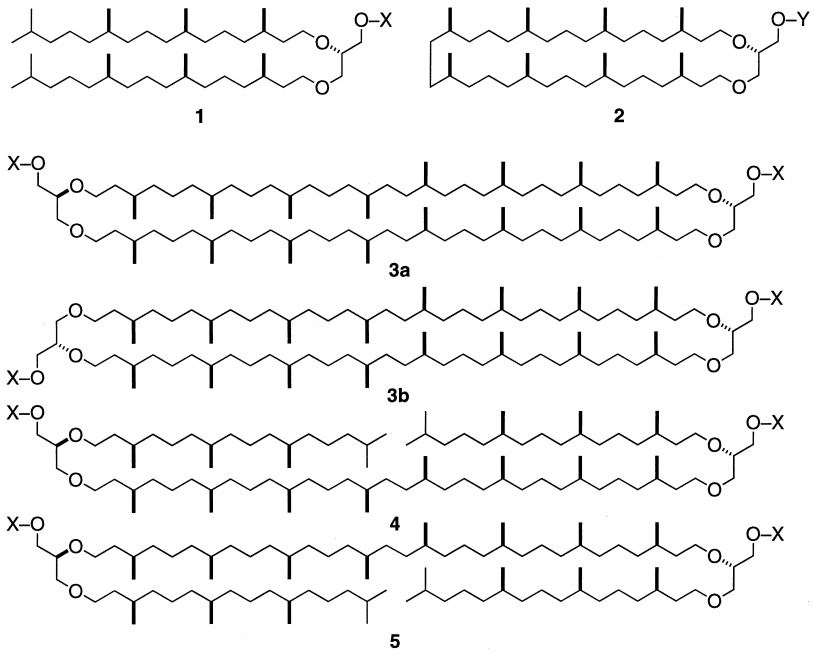

headgroups).[4] These lipids can be expected, by virtue of their obvious amphiphilicity, to form organized systems in which the very long chain is extended across the membrane,[5] leading to molecular assemblies structurally similar to the classical bilayers of eucaryotic biomembranes but linked by C–C bonds in mid-membrane. Indeed, formation of multilamellar liposomes has been reported with the native lipid mixture isolated from *Sulfolobus acidocaldarius*,[6] whereas some close derivatives of **3** form predominantly nonlamellar assemblies such as cubic phases.[7] Menger and Chen have synthesized a fully demethylated 72-membered macrocyclic archæal lipid analogue based on *n*-alkyl chains, rather than polyprenyl, which did not form vesicular systems.[8] Some models of natural acyclic bola-amphiphilic lipids have also been synthesized and their phase properties have been reported.[9]

We have previously shown that phosphates instead of phosphatidylcholines or other complex headgroups are efficient for membrane formation with polyprenyl derivatives, both with two chains[10a] or with only one,[10b] and we have hypothesized that these polyprenyl phosphates may have been the most primitive phospholipids. As diphytanylglyceryl phosphate **1** easily formed vesicles,[11] we now study the phosphates **3ab** ($X = PO_3H_2$, 1:1 mixture [molar] of **3a** and **3b**), **4** ($X = PO_3H_2$), and **5** ($X = PO_3H_2$).

Abstract in French: *Les diphosphates acycliques bola-amphiphiliques de Sulfolobus (une archéobactérie) ont été pour la première fois synthétisés stéréosélectivement. Les propriétés d'auto-organisation de ces lipides ont été comparées avec celles de diphosphates macrocycliques possédant un squelette voisin. Le microscopie en contraste de phase et en fluorescence montre que les lipides dipolaires macrocycliques **3** donnent spontanément des vésicules géantes, tandis que les lipides acycliques **4** et **5** ne forment pas de vésicules par eux-mêmes. L'étude par diffraction de rayons X (Synchrotron DESY) montre que le diphosphate **4** donne un système de couches parallèles distantes d'environ 4,25 nm. Par addition de phytanol, de phytol ou de géranylgéraniol, des vésicules géantes sont été formées rapidement à partir de **4** et de **5**. L'addition de n-hexadécanol ou de cholestérol n'induit pas la formation de vésicules.*

Results

Synthesis

In order to compare the membrane properties of the 72-membered macrocyclic diphosphates **3ab** ($X = PO_3H_2$) and their acyclic counterparts **4** ($X = PO_3H_2$) and **5** ($X = PO_3H_2$), we have synthesized these lipids according to Scheme 1.

72-Membered macrocyclic diphosphates 3ab: We have recently developed a convenient synthesis of the 72-membered macrocyclic diol **3ab** (X = H, mixture of 1:1 molar ratio of **3a** and **3b**).[12] In the presence of 4-dimethylaminopyridine (DMAP) and pyridine, a mixture of the diols **3a** and **3b** (X = H)[13] was treated with diphenylphosphoryl chloride[14] in benzene to give the corresponding bisphospho-triesters. This mixture was subjected to catalytic hydrogenolysis in the presence of platinum (from PtO_2) to afford the desired bisphosphoric acid derivatives **3a,b** ($X = PO_3H_2$, 72% yield).

Acyclic bola-amphiphilic diphosphates 4 and 5: The bola-amphiphilic phosphate **4**, structurally analogous to the macrocyclic lipid **3ab** but not macrocyclic, has also been found in *Sulfolobus solfataricus*.[20] In this lipid, each of the polar headgroups is linked to two chains, a C-40 bisphytanyl and a C-20 phytanyl one. It was synthesized as follows.

Phytanol (**6**) was oxidized under Swern conditions to give in 82% yield aldehyde **7**, which was treated with *sn*-1-*O*-benzylglycerol **8**[15] in the presence of toluene-*p*-sulfonic acid and $MgSO_4$ to give quantitatively the acetal **9** as a diastereomeric mixture. The acetal **9** was treated with diisobutylaluminum hydride (DIBAL-H) to give a mixture of positionally isomeric monoalkylated benzylglycerol derivatives, which were easily separated by silica–gel column chromatography to afford the *sn*-2-*O*-alkylated benzylglycerol **10** and the *sn*-3-*O*-alkylated benzylglycerol **11** in 48 and 50% yields, respectively.[16] The *sn*-2-*O*-alkylated glycerol **10** was further alkylated via its sodium alkoxide with **17**[17] to afford 2,3-*O*-disubstituted *sn*-1-*O*-benzylglycerol **12** in 85% yield. Subsequent deprotection of the TBS group, Swern oxidation, and Wittig olefination afforded the half-size diether **15**. This was dimerized by metathesis using the ruthenium-alkylidene complex, $[RuCl_2(=CHPh)(PCy_3)_2]$ developed by Grubbs

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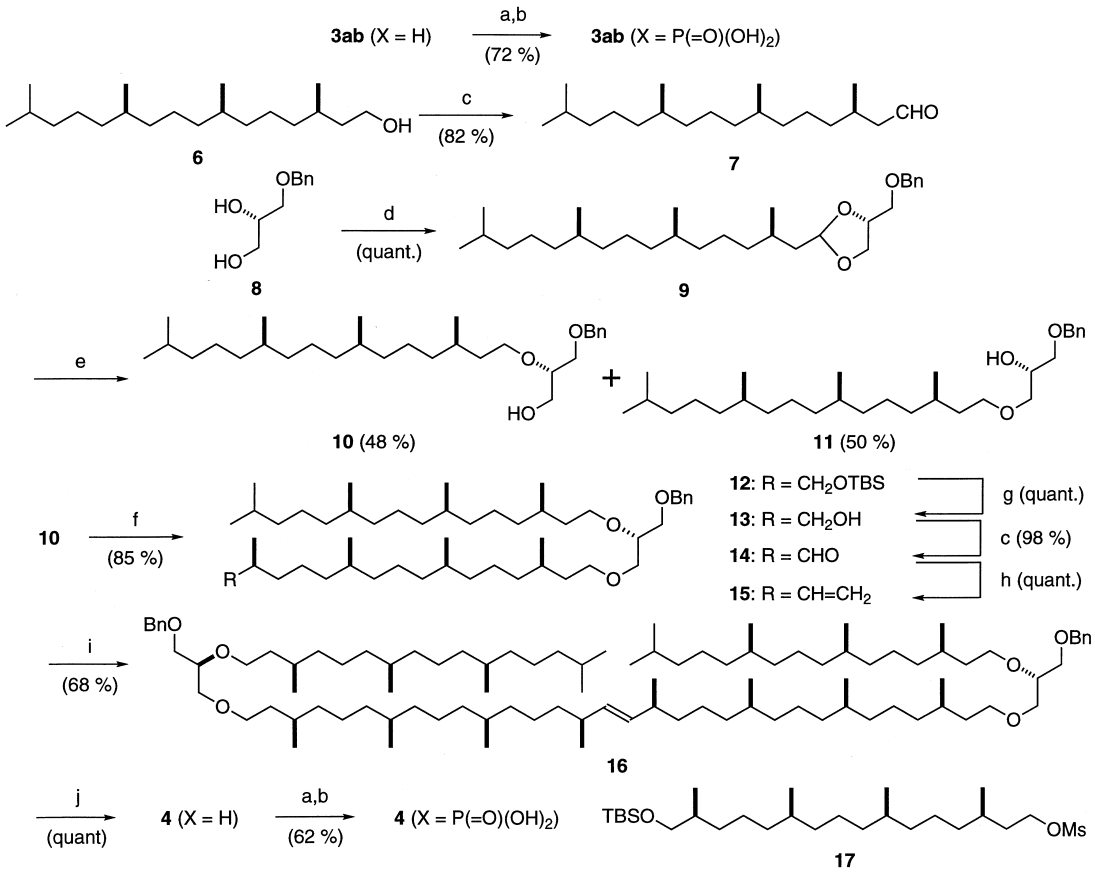
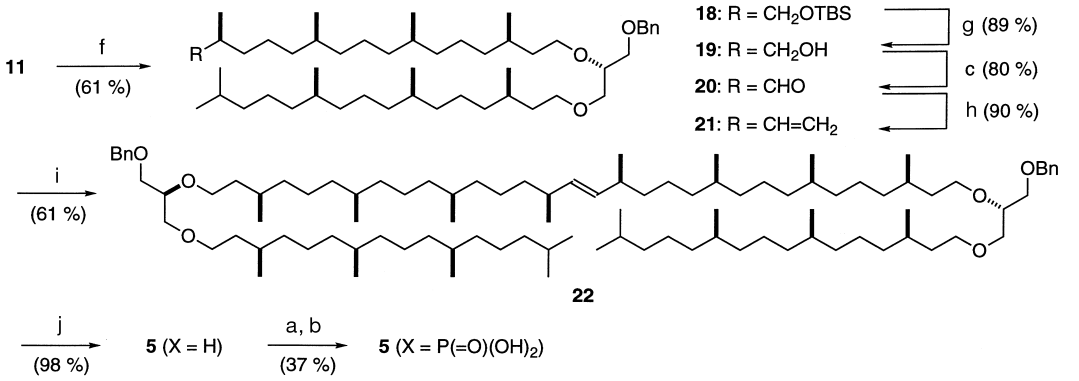


Scheme 1. Synthesis of the dipolar lipids **3ab** ($X = PO_3H_2$), **4** ($X = PO_3H_2$) and **5** ($X = PO_3H_2$). Reagents: a) $(PhO)_2P(=O)Cl$, DMAP/Py/benzene; b) H_2, PtO_2/HOAc; c) Swern oxidation; d) compound **7**, *p*-TsOH, $MgSO_4/CH_2Cl_2$; e) DIBAL-H/toluene; f) NaH, compound **17**/DMSO; g) TBAF/THF; h) $Ph_3P{=}CH_2$/THF; i) $[RuCl_2(=CHPh)(PCy_3)_2]/CH_2Cl_2$; j) H_2, 10 % Pd-C/EtOAc/HOAc. TBAF = tetrabutylammonium fluoride.



Scheme 2. Synthesis of the dipolar lipid **5** ($X = PO_3H_2$). Reagents see Scheme 1.

et al.[18] The reaction proceeded smoothly to yield the unsaturated tetraether **16** in 68% yield. Reduction of the double bond and deprotection of the benzyl groups of **16** by catalytic hydrogenation afforded **4** ($X = OH$) quantitatively. Finally, phosphorylation as described above gave the acyclic tetraether core lipid **4** ($X = PO_3H_2$) in 62% yield.

The acyclic tetraether core lipid **5** ($X = PO_3H_2$) was synthesized from **11** in the same manner (Scheme 2).

Optical microscopy: "Giant" vesicles, with a diameter of 10 μm or more, can be observed in situ, in water, with an optical microscope working in the Nomarsky mode.[19] By addition of Nile Red, a neutral lipophilic fluorescent probe, one can visualize more clearly the image of the membrane around the vesicles in the fluorescence mode. When the 72-membered macrocyclic phosphates **3ab** ($X = PO_3H_2$), with the same core structures as the mixture of lipids found in thermophilic Archæa, were dispersed in a neutral buffer, they easily formed giant vesicles (50–60 μm) (Figure 1).

Lipid **4** ($X = PO_3H_2$), when placed on a microscope slide and covered by a buffer (Tris–HCl or Glycine–NaOH), did *not* form vesicles. Mild sonication, or heating to 45 °C, did not give any sign of formation of vesicles. However, to our surprise, formation of giant vesicles started very rapidly when

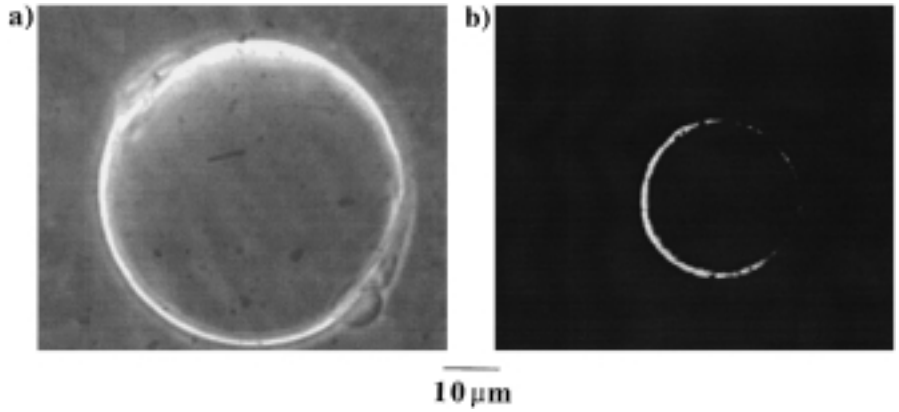
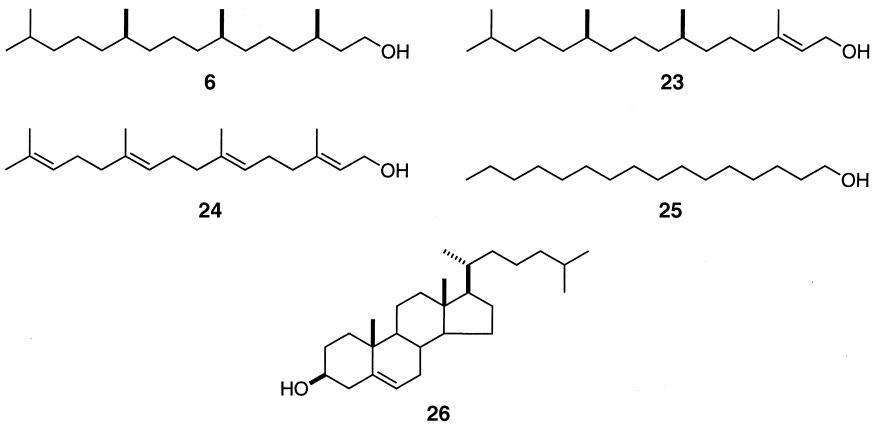


Figure 1. Phase contrast image (left) and fluorescent microscopic image (right) of giant vesicles from the 72-membered macrocyclic dipolar phosphates **3ab** ($X = PO_3H_2$).

we treated with the same buffer a film of **4** premixed with a 2–3 molar excess of phytanol (**6**), phytol (**23**) or geranylgeraniol (**24**). These vesicles grew in size with time, apparently by capture of further material from unorganized reservoirs as shown in Figure 2. *n*-Hexadecanol (**25**) or cholesterol (**26**), of similar polarity and dimensions as **6**, **23**, and **24** (as judged from scale molecular models), but nevertheless of different shapes, were also tested as additives, and found not to induce vesicle formation. In addition, the regioisomeric bola-amphiphilic lipid **5** did not give giant vesicles when pure, but only after addition of phytanol.



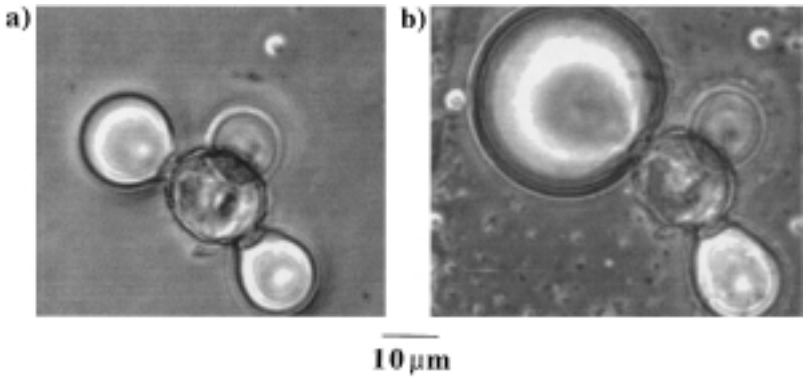


Figure 2. a) Phase contrast image of a giant vesicle from the lipid **4**/phytanol system (1/2 molar ratio). The vesicle starts growing almost immediately from the lipid droplet at the center of the image. b) Same frame photographed after one day, showing that the size of the vesicle has increased. The droplet at the center of the image appears to be the source of the new material required for the growth of the vesicle. Bar: 10 μm.

The insertion of phytanol, phytol, or geranylgeraniol (which themselves, when pure, did not form vesicles) into the membrane of vesicles of **4** is of course made possible by the nearly identical lipophilic parts of these molecules. The additives may favor the formation of vesicles mainly by reduction of the electrostatic interactions between the ionized headgroups, and also act as space-filling partners, facilitating the curvature of the system to form vesicles. Two similar cases of vesicle formation induced by additives have been reported.[7c, 21]

Synchrotron X-ray diffraction: We and others[11] have shown that the simplest phosphate **1** easily forms giant vesicles, however, the diffraction pattern unexpectedly showed well-expressed hexagonal structures over the entire temperature range measured (from 5 °C to 87 °C), with a distance between cylinders of 6.23 nm at 20 °C and 5.76 nm at 87 °C. This apparent contradiction, may be due to two differences: the synchrotron study was run at a 10^3 higher concentration of lipid, in pure water (pH 7) instead of a pH 7.8–8.4 buffer. This may be checked when we can obtain beam time.

The diffraction pattern of phosphate **4** and water, without added phytanol, showed only, by a small and broad reflection, a lamellar structure with a repeat distance of 4.25 nm at 4 °C and 4.18 at 50 °C, but with only a few layers. This interlamellar distance is compatible with the thickness expected for a system made of molecules such as **4**, fully extended.

Discussion

These studies suggest several remarks. First in the context of our hypothesis of the primitiveness of membranes of polyterpenyl phosphates, our observations may have some significance: assuming polyprenols to

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be formed prebiotically, it would not be necessary to phosphorylate them fully to induce vesicle formation, but only to about 1/4–1/3. It may even have been advantageous to have had, at an early stage of evolution of Archæa, an incomplete conversion to the phosphates of the precursors of the present complex membrane constituents. In fact, it would appear warranted to study more critically the mixture of polar lipids from an archæal organism, to check whether or not they contain non-phosphorylated polyprenols alongside the highly polar phospholipids already characterized. It may also be possible that these mixtures contain phospholipids still with simple phosphate headgroups, as we had postulated earlier for "primitive" microorganisms.[10]

The factors governing the spontaneous formation of vesicles or cubic phases from archæal lipids and water, are not clear,[7] and it seems that so far spontaneous vesicle formation has only been described from the native *mixture* (headgroups and chains) of polar lipids from *Sulfolobus acidocaldarius*.[6] To the best of our knowledge, this is therefore the first report of the formation of vesicles from a defined stereochemically pure archæal polar lipid mixture, which differs from the natural ones only by its phosphate headgroups (instead of phosphocholines, for example).

Finally, it will also be interesting to refine the observation made here of a very high selectivity of "recognition" of the nonpolar part of vesicle-forming phospholipids, and of membrane-improving additives.

Experimental Section

Synthesis: All reactions, except for catalytic hydrogenations, were carried out in an inert (Ar or N_2) atmosphere. 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 and/or a LA-400 spectrometers. ^{31}P NMR spectra were recorded on a JEOL LA-400 spectrometer. Deuteriochloroform (99.8 atom % enriched, Merck) was used as the NMR solvent, unless otherwise indicated. ^{1}H NMR and ^{13}C NMR chemical shifts are 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 spectroscopy. Column chromatography was carried out on Kieselgel 60 (70–230 mesh or 230–400 mesh, Merck). THF was distilled from sodium/benzophenone ketyl prior to use. Triethylamine was distilled from potassium hydroxide. DMSO, CH_2Cl_2, benzene, and toluene were distilled from calcium hydride.

(2*S*,7*R*,11*R*,15*S*,19*S*,22*S*,26*S*,30*R*,34*R*,38*S*,43*R*,47*R*,51*S*,55*S*,58*S*,62*S*,66*R*,70*R*)-2,38-Bis(phosphoryloxymethyl)-7,11,15,19,22,26,30,34,43,47,51,55,58,62,66,70-hexadecamethyl-1,4,37,40-tetraoxacyclodoheptacontane and (2*S*,7*R*,11*R*,15*S*,19*S*,22*S*,26*S*,30*R*,34*R*,38*S*,43*R*,47*R*,51*S*,55*S*,58*S*,62*S*,66*R*,70*R*)-2,39-bis(phosphoryloxymethyl)-7,11,15,19,22,26,30,34,43,47,51,55,58,62,66,70-hexadecamethyl-1,4,37,40-tetraoxacyclodoheptacontane [3ab (X = PO_3H_2)]: Diphenylphosphoryl chloride (145 μL, 0.70 mmol) was added dropwise to a mixture of **3ab** (X = H)[13] (35 mg, 27 μmol) and DMAP (14 mg, 0.12 mmol) in pyridine (0.5 mL) and benzene (2 mL) at room temperature, and the mixture was stirred at room temperature for 12 h. Aqueous HCl (2 M, 1 mL) was added and the mixture was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with benzene/EtOAc (25:1) to give the bisphosphotriester (46 mg, 95 %) as an oil. A mixture of bisphosphotriester (43 mg, 25 μmol) and PtO_2 (18 mg) in acetic acid (6 mL) was stirred at room temperature under a hydrogen atmosphere for 19 h. The catalyst was filtered through a pad of Celite and washed with $CHCl_3/CH_3OH$ (2:1 *v*/*v*). The filtrate and washings were concentrated to dryness. The residue was purified over Sephadex®LH-20 with $CHCl_3/CH_3OH$ (2:1 *v*/*v*) to give the bisphosphoric acid **3ab** (X = P(=O)(OH)$_2$) (27 mg, 72 %) as a hygroscopic wax. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 8:1): δ = 0.84–0.89 (m, 48H), 1.00–1.70 (m, 104H), 3.45–3.70 (m, 14H), 3.99 (br, 4H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ = 8:1): δ = 19.52, 19.63, 19.72, 24.27, 24.35, 29.59, 29.74, 32.63, 32.68, 32.91, 34.11, 36.47, 36.74, 37.26, 65.23, 68.81, 69.98, 70.25, 77.52 (d, J = 7.4 Hz); ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ = 8:1): δ = 0.79; elemental analysis (%) calcd for $C_{86}H_{174}O_{12}P_2$: C 70.63, H 11.99; found: C 70.90, H 12.21.

(3*R*,7*R*,11*R*)-3,7,11,15-Tetramethylhexadecanal (7): To a stirred solution of oxalyl chloride (29.0 mL, 2 M in CH_2Cl_2, 58.0 mmol) in CH_2Cl_2 (40 mL) was slowly added DMSO (5.50 mL, 77.5 mmol) at −78 °C. The mixture was stirred for 35 min. A solution of **6** (5.78 g, 19.4 mmol) in CH_2Cl_2 (44 mL) was added dropwise over 5 min. The mixture was stirred for 20 min, warmed to −25 °C, and stirring was continued for 2.5 h. The mixture was cooled to −78 °C, and Et_3N (25.0 mL, 179 mmol) was added dropwise over 5 min. The mixture was gradually warmed to room temperature, and a saturated aqueous solution of NH_4Cl was added. The mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane/EtOAc (7:1) to give aldehyde **7** (4.73 g, 82 %) as an oil. $[\alpha]_D^{25}$ +8 (*c* 0.83, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 0.85 (d, J = 6.6 Hz, 6H), 0.87 (d, J = 6.8 Hz, 6H), 0.96 (d, J = 6.6 Hz, 3H), 1.00–1.43 (m, 20H), 1.52 (m, 1H), 2.05 (m, 1H), 2.22 (ddd, J = 2.7, 7.8 and 16.1 Hz, 1H), 2.40 (ddd, J = 2.0, 2.8 and 16.0 Hz, 1H), 9.76 (t, J = 2.2 Hz, 1H); ^{13}C NMR (100 MHz): δ = 19.72, 19.74, 20.02, 22.62, 22.71, 24.37, 24.44, 24.78, 27.97, 28.20, 32.73, 32.77, 37.06, 37.25, 37.26, 37.34, 37.41, 39.35, 51.07, 203.21; IR (neat): $\tilde{\nu}$ = 1379, 1464, 1730, 2870, 2927, 2954 cm^{-1}; elemental analysis (%) calcd for $C_{20}H_{40}O$: C 81.01, H 13.60; found: C 80.91, H 13.72.

(4*R*)-4-Benzyloxymethyl-2-[(2*R*,6*R*,10*R*)-2,6,10,14-tetramethyl pentadecanyl]-1,3-dioxolane (9): A mixture of **7** (4.21 g, 14.2 mmol), 1-*O*-benzyl-*sn*-glycerol (**8**)[15] (2.85 g, 15.6 mmol), toluene-*p*-sulfonic acid (130 mg, 0.755 mmol), and $MgSO_4$ (2.23 g, 18.5 mmol) in CH_2Cl_2 (70 mL) was stirred for 4.5 h at room temperature. The mixture was filtered, EtOAc and saturated aqueous $NaHCO_3$ were added to the filtrate, and the organic phase was separated. The aqueous phase was extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane/EtOAc (20:1) to give an oily **9** as a 7:3 mixture of diastereomers (6.52 g, quant.). ^{1}H NMR (300 MHz): δ = 0.83–0.88 (m, 12H), 0.93 (d, J = 6.6 Hz, 3H), 0.97–1.74 (m, 25H), 3.41–3.59 (m, 2H), 3.64 (dd, J = 6.8 and 8.0 Hz, 0.3H), 3.79 (dd, J = 5.1 and 8.3 Hz, 0.7H), 3.90 (dd, J = 7.1 and 8.1 Hz, 0.7H), 4.12 (dd, J = 6.8 and 8.3 Hz, 0.3H), 4.18–4.30 (m, 1H), 4.57 (s, 0.6H), 4.58 (s, 1.4H), 4.94 (t, J = 4.9 Hz, 0.7H), 5.03 (dd, J = 4.4 and 5.6 Hz, 0.3H), 7.25–7.34 (m, 5H); ^{13}C NMR (75 MHz): δ = 19.76, 19.90, 19.98, 22.62, 22.71, 24.18, 24.45, 24.78, 27.96, 29.27, 32.78, 37.26, 37.27, 37.37, 37.44, 37.63, 37.72, 39.36, 41.07, 41.19, 67.45, 70.53, 71.11, 73.48, 74.37, 74.64, 103.80, 104.35, 127.66, 127.71, 127.73, 128.39, 137.96; IR (neat): $\tilde{\nu}$ = 698, 735, 758, 1030, 1103, 1128, 1377, 1462, 2868, 2925, 2952 cm^{-1}; elemental analysis (%) calcd for $C_{30}H_{52}O_3$: C 78.21, H 11.38; found: C 78.41, H 11.67.

1-*O*-Benzyl-2-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-glycerol (10) and 1-*O*-benzyl-3-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethyl hexadecanyl]-*sn*-glycerol (11): A solution of DIBAL-H (42.0 mL, 1 M in toluene, 42.0 mmol) was added to a solution of **9** (6.38 g, 13.8 mmol) in toluene (30 mL) at 0 °C. The mixture was stirred for 5 min at the same temperature, and then at room temperature for 16 h. The reaction was quenched by addition of saturated aqueous NH_4Cl at 0 °C. After 10 min, diehtyl ether and HCl (2 N) were added. The diethyl ether layer was separated and the aqueous phase was extracted three times with ether. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and concentrated to dryness. The residue was purified by flash chromatography over silica gel with hexane/EtOAc (15:1–7:1) to give a more polar product **10** (3.08 g, 48 %) and a less polar product **11** (3.19 g, 50 %). Compound **10**: $[\alpha]_D^{25}$ −8 (*c* = 1.07, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 0.84 (d, J = 6.6 Hz, 6H), 0.86 (d, J = 6.8 Hz, 6H), 0.87 (d, J = 6.6 Hz, 3H), 1.01–1.75 (m, 24H), 2.13 (br, 1H), 3.52–3.75 (m, 7H), 4.54 (s, 2H), 7.25–7.37 (m, 5H); ^{13}C NMR (100 MHz): δ = 19.67, 19.73, 19.74, 22.60, 22.70, 24.33, 24.45, 24.77, 27.95, 29.81, 32.77, 37.05, 37.27, 37.33, 37.37, 37.43, 37.47, 39.35, 62.87, 68.67, 70.00, 73.50, 78.47, 127.61, 127.68, 128.39, 138.00; IR (neat): $\tilde{\nu}$ = 698, 735, 820, 1099, 1377, 1462, 2868, 2924, 2954, 3454 cm^{-1}; elemental analysis (%) calcd for $C_{30}H_{54}O_3$: C 77.87, H 11.76; found: C 77.67,

H 11.98. Compound **11**: $[\alpha]_D^{24}$ −0.4 (c = 1.15, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 0.84 (d, J = 6.6 Hz, 6H), 0.86 (d, J = 6.6 Hz, 6H), 0.87 (d, J = 6.6 Hz, 3H), 1.00–1.80 (m, 24H), 2.48 (br, 1H), 3.44–3.57 (m, 6H), 3.98 (m, 1H), 4.56 (s, 2H), 7.26–7.37 (m, 5H); ^{13}C NMR (100 MHz): δ = 19.70, 19.74, 19.76, 22.62, 22.71, 24.35, 24.46, 24.78, 27.97, 29.90, 32.79, 36.57, 37.28, 37.34, 37.39, 37.44, 37.49, 39.37, 69.53, 69.98, 71.40, 71.82, 73.44, 127.70, 128.41, 138.03; IR (neat): $\tilde{\nu}$ = 698, 735, 750, 1115, 1377, 1462, 2868, 2925, 2952, 3448 cm^{-1}; elemental analysis (%) calcd for $C_{30}H_{54}O_3$: C 77.87, H 11.76; found: C 77.76, H 12.06.

1-*O*-Benzyl-2-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-3-O-[(3*R*,7*R*,11*S*,15*S*)-16-*tert*-butyldimethylsilyloxy-3,7,11,15-tetramethylhexadecanyl]-*sn*-glycerol (12): To prewashed NaH (71.7 mg, 1.79 mmol) in DMSO (2 mL) was added a solution of **10** (261 mg, 0.564 mmol) in DMSO (3.5 mL). The mixture was stirred for 30 min at room temperature and a solution of the mesylate **17**[17] (264 mg, 0.520 mmol) in DMSO (2.5 mL) was added. The mixture was stirred at room temperature for 1 h and at 40 °C for 23 h. After cooling to 0 °C, water (5 mL) was added to the mixture. The mixture was extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane/EtOAc (20:1) to give **12** (385 mg, 85%) as an oil. $[\alpha]_D^{28}$ +1.5 (c = 1.77, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 0.04 (s, 6H), 0.84 (d, J = 6.6 Hz, 12H), 0.87 (d, J = 6.6 Hz, 15H), 0.89 (s, 9H), 1.00–1.70 (m, 48H), 3.34 (dd, J = 6.8 and 9.8 Hz, 1H), 3.43–3.66 (m, 10H), 4.56 (s, 2H), 7.26–7.34 (m, 5H); ^{13}C NMR (100 MHz): δ = −5.35, 16.81, 18.35, 19.68, 19.72, 19.75, 22.63, 22.72, 24.36, 24.39, 24.47, 24.79, 25.96, 27.97, 29.81, 29.89, 32.76, 32.79, 32.80, 33.51, 35.76, 36.64, 37.09, 37.29, 37.40, 37.45, 37.53, 39.36, 68.42, 68.87, 69.97, 70.31, 70.78, 73.35, 77.94, 127.48, 127.57, 128.29, 138.42; IR (neat): $\tilde{\nu}$ = 837, 1101, 1252, 1377, 1462, 2858, 2927, 2954 cm^{-1}; elemental analysis (%) calcd for $C_{56}H_{108}O_4Si$: C 77.00, H 12.46; found: C 76.71, H 12.68.

1-*O*-Benzyl-2-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-3-*O*-[(3*R*,7*R*,11*S*,15*S*)-16-hydroxy-3,7,11,15-tetramethylhexadecanyl]-*sn*-glycerol (13): A solution of tetrabutylammonium fluoride (1.00 mL, 1M in THF, 1.00 mmol) was added to a solution of **12** (385 mg, 0.440 mmol) in THF (10 mL). The mixture was stirred for 8 h at room temperature and concentrated in vacuo. The residue was chromatographed over silica gel with hexane/EtOAc (7:1–5:1) to give alcohol **13** (333 mg, quant.) as an oil. $[\alpha]_D^{27}$ +0.5 (c = 0.767, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 0.83–0.93 (m, 24H), 0.92 (d, J = 6.8 Hz, 3H), 1.00–1.70 (m, 48H), 3.39–3.66 (m, 11H), 4.55 (s, 2H), 7.26–7.34 (m, 5H); ^{13}C NMR (100 MHz): δ = 16.63, 19.68, 19.72, 19.76, 22.62, 22.71, 24.36, 24.40, 24.44, 24.47, 24.79, 27.97, 29.83, 29.91, 32.76, 32.79, 32.80, 33.49, 35.78, 36.64, 37.11, 37.29, 37.30, 37.36, 37.38, 37.40, 37.46, 37.53, 39.37, 68.39, 68.89, 69.97, 70.34, 70.81, 73.36, 77.95, 127.48, 127.57, 128.29, 138.43; IR (neat): $\tilde{\nu}$ = 1113, 1377, 1462, 2868, 2925, 2952, 3423 cm^{-1}; elemental analysis (%) calcd for $C_{50}H_{94}O_4$: C 79.09, H 12.48; found: C 78.80, H 12.46.

1-*O*-Benzyl-2-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-3-*O*-[(3*R*,7*R*,11*S*,15*S*)-15-formyl-3,7,11,15-tetramethylpentadecanyl]-*sn*-glycerol (14): To a stirred solution of oxalyl chloride (1.00 mL, 2M in CH_2Cl_2, 2.00 mmol) in CH_2Cl_2 (6 mL) was slowly added DMSO (200 μL, 2.82 mmol) at −78 °C. The mixture was stirred for 30 min. A solution of **13** (333 mg, 0.439 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 10 min. The mixture was stirred for 25 min, warmed to −25 °C, and stirring was continued for 4 h. The mixture was recooled to −78 °C, Et_3N (1.00 mL, 7.17 mmol) was added dropwise, and the mixture was gradually warmed to room temperature. Saturated aqueous NH_4Cl solution (3 mL) was added, and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane/EtOAc (20:1) to give aldehyde **14** (325 mg, 98%) as an oil. $[\alpha]_D^{27}$ +8 (c = 0.667, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 0.83–0.87 (m, 24H), 1.09 (d, J = 7.1 Hz, 3H), 1.00–1.75 (m, 48H), 2.34 (m, 1H), 3.42–3.66 (m, 9H), 4.55 (s, 2H), 7.26–7.34 (m, 5H), 9.61 (d, J = 2.2 Hz, 1H); ^{13}C NMR (100 MHz): δ = 13.37, 19.65, 19.69, 19.72, 19.75, 19.77, 22.63, 22.72, 24.37, 24.42, 24.45, 24.48, 24.79, 27.98, 29.83, 29.92, 30.89, 32.66, 32.80, 32.82, 36.65, 37.01, 37.12, 37.30, 37.34, 37.39, 37.41, 37.47, 37.54, 39.38, 46.35, 68.89, 69.98, 70.35, 70.82, 73.36, 77.97, 127.49, 127.57, 128.30, 138.46, 205.36; IR (neat): $\tilde{\nu}$ = 696, 735, 1115, 1377, 1462, 1730, 2860, 2925, 2952 cm^{-1}; elemental analysis (%) calcd for $C_{50}H_{92}O_4$: C 79.30, H 12.25; found: C 79.02, H 11.98.

1-*O*-Benzyl-2-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-3-*O*-[(3*R*,7*R*,11*S*,15*S*)-3,7,11,15-tetramethylheptadec-16-enyl]-*sn*-glycerol (15): To a suspension of methyltriphenylphosphonium bromide (310 mg, 0.868 mmol) in THF (7 mL) was added *n*BuLi (550 μL, 1.50M in hexane, 0.825 mmol) at −78 °C, and the mixture was stirred at the same temperature for 20 min and at room temperature for 20 min. The mixture was recooled to −78 °C and a solution of aldehyde **14** (324 mg, 0.428 mmol) in THF (5 mL) was added dropwise over 5 min. The mixture was stirred at −78 °C for 15 min and at −25 °C for 1 h. Saturated aqueous NH_4Cl solution (2 mL) was added, and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane/EtOAc (50:1–30:1) to give olefin **15** (324 mg, quant.) as an oil. $[\alpha]_D^{27}$ +5 (c = 0.967, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 0.83–0.87 (m, 24H), 0.98 (d, J = 6.6 Hz, 3H), 1.00–1.70 (m, 47H), 2.11 (m, 1H), 3.42–3.66 (m, 9H), 4.55 (s, 2H), 4.89 (ddd, J = 1.7, 2.0 and 10 Hz, 1H), 4.94 (ddd, J = 1.2, 1.7 and 17 Hz, 1H), 5.69 (ddd, J = 7.6, 10 and 17 Hz, 1H), 7.26–7.34 (m, 5H); ^{13}C NMR (100 MHz): δ = 19.69, 19.72, 19.75, 19.76, 20.23, 22.62, 22.72, 24.37, 24.49, 24.63, 24.79, 27.98, 29.83, 29.92, 32.76, 32.80, 32.82, 36.65, 36.99, 37.12, 37.30, 37.40, 37.47, 37.54, 37.75, 39.38, 68.89, 69.98, 70.35, 70.82, 73.36, 77.97, 112.19, 127.49, 127.58, 128.30, 138.46, 145.01; IR (neat): $\tilde{\nu}$ = 696, 733, 908, 1115, 1377, 1462, 1641, 2866, 2925, 2952 cm^{-1}; elemental analysis (%) calcd for $C_{51}H_{94}O_3$: C 81.10, H 12.54; found: C 81.24, H 12.79.

3,3′-*O*-[(3*R*,7*R*,11*S*,15*S*,18*S*,22*S*,26*R*,30*R*)-3,7,11,15,18,22,26,30-Octamethyldotriacont-16-ene-1,32-diyl]-1,1′-di-*O*-benzyl-2,2′-di-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-diglycerol (16): A mixture of olefin **15** (195 mg, 0.258 mmol) and $[RuCl_2(=CHPh)(PCy_3)_2]$ (42.3 mg, 51.4 μmol) in CH_2Cl_2 (5.1 mL) was refluxed for 33 h. After concentration of the mixture, the resulting residue was purified by flash chromatography over silica gel with hexane/EtOAc (30:1) to afford **16** (131 mg, 68%) and unreacted **15** (51 mg, 26%). Compound **16**: $[\alpha]_D^{27}$ +8 (c = 0.515, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 0.82–0.87 (m, 48H), 0.94 (d, J = 6.6 Hz, 6H), 1.00–1.70 (m, 94H), 2.02 (br, 2H), 3.42–3.66 (m, 18H), 4.55 (s, 4H), 5.17 (dd, J = 2.2 and 4.6 Hz, 2H), 7.24–7.34 (m, 10H); ^{13}C NMR (100 MHz): δ = 19.69, 19.71, 19.75, 19.77, 21.18, 22.63, 22.72, 24.36, 24.48, 24.51, 24.74, 24.79, 27.97, 29.82, 29.92, 32.76, 32.80, 32.81, 32.84, 36.65, 36.71, 37.11, 37.30, 37.40, 37.46, 37.54, 39.37, 68.88, 69.97, 70.34, 70.81, 73.36, 77.96, 127.48, 127.56, 128.29, 134.57, 138.44; IR (neat): $\tilde{\nu}$ = 696, 733, 1115, 1377, 1462, 2866, 2925, 2952 cm^{-1}; elemental analysis (%) calcd for $C_{100}H_{184}O_6$: C 81.02, H 12.51; found: C 80.99, H 12.77.

3,3′-*O*-[(3*R*,7*R*,11*S*,15*S*,18*S*,22*S*,26*R*,30*R*)-3,7,11,15,18,22,26,30-Octamethyldotriacontane-1,32-diyl]-2,2′-di-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-diglycerol [4 (X = H)]: A mixture of **16** (131 mg, 88.2 μmol) and 10% Pd-C (106 mg) in EtOAc (40 mL) was stirred for 18 h under an atmospheric pressure of hydrogen at room temperature. The mixture was filtered through a pad of Celite and washed with EtOAc. The filtrate and washings were concentrated to dryness. The residue was chromatographed over silica gel with hexane/EtOAc (7:1) to give **4** (X = H) (115 mg, quant.) as an oil. $[\alpha]_D^{28}$ +9 (c = 0.743, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 0.84–0.89 (m, 54H), 1.00–1.70 (m, 100H), 2.21 (br, 2H), 3.45–3.74 (m, 18H); ^{13}C NMR (100 MHz): δ = 19.67, 19.69, 19.75, 22.62, 22.72, 24.36, 24.46, 24.79, 27.96, 29.83, 29.88, 32.79, 33.05, 34.32, 36.58, 37.07, 37.28, 37.35, 37.39, 37.42, 37.44, 37.49, 37.57, 39.36, 63.07, 68.63, 70.15, 70.94, 78.30; IR (neat): $\tilde{\nu}$ = 1049, 1117, 1377, 1462, 2868, 2925, 2952, 3448 cm^{-1}; elemental analysis (%) calcd for $C_{86}H_{174}O_6$: C 79.19, H 13.45; found: C 79.13, H 13.71.

3,3′-*O*-[(3*R*,7*R*,11*S*,15*S*,18*S*,22*S*,26*R*,30*R*)-3,7,11,15,18,22,26,30-Octamethyldotriacontane-1,32-diyl]-2,2′-di-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-diglycero-1,1′-bisphosphoric acid [4 (X = PO_3H_2)]: Diphenylphosphoryl chloride (100 μL, 0.48 mmol) was added dropwise to a mixture of diol **4** (X = H) (26 mg, 20 μmol) and DMAP (13 mg, 0.11 mmol) in pyridine (0.5 mL) and benzene (2 mL) at room temperature, and the mixture was stirred at room temperature for 6 h. Aqueous HCl (2M, 1 mL) was added and the mixture was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with benzene/EtOAc (25:1) to give bisphosphotriester (34 mg, 97%) as an oil. A mixture of bisphosphotriester (31 mg, 18 μmol) and PtO_2 (17 mg) in acetic acid (5 mL) was stirred at room temperature under a hydrogen atmosphere for 10 h. The catalyst was filtered through a pad of Celite and washed with $CHCl_3/CH_3OH$ (2:1 *v*/*v*). The filtrate and washings were combined and concentrated to dryness. The residue was purified over Sephadex®LH-20 with $CHCl_3/CH_3OH$ (2:1 *v*/*v*)

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to give bisphosphoric acid **4** (X = P(=O)(OH)$_2$) (17 mg, 64 %) as a hygroscopic wax. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (8:1)): $\delta = 0.84 - 0.89$ (m, 54 H), 1.00 – 1.70 (m, 100 H), 3.45 – 3.70 (m, 14 H), 3.98 (br, 4 H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ = 8:1): $\delta = 19.42$, 19.45, 19.54, 19.63, 22.41, 22.51, 24.22, 24.33, 24.63, 27.80, 29.52, 29.65, 29.78, 32.67, 32.89, 34.10, 36.47, 36.75, 37.13, 37.26, 37.28, 37.31, 37.41, 39.51, 65.04 (d, $J = 5.8$ Hz), 68.81, 70.01, 70.24, 77.56 (d, $J = 7.4$ Hz); ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ = 8:1): $\delta = 2.64$; elemental analysis (%) calcd for $C_{86}H_{176}O_{12}P_2$: C 70.54, H 12.11; found: C 70.83, H 12.21.

1-*O*-Benzyl-2-*O*-[(3*R*,7*R*,11*S*,15*S*)-16-*tert*-butyldimethylsilyloxy-3,7,11,15-tetramethylhexadecanyl]-3-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-glycerol (18): Compound **11** (571 mg, 1.23 mmol) was treated in the same manner as described for the preparation of **12** to give **18** (656 mg, 61 %) as an oil. $[\alpha]_D^{28}$ +0.1 ($c = 0.887$, $CHCl_3$); ^{1}H NMR (400 MHz): $\delta = 0.04$ (s, 6 H), 0.84 (d, $J = 6.6$ Hz, 12 H), 0.87 (d, $J = 6.6$ Hz, 15 H), 0.89 (s, 9 H), 1.00 – 1.70 (m, 48 H), 3.34 (dd, $J = 6.8$ and 9.8 Hz, 1 H), 3.43 – 3.66 (m, 10 H), 4.56 (s, 2 H), 7.26 – 7.34 (m, 5 H); ^{13}C NMR (100 MHz): $\delta = -5.34$, 16.82, 18.37, 19.69, 19.72, 19.76, 22.63, 22.72, 24.38, 24.41, 24.49, 24.80, 25.97, 27.98, 29.85, 29.92, 32.78, 32.80, 32.83, 33.54, 35.78, 36.66, 37.13, 37.30, 37.40, 37.43, 37.47, 37.54, 37.56, 39.38, 68.44, 68.91, 69.99, 70.38, 70.83, 73.37, 77.98, 127.49, 127.58, 128.30, 138.47; IR (neat): $\tilde{\nu} = 775$, 837, 1101, 1252, 1377, 1462, 2858, 2927, 2952 cm^{-1}; elemental analysis (%) calcd for $C_{56}H_{108}O_4Si$: C 77.00, H 12.46; found: C 76.74, H 12.76.

1-*O*-Benzyl-2-*O*-[(3*R*,7*R*,11*S*,15*S*)-16-hydroxy-3,7,11,15-tetramethylhexadecanyl]-3-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-glycerol (19): Compound **18** (545 mg, 0.623 mmol) was treated in the same manner as described for the preparation of **13** to give alcohol **19** (423 mg, 89 %) as an oil. $[\alpha]_D^{27}$ +0.1 ($c = 1.01$, $CHCl_3$); ^{1}H NMR (400 MHz): $\delta =$ 0.83 – 0.87 (m, 24 H), 0.92 (d, $J = 6.6$ Hz, 3 H), 1.00 – 1.70 (m, 48 H), 3.39 – 3.66 (m, 11 H), 4.55 (s, 2 H), 7.26 – 7.34 (m, 5 H); ^{13}C NMR (100 MHz): $\delta =$ 16.64, 19.69, 19.72, 19.77, 22.63, 22.72, 24.37, 24.40, 24.44, 24.48, 24.80, 27.98, 29.83, 29.91, 32.76, 32.80, 32.82, 33.50, 35.79, 36.65, 37.11, 37.30, 37.31, 37.39, 37.41, 37.46, 37.53, 37.55, 39.37, 68.40, 68.90, 69.98, 70.34, 70.81, 73.36, 77.96, 127.49, 127.58, 128.30, 138.44; IR (neat): $\tilde{\nu} = 1113$, 1377, 1462, 2868, 2925, 2952, 3444 cm^{-1}; elemental analysis (%) calcd for $C_{50}H_{94}O_4$: C 79.09, H 12.48; found: C 78.81, H 12.50.

1-*O*-Benzyl-2-*O*-[(3*R*,7*R*,11*S*,15*S*)-15-formyl-3,7,11,15-tetramethylpentadecanyl]-3-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-glycerol (20): Compound **19** (371 mg, 0.488 mmol) was treated in the same manner as described for **14** to give aldehyde **20** (299 mg, 80 %) as an oil. $[\alpha]_D^{28}$ +7 ($c = 0.880$, $CHCl_3$); ^{1}H NMR (400 MHz): $\delta = 0.83 - 0.87$ (m, 24 H), 1.09 (d, $J = 7.1$ Hz, 3 H), 1.00 – 1.75 (m, 48 H), 2.34 (ddd, $J = 1.7$, 6.8 and 13.4 Hz, 1 H), 3.42 – 3.66 (m, 9 H), 4.55 (s, 2 H), 7.26 – 7.34 (m, 5 H), 9.61 (d, $J = 2.0$ Hz, 1 H); ^{13}C NMR (100 MHz): $\delta = 13.33$, 19.63, 19.66, 19.70, 19.73, 22.60, 22.69, 24.34, 24.39, 24.42, 24.45, 24.77, 27.93, 29.79, 29.86, 30.85, 32.62, 32.76, 32.78, 36.61, 36.97, 37.07, 37.26, 37.31, 37.35, 37.37, 37.42, 37.49, 37.51, 39.34, 46.31, 68.84, 69.93, 70.29, 70.76, 73.31, 77.92, 127.45, 127.54, 128.26, 138.39, 205.29; IR (neat): $\tilde{\nu} = 665$, 696, 735, 1115, 1377, 1462, 1730, 2860, 2925, 2952 cm^{-1}; elemental analysis (%) calcd for $C_{50}H_{92}O_4$: C 79.30, H 12.25; found: C 79.34, H 12.23.

1-*O*-Benzyl-2-*O*-[(3*R*,7*R*,11*S*,15*S*)-3,7,11,15-tetramethylheptadec-16-enyl]-3-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-glycerol (21): Compound **20** (203 mg, 0.268 mmol) was treated in the same manner as described for **15** to give olefin **21** (183 mg, 90 %) as an oil. $[\alpha]_D^{28}$ +5 ($c =$ 0.750, $CHCl_3$); ^{1}H NMR (400 MHz): $\delta = 0.83 - 0.87$ (m, 24 H), 0.98 (d, $J =$ 6.6, 3 H), 1.00 – 1.70 (m, 47 H), 2.11 (m, 1 H), 3.42 – 3.66 (m, 9 H), 4.55 (s, 2 H), 4.89 (ddd, $J = 1.7$, 2.0 and 10 Hz, 1 H), 4.94 (ddd, $J = 1.2$, 1.7 and 17 Hz, 1 H), 5.69 (ddd, $J = 7.6$, 10 and 17 Hz, 1 H), 7.26 – 7.34 (m, 5 H); ^{13}C NMR (100 MHz): $\delta = 19.69$, 19.72, 19.75, 19.76, 20.23, 22.63, 22.72, 24.37, 24.49, 24.63, 24.80, 27.98, 29.83, 29.92, 32.76, 32.80, 32.82, 36.65, 36.99, 37.12, 37.30, 37.40, 37.48, 37.54, 37.76, 39.38, 68.89, 69.98, 70.35, 70.82, 73.36, 77.97, 112.20, 127.49, 127.58, 128.30, 138.46, 145.01; IR (neat): $\tilde{\nu} = 665$, 696, 733, 908, 1115, 1377, 1462, 1639, 2866, 2925, 2952 cm^{-1}; elemental analysis (%) calcd for $C_{51}H_{94}O_3$: C 81.10, H 12.54; found: C 80.80, H 12.77.

2,2′-*O*-[(3*R*,7*R*,11*S*,15*S*,18*S*,22*S*,26*R*,30*R*)-3,7,11,15,18,22,26,30-Octamethyldotriacont-16-ene-1,32-diyl]-1,1′-di-*O*-benzyl-3,3′-di-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-diglycerol (22): Compound **21** (85.0 mg, 0.113 mmol) was treated in the same manner as described for the preparation of **16** to give *E*-olefin **22** (51 mg, 61 %) and unreacted **21** (30 mg, 36 %) as an oil. Compound **22**: $[\alpha]_D^{24}$ +9 ($c = 0.510$, $CHCl_3$); ^{1}H NMR (400 MHz): $\delta = 0.82 - 0.87$ (m, 48 H), 0.94 (d, $J = 6.6$ Hz, 6 H), 1.00 – 1.70 (m, 94 H), 2.03 (br, 2 H), 3.42 – 3.66 (m, 18 H), 4.55 (s, 4 H), 5.18 (dd, $J = 2.2$ and 4.6 Hz, 2 H), 7.25 – 7.33 (m, 10 H); ^{13}C NMR (100 MHz): $\delta = 19.69$, 19.72, 19.76, 19.77, 21.18, 22.63, 22.72, 24.38, 24.48, 24.52, 24.74, 24.80, 27.98, 29.84, 29.92, 32.77, 32.80, 32.82, 32.85, 36.66, 36.71, 37.12, 37.30, 37.39, 37.42, 37.44, 37.46, 37.54, 37.56, 39.38, 68.89, 69.97, 70.36, 70.81, 73.36, 77.98, 127.48, 127.57, 128.29, 134.58, 138.46; IR (neat): $\tilde{\nu} = 696$, 733, 1115, 1377, 1462, 2866, 2925, 2952 cm^{-1}; elemental analysis (%) calcd for $C_{100}H_{184}O_6$: C 81.02, H 12.51; found: C 81.31, H 12.58.

2,2′-*O*-[(3*R*,7*R*,11*S*,15*S*,18*S*,22*S*,26*R*,30*R*)-3,7,11,15,18,22,26,30-Octamethyldotriacontane-1,32-diyl]-3,3′-di-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-diglycerol [5 (X = H)]: Compound **22** (100 mg, 0.0677 mmol) was treated in the same manner as described for the preparation of **4** (X = H) to give **5** (X = H) (87 mg, 98 %) as an oil. $[\alpha]_D^{27}$ +9 ($c = 0.533$, $CHCl_3$); ^{1}H NMR (400 MHz): $\delta = 0.84 - 0.89$ (m, 54 H), 1.00 – 1.70 (m, 100 H), 2.19 (t, $J = 6.1$ Hz, 2 H), 3.44 – 3.75 (m, 18 H); ^{13}C NMR (100 MHz): $\delta = 19.67$, 19.70, 19.72, 19.75, 22.62, 22.71, 24.36, 24.47, 24.79, 27.97, 29.86, 29.89, 32.80, 33.06, 33.14, 34.33, 34.43, 36.59, 37.08, 37.29, 37.35, 37.38, 37.40, 37.44, 37.49, 37.52, 37.58, 39.37, 63.10, 68.65, 70.16, 70.95, 78.30; IR (neat): $\tilde{\nu} = 1049$, 1115, 1377, 1462, 2868, 2925, 2952, 3458 cm^{-1}; elemental analysis (%) calcd for $C_{86}H_{174}O_6$: C 79.19, H 13.45; found: C 79.18, H 13.61.

2,2′-*O*-[(3*R*,7*R*,11*S*,15*S*,18*S*,22*S*,26*R*,30*R*)-3,7,11,15,18,22,26,30-Octamethyldotriacontane-1,32-diyl]-3,3′-di-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-diglycero-1,1′-bisphosphoric acid [5 (X = P(=O)(OH)$_2$)]: Compound **5** (X = H) (25 mg, 19 μmol) was treated in the same manner as described for the preparation of **4** to give bisphosphotriester (29 mg, 86 %), part of which (25 mg, 14 μmol) was further treated to afford **5** (X = P(=O)(OH)$_2$) (9 mg, 43 %) as a hygroscopic wax. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (2:1)): $\delta = 0.84 - 0.89$ (m, 54 H), 1.00 – 1.70 (m, 100 H), 3.45 – 3.70 (m, 14 H), 3.98 (br, 4 H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ (2:1)): $\delta = 19.22$, 19.32, 19.38, 22.17, 22.27, 24.05, 24.13, 24.45, 27.63, 29.33, 29.44, 29.57, 32.47, 32.71, 33.94, 36.27, 36.58, 36.93, 37.09, 37.21, 39.03, 65.07 (br), 68.68, 69.83, 70.07, 77.30 (br); ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ = 2:1): $\delta = 0.57$; elemental analysis (%) calcd for $C_{86}H_{176}O_{12}P_2$: C 70.54, H 12.11; found: C 70.24, H 11.94.

Microscopy: The self-organization of the amphiphiles **1, 3ab**, **4**, and **5** in water was studied by phase contrast and fluorescence microscopy. Giant vesicles were prepared from the lipids following reported procedures.[2, 22] The lipid (0.5 mg) was hydrated in Tris – HCl buffer (3.2 mL, 0.05 M) at pH 7.8 or glycine – NaOH buffer (0.05 M) at pH 8.4 (conditions leading to about 50 % dianion). For lipid **4**, a 2 – 3 molar excess of neat phytanol (**6**), phytol (**23**), or geranylgeraniol (**24**) was added to the lipid film and hydrated as usual. Fluorescence microscopy was used to confirm vesicle formation: the lipid film was prepared with a $CHCl_3$/MeOH solution of Nile Red and, after hydration of the film, the plate was observed in a fluorescence microscope.

Phase contrast and fluorescence microscopies were carried out with an inverted microscope (Axiovert 135, Carl Zeiss) equipped with a charge-coupled device camera (C2400 – 75i, Hamamatsu Photonics). The images were stored and processed on a 7500/100 PowerMacintosh connected to an image processor (Argus-20, Hamamatsu Photonics).

X-ray diffraction: The samples of lipids **1** or **4** (2 mg) were suspended in Millipore "pure" water (50 μL, pH 7.0) and transferred into thin-walled glass tubes and mounted on a Peltier-controlled sample holder with X-ray transparent windows. Diffraction patterns were recorded on the A2 double focusing monochromator-mirror camera[23] at Hasylab, at the Deutsches Elektronen Synchrotron (DESY) in Hamburg, on the storage ring Doris. The wavelength was fixed at 0.15 nm. We used a data acquisition system allowing simultaneous recording of reflections at different angular regimes.[24, 25] A fast solenoid-driven lead shutter controlled by the data acquisition system was used to prevent irradiation of the sample when no diffraction data were collected. For further details on the X-ray diffraction technique and data analysis, see refs.[26, 27]

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