Synthesis and Thermotropic Properties of Macrocyclic Lipids Related to Archaebacterial Membranes

F. M. Menger,*,† X. Y. Chen,† S. Brocchini,† H. P. Hopkins,*,‡ and D. Hamilton‡

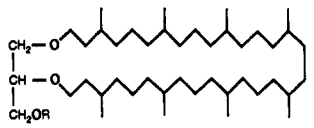
Contribution from the Departments of Chemistry, Emory University, Atlanta, Georgia 30322, and Georgia State University, Atlanta, Georgia 30303

Received February 17, 1993

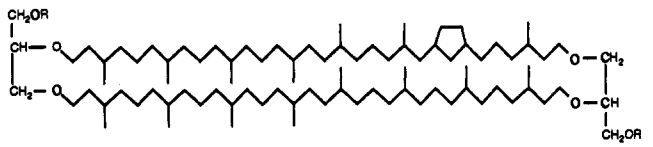
Abstract: Macrocyclic phospholipids containing 32–44 ring atoms were synthesized by a route involving a high-temperature Glaser oxidation as the key step. These lipids are analogous to mammalian phospholipids except a single extra carbon–carbon bond joins the chain termini. The new lipids offered, therefore, an opportunity to examine thermotropic properties of their membranes when the chains within a given molecule are unable to move independently of one another. It was concluded that chain "tethering" (a) raises the transition temperatures substantially for all but the shortest lipids, (b) lowers enthalpies of transition by, in part, reducing the number of gauche C–C linkages created during the melting process, and (c) lowers entropies of transition by impeding motional freedom within the liquid-crystalline phase. Molecular mechanics calculations on the macrocyclic lipids are described briefly.

Introduction

Archaebacterial lipids,[1–4] such as I[5] and II,[6] possess two structural features not usually found in the lipids of other organisms: (a) The hydrocarbon chains are connected to the



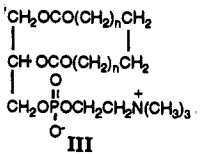
I



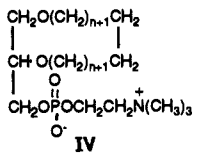
II

glycerol backbone by means of ether, rather than ester, linkages.[7] (b) The hydrocarbon chains are frequently joined to form a macrocyclic ring. Ether linkages are of obvious advantage in that they provide hydrolytic stability of the lipid at the high temperatures endured by thermophilic archaebacteria. It is less clear, however, what advantage (if any) is imparted by the macrocyclic ring. This uncertainty led us to prepare and examine both diester and diether lipids containing giant rings.[8]

We have synthesized a series of macrocyclic phospholipids shown below. The number n equaled 12, 14, 16, and 18 leading to rings containing a total of 32, 36, 40, and 44 atoms, respectively. Only two previous reports of such macrocyclic "models" of archaebacterial lipids have been published.[9,10] As will be shown,



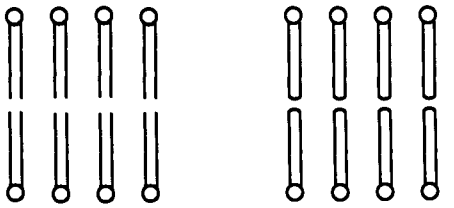
III



IV

our synthetic pathway includes a key step, the macrocyclization, that proceeds at an unusually high yield, thus making the materials more generally available.

Macrocyclic phospholipids are interesting apart from their structural similarity to archaebacterial natural products. In mammalian lipids, chains are unconnected and free to move independently. Joining the chains *via* an extra carbon–carbon bond, as in III and IV, destroys this independence. Anti/gauche ratios, flip-flop rates, interdigitation, rotational and translational motions, etc., could all be affected. These in turn could have a bearing on lipid packing and overall membrane structure and, consequently, on membrane stability and permeability. The fact that, until now, only a few "structure-activity" comparisons between "tethered" and "untethered" phospholipids have been



carried out reflects, no doubt, the past inaccessibility of the required compounds. In the present article, we focus primarily on the thermotropic behavior of the cyclic lipids because calorimetry is a particularly informative method for assessing membrane behavior and because we have had previous experience with the thermochemistry of branched lipids.[11]

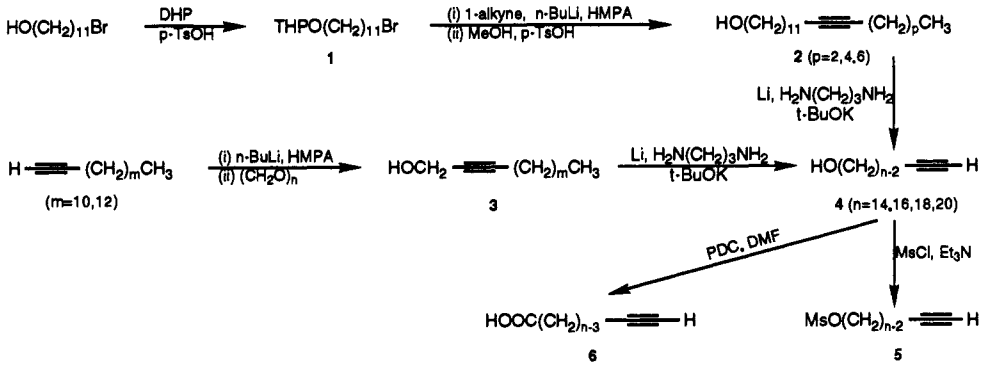
We should mention, in concluding this introduction, that synthetic compounds serving as archaebacterial "models", par-

† Emory University.
‡ Georgia State University.

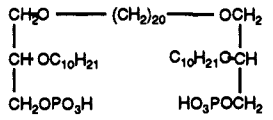
(1) Poulter, C. D.; Aoki, T.; Daniels, L. *J. Am. Chem. Soc.* **1988**, *110*, 2620.
(2) Kakinuma, K.; Yamagishi, M.; Fujimoto, Y.; Ikekawa, N.; Oshima, T. *J. Am. Chem. Soc.* **1990**, *112*, 2740.
(3) De Rosa, M.; Gambacorta, A. *Prog. Lipid Res.* **1988**, *27*, 153.
(4) Heathcock, C. H.; Finkelstein, B. L.; Aoki, T.; Poulter, C. D. *Science* **1985**, *229*, 862.
(5) Sprott, G. D. *J. Bioenerg. Biomembr.* **1992**, *24*, 555.
(6) Yamauchi, K.; Moriya, A.; Kinoshita, M. *Biochem. Biophys. Acta* **1989**, *1003*, 151.
(7) De Rosa, M.; De Rosa, S.; Gambacorta, A. *J. Chem. Soc., Chem. Commun.* **1977**, 514.
(8) For a preliminary account of this work, see: Menger, F. M.; Brocchini, S.; Chen, X. Y. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1492.
(9) Hébert, N.; Beck, A.; Lennox, R. B.; Just, G. *J. Org. Chem.* **1992**, *57*, 1777.
(10) Yamauchi, K.; Yamamoto, I.; Kinoshita, M. *J. Chem. Soc., Chem. Commun.* **1988**, 445.
(11) Menger, F. M.; Wood, M. G., Jr.; Zhou, Q. Z.; Hopkins, H. P.; Fumero, J. *J. Am. Chem. Soc.* **1988**, *110*, 6804.

0002-7863/93/1515-6600$04.00/0

Scheme I



ticularly the so-called bolaform amphiphiles (e.g. V),[12] comprise



V

a vibrant area of biomimetic chemistry. This work has been spearheaded by, among others, Führhop,[13] Moss,[14] Regen,[15] Thompson,[16] and Yamauchi.[17] Using the same synthetic strategy developed for III and IV, we can now prepare bolaform-type lipids in which the two free chains are further joined to create a giant ring with as many as 72 atoms. Since the bolaforms, and our various cyclic analogs, simulate natural lipids designed to withstand extreme conditions, it is possible that the materials will ultimately find applications in liposomal drug delivery and other aspects of biotechnology.[18]

Experimental Section

General Procedures. Melting points were determined with a Thomas Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a QE-300 MHz FT high-resolution spectrometer using a ^1H or ^{13}C solvent peak as an internal reference (*e.g.* 7.26 ppm for $CDCl_3$ and the $CDCl_3$ triplet at 77.00 ppm) unless otherwise noted. Fast atom bombardment (FAB) mass spectra were obtained on a VG 70-S mass spectrometer using *m*-nitrobenzyl alcohol (NBA) as the matrix. Elemental analyses were performed at Atlantic Microlab, Inc., P.O. Box 2288, Atlanta, Georgia. Fisher silica gel (200–425 mesh, type 60A) was used for column chromatography. Analytical TLC was performed with glass plates coated with silica gel 60 F_{254} (Merck). D-α,β-*O*-Isopropylideneglycerol, 2-chloro-2-oxo-1,3,2-dioxaphospholane, and potassium hydroxide (powder) were purchased from Fluka. Other chemicals were obtained from Aldrich Chemical Corp. or Farchan Ltd and were used as received. Solvents were of reagent grade and were distilled under N_2 before use: THF from sodium benzophenone ketyl, CH_2Cl_2 from P_2O_5, CCl_4 from CaH_2, benzene from Na, triethylamine from $LiAlH_4$, 1,3-diaminopropane from BaO. All reactions were performed under an inert atmosphere (N_2 or Ar) unless otherwise indicated.

Synthesis. The multistep synthesis with numbered compounds is given in Schemes I and II. A preliminary account of the synthesis has already been published.[8]

1-Bromo-11-(tetrahydropyranyloxy)undecane (1).[19] To an ice-cooled stirred solution of 11-bromo-1-undecanol (10.1 g, 40 mmol) and 3,4-dihydro-2*H*-pyran (DHP) (18 mL, 0.20 mol) in 100 mL of CH_2Cl_2 was added *p*-toluenesulfonic acid monohydrate (96 mg, 0.50 mmol). The solution was stirred for 10 min at 0–5 °C, then at room temperature for 1.5 h. The mixture was diluted with 150 mL of ether, washed successively with water, saturated $NaHCO_3$ and brine, and then dried over K_2CO_3/Na_2SO_4 and rotoevaporated. The crude product was distilled under reduced pressure with a small amount of solid Na_2CO_3 to give 11.6 g (153–160 °C, 0.2–0.3 mm Hg; 87%) of the desired product (R_f = 0.83 in ethyl acetate–hexane (1:4)).

12-Octadecyn-1-ol (2a).[20,19] A 250-mL three-neck round-bottom flask fitted with a thermometer, septum, glass stopper, and magnetic stirring bar was charged with 1-heptyne (9.61 g, 0.10 mol) and hexamethylphosphoramide (HMPA) (50 mL). This solution was cooled to 0–5 °C and stirred while *n*-BuLi (1.6 M solution in hexane, 65 mL, 0.104 mol) was slowly added by syringe at a rate to maintain the temperature of the reaction mixture below 5 °C. After completing the addition of *n*-BuLi, the red-colored reaction mixture was stirred for an additional 30 min. The hexane was then removed under high vacuum using two dry ice traps. The HMPA solution of 1-lithio-1-heptyne was again cooled to 0 °C, and 1-bromo-11-(tetrahydropyranyloxy)undecane (**1**) (36.8 g, 0.11 mol) was added slowly. The solution was then stirred overnight at room temperature. Ethyl acetate (100 mL) followed by water (250 mL) was added and the mixture transferred to a 500-mL separatory funnel. The layers were separated, and the aqueous layer was extracted twice more with ethyl acetate. The combined ethyl acetate volumes were washed with water and brine, and then dried over Na_2SO_4. Rotoevaporation of the ethyl acetate gave a brown-colored oil to which methanol (150 mL) and *p*-toluenesulfonic acid monohydrate (usually 0.2–0.5 g, until the reaction solution became acidic to universal pH, pH = 3–4) were added. The reaction mixture was stirred for 1.5 h. Saturated $NaHCO_3$ (50 mL) and water (100 mL) were then added, and the solution was extracted three times with ethyl acetate. The combined ethyl acetate was washed with water and brine, and dried over Na_2SO_4. Rotoevaporation of solvent gave an oily residue which was purified by silica gel chromatography, eluting with ethyl acetate–hexane (1:4 *v/v*, R_f = 0.38) to yield 12-octadecyn-1-ol (**2a**) (19.1 g, 72% from heptyne) as a semi-solid. ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (m, 3H), 1.24 (s, 16H), 1.42–1.54 (m, 8H), 2.10 (m, 4H), 3.59 (dt-like, 2H).

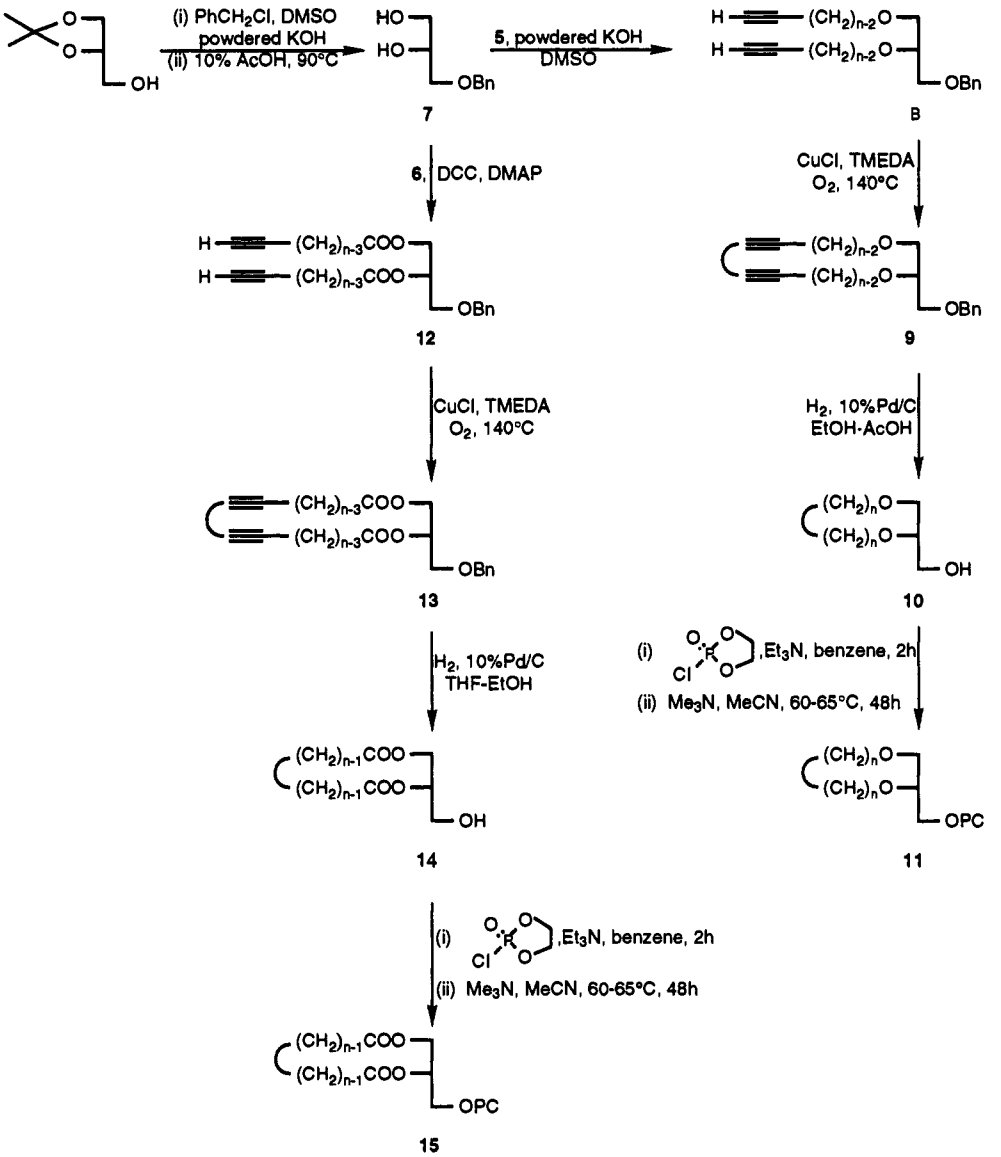
12-Hexadecyn-1-ol (2b). The same procedure as for compound **2a** was followed. Thus 1-pentyne (5.0 g, 73 mmol) was reacted with 1-bromo-11-(tetrahydropyranyloxy)undecane (**1**) (27.0 g, 80.6 mmol) to give 12-hexadecyn-1-ol (**2b**) (13.3 g, 76% based on starting 1-pentyne) as an oil. ^1H NMR (300 MHz, $CDCl_3$): δ 0.94 (m, 3H), 1.25 (s, 12H), 1.43–1.53 (m, 8H), 2.10 (m, 4H), 3.60 (dt-like, 2H).

12-Eicosyn-1-ol (2c). The same procedure as for compound **2a** was followed. Thus 1-nonyne (2.86 g, 23 mmol) was reacted with 1-bromo-11-(tetrahydropyranyloxy)undecane (**1**) (8.13 g, 24 mmol) to give 12-eicosyn-1-ol (**2c**) (5.06 g, 75% based on starting 1-nonyne) as a solid. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t-like, 3H), 1.27 (s, 20H), 1.35–1.59 (m, 8H), 2.13 (t, J = 6.8 Hz, 4H), 3.64 (t, J = 6.5 Hz, 2H).

2-Hexadecyn-1-ol (3a). A 100-mL three-neck round-bottom flask fitted with a thermometer, septum, glass stopper, and magnetic stirring bar was charged with 1-pentadecyne (4.9 g, 23 mmol) and hexamethylphosphoramide (50 mL). This solution was cooled to 0–5 °C and stirred while *n*-BuLi (2.5 M solution in hexane, 10.4 mL, 26 mmol) was slowly added by syringe at a rate to maintain the temperature of the reaction mixture below 5 °C. After complete addition of *n*-BuLi, the red-colord reaction mixture was stirred for an additional 30 min and paraformaldehyde (1.02 g, 34 mmol) in hexamethylphosphoramide (10 mL) was added. The solution was then stirred overnight at room temperature. The mixture was diluted with water (250 mL), poured into a 500-mL

(12) Thompson, D. H.; Wong, K. F.; Humphry-Baker, R.; Wheeler, J. J.; Kim, J.-M.; Rananavare, S. B. *J. Am. Chem. Soc.* **1992**, *114*, 9035.
(13) Fuhrhop, J.-H.; Hungerbühler, H.; Siggel, U. *Langmuir* **1990**, *6*, 1295.
(14) Moss, R. A.; Li, J.-M. *J. Am. Chem. Soc.* **1992**, *114*, 9227.
(15) Nagawa, Y.; Regen, S. L. *J. Am. Chem. Soc.* **1991**, *113*, 7237.
(16) Kim, J.-M.; Thompson, D. H. *Langmuir* **1992**, *8*, 637.
(17) Yamauchi, K.; Sakamota, Y.; Moriya, A.; Yamada, K.; Hosokawa, T.; Higuchi, T.; Kinoshita, M. *J. Am. Chem. Soc.* **1990**, *112*, 3188.
(18) Gilmour, D. J. *Chem. Ind.* **1990**, 285.
(19) Bernady, K. F.; Floyd, M. B.; Poletto, J. F.; Weiss, M. J. *J. Org. Chem.* **1979**, *44*, 1438.
(20) Brattesani, D. N.; Heathcock, C. H. *Synth. Commun.* **1973**, *3*, 245.

Scheme II



separatory funnel, and extracted three times with ethyl acetate. The combined ethyl acetate volumes were washed with water and brine, dried over Na_2SO_4, and rotoevaporated. A semi-solid residue was purified by silica gel chromatography, eluting with ethyl acetate–hexane (1:4 v/v) to give 2-hexadecyn-1-ol (**3a**) as a white solid (4.45 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t-like, 3H), 1.26 (s, 20H), 1.50 (m, 2H), 2.21 (m, 2H), 4.25 (t, J = 1.7 Hz, 2H).

2-Tetradecyn-1-ol (3b). The same procedure as for compound **3a** was followed. Thus 1-tridecyne (7.0 g, 39 mmol) was converted to 2-tetradecyn-1-ol (**3b**) in a yield of 6.97 g (85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t-like, 3H), 1.26 (s, 16H), 1.50 (q, J = 7.2 Hz, 2H), 1.67 (s, 1H), 2.20 (t, J = 7.1 Hz, 2H), 4.24 (s, 2H).

17-Octadecyn-1-ol (4a).[21] A mixture of lithium metal (1.20 g, 0.17 mol, washed free from mineral oil with hexane before using) and 40 mL of 1,3-diaminopropane in a 250-mL single-neck round-bottom flask was stirred vigorously and heated to 70 °C in an oil bath for about 1.5 h until the blue color had discharged and a white suspension had formed. The resulting mixture was cooled to room temperature, and potassium *tert*-butoxide (11.9 g, 0.10 mol) was added in one portion, affording a pale yellow mixture. After the mixture was stirred for 15 min, 12-octadecyn-1-ol (**2a**) (7.06 g, 26 mmol) was added and the color changed to deep red. Frequently the reaction mixture became temporarily too viscous to stir and it was necessary to add more 1,3-diaminopropane. The mixture was stirred for 2 h, quenched with ice–water, and poured into a 500-mL separatory funnel. The product was extracted three times with ethyl acetate, and the ethyl acetate volumes were combined and washed with water and brine. Rotoevaporation of the dried solution (over Na_2SO_4) gave a semi-solid yellow-brown residue which was further purified by silica gel chromatography, eluting with ethyl acetate–hexane (1:4 v/v). The desired product was recrystallized from pentane as a white solid (3.36 g, 48%). Mp: 59–60.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 24H), 1.47–1.58 (m, 4H), 1.93 (t, J = 2.6 Hz, 1H), 2.17 (dt, J = 2.7, 7.6 Hz, 2H), 3.63 (t, J = 6.6 Hz, 2H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.39, 25.73, 28.49, 28.76, 29.10, 29.42, 29.50, 29.60, 29.64, 32.80, 63.08, 68.00, 84.81. Anal. Calcd for $C_{18}H_{34}O$: C, 81.21; H, 12.78. Found: C, 81.22; H, 12.83. LRMS (FAB): m/z 267 (M + H)$^+$, 273 (M + Li)$^+$.

15-Hexadecyn-1-ol (4b). Mp: 49–50.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 20H), 1.47–1.58 (m, 4H), 1.93 (t, J = 2.6 Hz, 1H), 2.17 (dt, J = 2.4, 7.1 Hz, 2H), 3.63 (t, J = 6.6 Hz, 2H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.38, 25.72, 28.48, 28.75, 29.09, 29.42, 29.48, 29.58, 29.60, 32.79, 63.07, 68.00, 84.80. Anal. Calcd for $C_{16}H_{30}O$: C, 80.67; H, 12.60. Found: C, 80.44; H, 12.55. LRMS (FAB): m/z 239 (M + H)$^+$, 245 (M + Li)$^+$.

13-Tetradecyn-1-ol (4c). Mp: 40.5–42.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 16H), 1.47–1.58 (m, 4H), 1.93 (t, J = 2.3 Hz, 1H), 2.17 (dt, J = 2.3, 7.1 Hz, 2H), 3.63 (t, J = 6.6 Hz, 2H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.31, 25.68, 28.41, 28.68, 29.03, 29.36, 29.41, 29.49, 32.70, 62.84, 67.99, 84.69. Anal. Calcd for $C_{14}H_{26}O$: C, 79.94; H, 12.46. Found: C, 79.97; H, 12.44. LRMS (FAB): m/z 211 (M + H)$^+$, 217 (M + Li)$^+$.

19-Eicosyn-1-ol (4d). Mp: 67.0–69.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 28H), 1.47–1.58 (m, 4H), 1.93 (t, J = 2.6 Hz, 1H), 2.17 (dt, J = 2.4, 7.2 Hz, 2H), 3.63 (dd, J = 6.3, 12.0 Hz, 2H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.39, 25.73, 28.49, 28.76, 29.09, 29.42,

(21) Abrams, S. R. *Can. J. Chem.* **1984**, *62*, 1333.

29.50, 29.66, 32.80, 63.07, 68.00, 84.80. Anal. Calcd for $C_{20}H_{38}O$: C, 81.56; H, 13.00. Found: C, 81.55; H, 12.98. LRMS (FAB): m/z 301 $(M + Li)^+$.

17-Octadecyn-1-ylmethanesulfonate (5a). To a 100-mL three-neck round-bottom flask fitted with a 60-mL pressure-equalizing dropping funnel, a thermometer, and a N_2 inlet were added 17-octadecyn-1-ol (**4a**) (1.55 g, 5.8 mmol), triethylamine (1.4 mL, 10 mmol), and methylene chloride (50 mL). A solution of methanesulfonyl chloride (0.80 mL, 10 mmol) in 10 mL of methylene chloride in the dropping funnel was added to the cooled (0 °C) vigorously stirred mixture over a 30–45-min period. After 15 min, the ice bath was removed and stirring was continued for an additional hour at room temperature. Then the solution was diluted with ethyl ether and transferred to a separatory funnel, where it was washed with H_2O, 5% HCl, saturated $NaHCO_3$, and brine. Drying over $MgSO_4$ followed by rotoevaporation of the solvent gave the desired product (1.83 g, 92%) as a solid without further purification. ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 22H), 1.37 (m, 2H), 1.52 (q, J = 7.2 Hz, 2H), 1.75 (q, J = 7.2 Hz, 2H), 1.94 (t, J = 2.7 Hz, 1H), 2.18 (dt, J = 2.7, 7.0 Hz, 2H), 3.00 (s, 3H), 4.22 (t, J = 6.6 Hz, 2H).

15-Hexadecyn-1-ylmethanesulfonate (5b). ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 18H), 1.39 (m, 2H), 1.53 (q, J = 6.9 Hz, 2H), 1.75 (q, J = 6.9 Hz, 2H), 1.94 (t, J = 2.4 Hz, 1H), 2.18 (dt, J = 2.7, 7.1 Hz, 2H), 3.00 (s, 3H), 4.22 (t, J = 6.6 Hz, 2H).

13-Tetradecyn-1-ylmethanesulfonate (5c). ^1H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 14H), 1.38 (m, 2H), 1.52 (q, J = 6.9 Hz, 2H), 1.74 (q, J = 6.9 Hz, 2H), 1.94 (t, J = 2.4 Hz, 1H), 2.18 (dt, J = 2.7, 7.0 Hz, 2H), 3.00 (s, 3H), 4.22 (t, J = 6.6 Hz, 2H).

19-Eicosyn-1-ylmethanesulfonate (5d). ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 26H), 1.37 (m, 2H), 1.52 (q-like, 2H), 1.75 (q, J = 7.2 Hz, 2H), 1.94 (t, J = 2.4 Hz, 1H), 2.18 (dt, J = 2.7, 7.2 Hz, 2H), 3.00 (s, 3H), 4.22 (t, J = 6.4 Hz, 2H).

17-Octadecyn-1-oic acid (6a).[22] To a solution of 17-octadecyn-1-ol (**4a**) (3.2 g, 12 mmol) in anhydrous dimethylformamide (DMF) (70 mL) in a dry 250-mL single-neck round-bottom flask was added pyridinium dichromate (PDC) (15.8 g, 42 mmol), and the mixture was stirred for 9–10 h. Ethyl acetate (200 mL) was poured into the reaction mixture and then transferred to a 1000-mL separatory funnel which contained water (200 mL) and 10% HCl (50 mL). After gentle swirling and mixing, the aqueous layer was separated and extracted with ethyl acetate (3×). The ethyl acetate volumes were combined, washed with water and brine, dried over Na_2SO_4, and rotoevaporated. The residue was purified by silica gel chromatography, eluting with ethyl acetate–hexane (1:4 v/v), and recrystallized in methanol to give the desired product (2.9 g, 86%) as a white solid. Mp: 64–65 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 22H), 1.48–1.65 (m, 4H), 1.94 (t, J = 2.6 Hz, 1H), 2.18 (dt, J = 2.6, 7.1 Hz, 2H), 2.35 (t, J = 7.5 Hz, 2H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.38, 24.65, 28.48, 28.75, 29.04, 29.10, 29.22, 29.41, 29.48, 29.59, 29.61, 34.07, 68.00, 84.76, 180.28. Anal. Calcd for $C_{18}H_{32}O_2$: C, 77.14; H, 11.43. Found: C, 77.02; H, 11.47. LRMS (FAB): m/z 281 $(M + H)^+$, 287 $(M + Li)^+$.

15-Hexadecyn-1-oic acid (6b). Mp: 51.4–53 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 18H), 1.48–1.65 (m, 4H), 1.94 (t, J = 2.1 Hz, 1H), 2.18 (dt, J = 2.4, 7.1 Hz, 2H), 2.35 (t, J = 7.4 Hz, 2H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.36, 24.63, 28.46, 28.73, 29.02, 29.07, 29.20, 29.38, 29.46, 29.54, 34.08, 68.01, 84.72, 180.50. Anal. Calcd for $C_{16}H_{28}O_2$: C, 76.19; H, 11.11. Found: C, 75.93; H, 11.03. LRMS (FAB): m/z 253 $(M + H)^+$, 259 $(M + Li)^+$, 265 $(M + 2Li - H)^+$.

13-Tetradecyn-1-oic acid (6c). Mp: 44.5–46 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 14H), 1.48–1.62 (m, 4H), 1.92 (t, J = 2.4 Hz, 1H), 2.16 (dt, J = 2.3, 6.8 Hz, 2H), 2.33 (t, J = 7.4 Hz, 2H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.36, 24.62, 28.45, 28.71, 29.00, 29.05, 29.18, 29.35, 29.42, 29.46, 34.07, 68.02, 84.72, 180.39. Anal. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78. Found: C, 75.08; H, 10.81. LRMS (FAB): m/z 225 $(M + H)^+$, 231 $(M + Li)^+$.

3-Benzyl-*sn*-glycerol (7).[23,24] To a dry 250-mL single-neck round-bottom flask were added D-α,β-*O*-isopropylideneglycerol (6.0 g, 45 mmol), benzyl chloride (5.7 mL, 50 mmol), dry powdered KOH (25.2 g, 0.45 mol), and DMSO (75 mL). The solution was stirred for 2–3 h at room temperature, then poured into a 500-mL separatory funnel. Ethyl acetate (150 mL), 10% HCl (50 mL), and water (250 mL) were added, and the aqueous layer was separated and extracted with ethyl acetate (2×). The ethyl acetate volumes were combined, washed with water and brine, and dried over Na_2SO_4. Rotoevaporation of the ethyl acetate solution gave a lightly yellow residue to which 10% acetic acid (100 mL) was added, and the solution was stirred at 80–95 °C until homogeneous (2–3 H). After the mixture was cooled to room temperature, ethyl acetate (100 mL) was added and the solution poured into a 500-mL separatory funnel. The aqueous layer was extracted twice more with ethyl acetate, then the combined ethyl acetate volumes were washed with water, saturated $NaHCO_3$, and brine, and then dried over Na_2SO_4. Rotoevaporation of the ethyl acetate solution gave the crude product as an oil (5.4 g, 66% based on starting isopropylideneglycerol) which was essentially pure (^1H NMR). Further purification was achieved with silica gel chromatography, eluting with ethyl acetate–petroleum ether (1:2 v/v, R_f = 0.16). ^1H NMR (300 MHz, $CDCl_3$): δ 3.01 (s, 2H), 3.52–3.69 (m, 4H), 3.87 (m, 1H), 4.53 (s, 2H), 7.33 (m, 5H).

1,2-Di-*O*-(17-octadecynyl)-3-benzyl-*sn*-glycerol (8a).[23] To a 200-mL dry single-neck round-bottom flask were added 3-benzyl-*sn*-glycerol (**7**) (0.95 g, 5.2 mmol), dry powdered KOH (1.75 g, 31 mmol), and DMSO (50 mL). After 15 min of stirring, 17-octadecyn-1-ylmethanesulfonate (**5a**) (4.07 g, 12 mmol) was added. The reaction was allowed to continue for 2.5 h. Ethyl acetate, 5% HCl, and water were added, and the mixture was transferred to a 500-mL separatory funnel. The aqueous layer was separated and extracted with ethyl acetate (2×), and the combined ethyl acetate phases were washed with water and brine. Drying over Na_2SO_4 and rotoevaporation of the ethyl acetate gave an oily residue which was purified by silica gel chromatography, eluting with ethyl acetate–hexane (1:6 v/v) to give the desired product (3.19 g, 90%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 48H), 1.52 (m, 8H), 1.93 (t, J = 2.4 Hz, 2H), 2.18 (m, 4H), 3.40–3.59 (m, 9H), 4.55 (s, 2H), 7.33 (m, 5H).

1,2-Di-*O*-(15-hexadecynyl)-3-benzyl-*sn*-glycerol (8b). ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 40H), 1.52 (m, 8H), 1.94 (t, J = 2.4 Hz, 2H), 2.18 (m, 4H), 3.40–3.59 (m, 9H), 4.55 (s, 2H), 7.34 (m, 5H).

1,2-Di-*O*-(13-tetradecynyl)-3-benzyl-*sn*-glycerol (8c). ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 32H), 1.52 (m, 8H), 1.93 (t, J = 2.7 Hz, 2H), 2.18 (m, 4H), 3.40–3.60 (m, 9H), 4.55 (s, 2H), 7.34 (m, 5H).

1,2-Di-*O*-(19-eicosynyl)-3-benzyl-*sn*-glycerol (8d). ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 56H), 1.52 (m, 8H), 1.93 (t, J = 2.6 Hz, 2H), 2.18 (dt, J = 2.4, 7.2 Hz, 4H), 3.40–3.59 (m, 9H), 4.55 (s, 2H), 7.33 (m, 5H).

1,2-Di-*O*-(17-octadecyn-1-oyl)-3-benzyl-*sn*-glycerol (12a).[25] A dry 50-mL single-neck round-bottom flask was charged with 3-benzyl-*sn*-glycerol (**7**) (0.50 g, 2.7 mmol), 17-octadecyn-1-oic acid (**6a**) (1.6 g, 5.7 mmol), 4-(dimethylamino)pyridine (DMAP) (0.77 g, 6.3 mmol), and dry carbon tetrachloride (40 mL). This solution was stirred and cooled (0 °C), and then a solution of 1,3-dicyclohexylcarbodiimide (DCC) (1.3 g, 6.3 mmol) in carbon tetrachloride (2 mL) was added. After 3–4 h, the 1,3-dicyclohexylurea was filtered off and rinsed with chloroform, and solvents were rotoevaporated. The residue was purified by silica gel chromatography, eluting with acetate–hexane (1:6 v/v), and triturated in cold methanol (−10 to 0 °C) to give the desired product as a white solid (1.54 g, 81%). Mp: 38.5–39.5 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 44H), 1.47–1.61 m, 8H), 1.93 (t, J = 2.4 Hz, 2H), 2.17 (dt, J = 2.4, 7.1 Hz, 4H), 2.25–2.34 (m, 4H), 3.58 (d, J = 5.1 Hz, 2H), 4.15–4.37 (m, 2H), 4.53 (d, J = 3.4 Hz, 2H), 5.24 (q-like, 1H), 7.31 (m, 5H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.38, 24.86, 24.93, 28.48, 28.74, 29.09, 29.26, 29.48, 29.60, 29.63, 34.09, 34.30, 62.62, 68.00, 68.23, 69.97, 73.27, 84.76, 127.58, 127.74, 128.38, 137.68, 173.06, 173.35. Anal. Calcd for $C_{46}H_{74}O_5$: C, 78.14; H, 10.55. Found: C, 78.44; H, 10.87. LRMS (FAB): m/z 707 $(M + H)^+$, 713 $(M + Li)^+$.

1,2-Di-*O*-(15-hexadecyn-1-oyl)-3-benzyl-*sn*-glycerol (12b). Semisolid. ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 36H), 1.47–1.60 (m, 8H), 1.93 (t, J = 2.6 Hz, 2H), 2.17 (dt, J = 2.4, 7.2 Hz, 4H), 2.24–2.34 (m, 4H), 3.58 (d, J = 5.1 Hz, 2H), 4.15–4.36 (m, 2H), 4.53 (d, J = 3.6 Hz, 2H), 5.24 (q-like, 1H), 7.31 (m, 5H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.38, 24.86, 24.94, 28.48, 28.75, 29.10, 29.27, 29.46, 29.49, 29.59, 34.10, 34.32, 62.63, 68.01, 68.25, 69.98, 73.28, 84.78, 127.60, 127.76, 128.40, 137.68, 173.09, 173.38. Anal. Calcd for $C_{42}H_{66}O_5$: C, 77.53; H, 10.15. Found: C, 77.59; H, 10.26. LRMS (FAB): m/z 651 $(M + H)^+$, 657 $(M + Li)^+$.

1,2-Di-*O*-(13-tetradecyn-1-oyl)-3-benzyl-*sn*-glycerol (12c). Oil. ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 28H), 1.47–1.61 (m, 8H), 1.93 (t, J = 2.6 Hz, 2H), 2.17 (dt, J = 2.6, 7.3 Hz, 4H), 2.24–2.34 (m, 4H), 3.58 (d, J = 5.1 Hz, 2H), 4.14–4.37 (m, 2H), 4.53 (d, J = 3.6 Hz, 2H), 5.23 (m, 1H), 7.32 (m, 5H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.29, 24.58, 24.76, 24.84, 25.36, 28.38, 28.64, 28.99, 29.16, 29.33, 29.37, 29.43, 29.99, 33.99, 34.82, 62.53, 68.00, 68.15, 69.90, 73.18, 84.59, 127.50, 127.95, 128.29, 137.61, 172.93, 173.22. Anal. Calcd for $C_{38}H_{58}O_5$: C, 76.72;

(22) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.
(23) Johnstone, R. A. W.; Rose, M. E. *Tetrahedron* **1979**, *35*, 2169.
(24) Howe, R. J.; Malkin, T. *J. Chem. Soc.* **1951**, 2663.
(25) Duralski, A. A.; Spooner, P. J. R.; Watts, A. *Tetrahedron Lett.* **1989**, *30*, 3585.

H, 9.83. Found: C, 76.90; H, 10.09. LRMS (FAB): m/z 595 (M + H)$^+$, 601 (M + Li)$^+$.

C_{18} Macrocyclic Ether (9a). To a three-neck, 500-mL round-bottom flask equipped with a magnetic stirring bar a condensor, a rubber stopper with a glass tube, and a septum were added CuCl (0.35 g, 3.5 mmol), and *N,N,N',N'*-tetramethylethylenediamine (TMEDA) (0.55 mL, 3.6 mmol) in 300 mL of xylene. The solution was heated to 140 °C in an oil bath, while oxygen was gently bubbled into it through the glass tube. During the heating period, the solution first became light green, then dark olive green. When the temperature reached 125–130 °C, a xylene-insoluble oily substance settled along the inner surface of the reaction flask. As the temperature continued to rise, the reaction mixture became clear light green and then turned dark again. A solution of compound **8a** (0.95 g, 1.4 mmol) in 40 mL of xylene was added dropwise to the heated xylene solution with the aid of a syringe pump (50-mL syringe) over a period of 4–5 h. Following completion of the addition, heat and oxygen were turned off. The xylene was rotoevaporated, 10% HCl (150 mL) was added to the residue, and the mixture was extracted with ethyl acetate (100 mL) (3×). The combined ethyl acetate phases were washed with saturated $NaHCO_3$, water, and brine and dried over Na_2SO_4. Rotoevaporation of the ethyl acetate gave a residue which was purified by silica gel chromatography, eluting with ethyl acetate–hexane (1:6 v/v) to give the desired macrocyclic product (**9a**) (0.74 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 48H), 1.57 (m, 8H), 2.25 (t, J = 6.6 Hz, 4H), 3.42–3.61 (m, 9H), 4.55 (s, 2H), 7.33 (m, 5H).

C_{16} Macrocyclic Ether (9b). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (s, 40H), 1.55 (m, 8H), 2.26 (t, J = 6.0 Hz, 4H), 3.43–3.62 (m, 9H), 4.56 (s, 2H), 7.33 (m, 5H).

C_{14} Macrocyclic Ether (9c). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (s, 32H), 1.55 (m, 8H), 2.26 (t, J = 5.7 Hz, 4H), 3.45–3.63 (m, 9H), 4.56 (s, 2H), 7.33 (m, 5H).

C_{20} Macrocyclic Ether (9d). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 56H), 1.55 (m, 8H), 2.25 (t, J = 6.3 Hz, 4H), 3.42–3.60 (m, 9H), 4.55 (s, 2H), 7.33 (m, 5H).

1,2-*O*-(17,19-Hexatriacontadiyne-1,36-dioyl)-3-benzyl-*sn*-glycerol (13a). Solid. Mp: 47–49 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 44H), 1.40–1.62 (m, 8H), 2.23–2.34 (m, 8H), 3.58 (d, J = 5.2 Hz, 2H), 4.18–4.40 (m, 2H), 4.54 (d, J = 2.1 Hz, 2H), 5.26 (m, 1H), 7.31 (m, 5H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 19.11, 24.88, 24.94, 28.02, 28.46, 28.92, 29.04, 29.06, 29.21, 29.27, 29.35, 29.44, 29.50, 29.55, 34.16, 34.34, 62.77, 65.46, 68.23, 69.96, 73.28, 127.59, 127.75, 128.39, 137.65, 173.06, 173.37. Anal. Calcd for $C_{46}H_{72}O_5$: C, 78.40; H, 10.22. Found: C, 78.29; H, 10.27. LRMS (FAB): m/z 705 (M + H)$^+$, 711 (M + Li)$^+$.

1,2-*O*-(15,17-Dotriacontadiyne-1,32-dioyl)-3-benzyl-*sn*-glycerol (13b). Semi-solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 36H), 1.36–1.61 (m, 8H), 2.22–2.34 (m, 8H), 3.58 (d, J = 5.2 Hz, 2H), 4.16–4.40 (m, 2H), 4.53 (d, J = 2.1 Hz, 2H), 5.25 (m, 1H), 7.31 (m, 5H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 19.06, 24.83, 24.88, 27.93, 28.34, 28.84, 28.87, 28.99, 29.02, 29.10, 29.13, 29.20, 29.24, 29.27, 29.37, 29.43, 34.12, 34.29, 62.72, 65.49, 68.20, 69.93, 73.23, 127.54, 127.70, 128.34, 137.62, 173.00, 173.31. Anal. Calcd for $C_{42}H_{64}O_5$: C, 77.78; H, 9.87. Found: C, 77.55; H, 9.89. LRMS (FAB): m/z 649.5 (M + H)$^+$, 655.5 (M + Li)$^+$.

1,2-*O*-(13,15-Octacosadiyne-1,28-dioyl)-3-benzyl-*sn*-glycerol (13c). Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 28H), 1.42–1.60 (m, 8H), 2.21–2.33 (m, 8H), 3.57 (d, J = 5.1 Hz, 2H), 4.16–4.39 (m, 2H), 4.52 (s, 2H), 5.25 (m, 1H), 7.30 (m, 5H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 18.99, 24.74, 24.79, 27.82, 28.19, 28.23, 28.73, 28.78, 28.94, 28.98, 29.12, 29.21, 29.24, 34.06, 34.23, 62.64, 65.54, 68.17, 69.89, 73.18, 127.49, 127.66, 128.30, 137.59, 172.92, 173.24. Anal. Calcd for $C_{38}H_{56}O_5$: C 77.02; H, 9.45. Found: C, 76.75; H, 9.47. LRMS (FAB): m/z 593 (M + H)$^+$, 599 (M + Li)$^+$.

1,2-*O*-(Hexatriacontane-1,36-diyl)-*sn*-glycerol (10a).[26] To a 25-mL single-neck round-bottom flask with a magnetic stirring bar were added compound **9a** (0.40 g, 0.59 mmol), palladium (10% on carbon, 0.2 g, 50% w/w), and ethanol–acetic acid (12 mL, 5:1 v/v). Hydrogenolysis was conducted at room temperature at slight positive pressure using hydrogen supplied but a buret driven by oil. Reaction progress was indicated by the uptake of hydrogen. After 2 h, the reaction was completed. The mixture was diluted with chloroform and filtered. The filtrate was concentrated to furnish crude product which was purified by silica gel chromatography, eluting with ethyl acetate–hexane (1:4 v/v). Further purification was achieved by recrystallization from methanol to give the pure product (0.24 g, 67%) as a white solid. Mp: 67.0–69.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 64H), 1.56 (m, 4H), 2.17 (t, J = 6.0 Hz, 1H), 3.42–3.74 (m, 9H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 26.02, 26.05, 29.11, 29.16, 29.21, 29.28, 29.33, 29.39, 29.50, 29.70, 30.01, 63.04, 70.39, 71.15, 71.75, 78.41. Anal. Calcd for $C_{39}H_{78}O_3$: C, 78.72; H, 13.21. Found: C, 78.48; H, 13.09. LRMS (FAB): m/z 595.6 (M + H)$^+$.

1,2-*O*-(Dotriacontane-1,32-diyl)-*sn*-glycerol (10b). Mp: 59.5–60.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 56H), 1.56 (m, 4H), 2.14 (t, J = 6.6 Hz, 1H), 3.42–3.71 (m, 9H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 26.00, 26.04, 28.99, 29.05, 29.12, 29.21, 29.29, 29.36, 29.42, 29.46, 29.54, 29.70, 63.04, 70.40, 71.19, 71.74, 78.42. Anal. Calcd for $C_{35}H_{70}O_3$: C, 78.00; H, 13.09. Found: C, 77.75; H, 12.94. LRMS (FAB): m/z 546 (M + Li)$^+$.

1,2-*O*-(Octacosane-1,28-diyl)-*sn*-glycerol (10c). Mp: 49.0–50.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 48H), 1.56 (m, 4H), 2.21 (brs, 1H), 3.41–3.72 (m, 9H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 25.96, 26.00, 28.78, 28.81, 28.91, 28.99, 29.11, 29.26, 29.32, 29.34, 29.38, 29.47, 29.51, 29.61, 29.68, 29.97, 62.98, 70.37, 71.18, 71.72, 78.45. Anal. Calcd for $C_{31}H_{62}O_3$: C, 77.12; H, 12.94. Found: C, 76.89; H, 12.84. LRMS (FAB): m/z 483.5 (M + H)$^+$.

1,2-*O*-(Tetracontane-1,40-diyl)-*sn*-glycerol (10d). Mp: 75.0–76.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 72H), 1.56 (m, 4H), 2.15 (m, 1H), 3.42–3.74 (m, 9H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 26.03, 26.06, 29.22, 29.25, 29.28, 29.34, 29.37, 29.43, 29.51, 29.54, 29.70, 29.81, 63.06, 70.40, 71.14, 71.77, 78.38. Anal. Calcd for $C_{43}H_{86}O_3$: C, 79.32; H, 13.31. Found: C, 79.40; H, 13.38. LRMS (FAB): m/z 652 (M + H)$^+$, 658 (M + Li)$^+$.

1,2-*O*-(Hexatriacontane-1,36-dioyl)-*sn*-glycerol (14a). A solution of compound **13a** (0.23 g, 0.33 mmol) in THF–ethanol (10 mL, 4:1 v/v) was hydrogenated in the presence of 10% Pd/C (0.11 g, 50% w/w) at 50-psi pressure of H_2 for 2 h. The catalyst was filtered off and washed with $CHCl_3$. After evaporation of the solvents, the crude product was recrystallized from acetone to give the pure product (0.12 g, 59%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 60H), 1.62 (m, 4H), 2.01 (t-like, 1H), 2.33 (q, J = 7.2 Hz, 4H), 3.73 (t, J = 5.7 Hz, 2H), 4.26–4.32 (m, 2H), 5.10 (m, 1H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 24.92, 24.96, 28.85, 28.92, 28.96, 29.03, 29.07, 29.11, 29.21, 29.30, 29.42, 29.45, 29.54, 34.16, 34.33, 61.60, 62.18, 72.16, 173.42, 173.75. LRMS (FAB): m/z 623.7 (M + H)$^+$, 629.7 (M + Li)$^+$.

1,2-*O*-(Dotriacontane-1,32-dioyl)-*sn*-glycerol (14b). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 52H), 1.61 (m, 4H), 2.10 (brs, 1H), 2.33 (q-like, 4H), 3.72 (d, J = 5.1 Hz, 2H), 4.23–4.33 (m, 2H), 5.10 (m, 1H). ^{13}G NMR (75.1 MHz, $CDCl_3$): δ 24.90, 24.95, 28.71, 28.77, 28.84, 28.86, 29.00, 29.05, 29.09, 29.12, 29.18, 29.22, 29.24, 29.38, 29.46, 29.64, 29.68, 34.15, 34.32, 61.53, 62.14, 72.14, 173.43, 173.74. LRMS (FAB): m/z 567.68 (M + H)$^+$, 574 (M + Li)$^+$.

1,2-*O*-(Octacosane-1,28-dioyl)-*sn*-glycerol (14c). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 44H), 1.61 (m, 4H), 2.13 (s, 1H), 2.33 (q-like, 4H), 3.72 (t, J = 4.2 Hz, 2H), 4.21–4.37 (m, 2H), 5.10 (m, 1H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 24.90, 24.95, 28.51, 28.60, 28.68, 28.71, 28.85, 28.90, 29.00, 29.14, 29.18, 29.30, 34.15, 34.32, 61.53, 62.25, 72.16, 173.45, 173.76. LRMS (FAB): m/z 517 (M + Li)$^+$.

1,2-*O*-(Hexatriacontane-1,36-diyl)-*sn*-glycero-3-phosphatidylcholine (11a).[27] To a cooled solution (0 °C) of compound **10a** (0.208 g, 0.35 mmol) and triethylamine (67.9 μL, 0.49 mmol) in dry benzene (4 mL) was added dropwise 2-chloro-2-oxo-1,3,2-dioxaphospholane (38.6 μL, 0.42 mmol). The mixture was stirred at 0 °C for a few minutes and then at room temperature for 2 h. The crystalline Et_3N·HCl was removed by filtration, and the solvent was evaporated in vacuo to give a white solid which was used in the next step immediately.

The above solid was transferred to a pressure tube with anhydrous acetonitrile (12 mL), and the pressure tube was cooled in a dry ice–acetone bath. Anhydrous trimethylamine (1 mL) was then added with a syringe. The tube was sealed and heated in an oil bath at 60–65 °C for 48 h. The tube was cooled and opened, the white solid was dissolved in chloroform, and the solution was transferred to a round-bottom flask. Rotoevaporation of solvents gave the crude product which was purified by silica gel chromatography (80–200 mesh, about 7 g) while eluting with chloroform–methanol according to the following gradient (50-mL fractions were taken):

%$CHCl_3$	%MeOH	volume (mL)
80	20	50
60	40	100
40	60	100
20	80	200
10	90	150

(26) Yamauchi, K.; Une, F.; Tabata, S.; Kinoshita, M. *J. Chem. Soc., Perkin Trans. 1* **1986**, 765.

(27) Bhatia, S. K.; Hajdu, J. *Synthesis* **1989**, 16.

The pure desired product was isolated in 44% yield (116 mg). Its purity was judged by TLC (silica TLC plates developed with $CHCl_3$:CH_3OH:H_2O = 65:25:5 and dyed with I_2) having only one spot. ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 64H), 1.51 (m, 4H), 3.36 (s, 9H), 3.40–3.59 (m, 7H), 3.77–3.83 (m, 4H), 4.28 (brs, 2H). Anal. Calcd for $C_{44}H_{90}O_6NP\cdot H_2O$: C, 67.91; H, 11.92; N, 1.80. Found: C, 67.77; H, 11.77; N, 1.80. LRMS (FAB): m/z 761 $(M + H)^+$, 767 $(M + Li)^+$.

1,2-*O*-(Dotriacontane-1,32-diyl)-*sn*-glycero-3-phosphatidylcholine (11b). ^1H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 56H), 1.52 (m, 4H), 3.40 (s, 9H), 3.42–3.62 (m, 7H), 3.80–3.87 (m, 4H), 4.32 (brs, 2H). Anal. Calcd for $C_{40}H_{82}O_6NP\cdot H_2O$: C, 66.54; H, 11.73; N, 1.94. Found: C, 66.69; H, 11.73; N, 1.90. LRMS (FAB): m/z 705 $(M + H)^+$, 711 $(M + Li)^+$.

1,2-*O*-(Octacosane-1,28-diyl)-*sn*-glycero-3-phosphatidylcholine (11c). ^1H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 48H), 1.52 (m, 4H), 3.36 (s, 9H), 3.40–3.78 (m, 7H), 3.81–3.84 (m, 4H), 4.28 (brs, 2H). Anal. Calcd for $C_{36}H_{74}O_6NP\cdot H_2O$: C, 64.93; H, 11.50; N, 2.10. Found: C, 65.01; H, 11.47; N, 2.12. LRMS (FAB): m/z 649 $(M + H)^+$.

1,2-*O*-(Tetracontane-1,40-diyl)-*sn*-glycero-3-phosphatidylcholine (11d). ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 72H), 1.52 (m, 4H), 3.40 (s, 9H), 3.42–3.61 (m, 7H), 3.80–3.89 (m, 4H), 4.35 (m, 2H). LRMS (3-NBA): m/z 816.5 $(M + H)^+$, 822.5 $(M + Li)^+$.

1,2-*O*-(Hexatriacontane-1,36-dioyl)-*sn*-glycero-3-phosphatidylcholine (15a). ^1H NMR (300 MHz, $CDCl_3$:CD_3OD = 2:1, TMS as internal reference): δ 1.19 (s, 60H), 1.53 (m, 4H), 2.20 (m, 4H), 3.13 (s, 9H), 3.52 (m, 2H), 3.87–4.17 (m, 6H), 5.15 (m, 1H). ^{13}C NMR (75.1 MHz, $CDCl_3$:CD_3OD = 2:1, TMS as internal reference): δ 24.03, 24.08, 27.70, 27.74, 27.78, 27.84, 27.90, 27.99, 28.06, 28.15, 28.21, 28.32, 28.37, 28.41, 28.45, 28.53, 28.57, 28.66, 28.76, 33.26, 33.38, 53.18, 58.14, 61.89, 62.73, 65.59, 69.52, 172.74, 173.10. LRMS (FAB): m/z 788 $(M + H)^+$, 794 $(M + Li)^+$.

1,2-*O*-(Dotriacontane-1,32-dioyl)-*sn*-glycero-3-phosphatidylcholine (15b). ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 52H), 1.57 (brs, 4H), 2.28 (m, 4H), 3.38 (S, 9H), 3.83–4.43 (m, 8H), 5.20 (m, 1H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 24.92, 25.02, 28.50, 28.56, 28.61, 28.67, 28.75, 28.85, 28.93, 29.01, 29.12, 29.17, 29.20, 29.28, 29.35, 29.42, 29.47, 29.52, 29.60, 29.66, 34.20, 34.39, 54.48, 59.26, 63.06, 63.39, 66.34, 70.56, 173.20, 173.52. LRMS (FAB): m/z 733 $(M + H)^+$, 739 $(M + Li)^+$.

1,2-*O*-(Octacosane-1,28-dioyl)-*sn*-glycero-3-phosphatidylcholine (15c). ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (s, 44H), 1.57 (brs, 4H), 2.27 (m, 4H), 3.36 (s, 9H), 3.80–4.41 (m, 8H), 5.20 (m, 1H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 24.90, 25.01, 28.23, 28.26, 28.33, 28.41, 28.46, 28.57, 28.70, 28.83, 28.91, 29.05, 29.14, 29.24, 29.31, 29.41, 29.45, 34.17, 34.36, 54.32, 59.26, 63.01, 63.34, 66.27, 70.50, 173.17, 173.51. LRMS (FAB): m/z 676 $(M + H)^+$, 682 $(M + Li)^+$.

DSC Sample Preparation. Multilamellar suspensions of the lipid samples were prepared by dissolving small amounts (2–4 mg) of each in methanol–chloroform (50:50 by volume methanol, 99.9% ACS certified grade, and chloroform, HPLC grade, filtered through a 0.5-μm filter), and this solution was placed in a 250-mL round-bottom flask. The bulk of the methanol–chloroform was evaporated with a stream of nitrogen, leaving the lipid as a thin film on the walls of the flask. The remaining solvent was removed by subjecting the lipid film to high vacuum for at least 3 h. Each sample was then suspended in a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (10 mM HEPES, 160 mM KCl, 0.1 mM EDTA, pH 7.5) by placing the flask for several minutes in a water bath at a temperature at least 10 °C above the T_c for the gel-to-liquid-crystal phase transition, followed by vortexing the sample for 5 min with a Vortex, Jr. mixer (Scientific Industries, Inc.). The heating of the lipid and buffer was necessary to ensure full hydration of the lipid bilayers.

Phosphate Analysis. Magnesium nitrate hexahydrate and ammonium molybdate(VI) tetrahydrate were purchased from Aldrich Chemical Co. Ascorbic acid was obtained from J. T. Baker Co. A Bausch & Lomb Spectronic 2000, which was calibrated with a holmium oxide glass filter, was used to read all absorbances. Standard curves were made from solutions with known concentrations of KH_2PO_4; absorbances were determined relative to a blank composed of all components except a source of phosphate. As soon as a DSC run was completed, the suspension was agitated to ensure uniformity by drawing it in and out of a syringe several times. An aliquot (100 μL) was then transferred immediately to each of eight screw-cap test tubes to which was added 0.3 mL of 5% magnesium nitrate in a 95% ethanol solution (prepared fresh daily). After evaporation to dryness, the tubes were heated over a Fischer burner until the emission of brown fumes had subsided. Each tube, after receiving 0.6 mL of 0.5 N HCl, was capped and heated in a boiling water bath for 30 min. Next, the tubes received 1.4 mL of a solution containing six parts of 0.42% $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 1 N H_2SO_4 and one part of 10% aqueous ascorbic acid. This was followed by capping, vortexing for 15 s, and incubating at 45 °C for 30 min. A 1.0-mL aliquot of this solution was diluted to 5.0 mL with deionized water and vortexed for 30 s and the absorbance read at 820 nm. Phosphate concentrations were found to be in the 0.9–3.2 ± 0.06 mM range. No difference was observed when phosphate analyses were carried out before and after the DSC runs.

Differential Scanning Calorimetry. All calorimetric measurements were performed with a MC-2 differential scanning calorimeter purchased from Microcal, Inc., and interfaced to an IBM personal computer. This instrument consists of two cells, one for the sample, and one for the reference buffer or solvent. The cells were heated at a constant rate and in such a way that a zero temperature difference was maintained between the two. When a phase change occurred, the resulting temperature imbalance between the cells was detected by means of an array of thermocouples between the cells, and more heat was added to the lagging cell in order to equalize the temperature in both cells. The excess power applied to the lagging cell, recorded digitally at uniform temperature intervals, was proportional to the excess heat capacity. A scan rate of ~60 °C/h was used in experiments involving the cyclic lipids. However, in order to record accurately the sharp endotherms characteristic of the gel-to-liquid-crystal transitions of the "untethered" diacylphosphatidylcholine samples, it was necessary to scan at the rate of 2.5–3.0 °C/h.

Previous studies on biological systems have used the difference between the heat capacity in a reference state (*i.e.* where the lipid is entirely in the gel or liquid-crystal phase) and the observed heat capacity. This difference has been shown to be virtually identical to the difference calculated directly from the experimental ΔC_p curves as described in the following procedure.[28]

Heat capacity data, originally recorded in units of mcal/min, were converted into mcal/deg by dividing each point by the scan rate. A baseline, recorded with buffer in both the sample and reference cells and also normalized to mcal/deg, was subtracted from each heat capacity curve to eliminate any variations due to mismatch between the two cells. Finally, individual data points were divided by the number of millimoles of lipid, determined from the phosphate analysis, thereby converting the heat capacity (after division by 1000) to (kcal/deg)/mol.

Excess heat capacity data for the transitions were filtered digitally by the method of splines. Thus, the normalized curves were established by connecting the baseline before and after the transition and then performing a point-by-point subtraction from this straight line. Transition enthalpies were calculated by numerical integration of the resulting heat capacity values as a function of temperature (*i.e.* $\Delta H = \int C_p\, dt$).

Main transition entropies ΔS_c, in units of cal/(mol K), were estimated with the assumption that at T_c the two phases are at equilibrium in which case $\Delta S_c = \Delta H_c/T$, where ΔH_c is expressed in cal/mol and T in K.

Results and Discussion

Multistep syntheses of the tethered phospholipids (Schemes I and II) are detailed in the Experimental Section. Reported yields are representative of those obtained from repeated runs carried out by two independent experimenters. The key reaction, among the more prosaic ones, is a high-temperature Glaser oxidation (**12** to **13** in Scheme II) that served to close the macrocyclic ring in surprisingly high yield. Normally, of course, yields in giant-ring closures can be adversely affected by an intermolecular coupling that competes with the desirable intramolecular process. Yields were, in fact, unacceptably low when classical low-temperature Glaser conditions were employed (even with a slow injection of one of the reagents to maintain "high dilution"). Upon heating the Glaser system to 140 °C, however, we achieved the high yields listed in Table I. Note that the smaller numbers in the table represent reactions carried out early on in the project when the conditions had not yet been "fine-tuned". Thus, the larger yields in the table (70–85%) are more indicative of those that can be expected if our "recipe" is duplicated.

We do not understand why high temperature augments the Glaser yields. Our experience has been, however, that long chains generally impede reaction rates even when the chain atoms lie remote from the site of chemical change. Perhaps the chains coil about the reaction center, thereby inhibiting the approach of reagents. High temperature would, of course, tend to uncoil the chains and expose the reactive sites. It should be mentioned in

(28) Privalov, P. L.; Potehkin, T. *Methods Enzymol.* **1986**, *131*, 4.

Table I. Yields for High-Temperature Glaser Cyclization of Seven Giant Rings[a,b]

lipid type	ring size	yield, %
diester	32	52
diester	36	64
diester	40	60, 68
diether	32	78, 79
diether	36	83
diether	40	77, 78, 85
diether	44	71

[a] Reactions were run with 0.9–2.8 g of diyne as described in the text. Yields represent the amount of material after purification by column chromatography. [b] The high macrocyclization yields are rationalizeable in terms of theoretical considerations advanced by Mandolini, L. *Adv. Phys. Org. Chem.* **1986**, *22*, 1.

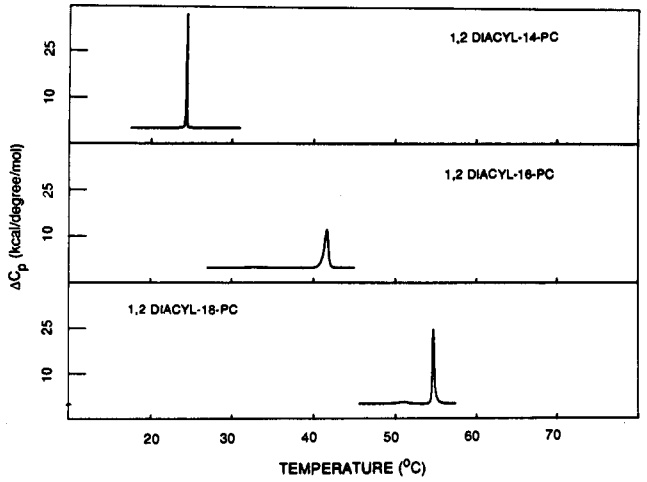
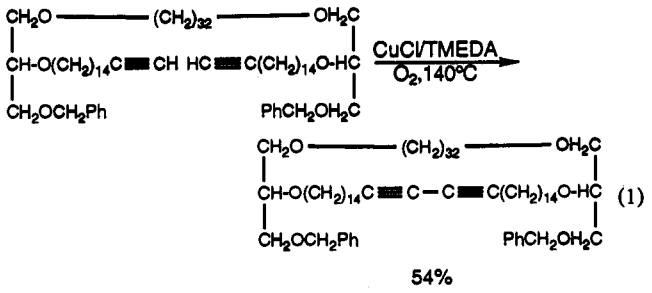


Figure 1. Excess heat capacity curves derived from DSC thermograms for multilamellar suspensions in HEPES buffer (10 mM HEPES, 160 mM KCl, 0.1 M EDTA, pH = 7.5) of diacylphosphatidylcholines (untethered diesters). The number before "PC" refers to the total number of carbons in the saturated fatty acid composing the lipid. Top: [lipid] = 9.4 mM, scan rate = 2.5 °C/h. Middle: [lipid] = 1.2 mM, scan rate = 58 °C/h. Bottom: [lipid] = 1.7 mM, scan rate = 96 °C/h.

this regard that recent work of others has described macrocyclic ring closures under more conventional Glaser conditions.[9] In our hands and with our particular compounds, these conditions led to considerable amounts of starting material and byproduct.

A particularly promising indicant that our high-temperature variation of the Glaser oxidation might have general applicability in constructing macrocyclic biomolecules comes from a recent synthesis of a "bolaform" lipid (eq 1). Yields of 54–66% for



closing rings with as many as 72 atoms have now been obtained. The reaction, therefore, permits the eventual study of highly interesting "double phospholipids" whose properties will, when the work is completed, be the subject of another manuscript.

Plots of excess heat capacity *vs.* temperature for the untethered and tethered diacylphosphatidylcholines are shown in Figures 1 and 2, respectively. Note the striking difference in the ordinate scales: 0–25 (kcal/deg)/mol for the untethered diesters and 0–1.5 (kcal/deg)/mol for the tethered diesters. Corresponding plots for the untethered and tethered dialkylphosphatidylcholines are

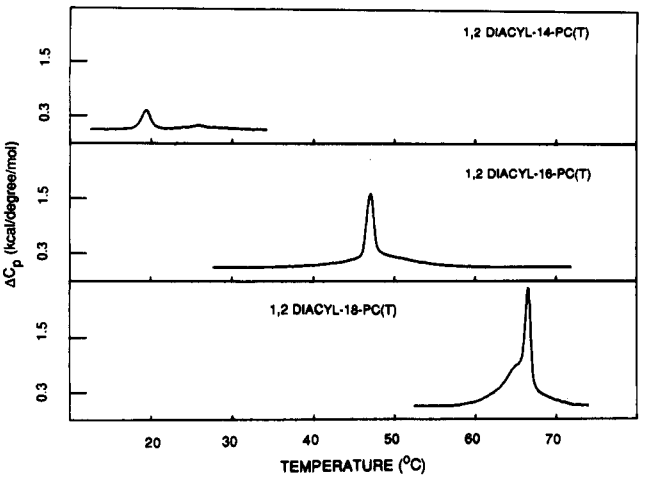


Figure 2. Excess heat capacity curves for tethered diacylphosphatidylcholines. The number before "PC(T)" refers to the total number of carbons in the saturated fatty acid composing the lipid before the two fatty acids are linked at their termini. All scans were run at 1.2–1.6 mM lipid and a scan rate of 55 °C/h.

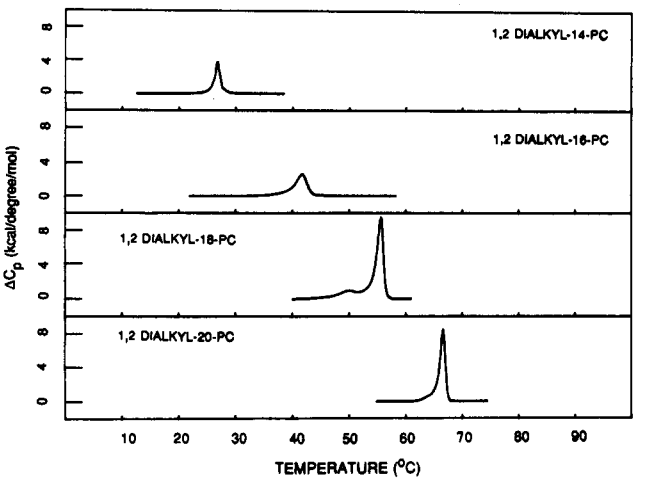


Figure 3. Excess heat capacity curves for untethered dialkylphosphatidylcholines. The number before "PC" refers to the total number of carbons in each saturated ether chain. Scans were run at 55 °C/h and 1.1–3.0 mM lipid.

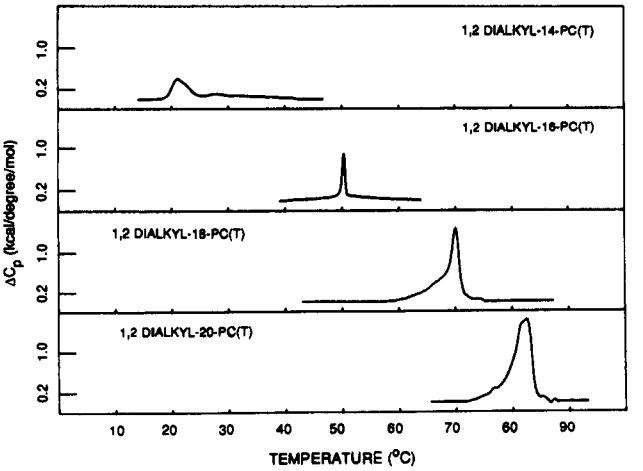


Figure 4. Excess heat capacity curves for tethered dialkylphosphatidylcholines. The number before "PC(T)" refers to the total number of carbons in each saturated ether chain prior to their being connected at their termini. Scans were run at 55 °C/h and 0.7–1.8 mM lipid.

given in Figures 3 and 4. The ordinate scales for the latter two graphs are also very different: 0–8 (kcal/deg)/mol for the untethered diethers and 0–1.0 (kcal/deg)/mol for the tethered diethers.

Table II. Thermodynamic Parameters for Thermotropic Phase Changes in Untethered and Tethered 1,2-Diacyl- and 1,2-Dialkylphosphatidylcholines with Linear Saturated Chains of 14–20 Carbons[a]

chain	T_p	ΔH_p	T_c	ΔH_c	ΔS_c
		Untethered 1,2-Diacyl[b]			
C_{14}	15.1	1.3	23.9	5.3	17.8
C_{16}	32.8	0.5	41.6	8.1	25.7
C_{18}	51.0	0.9	54.7	9.8	27.0
		Tethered 1,2-Diacyl[c]			
C_{14}			19.4	0.6	2.1
C_{16}	11.0	0.4	47.0	4.2	13.1
C_{18}			66.6	6.0	17.1
		Untethered 1,2-Dialkyl[b]			
C_{14}			26.9	5.4	17.8
C_{16}			41.7	8.4	26.7
C_{18}	50.2	2.0	55.6	9.6	29.2
C_{20}	64.9	2.6	66.5	10.4	30.6
		Tethered 1,2-Dialkyl[c]			
C_{14}			21.0	1.2	3.9
C_{16}	14.8	3.2	50.4	2.8	8.5
C_{18}	26.0	2.0	70.1	5.1	14.6
C_{20}	35.0	0.3	82.6	6.9	19.4

[a] T_p and ΔH_p refer to the pretransition temperature (°C) and enthalpy (kcal/mol), respectively. T_c, ΔH_c, and ΔS_c refer to the main transition temperature (°C), enthalpy (kcal/mol), and entropy (cal/(mol K)), respectively. [b] The untethered lipid data are known (ref 29), but were repeated here so that untethered and tethered lipids can be compared under identical experimental conditions. Agreement with the literature is satisfactory. [c] A C_{14} tethered lipid, for example, refers to a chain length of 14 for each chain prior to their being joined in a macrocyclic ring.

Phase-transition temperatures in Celsius are recorded for "pretransitions" (T_p) and "main transitions" (T_c) in Table II. These temperatures represent the observed maxima in the excess heat capacity *vs.* temperature profiles. Owing to their weak intensity and uncertain molecular origin, many of the pretransition peaks have not been incorporated into the graphs. Table II also lists the enthalpy changes in kcal/mol associated with the pretransitions (ΔH_p) and main transitions (ΔH_c).

Although thermotropic properties are particularly sensitive to impurities, two facts lend confidence to the data in Table II: (a) The synthetic lipids were chromatographed repeatedly on silica gel columns until TLC analyses indicated an absence of detectable impurities. The resulting lipids were subjected to calorimetric studies within 2 days of purification. (b) The data in Table II show well-behaved variations with chain length indicative of materials free from disrupting impurities. For example, the thermodynamic parameters for untethered diesters correspond closely to the corresponding data for the untethered diethers, as do the parameters for tethered diesters and diethers. Both transition temperatures and enthalpies increase smoothly with chain length. We estimate that main transition temperatures are accurate to ±0.2 °C and enthalpies to ±0.5 kcal/mol. Data for the untethered lipids are in reasonable agreement with those in the literature.[29]

It should be stated at the outset that our discussion will focus primarily on the main transition (the so-called $P_\beta \rightarrow L_\alpha$ process known to represent the "melting" or disordering of the hydrocarbon tails).[30] Our objective was to study the effect of a single additional carbon–carbon bond, joining the two chains at their termini, on the main transition, which is, by far, the most widely studied thermodynamic event in bilayer chemistry. Pretransitions, although very interesting, have been recorded in Table II mainly for the sake of thoroughness.

One notable feature of Table II, already alluded to, is that untethered diesters and diethers (as well as tethered diesters and diethers) of equal chain length (or ring size) have similar T_c values. For example, T_c's of the C_{16} untethered diester and diether lipids are 41.6 and 41.7 °C, respectively. Tethered diester and diether lipids with "chain lengths" of 16 carbons (defined hereafter as the number of carbons in the two fatty acids *prior* to their connection) have main transition temperatures of 47.0 and 50.4 °C. Thus, T_c seems rather unresponsive to the type of linkage, ester or ether, which attaches the chains or ring to the glycerol backbone. This is consistent with past data[29] and with the contention that T_c reflects conformational changes among the chains themselves. Below T_c, in the "gel phase", the chains exist as parallel, fully extended chains with few if any gauche conformations. Above T_c, in the "liquid-crystalline phase", lipids assume a more disordered packing with anywhere from 2–6 gauche conformations per chain.[31,32]

A more interesting, and far less predictable, relationship among the T_c data of Table II relates to the comparison between corresponding untethered and tethered lipids. With the exception of the short C_{14} lipids (which show low and poorly understood transition entropies), tethering invariably elevates the T_c values.[33] The longer the chain length, the greater the difference between tethered and untethered. In the C_{20} series, the difference between the two reaches 16 °C, a substantial effect considering that, in gross structural terms, only a single carbon–carbon bond distinguishes them. Furthermore, it is unusual that a structural perturbation *raises* T_c over and above that achieved by linear and saturated fatty acids. By way of comparison,[29] a single *trans* double bond at C(9) of chain 2 in distearoylphosphatidylcholine lowers T_c from 55 to 26 °C; a single *cis* double bond at the same position drops T_c further to +3 °C. A single methyl group on C(16) of chain 2 of distearoylphosphatidylcholine reduces T_c from 55 to 39 °C.[11]

It is tempting to speculate that ring formation in archaebacterial lipids is related, in part, to the need for many of them to maintain membrane integrity at high temperatures.[34] As is evident from lipids **I** and **II**, archaebacteria, in contrast to higher organisms, utilize the isoprenyl pathway to synthesize their lipids. This results in lipids that are methylated on every fifth carbon, a structural feature that would seem to impair lipid packing and reduce thermal stability.[35] Archaebacteria may have countered the stability problem, that chance biosynthetic factors imposed on them, by forming giant rings. One would predict on this basis that "double phospholipids" (see eq 1), bearing a giant ring but no methyl substituents, should manifest an unusual thermal stability with possible practical ramifications,[5,18] and this prediction is now being tested.

Transition enthalpies in Table II show a steady increase with chain length and T_c within both the tethered and untethered families. A positive correlation between ΔH_c and T_c is the usual and expected behavior.[29] But, remarkably, the tethered lipids, despite their high T_c's, are characterized by ΔH_c values that are consistently lower (by about 4 kcal/mol) than their untethered counterparts. It is these low ΔH_c values that gives rise to the *Y*-scale disparity in the heat capacity curves (Figures 1–4). We are thus faced with the dilemma of explaining why tethering causes transition temperatures to increase while, simultaneously, the transition enthalpies to decrease substantially. In order to do so, we must explore the molecular basis of the gel-to-liquid-crystalline transition.

The gel-to-liquid-crystalline transition is, in general, a highly cooperative event. The process resembles a simple melting of an

(29) Marsh, D. *CRC Handbook of Lipid Bilayers*; CRC Press: Boca Raton, FL, 1990; pp 136, 149. Note that the nonexistence of a transition point for macrolide bolaamphiphiles has been already reported: Fuhrhop, J.-H.; David, H.-H.; Mathieu, J.; Liman, U.; Winter, H.-J.; Boekema, E. *J. Am. Chem. Soc.* **1986**, *108*, 1785.

(30) Lipka, G.; Chowdhry, B. Z.; Sturtevant, J. M. *J. Phys. Chem.* **1984**, *88*, 5401.

(31) Seelig, A.; Seelig, J. *Biochemistry* **1974**, *13*, 4839.

(32) Trauble, H.; Haynes, D. H. *Chem. Phys. Lipids* **1971**, *7*, 324.

(33) Reference 10 reports a T_c of 42.6 °C for the tethered C_{16} diester compared to our 47.0 °C (Table II). The source of the discrepancy is not known.

(34) Brock, T. D., Ed. *Thermophiles, General Molecular and Applied Microbiology*; Wiley: New York, 1986.

(35) Lewis, R. N. A. H.; Sykes, B. D.; McElhaney, R. N. *Biochemistry* **1987**, *26*, 4036.

n-alkane in which stable microdomains nucleate the interconversion of one phase into the other.[36] Two major energy components are responsible for energy absorption at the main phase transition: a reduction in van der Waals interactions among the chains and an increase in the number of gauche C–C linkages.[37] As T_c is exceeded, the average number of gauche kinks per molecule increases from 0 to 2–6 (the variation arising from different reports on the subject).[31,32] According to spin-label data,[38] the probability of a gauche conformation markedly increases beyond the midpoint of the chain. During the order-to-disorder transition, a bilayer increases in molecular area by 6 Å^2 while concurrently decreasing its thickness by 20% and increasing its head-group hydration by several water molecules per lipid.[39,40]

Let us now consider (in as much as it is known) the sequence of events as a gel phase transforms into a liquid-crystalline phase. When the temperature is raised on a lipid bilayer, attractive van der Waals interactions among the chains are destroyed, leading to pockets of disorder among the otherwise well-aligned array of molecules. Near the phase-transition temperature, populations of disordered microdomains allow a rapid, cooperative, and precipitous melting of the entire bilayer. This phase transition is accompanied by an expansion of the membrane and an increase in space-filling gauche linkages among the chains. The overall absorption of energy corresponds to the loss of van der Waals attractions plus 0.9 kcal/mol for each new gauche linkage. Other energy terms have been identified, but these are either insignificant or else would likely cancel when comparing tethered and untethered lipids.

If the foregoing is a more-or-less accurate scenario, then one can see why, in general, ΔH_c and T_c are positively correlated for a series of homologous compounds. T_c reflects the number of van der Waals contacts that must be broken to initiate the subsequent "catastrophic" rearrangement of molecules known as a phase transition. ΔH_c reflects (a) the energy content of all broken van der Waals contacts when the gels transforms into a liquid-crystalline phase and (b) the energy of all anti-to-gauche rotamerizations. Distearoylphosphatidylcholine (T_c = 55 °C, ΔH_c = 9.8 kcal/mol) has a higher transition temperature and enthalpy than dimyristoylphosphatidylcholine (T_c = 24 °C, ΔH_c = 5.3 kcal/mol) largely because a longer chain, with its greater number of van der Waals contacts, elevates both T_c and ΔH_c.

The fact that tethered lipids usually have higher T_c values than their corresponding untethered analogs suggests (a) tethering does not substantially impair packing within the gel phase and (b) disordered microdomains, which precede the main phase transition, are more difficult to form in tethered systems. Why should this be? The simplest explanation is that the tethered lipids are blocked from achieving disordered microdomains *via* chain/chain separations within single molecules. Local disorder must arise mainly from destruction of *inter*molecular lipid/lipid contacts. With a major mechanism of disorder not available to the tethered lipids, their T_c increases.

The extraordinarily low ΔH_c values of the tethered lipids could, in principle, originate from either of two effects: (a) tethering could adversely affect the mode of packing of the hydrocarbon chains in the gel state or (b) macrocyclization prevents the formation of 2–6 new gauche bonds characteristic of melting in acyclic lipids.[31,32] We greatly favor the second rationale. First, the T_c data favor (as already mentioned) a nondisrupted packing within the gel state of the tethered lipids. Second, the average difference between ΔH_c for tethered and untethered lipids in Table II (ca. 4 kcal/mol) corresponds to about four gauche linkages per molecule. This is the number of gauche linkages formed when an untethered lipid undergoes a phase transition. It appears, therefore, that macrocyclization has enabled us to segregate the two major components of ΔH_c (*i.e.* van der Waals attraction and C–C bond rotation) by sharply reducing or eliminating the contribution usually made by chain kinking.

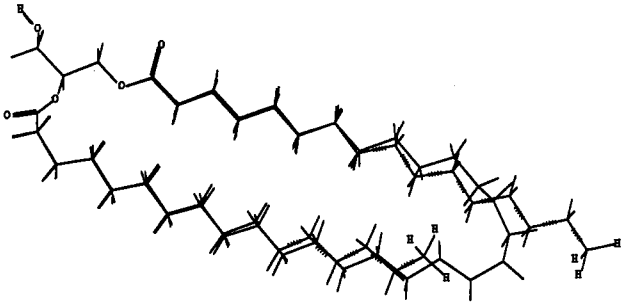


Figure 5. Energy-minimized structure of a tethered distearoyl lipid superimposed upon the X-ray-based structure of the untethered lipid (the latter being indicated by the hashed bonds and terminal methyl groups).

Entropies of transition (Table II) are in concert with the preceding picture. Tethered lipids, structurally unable to achieve internal chain separation (apart from possible buckling of the ring), are invariably associated with lower entropies than their untethered counterparts. In effect, the chains are immobilized in the liquid-crystalline phase, leading to less favorable entropic changes.

In summary, tethering has three effects: (a) it raises the transition temperatures for all but the shortest lipids, (b) it lowers enthalpies of transition by, we presume, reducing the number of gauche C–C bonds created during the melting process, and (c) it lowers entropies of transition by preventing two chains of a single molecule from engaging in independent motion within the liquid-crystalline phase.

A brief molecular mechanics examination of the cyclic lipids supports the picture portrayed above. Figure 5 shows an energy-minimized C_{16} cyclic diester superimposed upon the acyclic diester with the identical number of carbons. The overlap (apart from a "loose" ethyl group of the latter) is good, suggesting that the cyclic lipids would have minimal packing problems in the gel state. Placing gauche linkages at the C(5)–C(6) and C(7)–C(8) bonds of chain 1 of the acyclic lipid costs only 1.3 kcal/mol. The same kink in the cyclic analog costs 5.8 kcal/mol. Creating chain disorder in the cyclic case is, therefore, energetically expensive and probably not an important factor in the gel-to-liquid-crystal transitions of the tethered lipids. As discussed, this fact is critical to the thermotropic properties of the membranes.

We might remark, in conclusion, that lipid chemistry can look forward to further major progress by two obvious routes: (a) new methodologies and (b) new lipids. For those who prefer not to wait for new instrumentation to appear on the scene, there is only one recourse: synthesize new lipids with interesting and informative structural elements. This was the approach taken here and in our previous work.[11] The problem with the approach, of course, is that it involves tedious synthetic work that is not an option for more biologically or physically oriented chemists. It is clear, therefore, that much interesting work in the future will arise from collaborative efforts.

Acknowledgment. This work was supported by the National Institutes of Health and the Dow Chemical Co.

(36) Hauser, H.; Pascher, I.; Pearson, R. H.; Sundell, S. *Biochem. Biophys. Acta* **1981**, *650*, 21.
(37) Nagle, J. F. *J. Chem. Phys.* **1973**, *58*, 252.
(38) Hubbell, W. L.; McConnell, H. M. *J. Am. Chem. Soc.* **1971**, *93*, 314.
(39) Ruocco, M. J.; Shipley, G. G. *Biochem. Biophys. Acta* **1982**, *691*, 309.
(40) Nagle, J. F. *Annu. Rev. Phys. Chem.* **1980**, *31*, 157.