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Specific Deuterium Labeling of Archaeal 36-Membered Macrocyclic Diether Lipid

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Described in this paper is a practical synthesis of the specifically deuterated 36-membered macrocyclic diether lipid **4** and its acyclic deuterated counterpart **5**, both of which are essential to the detailed biophysical studies of the conformational order of phospholipids in vesicles. The synthesis of **4** was principally composed of the preparation of deuterated diether **15**, subsequent macrocyclization of the crucial dialdehyde **21** by McMurry coupling, and final transformation into the phosphocholine derivative. The acyclic deuterated counterpart **5** was also synthesized from the common intermediary tosylate **16**.

Archaea (archaebacteria) have been attracting considerable attention from both biochemical and evolutionary perspectives. It is established that these bacteria are distinct from prokaryotes and eukaryotes, and are now classified in a third independent domain.[1] Archaea usually inhabit extraordinary environments such as high temperature, highly acidic, and/or salt-rich conditions, or the complete absence of oxygen. The characteristic feature of archaea is found in the chemical structure of its core membrane lipids. The basic core structure of the archaeal membrane is 2,3-di-*O*-phytanyl-*sn*-glycerol **1**, as shown in Fig. 1.[2] The most striking feature of archaeal membrane lipids is found in the presence of macrocyclic structures as large as 36- and 72-membered rings of thermoacidophilic archaea and methanogens (**2** and **3ab**), as also shown in Fig. 1.[2] Recently, we have addressed the first syntheses of the macrocyclic 36-membered and 72-membered lipids by using McMurry coupling[3] as a key reaction of macrocyclization.[4]

These macrocyclic lipids may play some important role *per se* in adaptation to the extreme environments. Several modeling and synthetic studies have been reported so far to investigate the stability, fluidity, and permeability of the archaeal membrane lipids especially in terms of their thermostability, since these lipids may provide a clue to the development of heat-resistant membranes.[5] Along this line, we recently synthesized a novel macrocyclic phosphate, dotriacontan-1,32-diyl phosphate, as a model of archaeal macrocyclic lipids, and discussed the bilayer and monolayer properties with the comparative experiments using specifically deuterated cyclic and acyclic lipids. It appeared consequently that the covalent binding at the terminal position of the hydrophobic chains affects the entire conformation of the model lipids.[6] We have now turned our attention to the properties of membranes composed of natural archaeal macrocyclic lipids.

^{2}H NMR spectroscopy and small-angle neutron scattering are powerful tools for detailed biophysical studies of conformational and motional properties,[7] and the organized structures in vesicles,[8] respectively. Prerequisite for these studies is to develop a convenient synthetic method for specifically deuterated macrocyclic lipids in a significant scale. Smith et al. previously reported the molecular order and dynamics of diphytanylglycerol phospholipids using the specifically deuterated lipids at the 1-, 2-, and 3-positions of the phytanyl moiety, by comparing them with *n*-alkyl and *n*-acyl chains of phosphatidic acids by ^{2}H and ^{31}P NMR.[5c] It was pointed out accordingly that branched methyl groups affect both the molecular order and dynamics and that the ether linkages increase the segmental order. However, the conformational and motional properties of the inner regions of bilayer composed of archaeal lipids have yet to be described. Especially, no investigation has appeared concerning the archaeal 36-membered macrocyclic lipid due to the lack of a sufficient supply of appropriately labeled probes. In this paper, we wish to report a practical synthesis of specifically deuterium labeled 36-membered diether phospholipid **4** and its acyclic counterpart **5** (Fig. 2).

Results and Discussion

To address the significance of the macrocyclic structure of the archaeal 36-membered lipid on the molecular order and dynamics, site-specific deuterium labeling at the inner regions of the lipid bilayer appeared to be necessary. Site-specific labeling of archaeal lipids should provide particular information to such positions. Furthermore, comparative studies between the macrocyclic lipid and its acyclic counterpart with specific deuterium labeling at similar positions is indispensable for detailed biophysical analysis. We, therefore, envisioned the specifically deuterium labeled 36-membered diether phospholipid **4** and its acyclic counterpart **5** as targeted probes.

The synthesis of **4** started from the previously prepared C_{10} sulfone **6**[4b] as shown in Scheme 1. The lithium carbanion

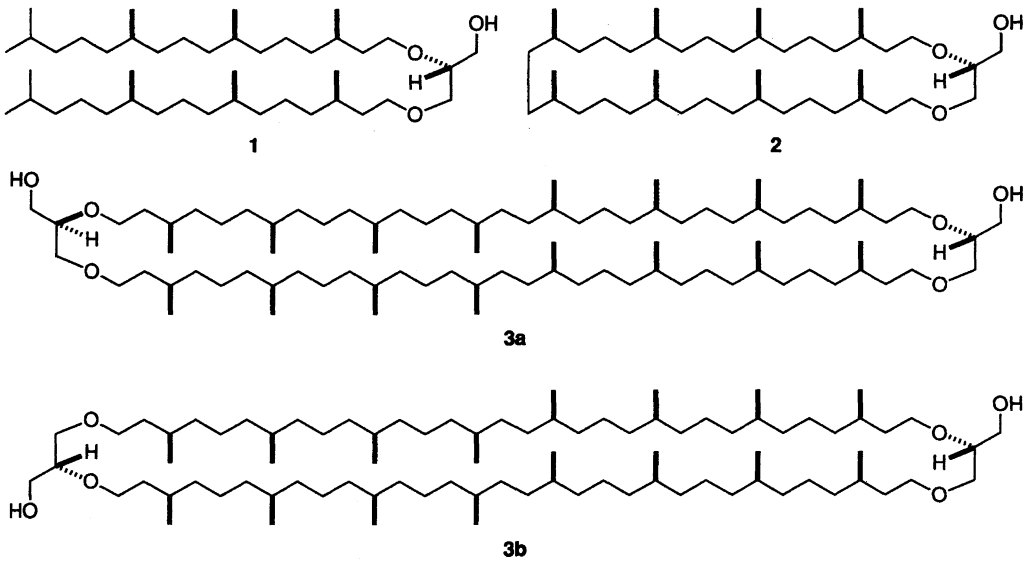


Fig. 1. Typical structures of archaeal core membrane lipids (**1**, **2**, and **3ab**).

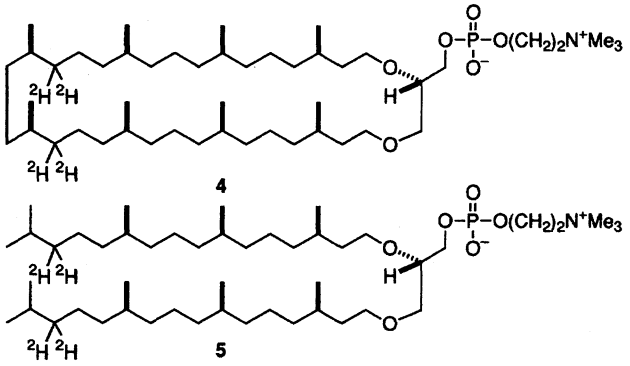


Fig. 2.

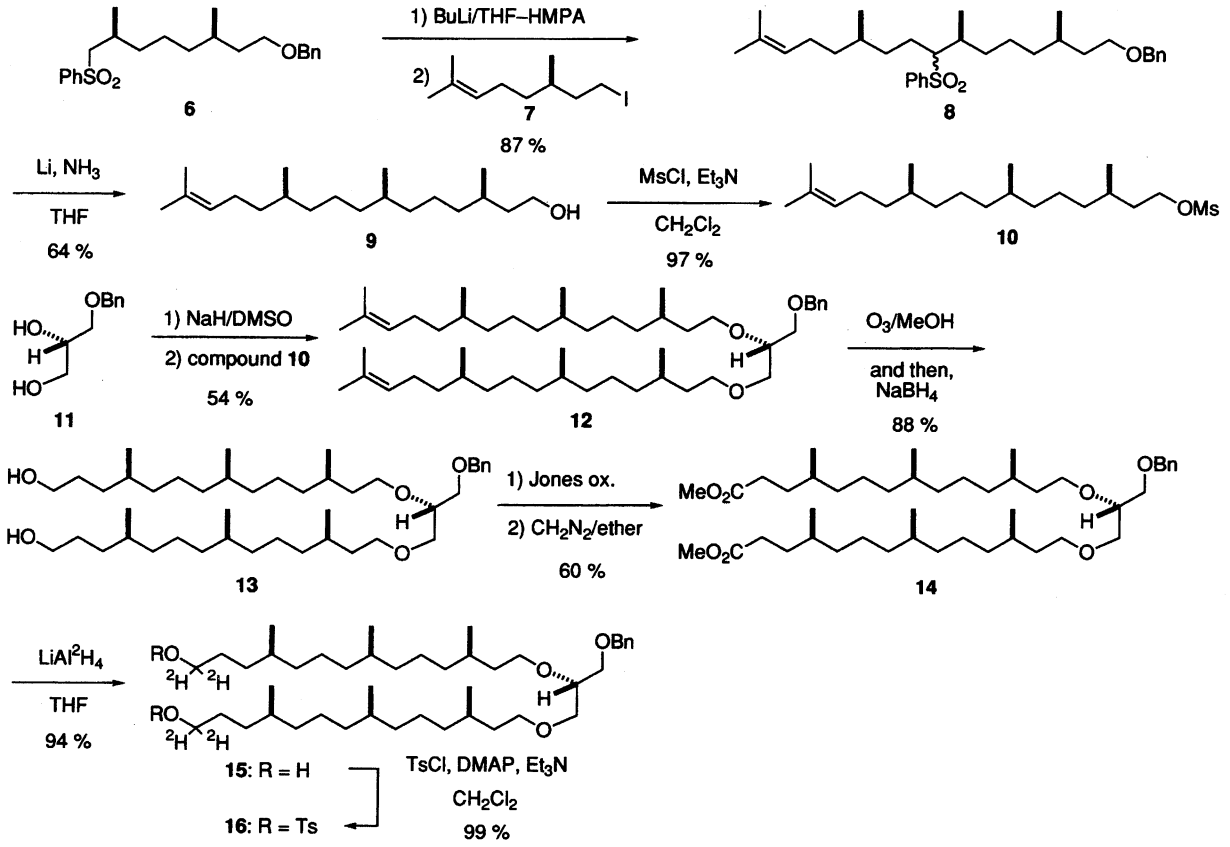
prepared from **6** was treated with (*R*)-citronellyl iodide **7**[9] in THF-HMPA at −25 °C to furnish a coupled product **8** in 87% yield. The phenylsulfonyl and benzyl groups were reductively removed under the Birch conditions to give an intermediary C_{20} unit **9** in 64% yield. The *sn*-2-*O*-alkylated glycerol **11**[10] was alkylated via its sodium alkoxide with the mesylate derivative **10** prepared from the C_{20} unit **9** to afford 2,3-*O*-disubstituted *sn*-1-*O*-benzylglycerol **12** in 54% yield. To introduce deuterium, the terminal double bonds of **12** were oxidatively transformed into ester groups by a series of reactions from ozonolysis, sodium borohydride (tetrahydroborate) reduction, Jones oxidation, and esterification with diazomethane to afford diester **14**. The diester **14** was then treated with $LiAl^2H_4$ to give the desired diol-d_4 **15** in an excellent yield. The deuterium content was estimated at this stage to be 99% by ^1H NMR. The deuterated alcohol **15** was converted in a conventional way to ditosylate **16** in 99% yield. The ditosylate **16** appeared to be versatile and was used as a common precursor to both **4** and **5**.

Next, we applied the previously described methodology for preparation of a C_{10} unit[4b] from the intermediary ditosylate **16** (Scheme 2). The tosyloxy groups of **16** were displaced by treatment with lithium bromide in DMF to afford dibromide-d_4 **17**. Coupling of **17** with acetylide anion derived from tetrahydro-2-(2-propynyloxy)-2*H*-pyran in THF-HMPA, followed by acid hydrolysis, gave bis-alkynyl alcohol-d_4 **18** in 92% yield. Reduction of **18** with $LiAlH_4$ gave bis-(*E*)-allylic alcohol-d_4 **19** with high selectivity in 48% yield. The bis-allylic alcohol-d_4 **19** was subjected to Sharpless asymmetric epoxidation[11] to give diepoxide-d_4 **20** in 59% yield. Stereoselective epoxide opening with trimethylaluminum in CH_2Cl_2,[12] followed by treatment with sodium periodate gave dialdehyde-d_4 **21** in 95% yield.

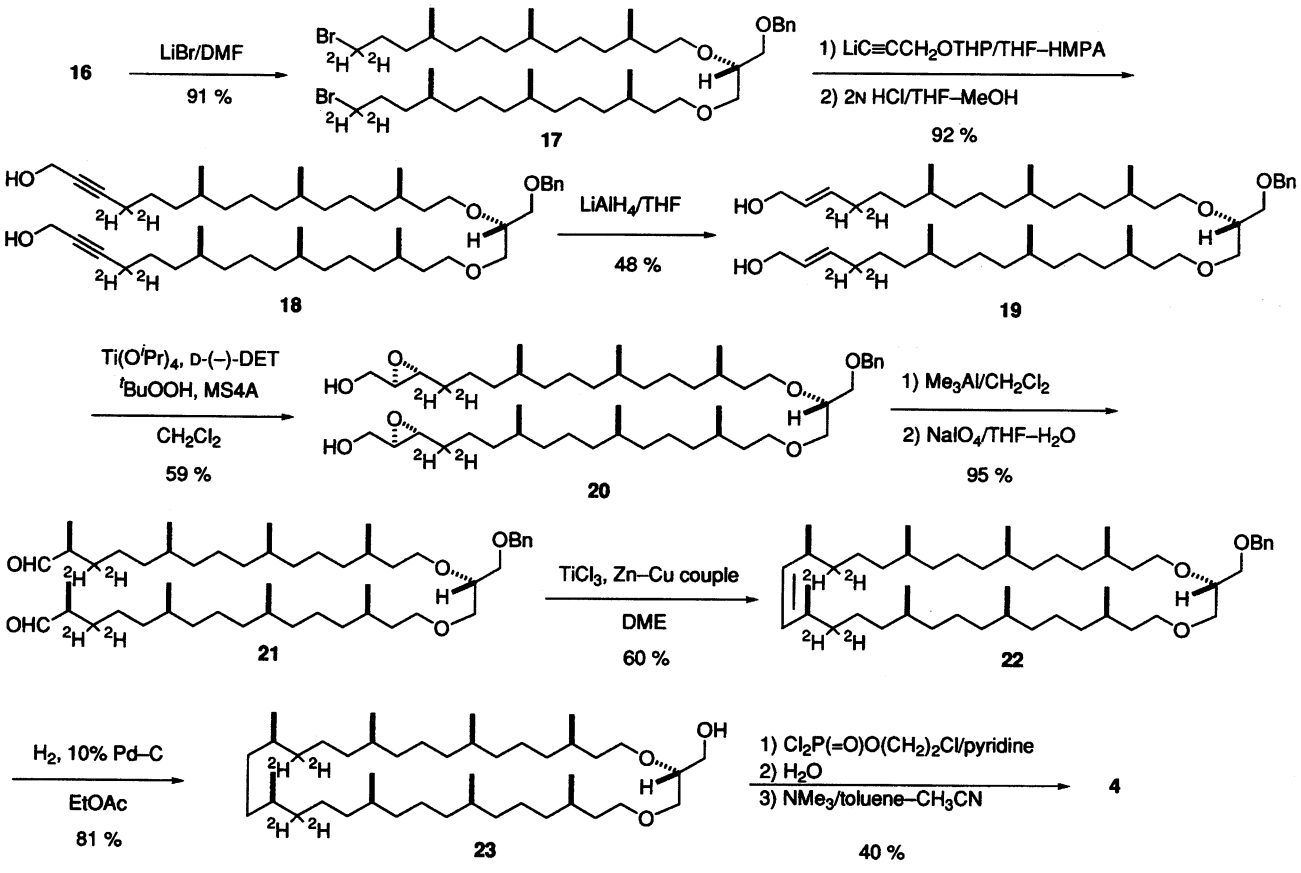
The dialdehyde-d_4 **21** underwent the key reaction of an intramolecular McMurry coupling under high dilution conditions as reported previously[4] to yield a 36-membered olefinic compound **22** in 60% yield. Reduction of the double bond and deprotection of the benzyl group were simultaneously performed by catalytic hydrogenation in the presence of Pd–C to give deuterated 36-membered core lipid-d_4 **23** in 81% yield. The final transformation of **23** into a phosphocholine derivative **4** was carried out by the previously described manipulation[4b] in 40% yield.

The deuterated acyclic counterpart **5** was synthesized from the intermediary ditosylate **16** as shown in Scheme 3. The coupling reaction of ditosylate **16** with isopropylmagnesium bromide in the presence of dilithium tetrachlorocuprate(II)[13] proceeded smoothly to provide a deuterated diphytanylglycerol derivative **24** in 87% yield. Catalytic hydrogenation of **24** gave a specifically deuterated diphytanylglycerol core **25** in 92% yield. Subsequently, **25** was transformed into a phosphocholine derivative **5** in 52% yield.

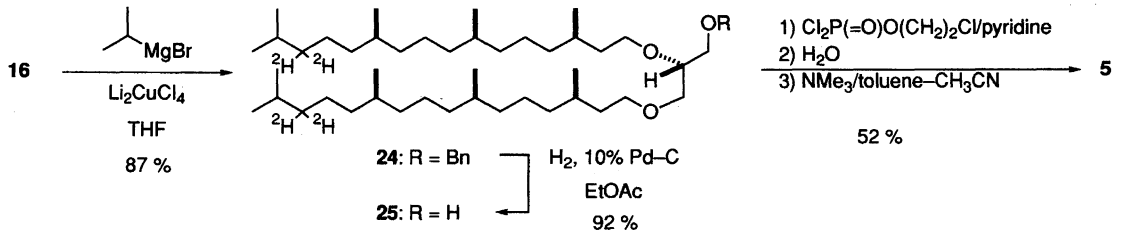
In summary, we have accomplished the synthesis of the specifically deuterium labeled archaeal diether phospholipids **4** and **5**. These deuterated lipids must be useful for investigations to get more insight into the characteristics of the thermophilic archaeal macrocyclic diether lipids. Further studies using these deuterated lipids are currently in progress and will be reported in due course.



Scheme 1.



Scheme 2.



Scheme 3.

Experimental

IR spectra were taken on a Horiba FT-710 Fourier transform infrared spectrometer. Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. ^{1}H NMR, ^{2}H NMR, and ^{13}C NMR spectra were recorded on JEOL LA-300 and/or LA-400 spectrometers. Deuteriochloroform (99.8 atom% enriched, Merck) was used for the NMR solvent, unless otherwise indicated. NMR chemical shifts were reported as δ values based on internal TMS ($\delta_H = 0$) or solvent signal ($CDCl_3$ $\delta_C = 77.0$) as reference. Mass spectra were obtained by using a JEOL AX-505HA mass spectrometer. Column chromatography was done with a Kieselgel 60 (70—230 mesh or 230—400 mesh, Merck). All reactions, except for catalytic hydrogenation, were carried out in an inert (Ar or N_2) atmosphere. THF and DME were distilled from sodium/benzophenone ketyl. Pyridine and triethylamine were distilled from potassium hydroxide. DMF was distilled from $CaSO_4$. DMSO, CH_2Cl_2, HMPA, toluene, and CH_3CN were distilled from calcium hydride.

(3*R*,7*S*,11*R*)-1-Benzyloxy-3,7,11,15-tetramethyl-8-phenylsulfonyl-14-hexadecene (8). A 100-ml Schlenk tube was charged with (2*S*,6*R*)-8-benzyloxy-1-phenylsulfonyl-2,6-dimethyloctane (**6**) (1.50 g, 3.87 mmol)[4b] and degassed THF (15 ml). A solution of BuLi in hexane (2.55 ml, 1.59 M, 4.05 mmol; 1 M = 1 mol dm^{-3}) was added dropwise, and the mixture was stirred at −78 °C for 20 min, and then at 0 °C for 15 min. The mixture was recooled to −25 °C, and HMPA (6 ml) was added at the same temperature. After 25 min of stirring at −25 °C, a solution of (*R*)-citronellyl iodide (**7**) (1.18 g, 4.42 mmol) in degassed THF (11 ml) was added dropwise, and the mixture was stirred at −25 °C for 4 h. Saturated aqueous NH_4Cl 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 (10 : 1) to give oily sulfone (**8**) as a mixture of diastereomers (1.78 g, 87%). ^{1}H NMR (400 MHz) δ = 0.79 (d, J = 6.4 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H), 1.00—1.70 (m, 15H), 1.58 (s, 3H), 1.68 (s, 3H), 1.75—1.95 (m, 3H), 2.10—2.25 (m, 1H), 2.82—2.89 (dt, J = 1.7, 5.6 Hz, 1H), 3.42—3.52 (m, 2H), 4.49 (s, 2H), 5.04 (tt, J = 1.2, 7.1 Hz, 1H), 7.26—7.35 (m, 5H), 7.53—7.64 (m, 3H), 7.87—7.89 (m, 2H); IR (neat) 692, 733, 1086, 1099, 1146, 1304, 1446, 2856, 2925, 2956 cm^{-1}. Found: C, 75.54; H, 9.69%. Calcd for $C_{33}H_{50}O_3S$: C, 75.24; H, 9.57%.

(3*R*,7*R*,11*S*)-3,7,11,15-Tetramethylhexadec-14-en-1-ol (9). Small pieces of Li (3.0 g, 432 mmol) was added to dry liquid ammonia (ca. 300 ml) at −78 °C, and the mixture was stirred for 3.5 h. A solution of sulfone (**8**) (4.18 g, 7.93 mmol) in THF (60 ml) was added dropwise over 10 min at −78 °C. The mixture was stirred at −78 °C for 25 min, and then at 0 °C for 2 h. After the mixture was recooled to −78 °C, the reaction was quenched by addition of methanol and solid NH_4Cl. The mixture was allowed to warm to room temperature and was left overnight. The residue was diluted with ether and saturated aqueous NH_4Cl, and the aqueous phase was extracted with ether. The combined 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 alcohol (**9**) (1.50 g, 64%) as an oil. $[\alpha]_D^{27}$ +0.31° (*c* 0.43, $CHCl_3$); ^{1}H NMR (400 MHz) δ = 0.84 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 6.1 Hz, 3H), 0.90 (d, J = 6.4 Hz, 3H), 1.00—1.70 (m, 18H), 1.75—1.85 (m, 2H), 1.60 (s, 3H), 1.68 (s, 3H), 1.88—2.03 (m, 2H), 3.63—3.73 (m, 2H), 5.10 (tt, J = 1.2, 7.1 Hz, 1H); ^{13}C NMR (100 MHz) δ = 17.60, 19.64, 19.67, 19.76, 24.36, 24.40, 25.58, 25.70, 29.53, 32.44, 32.79, 37.10, 37.31, 37.37, 37.49, 39.97, 61.25, 125.10, 130.91; IR (neat) 1057, 1377, 1462, 2858, 2925, 2952, 3330 cm^{-1}. Found: C, 81.25; H, 13.36%. Calcd for $C_{20}H_{40}O$: C, 81.01; H, 13.60%.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*)-3,7,11,15-tetramethylhexadec-14-en-1-yl]-*sn*-glycerol (12). Methanesulfonyl chloride (480 μl, 6.20 mmol) was added dropwise to a mixture of alcohol (**9**) (1.51 g, 5.10 mmol) and Et_3N (2.15 ml, 15.4 mmol) in CH_2Cl_2 (15 ml) at 0 °C and the solution was stirred at 0 °C for 1 h. Water (10 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 (10 : 1) to give methanesulfonate (**10**) (1.86 g, 97%).

To a suspension of pre-washed NaH (186 mg, 7.77 mmol) in DMSO (10 ml) was added a solution of 1-*O*-benzyl-*sn*-glycerol (**11**) (443 mg, 2.43 mmol) in DMSO (9 ml) at 10 °C, and the mixture was stirred at room temperature for 2 h. A solution of the separately prepared mesylate (**10**) (1.78 g, 4.57 mmol) in DMSO (14 ml) was added at room temperature and the resulting was stirred at room temperature for 2 d. Water (20 ml) was added and the mixture was extracted with EtOAc. The combined organic extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane–EtOAc (100 : 1 to 20 : 1) to give a dialkylated glycerol derivative (**12**) (964 mg, 54%) as an oil. $[\alpha]_D^{26}$ −0.50° (*c* 0.35, $CHCl_3$); ^{1}H NMR (400 MHz) δ = 0.85 (d, J = 7.6 Hz, 6H), 0.86 (d, J = 6.6 Hz, 6H), 0.87 (d, J = 6.6 Hz, 6H), 1.00—1.70 (m, 34H), 1.60 (s, 6H), 1.68 (s, 6H), 1.75—1.88 (m, 4H), 1.90—2.10 (m, 4H), 3.44—3.66 (m, 9H), 4.56 (s, 2H), 5.10 (tt, J = 1.2, 7.1 Hz, 2H), 7.26—7.34 (m, 5H); ^{13}C NMR (100 MHz) δ = 17.62, 19.64, 19.68, 19.71, 19.76, 24.36, 24.42, 25.58, 25.72, 29.82, 29.90, 32.44, 32.81, 36.64, 37.11, 37.32, 37.39, 37.54, 68.88, 69.97, 70.32, 70.79, 73.35, 77.94, 125.10, 127.49, 125.57, 128.29, 130.92, 138.43; IR (neat) 698, 733, 1115, 1377, 1454, 1745, 2858, 2925, 2956 cm^{-1}. Found: C, 81.51; H, 12.50%. Calcd for $C_{50}H_{90}O_3$: C, 81.24; H, 12.27%.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*)-14-hydroxy-3,7,11-trimethyltetradecyl]-*sn*-glycerol (13). Ozone was introduced into a solution of (**12**) (800 mg, 1.08 mmol) in methanol (20 ml) at −78 °C for 1.5 h. $NaBH_4$ (120 mg, 3.17 mmol) was carefully added to the mixture at −78 °C, and the mixture was gradually warmed to room temperature and continuously stirred for 5 h. The solvent was removed in vacuo and the residue was diluted with ether

and 2 M HCl. The aqueous phase was extracted with ether. The combined organic phase was successively washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane–EtOAc (4 : 1 to 2 : 1) to give diol (**13**) (655 mg, 88%) as an oil. $[\alpha]_D^{20}$ +2.16° (*c* 0.97, $CHCl_3$); ^{1}H NMR (300 MHz) δ = 0.84 (d, *J* = 7.1 Hz, 6H), 0.86 (d, *J* = 7.1 Hz, 6H), 0.87 (d, *J* = 6.6 Hz, 6H), 1.50—1.85 (m, 42H), 3.38—3.64 (m, 13H), 4.55 (s, 2H), 7.26—7.34 (m, 5H); ^{13}C NMR (75 MHz) δ = 19.60, 19.66, 19.71, 19.76, 24.34, 24.38, 29.79, 29.86, 30.32, 32.59, 32.61, 32.74, 32.77, 32.87, 32.96, 36.59, 36.67, 37.05, 37.13, 37.19, 37.24, 37.29, 37.37, 37.41, 37.45, 37.48, 63.39, 68.87, 69.95, 70.28, 70.77, 73.33, 77.92, 127.48, 127.57, 128.28, 138.38; IR (neat) 698, 735, 752, 1061, 1113, 1377, 1462, 2860, 2925, 2951, 3356 cm^{-1}. Found: C, 76.57; H, 12.26%. Calcd for $C_{44}H_{82}O_5$: C, 76.47; H, 11.96%.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*)-13-methoxycarbonyl-3,7,11-trimethyltridecyl]-*sn*-glycerol (14). Jones' reagent was added dropwise to a solution of diol (**13**) (397 mg, 0.575 mmol) in acetone (25 ml) at 0 °C until the mixture turned red, and the mixture was further stirred at 0 °C for 4 h. Excess oxidant was destroyed by adding 2-propanol at 0 °C, and the resulting greenish solution was filtered over a pad of Celite while washing with ether. The combined organic solution was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The oily residue was treated with an excess of ethereal CH_2N_2 at 0 °C for 3 h. The mixture was concentrated to dryness, and the residue was chromatographed over silica gel with hexane–ether (4 : 1) to give diester (**14**) (258 mg, 60%) as an oil. $[\alpha]_D^{20}$ +3.35° (*c* 0.873, $CHCl_3$); ^{1}H NMR (300 MHz) δ = 0.83—0.88 (d, *J* = 6.6 Hz, 18H), 1.00—1.85 (m, 38H), 2.29 (dt, *J* = 9.2, 6.1 Hz, 4H), 3.66 (s, 6H), 3.44—3.66 (m, 9H), 4.55 (s, 2H), 7.26—7.34 (m, 5H); ^{13}C NMR (75 MHz) δ = 19.22, 19.29, 19.60, 19.66, 19.70, 19.71, 24.33, 29.81, 29.90, 31.88, 31.97, 32.42, 32.78, 36.63, 36.72, 36.96, 36.99, 37.10, 37.18, 37.27, 37.29, 37.36, 37.45, 37.50, 51.44, 68.87, 69.96, 70.34, 70.79, 73.34, 77.95, 127.47, 127.56, 128.28, 138.44, 174.56; IR (neat) 698, 735, 1115, 1169, 1377, 1462, 1741, 2860, 2925, 2952 cm^{-1}. Found: C, 73.66; H, 11.29%. Calcd for $C_{46}H_{82}O_7$: C, 73.95; H, 11.06%.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*)-14-hydroxy-3,7,11-trimethyl-[14,14-$^2H_2$]tetradecyl]-*sn*-glycerol (15). Lithium aluminum deuteride (61 mg, 1.46 mmol, 99 atom% enriched, Merck) was added portionwise to a solution of diester (**14**) (258 mg, 0.345 mmol) in THF (20 ml) at 0 °C, and the mixture was stirred at 0 °C for 12 h. Hydrochloric acid (2 M, 10 ml) was carefully added at 0 °C and mixture was extracted 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 chromatographed over silica gel with hexane–EtOAc (1 : 1) to give diol-d_4 (**15**) (225 mg, 94%). $[\alpha]_D^{28}$ +1.31° (*c* 0.41, $CHCl_3$); ^{1}H NMR (300 MHz) δ = 0.84 (d, *J* = 7.1 Hz, 6H), 0.86 (d, *J* = 7.1 Hz, 6H), 0.87 (d, *J* = 6.3 Hz, 6H), 1.00—1.85 (m, 42H), 3.43—3.65 (m, 9H), 4.55 (s, 2H), 7.26—7.34 (m, 5H); ^{13}C NMR (75 MHz) δ = 19.68, 24.34, 24.39, 29.80, 29.87, 30.13, 32.60, 32.63, 32.77, 32.83, 32.93, 36.61, 36.68, 37.06, 37.14, 37.31, 37.42, 37.45, 37.49, 62.65 (quintet, *J* = 21 Hz), 68.87, 69.96, 70.31, 70.79, 73.34, 77.94, 127.48, 127.57, 128.28, 138.40; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 3.60; IR (neat) 698, 735, 968, 1113, 1377, 1462, 2090, 2191, 2860, 2925, 2951, 3415 cm^{-1}. Found: C, 75.76; H+^{2}H, 12.03%. Calcd for $C_{44}H_{78}{}^2H_4O_5$: C, 76.03; H+^{2}H, 11.89%.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*)-3,7,11-trimethyl-14-tosyloxy-[14,14-$^2H_2$]tetradecyl]-*sn*-glycerol (16). To a solution of diol-d_4 (**15**) (220 mg, 0.316 mmol), DMAP (20 mg, 0.164 mmol), and Et_3N (440 µl, 3.16 mmol) in CH_2Cl_2 (10 ml) was added *p*TsCl (300 mg, 1.57 mmol) at 0 °C, and the mixture was stirred at 0 °C for 5 h. Water (10 ml) was added and the mixture was extracted with EtOAc. The organic phase was successively washed with 2 M HCl, saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated to dryness to give ditosylate-d_4 (**16**) (315 mg, 99%). The obtained material was used for the next step without further purification.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*)-14-bromo-3,7,11-trimethyl-[14,14-$^2H_2$]tetradecyl]-*sn*-glycerol (17). Ditosylate-d_4 (**16**) (215 mg, 0.214 mmol) and LiBr (300 mg, 3.45 mmol) were dissolved in DMF (8 ml), and the mixture was stirred at 50 °C for 11 h. Water (10 ml) was added and the mixture was extracted with EtOAc. The organic phase was washed with water, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane–EtOAc (20 : 1) to give dibromide-d_4 (**17**) (159 mg, 91%) as an oil. $[\alpha]_D^{26}$ +3.98° (*c* 0.67, $CHCl_3$); ^{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* = 6.4 Hz, 6H), 1.00—1.95 (m, 42H), 3.44—3.66 (m, 9H), 4.56 (s, 2H), 7.26—7.34 (m, 5H); ^{13}C NMR (100 MHz) δ = 19.62, 19.68, 19.72, 19.75, 24.37, 29.80, 29.88, 30.28, 32.22, 32.79, 33.87 (quintet, *J* = 23 Hz), 35.38, 36.62, 37.08, 37.18, 37.30, 37.35, 37.50, 37.52, 68.86, 69.95, 70.30, 70.78, 73.34, 77.93, 127.48, 127.56, 128.29, 138.42; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 3.38; IR (neat) 698, 758, 995, 1113, 1377, 1462, 2156, 2868, 2925, 2951 cm^{-1}. Found: C, 64.35; H+^{2}H, 9.86%. Calcd for $C_{44}H_{76}{}^2H_4Br_2O_3$: C, 64.38; H+^{2}H, 9.82%.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*)-17-hydroxy-3,7,11-trimethyl-[14,14-$^2H_2$]heptadec-15-yn-1-yl]-*sn*-glycerol (18). To a solution of tetrahydro-2-(2-propynyloxy)-2*H*-pyran (30.5 g, 217 mmol) in THF (8ml) was added BuLi in hexane (2.40 ml, 1.59 M, 3.82 mmol) at −78 °C, and the mixture was stirred at 0 °C for 30 min. The mixture was recooled to −78 °C, and then HMPA (4 ml) was added at the same temperature. After 25 min, a solution of dibromide-d_4 (**17**) (300 mg, 0.365 mmol) in THF (16 ml) was added dropwise at −78 °C, and the mixture was stirred at −78 °C for 20 min, and then at room temperature for 1 h. Saturated aqueous NH_4Cl (10 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 residual oil was treated with 2 M HCl (5 ml) in THF–methanol (1 : 1, 40 ml) at room temperature for 12 h. After removal of the solvent, the residue was chromatographed over silica gel with hexane–EtOAc (4 : 1—2 : 1) to give bis-alkynyl alcohol-d_4 (**18**) (258 mg, 92%) as an oil. $[\alpha]_D^{26}$ +2.83° (*c* 0.67, $CHCl_3$); ^{1}H NMR (400 MHz) δ = 0.84 (d, *J* = 6.4 Hz, 6H), 0.86 (d, *J* = 6.1 Hz, 6H), 0.87 (d, *J* = 6.6 Hz, 6H), 1.00—1.70 (m, 42H), 1.87 (br, 2H), 3.42—3.66 (m, 9H), 4.24 (br, 4H), 4.56 (s, 2H), 7.27—7.34 (m, 5H); ^{13}C NMR (100 MHz) δ = 18.45 (quintet, *J* = 20 Hz), 19.61, 19.65, 19.68, 19.74, 24.32, 24.36, 25.98, 29.77, 29.84, 32.38, 32.76, 36.13, 36.56, 37.02, 37.21, 37.31, 37.46, 37.48, 51.29, 68.86 69.94, 70.23, 70.73, 73.31, 77.90, 78.31, 86.49, 127.47, 127.56, 128.26, 138.33; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 2.15; IR (neat) 698, 756, 1043, 1111, 1377, 1462, 1739, 2098, 2193, 2245, 2866, 2925, 3417 cm^{-1}. Found: C, 77.99; H+^{2}H, 11.45%. Calcd for $C_{50}H_{82}{}^2H_4O_5$: C, 77.87; H+^{2}H, 11.24%.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*,15*E*)-17-hydroxy-3,7,11-trimethyl-[14,14-$^2H_2$]heptadec-15-en-1-yl]-*sn*-glycerol (19). A solution of bis-alkynyl alcohol-d_4 (**18**) (241 mg, 0.313 mmol) in THF (10 ml) was added dropwise to a suspension of $LiAlH_4$ (133 mg, 3.52 mmol) in THF (5 ml) at 0 °C over a period of 15 min. The mixture was stirred at room temperature for 20 h. Water (3 ml) was carefully added, and the mixture was diluted with ether and 2 M HCl. The organic phase was separated and the aqueous phase

was extracted with ether. The combined 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 hexane–EtOAc (2 : 1) to give bis-allylic alcohol-d_4 (**19**) (117 mg, 48%) as an oil. $[\alpha]_D^{26}$ −0.58° (*c* 0.49, $CHCl_3$); ^{1}H NMR (300 MHz) δ = 0.84 (d, *J* = 7.1 Hz, 6H), 0.85 (d, *J* = 5.6 Hz, 6H), 0.87 (d, *J* = 6.3 Hz, 6H), 1.00—1.75 (m, 42H), 3.44—3.64 (m, 9H), 4.08 (d, *J* = 4.9 Hz, 4H), 4.55 (s, 2H), 5.64 (dt, *J* = 15.4, 4.9 Hz, 2H), 5.70 (d, *J* = 15.4 Hz, 2H), 7.26—7.34 (m, 5H); ^{13}C NMR (75 MHz) δ = 19.68, 19.76, 24.35, 24.43, 26.45, 29.80, 29.88, 31.78 (m, *J* = 21 Hz), 32.66, 32.78, 36.48, 36.61, 37.07, 37.35, 37.50, 63.81, 68.87, 69.96, 70.29, 70.77, 73.34, 77.93, 127.48, 127.57, 128.28, 128.85, 133.46, 138.39; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 2.02; IR (neat) 698, 735, 754, 976, 1028, 1109, 1377, 1462, 2096, 2185, 2858, 2925, 2951, 3415 cm^{-1}. Found: C, 77.36; H+^{2}H, 11.87%. Calcd for $C_{50}H_{86}{}^2H_4O_5$: C, 77.47; H+^{2}H, 11.70%.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*,15*R*,16*R*)-15,16-epoxy-17-hydroxy-3,7,11-trimethyl-[14,14-$^2H_2$]heptadecyl]-*sn*-glycerol (20). To a suspension of molecular sieves 4A powder (211 mg) in CH_2Cl_2 (4 ml) were added tetraisopropyl orthotitanate (400 µl, 1.37 mmol) and D-(−)-diethyl tartrate (250 µl, 1.46 mmol) at −25 °C, and the mixture was stirred at −25 °C for 25 min. A solution of bis-allylic alcohol-d_4 (**20**) (180 mg, 0.232 mmol) in CH_2Cl_2 (4 ml) was added dropwise over a period of 5 min at −25 °C. After this was stirred for 25 min, *t*-butyl hydroperoxide in decane (5.5 M, 400 µl, 2.20 mmol) was added dropwise at the same temperature, and stirring was continued for 3 h at −25 °C. Aqueous 10% tartaric acid (5 ml) was added at −25 °C and the mixture was stirred at room temperature for 1 h. The mixture was diluted with ether and the organic phase was separated. The aqueous phase was extracted with ether. The combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was dissolved in ether (8ml) and 10% aqueous NaOH (5 ml) was added at 0 °C. After 30 min of stirring at 0 °C, two phases were separated and the aqueous phase was extracted with ether. The combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane–EtOAc (2 : 1) to give diepoxide-d_4 (**20**) (111 mg, 59%) as an oil. $[\alpha]_D^{23}$ +13.47° (*c* 0.48, $CHCl_3$); ^{1}H NMR (400 MHz) δ = 0.84 (d, *J* = 7.3 Hz, 6H), 0.86 (d, *J* = 6.8 Hz, 6H), 0.87 (d, *J* = 6.4 Hz, 6H), 1.00—1.90 (m, 42H), 2.92 (m, 2H), 2.95 (d, *J* = 2.0 Hz, 2H), 3.42—3.65 (m, 11H), 3.91 (d, *J* = 12.0 Hz, 2H), 4.56 (s, 2H), 7.26—7.34 (m, 5H); ^{13}C NMR (100 MHz) δ = 19.61, 19.67, 19.70, 19.75, 23.23, 24.35, 24.42, 29.79, 29.87, 31.09 (m *J* = 21 Hz), 32.71, 32.78, 36.60, 36.65, 37.06, 37.27, 37.35, 37.49, 37.51, 55.87, 58.37, 61.67, 68.86, 69.95, 70.27, 70.76, 73.33, 77.91, 127.48, 127.56, 128.29, 138.38; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 1.55; IR (neat) 698, 737, 752, 1030, 1105, 1377, 1462, 2110, 2195, 2860, 2925, 2951, 3440 cm^{-1}. Found: C, 74.27; H+^{2}H, 11.29%. Calcd for $C_{50}H_{86}{}^2H_4O_7$: C, 74.40; H+^{2}H, 11.24%.

1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*S*,15*S*)-15-formyl-3,7,11,15-tetramethyl-[14,14-$^2H_2$]pentadecyl]-*sn*-glycerol (21). A solution of trimethylaluminum in hexane (1.40 ml, 1.00 M, 1.41 mmol) was added dropwise to a solution of diepoxide-d_4 (**20**) (109 mg, 0.136 mmol) in CH_2Cl_2 (4 ml) at 0 °C during 5 min, and the mixture was stirred at 0 °C for 2 h. Methanol (1.5 ml) was carefully added at 0 °C and the mixture was diluted with ether and 2 M HCl. The organic layer was separated and the aqueous phase was extracted with ether. The combined organic layer was successively washed with saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was dissolved in THF (2.5 ml) and water (2.5 ml), and sodium periodate (156 mg, 0.731 mmol) was then added. The resulting mixture was stirred at room temperature for 2 h. The mixture was diluted with ether and saturated $Na_2S_2O_3$, and the phases were separated. The aqueous phase was extracted with ether. The combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane–EtOAc (4 : 1) to give dialdehyde-d_4 (**21**) (100 mg, 95%) as an oil. $[\alpha]_D^{26}$ +7.32° (*c* 0.39, $CHCl_3$); ^{1}H NMR (400 MHz) δ = 0.82—0.87 (m, 18H), 1.09 (d, *J* = 7.1 Hz, 6H), 1.00—1.75 (m, 42H), 2.33 (m, 2H), 3.44—3.66 (m, 9H), 4.56 (s, 2H), 7.26—7.34 (m, 5H), 9.62 (d, *J* = 2.0 Hz, 2H); ^{13}C NMR (75 MHz) δ = 13.27, 19.63, 19.66, 19.70, 19.73, 24.19, 24.34, 24.43, 29.67, 29.81, 29.85, 29.89, 30.29, 32.64, 32.79, 36.62, 36.93, 37.09, 37.32, 37.36, 37.52, 46.17, 68.86, 69.94, 70.33, 70.79, 73.33, 77.95, 127.46, 127.54, 128.27, 138.43, 205.32; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 1.32, 1.66; IR (neat) 698, 735, 1115, 1377, 1458, 1728, 2094, 2183, 2858, 2925, 2954 cm^{-1}. Found: C, 77.77%; H+^{2}H, 11.66%. Calcd for $C_{50}H_{86}{}^2H_4O_5$: C, 77.47; H+^{2}H, 11.70%.

(2*S*,7*R*,11*R*,15*S*,19*S*,22*S*,26*S*,30*R*,34*R*)-2-Benzyloxymethyl-7,11,15,19,22,26,30,34-octamethyl-1,4-dioxa-[18,18,23,23-$^2H_4$]cyclohexatriacont-20-ene (22). Powdered $TiCl_3$ (0.5 g, 3.3 mmol) and Zn–Cu couple (0.5 g, 7.7 mmol) were placed in a 100-ml Schlenk tube under argon atmosphere. DME (30 ml) was added and the mixture was refluxed for 2 h. A solution of dialdehyde-d_4 (**21**) (91 mg, 0.12 mmol) in DME (3 ml) was added to the refluxing slurry via a motor-driven syringe pump over a 44 h period. After an additional reflux for 18 h, the reaction mixture was cooled to room temperature. The mixture was treated with 20% aqueous K_2CO_3 (25 ml) at room temperature for 3.5 h, and then extracted several times with ether. The combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane–EtOAc (25 : 1) to give cyclic olefin-d_4 (**22**) (52 mg, 60%) as an oil. $[\alpha]_D^{26}$ +3.77° (*c* 0.47, $CHCl_3$); ^{1}H NMR (400 MHz) δ = 0.83 (d, *J* = 5.6 Hz, 6H), 0.84 (d, *J* = 5.6 Hz, 6H), 0.87 (d, *J* = 6.4 Hz, 6H), 0.94 (d, *J* = 6.6 Hz, 6H), 0.98—1.75 (m, 42H), 2.02 (br, 2H), 3.46—3.64 (m, 9H), 4.55 (s, 2H), 5.10 (m, 2H), 7.26—7.34 (m, 5H); ^{13}C NMR (100 MHz) δ = 19.76, 19.85, 21.76, 24.48, 24.86, 29.60, 29.71, 32.86, 36.62, 36.96, 37.16, 37.35, 37.47, 68.58, 69.69, 70.26, 71.42, 73.34, 77.92, 127.51, 127.57, 128.30, 134.94, 138.36; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 1.16, 1.19; IR (neat) 698, 733, 968, 1115, 1377, 1456, 1732, 2098, 2175, 2864, 2924, 2952 cm^{-1}. EI-MS *m/z* 743 (M^+), 727, 652 ($M^+ - CH_2Ph$), 637, 560. Found: C, 80.52; H+^{2}H, 12.45%. Calcd for $C_{50}H_{86}{}^2H_4O_3$: C, 80.80; H+^{2}H, 12.21%.

(2*R*,7*R*,11*R*,15*S*,19*S*,22*S*,26*S*,30*R*,34*R*)-2-Hydroxymethyl-7,11,15,19,22,26,30,34-octamethyl-1,4-dioxa-[18,18,23,23-$^2H_4$]cyclohexatriacontane (23). A mixture of cyclic olefin-d_4 (**22**) (50 mg, 0.0666 mmol) and 10% Pd–C (52 mg) in EtOAc (10 ml) was stirred for 17.5 h under atmospheric pressure of hydrogen at room temperature. The catalyst was filtered off through a pad of Celite and washed with EtOAc. The filtrate and washings were combined and concentrated to dryness. The residue was chromatographed over silica gel with hexane–EtOAc (10 : 1) to give alcohol-d_4 (**23**) (35 mg, 81%) as an oil. $[\alpha]_D^{28}$ +8.17° (*c* 0.78, $CHCl_3$); ^{1}H NMR (400 MHz) δ = 0.85 (d, *J* = 6.6 Hz, 12H), 0.88 (d, *J* = 6.6 Hz, 6H), 0.89 (d, *J* = 6.3 Hz, 6H), 1.00—1.75 (m, 48H), 2.17 (br, 1H), 3.46—3.72 (m, 9H); ^{13}C NMR (100 MHz) δ = 19.85, 19.93, 19.97, 20.03, 24.06, 24.14, 24.41, 29.72, 29.77, 32.67, 32.76, 32.85, 33.54, 34.12, 36.53, 37.00, 37.12, 37.26, 63.03, 68.53, 70.00, 71.18, 78.38; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 1.07, 1.21; IR (neat) 1055, 1117, 1377, 1462, 1732, 2092, 2173, 2856, 2925, 2956, 3444 cm^{-1}. EI-

MS *m/z* 655 (M^+), 637 ($M^+ - H_2O$), 624, 607. Found: C, 78.57; H+^{2}H, 13.16%. Calcd for $C_{43}H_{82}{}^2H_4O_3$: C, 78.84; H+^{2}H, 13.23%.

(2*S*,7*R*,11*R*,15*S*,19*S*,22*S*,26*S*,30*R*,34*R*)-7,11,15,19,22,26,30,34-Octamethyl-2-[2-(trimethylammonio)ethoxyphosphinatoxymethyl-1,4-dioxa-[18,18,23,23-$^2H_4$]cyclohexatriacontane (4). To a solution of alcohol-d_4 (**23**) (34 mg, 0.052 mmol) 130 mg, 0.198 mmol) in pyridine (2 ml) was added 2-chloroethyl phosphorodichloridate (75 μl, 0.58 mmol) at 0 °C. The mixture was stirred for 4 h at 0 °C. Water (10 ml) was added and the mixture was stirred at 0 °C for 30 min. Then, the mixture was acidified by adding 2 M HCl and extracted several times with $CHCl_3$. The combined organic extract was dried ($MgSO_4$), filtered, and concentrated to dryness. The residue obtained was chromatographed over silica gel with $CHCl_3$–methanol (15 : 1—7 : 1) to give a waxy residue (30 mg). The residue was dissolved in toluene (5 ml) and CH_3CN (3 ml), and the solution was transferred into a pressure bottle. The mixture was cooled to −78°C and dry trimethylamine (15 ml) was introduced. The mixture was heated at 50—60 °C for 3 d. After removal of the solvent, the residue was chromatographed over silica gel with $CHCl_3$–methanol (8 : 1) to $CHCl_3$–methanol–H_2O (65 : 25 : 4) to give phosphocholine-d_4 (**4**) (17 mg, 40%) as a hygroscopic wax. ^{1}H NMR (400 MHz, $CDCl_3 : CD_3OD$ = 5 : 1) δ = 0.85—0.89 (m, 24H), 1.00—1.70 (m, 48H), 3.22 (s, 9H), 3.49 (m, 3H), 3.55—3.70 (m, 6H), 3.88 (br, 2H), 4.23 (br, 2H); ^{13}C NMR (75 MHz, $CDCl_3 : CD_3OD$ = 4 : 1) δ = 19.33, 19.45, 19.47, 19.52, 19.57, 19.62, 19.67, 19.75, 23.56, 23.60, 24.13, 29.39, 29.55, 32.29, 32.34, 32.38, 32.49, 33.64, 36.35, 36.70, 36.80, 36.87, 36.93, 36.97, 37.04, 37.10, 37.17, 53.89, 58.56, 64.78, (d, J = 4.9 Hz), 66.21, 68.40, 69.68, 70.58, 77.66, (d, J = 8.2 Hz). High Resolution FAB-MS: Calcd for $C_{48}H_{95}{}^2H_4NO_6P$: (M^++H), 820.7461. Found: *m/z* 820.7460.

1-*O*-Benzyl-2,3-di-*O*-[(3*R*,7*R*,11*S*)-3,7,11,15-tetramethyl-[14,14-$^2H_2$]hexadecyl]-*sn*-glycerol (24). A solution of Li_2CuCl_4 in THF (2.70 ml), prepared from anhydrous LiCl (43 mg, 1.0 mmol) and anhydrous $CuCl_2$ (68 mg, 0.51 mmol) in THF (10 ml), was added to a solution of isopropylmagesium bromide in THF (20 ml, 1.58 M) at 0 °C, and then a solution of ditosylate-d_4 (**16**) in THF (15 ml) was added. The resulting mixture was stirred at 0 °C for 10 min, and saturated aqueous NH_4Cl (10 ml) was carefully added. The mixture was extracted with ether. The organic extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane–EtOAc (10 : 1) to give **24** (204 mg, 87%) as an oil. $[\alpha]_D^{24}$ +2.44° (*c* 0.83, $CHCl_3$); ^{1}H NMR (400 MHz) δ = 0.83—0.87 (m, 30H), 1.00—1.70 (m, 44H), 3.42—3.66 (m, 9H), 4.55 (s, 2H), 7.26—7.34 (m, 5H); ^{13}C NMR (100 MHz) δ = 19.75, 22.55, 22.64, 24.38, 24.48, 24.59, 27.79, 29.83, 29.91, 32.81, 36.66, 37.12, 37.25, 37.42, 37.47, 37.53, 38.49 (quintet, J = 19 Hz), 68.89, 69.98, 70.37, 70.83, 73.36, 77.98, 127.53, 127.58, 128.28, 138.46; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 1.12; IR (neat) 696, 733, 1115, 1377, 1462, 2087, 2173, 2866, 2925, 2952 cm^{-1}. Found: C, 80.07; H+^{2}H, 12.67%. Calcd for $C_{50}H_{90}{}^2H_4O_3$: C, 80.73; H+^{2}H, 12.68%.

2,3-Di-*O*-[(3*R*,7*R*,11*S*)-3,7,11,15-tetramethyl-[14,14-$^2H_2$]-hexadecyl]-*sn*-glycerol (25). A mixture of benzyl ether-d_4 (**24**) (176 mg, 0.235 mmol) and 10% Pd–C (116 mg) in EtOAc (30 ml) was stirred for 20 h at room temperature under an atmospheric pressure of hydrogen. The catalyst was separated by filtration with a pad of Celite and washed with EtOAc. The filtrate and washings were combined and concentrated to dryness. The residue was chromatographed over silica gel with hexane–EtOAc (10 : 1) to give alcohol-d_4 (**25**) (142 mg, 92%) as an oil. $[\alpha]_D^{26}$ +8.37° (*c* 0.43, $CHCl_3$); ^{1}H NMR (400 MHz) δ = 0.84—0.89 (m, 30H), 1.00—1.70 (m, 44H), 2.17 (t, J = 6.3 Hz, 1H), 3.44—3.75 (m, 9H); ^{13}C NMR (100 MHz) δ = 19.68, 19.70, 19.75, 19.76, 22.57, 22.66, 24.36, 24.47, 24.59, 27.79, 29.88, 29.92, 32.81, 36.61, 37.10, 37.26, 37.37, 37.41, 37.47, 37.51, 37.52, 38.49 (m, J = 19 Hz), 63.13, 68.66, 70.18, 70.98, 78.32; ^{2}H NMR (61.3 MHz, $CHCl_3$) δ = 1.11; IR (neat) 1051, 1117, 1365, 1377, 1462, 2087, 2173, 2868, 2925, 2952, 3442 cm^{-1}. EI-MS *m/z* 657 (M^+), 615. Found: C, 78.44; H+^{2}H, 13.49%. Calcd for $C_{43}H_{84}{}^2H_4O_3$: C, 78.59; H+^{2}H, 13.50%.

2,3-Di-*O*-[(3*R*,7*R*,11*S*)-3,7,11,15-tetramethyl-[14,14-$^2H_2$]-hexadecyl]-*sn*-glycero-1-phosphocholine (5). In the same manner as described in the synthesis of (**4**), alcohol-d_4 (**25**) (130 mg, 0.198 mmol) was converted to hygroscopic waxy phosphocholine-d_4 (**5**) (83 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$–CD_3OD = 8 : 1) δ = 0.83—0.87 (m, 30H), 1.00—1.41 (m, 36H), 1.46—1.64 (m, 8H), 3.23 (s, 9H), 3.47 (m, 3H), 3.616 (br, 6H), 3.90 (br, 2H), 4.24 (br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$–CD_3OD = 8 : 1) δ = 19.36, 19.45, 22.27, 22.36, 24.15, 24.24, 24.35, 27.55, 29.64, 29.74, 32.57, 36.46, 36.88, 37.01, 37.21, 37.34, 38.25 (quintet, J = 19 Hz), 54.02, 58.70 (d, J = 5.0 Hz), 64.89 (d, J = 5.8 Hz), 66.29, 68.67, 69.92, 70.45, 77.78 (d, J = 8.3 Hz). High Resolution FAB-MS: Calcd for $C_{48}H_{97}{}^2H_4NO_6P$: (M^++H), 822.7618. Found: *m/z* 822.7662.

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