Spanning or Looping? The Order and Conformation of Bipolar Phospholipids in Lipid Membranes Using ^{2}H NMR Spectroscopy

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Abstract: Solid-state ^{2}H NMR spectroscopy was used to study and characterize the conformation and order of bolaform lipid membranes. A series of ^{2}H-labeled bolaform phosphatidylcholines has been synthesized and their properties compared to a [D_4]dimyristoylphosphatidylcholine (DMPC) and a [D_8]-32 macrocyclic phosphatidylcholine. ^{31}P NMR measurements establish that the aqueous dispersions of these lipids adopt lamellar phases. Computational dePaking was used to extract the spectrum of the oriented system from spectra consisting of a superposition of randomly oriented domains in an unoriented sample. A large (> 90 %) and constant value for the normalized segmental order parameter (S_{mol}) was observed for all positions along the diacyl chain of the bolaform lipids and only a small population (< 10 %) of a less ordered conformer was observed. The less ordered conformer is assigned to the looping conformation on the basis of comparison with the deuterated macrocyclic phospholipid which has an enforced loop in its diacyl chain. The predominant population(> 90 %) of the bolaform lipids is assigned to a highly ordered, spanning conformer.

Keywords: conformation analysis · membranes · NMR spectroscopy · phospholipids

Introduction

The bipolar lipids found in archaebacterial membranes impart extreme stability to the membrane, allowing bacteria to withstand either high temperatures (thermophilic bacteria) or extremes of pH (acidophilic and alkaliphilic bacteria).[1] These bipolar lipids, generally termed bolaform lipids,[2] have been extensively studied and exhibit particularly interesting thermal properties and morphologies.[2, 3] The ability of bolaform lipids to preferentially form a bilayer spanning conformation compared to a looping conformation is the origin of much of the special stabilization of the resulting membranes (Figure 1).[4]

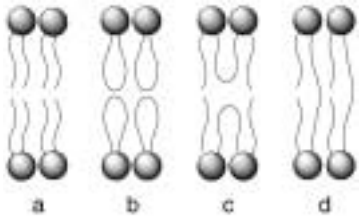

Figure 1. Schematic of membranes formed from: a) a diacyl phospholipid; b) a macrocyclic lipid; c) a looping conformation of a bolaform lipid; and d) a spanning conformation of a bolaform lipid.

Despite the importance of understanding membrane structure in relation to function, the relationships between conformer equilibria and membrane state have been difficult to detail. This difficulty arises because there has been no well-defined protocol available to determine the relative populations of spanning and looping conformers. In fact, it is often tacitly assumed without further study that the spanning conformer is exclusively adopted. We have addressed this problem by preparing a series of bolaform phospholipids and developing a ^{2}H NMR-based protocol which quantifies the spanning:looping ratio in aqueous bilayer dispersions.

Several studies provide evidence that macrocyclic bipolar lipids extracted from archaebacterial membranes preferentially adopt a spanning conformation to form a monolayer membrane.[5–8] In cases where there is not a macrocycle present, a looping conformer can however be prominent. For example, bipolar lipids with a photoactive linker for labeling membrane proteins exhibit a 1:1 spanning:looping

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ratio.[9] ^{2}H NMR studies have shown that a similar bipolar lipid, oriented within dimyristoylphosphatidylcholine multilayers, adopts a 9:1 spanning:looping ratio.[10, 11] A C_{32} bolaform lipid exhibits a 1.5:1 spanning to looping ratio.[3] There is also evidence that some tetraether bolaform amphiphiles favor, but do not exclusively adopt, a spanning conformation.[12–14] Finally, phospholipase A_2 hydrolysis and subsequent product analysis of a *sn*-1-tethered dithiol bolaform phosphatidylcholine established that there is a finite looping population in these vesicles.[15, 16]

The rate of spanning and looping interconversion, and whether the monitoring process reports steady state or dynamic populations is also important. Such an interconversion process has close parallels to the 'flip-flop' process implicated in the transbilayer movement of phospholipids. Hemi-macrocyclic bolaamphiphilic lipids (which do not favor a membrane-spanning conformation) have transbilayer dynamics similar to their related monopolar lipids.[3] On the other hand, bolaform lipids with a chain-stiffening biphenyl unit in the middle of the main chain favor a membrane-spanning conformation and show substantial resistance to transbilayer migration.[17] The complex relationships between chain composition and headgroups clearly influence both the nature of the conformers as well as their kinetics of interconversion.

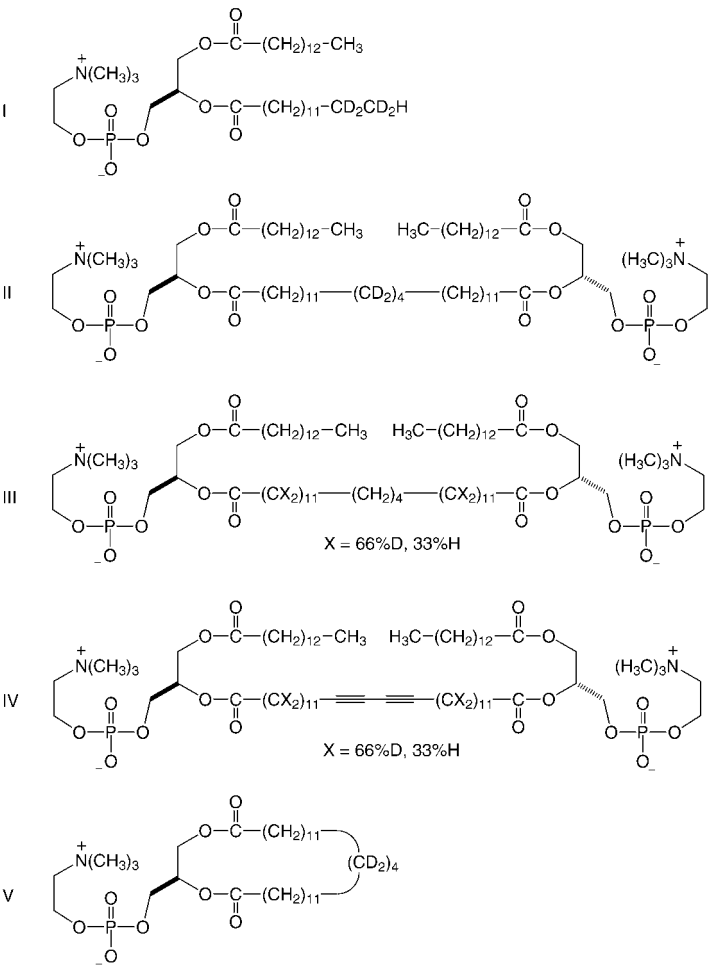
NMR techniques have been extensively used for the determination of lipid organization in membrane assemblies.[18] Order parameter versus chain position profiles derived from ^{2}H NMR data provide a particularly useful overview of the relationships between conformations and dynamics of lipid chains.[19–21] The quadrupolar splitting ($\Delta\nu$) in ^{2}H NMR spectroscopy is sensitive to the motions and orientations experienced by a labeled residue. A powder (or Pake) pattern represents the summation of the doublets generated from all orientations of a particular C–D bond with respect to the magnetic field. Lineshape analysis of solid-state ^{2}H NMR spectra thus allows one to probe acyl chain order in membranes in a noninvasive manner.[22] The process which computationally deconvolutes the signals obtained from non-oriented samples (dePaking) provides high-resolution ^{2}H NMR spectra of aggregated lipids.[23] DePakeing[24] effectively allows one to isolate the doublets which correspond to the 0° orientation (between the bilayer normal and the applied magnetic field) from all the other orientations.[25, 26]

In aqueous dispersions, the quadrupolar splitting is directly related to the C–D bond orientational order parameter S_{CD}. The normalized segmental order parameter S_{mol} is proportional to S_{CD} and is particularly useful when discussing the nature and location of chain segment motions. $S_{mol} = 0$ when there is an equal population of *trans* and *gauche* conformers (isotropic motion caused by a completely disordered state) and $S_{mol} = 1$ when a completely ordered, all-*trans* conformation is adopted by the chains.[8] The order parameter S_{mol} thus gives an average measure of order at a particular position along the labeled acyl chain. In general, the closer a C–D segment is to the headgroup, the more restricted will be its motion and the greater will be the value of the order parameter. The S_{mol} profile for a saturated phopholipid above its T_m (i.e. DMPC at 40 °C) exhibits a plateau ($S_{mol} = 0.5$) in the C2 to C6 region followed by a marked decrease in the order parameter as one moves towards the chain terminus. The small S_{mol} values reflect the progressive increase in the torsional and motional freedom (caused by reduced steric constraints) experienced by the chain past the first several carbon atoms.[27]

Our efforts have been directed towards determining the order characteristics of the spanning and looping states of bolaform lipids. This ^{2}H NMR investigation of saturated bipolar phospholipids was prompted by a study which distinguished between the looping and spanning conformations of saturated (α, ω)-dicarboxylic acids.[28]

Results and Discussion

In order to establish protocols and place our findings in the context of the literature reports of bolaform lipids, ([D_4]-13,13,14,14)-dimyristoylphosphatidylcholine ([D_4]DMPC; **I**) was synthesized and studied. Two of the three bipolar lipids used in this study are essentially two DMPC molecules coupled at the ω- and ω'-positions of the *sn*-2 acyl chains. Their acyl chains are deuterium labeled in either the middle of the linking C28 diacyl chain ([D_8]-13,13,14,14,15,15,16,16; **II**) or partially deuterated (66%) everywhere except in the middle of this chain ([H_8]-13,13,14,14,15,15,16,16; **III**). The



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third partially deuterated (66%) bipolar lipid is a synthetic precursor to **II**, and has a chain stiffening diacetylene moiety in the chain center (**IV**). Because a rigorous spectral assignment requires a 'reference' molecule whose acyl chains are forced to adopt a looping conformation, a ^{2}H-labeled 32-macrocycle analogue ([D_8]-13,13,14,14,15,15,16,16; **V**) of DMPC was synthesized and studied.

To initiate a discussion of lipid conformation, it is important to establish the gross morphology of the entire lipid assembly. ^{31}P NMR spectroscopy is particularly useful in distinguishing between lamellar (L_α) and hexagonal (H_{II}) phases.[29, 30] The negative chemical shift anisotropy ($\Delta\sigma$) observed in the ^{31}P NMR powder patterns of nondeuterated samples of **I**, **II**, and **V** are characteristic of a lamellar membrane morphology (Figure 2).[31] The thermal properties of lipid aggregates are

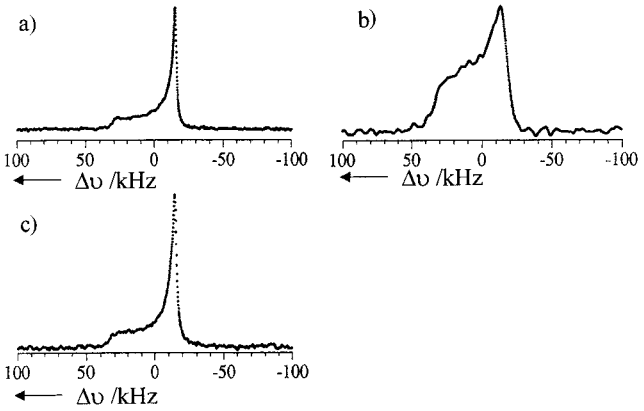

Figure 2. ^{31}P NMR powder patterns of a) **I** (at 40 °C, T_m+16.5 °C); b) **II** (nondeuterated) (at 70 °C, T_m+24.9 °C); and c) **V** (nondeuterated) (at 50 °C; T_m+27.5 °C).

also sensitive to chain packing and aggregate morphology. The main gel-to-liquid crystalline phase transition temperatures (T_m) of aqueous dispersions of **I**, **II**, **IV** (nondeuterated analogue), and **V** are 24.5, 45.1, 24.1, and 22.5 °C, respectively. Neither the [D_4]-substitution in **I** nor linking the *sn*-1 and *sn*-2 acyl chains as a macrocycle, **V**, affects the T_m value, given that T_m = 24.3 °C for DMPC.[32] The bolaform lipid **II**, on the other hand, has a significantly elevated T_m value (45.1 °C) as compared to that of DMPC. This is consistent with other bolaform lipids, where the tethering of the *sn*-2 chains raises the T_m value by 15 to 20 °C relative to the diacyl analogues.[8, 13, 15, 33] An enhancement of both hydrophobic and van der Waals interactions between adjacent acyl chains causes the T_m value to increase. Such an increase in the phase transition temperature suggests (but is not an unequivocal demonstration) that a substantial proportion of the bolaform adopts a spanning conformation. The diacetylene moiety within the bolaform lipid **IV** depresses the T_m by about 20 °C as compared to its saturated analogue **II.** This is as per expectations of the decrease of the T_m value caused by chain unsaturation.

A clear demonstration of the spanning/looping phenomenon in an aggregated state can be directly accessed by using ^{2}H NMR spectroscopy. All ^{2}H spectra were obtained above the T_m value to ensure that each sample is in a similar phase during measurement, that the spectra are well-resolved, and that there is adequate signal-to-noise (Figure 3). The dePaked spectra of aqueous dispersions of **I** and **V** define the appearance of the spectra of disordered chains and their S_{mol} values provide a means to compare the extent of order. The spectra, and thus order parameters, of **I** (S_{mol} = 0.06 and 0.26) and **V** (S_{mol} = 0.19 and 0.32) are quite similar. There are two S_{mol} values associated with each because each has labeling sites which experience a different degree of (dis)order. The small S_{mol} values of **I** for the terminal and penultimate chain positions are consistent with those observed for DMPC.[34] Because the ^{2}H labeling in **V** is located at C13 to C16 of the 32-macrocycle, the C–D bonds are forced to adopt a number of orientations with respect to the applied magnetic field (and membrane normal). The small S_{mol} values for **V** establish that the 'loop' inherent in the macrocycle does indeed force the C–D bonds to adopt a variety of orientations with respect to the bilayer normal. The turn in the macrocycle (Figure 1b) thus serves as the model of the turn in the looping bolaform conformer illustrated in Figure 1c.

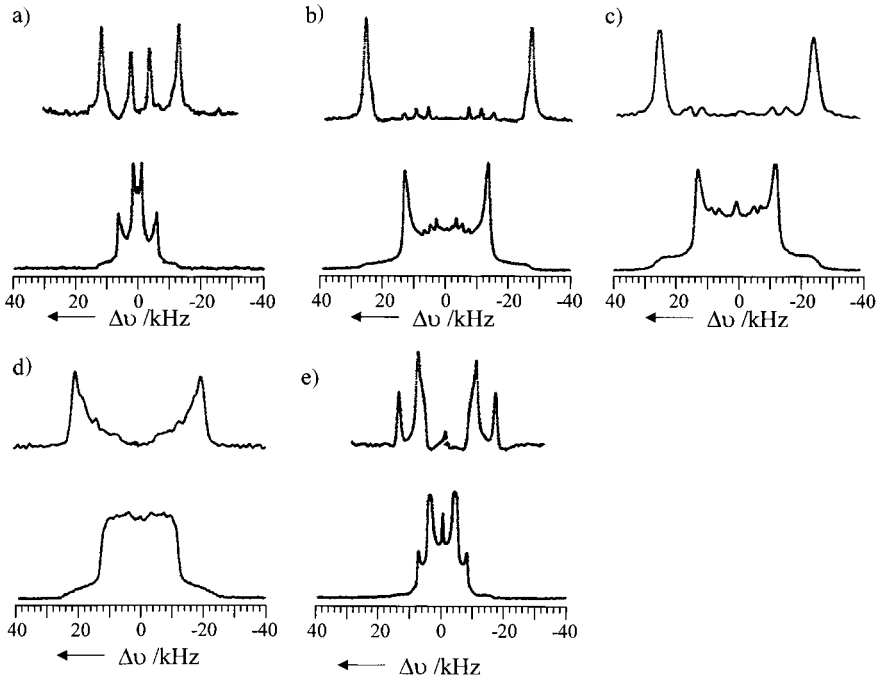

Figure 3. ^{2}H NMR powder patterns (lower) and corresponding dePaked spectra (upper) of unoriented hydrated lipids a) **I** (at 40 °C, T_m+16.5 °C); b) **II** (at 50 °C, T_m+4.9 °C); c) **III** (at 70 °C, ca. T_m+25 °C); d) **IV** (at 60 °C, T_m+35.9 °C); and e) **V** (at 40 °C, T_m+17.5 °C).

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Bolaform lipids **II, III**, and **IV** each have two components in their dePaked ^{2}H spectra. The dominant component has a large and constant S_{mol} value (0.56, 0.54, 0.50, respectively) over the entire length of the diacyl chain. An $S_{mol} = 0.5$ is observed only near the glycerol backbone of the conventional saturated phospholipids.[34] The fact that S_{mol} is invariant is especially noteworthy as it means that all segments of the diacyl chain are motionally equivalent in this conformation. S_{mol} of a spanning conformation is of course expected to be both large and relatively constant along the chain, given that both ends of the molecule are effectively 'pinned' to opposite sides of the membrane. This pinning greatly restricts the number of conformers the acyl chains can adopt at, for example, the chain midpoint (Figure 1d). This large and constant value of S_{mol} is thus a very useful, and general, diagnostic of the spanning conformation of bipolar lipids in a membrane matrix.

The minor component ($\Delta\nu = 17 - 42$ kHz) in the spectra of **II**–**IV** is associated with smaller S_{mol} values (0.13 to 0.33). The distinction between the spanning and looping conformers in **II**, **III**, and **IV** is made clear by using the behavior of **V** as a reference. The small $\Delta\nu$ values (and thus S_{mol}) observed in the spectra of **II**–**IV** and **V** allows assignment of the minor component to the looping conformer (Figure 1c). Of further note is the fact that the spectra of **II**, **III**, and **IV** are very similar although the positions of their labels are different. Although **IV** also has a chain-stiffening diacetylene in the middle of the diacyl chain, it still has a propensity to form a looping conformer. The C28 diacyl chain in **IV** is evidently long enough to accommodate a loop.

With the assignment of the spanning and looping conformers in hand, integration of the respective dePaked spectra provides their relative populations. In this lamellar morphology the great majority (>90%) of the bipolar lipid exists in the highly ordered spanning state and only a minor population (<10%) is associated with the disordered, looping state. There clearly is a natural tendency of the bolaform lipids to aggregate in an extended form which enhances both hydrophobic and van der Waals interactions and leads to the thermal stabilization observed in T_m measurements. The ^{2}H NMR protocol presented here thus provides a definitive link between the structural details of the lipids and the properties of the resulting organized assembly.

The order parameter can also be used to determine physical characteristics of the membrane. For example, it is possible to determine the relationship between the order parameter profile for a lamellar lipid phase and the lamellar hydrophobic thickness. The average hydrophobic thickness of the membrane ($\langle L \rangle$) can be estimated by using the relationship $\langle L \rangle = L_o[(1/2) - \langle S_{CD} \rangle]$, where L_o is the fully extended all-*trans* chain length and $\langle S_{CD} \rangle$ is the average chain order parameter.[40] The hydrophobic thickness of an all-*trans* acyl chain in DPPC is about 20 Å, whereas in the liquid-crystalline phase (>41 °C) it is about 14 Å. Therefore the *bilayer* hydrophobic thickness is expected to decrease by 12 Å on going from the gel state to the liquid-crystalline state.[41] Similarly for DMPC, the gel state all-*trans* bilayer hydrophobic thickness is about 35 Å and in the liquid-crystalline state it is expected to be about 24 Å.[42] With an order parameter of about 0.5 along the entire length of the hydrocarbon chain, the more ordered bolaform phospholipids (**II** or **III**) have a hydrophobic thickness of about 27 Å in the liquid-crystalline state. There is an increase of about 3 Å from a liquid-crystalline DMPC bilayer to a more ordered spanning conformation of **II** and **III** (which are, in essence, DMPC dimers). Interestingly, the bolaform lipid membrane thickness corresponds to the hydrophobic bilayer thickness of DPPC (ca. 28 Å) in the liquid-crystalline state. This is consistent with the observed correspondence between the thermal properties of **II** (T_m = 45.1 °C) and DPPC (T_m = 41.5 °C)[32] rather than DMPC (T_m = 24.3 °C).

These observations have important implications in the structure and function of archaebacterial membranes, their associated membrane proteins, and the manner in which phospholipids are accessible to membrane-associating enzymes such as phospholipase A_2. We are currently exploring how the kinetics and thermodynamics of the spanning/looping interconversion can be modulated by conditions and reagents. The ^{2}H NMR experiment will figure prominently in these studies.

Experimental Section

Materials: 1-Myristoyl-2-hydroxy-*sn*-glycero-3-phosphatidylcholine was obtained from Avanti Polar Lipids (Alabaster, AL). 3-Tetradecyn-l-ol was purchased from Farchan Laboratories (Gainesville, FL). D_2O and deuterium gas (99.99%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Wilkinson's catalyst (tris(triphenylphosphane)rhodium(I) chloride), 1,3-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), 1-hydroxybenzotriazole (HOBt), and deuterium-depleted water were obtained from Aldrich (Milwaukee, WI). 1,3-Diaminopropane (ca. 90% N-deuterated) was prepared according to a literature procedure.[39] The synthesis of 13-tetradecyn-1-oic acid, 13,15-octacosdiyn-1,28-dioic acid, and lipids **I**–**V** was based on a previous report on the synthesis of unusual macrocyclic and bolaform phosphatidylcholines.[35] Deuterium-labeled lipids were prepared by homogeneous catalytic deuteration with deuterium gas using Wilkinson's catalyst.[36] Heterogeneous catalysis proved to be an unsatisfactory method for the deuteration of straight-chain olefins due to double bond migration and exchange reactions that result in unspecific labelling.[37] Homogeneous catalysis for the preparation of specifically labelled fatty acids and lipids was based on a modified procedure of Chapman and Quinn.[38] Preparation of perdeuterated fatty acids relied on the deuteration of alkynols by isomerization of triple bonds using N-deuterated 1,3-diaminopropane as outlined by Abrams.[39] The degree of deuteration in the perdeuterated fatty alcohols and fatty acids was estimated by comparing proton integration values. The percent deuteration in the perdeuterated lipids **III** and **IV** was estimated using FAB mass spectrometry.

Myristic acid ([D_4]-13,13,14,14): 13-Tetradecyn-1-oic acid (220 mg, 0.98 mmol) was dissolved in freshly distilled, argon-saturated THF (7 mL) in a pressure reactor. Wilkinson's catalyst (100 mg, 0.11 mmol) was added and the reactor was evacuated and flushed with argon five times. The reactor was then flushed with deuterium gas and filled to a pressure of 1 psi. The mixture was stirred for 12 h at 35–40 °C. The solvent was then removed under vacuum and the residue was redissolved in hexanes. The resulting solution was filtered through several plugs of Celite and activated charcoal in order to remove all traces of the orange catalyst. The solvent was removed under vacuum to yield myristic acid ([D_4]-13,13,14,14) (60 mg, 0.26 mmol; 26%). ^{1}H NMR (499.84 MHz, $CDCl_3$): $\delta = 2.34$ (t, 2H; $HOOCC\underline{H}_2$), 1.63 (m, 2H; $HOOCCH_2C\underline{H}_2$), 1.26 (m, 18H; $C\underline{H}_2$), 1.21 (s, 1H; $CD_2\underline{H}$).

2-[Myristoyl ([D_4]-13,13,14,14)]-1-myristoyl-*sn*-glycero-3-phosphatidylcholine) (I): Myristic acid ([D_4]-13,13,14,14) (68 mg, 0.29 mmol), 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphatidylcholine (187 mg, 0.40 mmol),

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DCC (72 mg, 0.35 mmol), DMAP (92 mg, 0.75 mmol), and 1-hydroxybenzotriazole (101 mg, 0.75 mmol) were dissolved in dry methanol-free chloroform (15 mL) and refluxed under dry argon for 2 h. Additional DCC (72 mg, 0.35 mmoles) was added and the reaction was allowed to reflux an additional 20 hours. The solvent was removed under vacuum and the residue was chromatographed on silica (eluent: $CHCl_3$/MeOH/H_2O (70:26:4)). The purification yielded 100 mg (0.15 mmol) of **I** (52% with respect to the fatty acid starting material). R_f (silica, $CHCl_3$/MeOH/$H_2$0 (70:26:4)): 0.5; ^{1}H NMR (499.84 MHz, $CDCl_3$): $\delta = 5.18$ (m, 1H; C_2H), 4.38 (br d 5.21 1H; 1/2C_1H_2), 4.32 (br s 2H; CH_2OP), 4.12 (br dd, 1H; 1/2C_1H_2), 3.95 (br s, 2H; C_3H_2), 3.80 (br s, 2H; CH_2N^+), 3.34 (s, 9H; $(CH_3)_3N^+$), 2.30 (m, 4H; C_1OCOCH_2, C_2OCOCH_2), 1.58 (m, 4H; $C_1OCOCH_2CH_2$, $C_2OCOCH_2CH_2$), 1.25 (s, 38H; CH_2), 0.88 (t, 3H; CH_3), 0.82 (br s, 1H; CD_2H). ^{2}H NMR (76.73 MHz, $CDCl_3$): $\delta = 1.24$ (br s 2D; CD_2), 0.84 (br s 2D; CD_2H); ^{31}P NMR (81.0 MHz, $CDCl_3$:MeOD, 2:1): $\delta = -0.39$; FAB-MS (nitrobenzyl alcohol (NBA): *m/z*: 704 $[M+Na]^+$, 682 $[MH]^+$, 499 $[M - C_5H_{13}NO_4P]^+$.

Octacosa-1,28-dioic acid ([D_8]-13,13,14,14,15,15,16,16): 13,15-Octacosadiyn-1,28-dioic acid (280 mg, 0.63 mmol) was dissolved in freshly distilled, argon-saturated THF (7 mL). Wilkinson's catalyst (100 mg, 0.11 mmol) was added and the flask was flushed with deuterium gas and fitted with a balloon of deuterium gas. The mixture was stirred for 36 h at 35 °C. The resulting solution was filtered through a plug of Celite and activated charcoal. The solvent was then removed under vacuum and the residual catalyst was washed from the product with chloroform and acetone to yield octacosa-1,28-dioic acid ([D_8]-13,13,14,14,15,15,16,16) (115 mg, 0.25 mmol; 40%). ^{1}H NMR (499.84 MHz, $CDCl_3$/MeOD (2:1)): $\delta = 2.11$ (t, 4H; HOOCCH_2), 1.43 (m, 4H; HOOCCH_2CH_2), 1.50 (m, 36H: CH_2).

2,2′-(Octacosa-1,28-dioyl [D_8]-13,13,14,14,15,15,16,16-)bis(1-myristoyl-*sn*-glycero-3-phosphatidylcholine) (II): 1-Myristoyl-2-hydroxy-*sn*-glycero-3-phosphatidylcholine (347 mg, 0.74 mmol), octacosa-1,28-dioic acid ([D_8]-13,13,14,14,15,15,16,16) (115 mg, 0.25 mmol), DCC (57 mg, 0.28 mmol), DMAP (73.5, mg 0.60 mmol), and HOBt (81 mg, 0.60 mmol) were dissolved in dry methanol-free chloroform (15 mL) and refluxed under dry argon for 2 h. Additional DCC (57 mg, 0.28 mmol) was added and the reaction was refluxed for an additional 20 h. The solvent was removed under vacuum and the residue was chromatographed on silica (eluent: $CHCl_3$/MeOH/H_2O (40:50:10)). The purification yielded **II** (106 mg, 0.078 mmol, 31% with respect to fatty acid starting material). R_f (silica, $CHCl_3$/MeOH/$H_2$0 (70:26:4)): 0.1; ^{1}H NMR (499.84 MHz, $CDCl_3$/MeOD (2:1)): $\delta = 5.20$ (m, 2H; C_2H), 4.4 (br d, $J = 5.21$ Hz, 2H; 1/2C_1H_2), 4.32 (br s 4H; CH_2OP), 4.12 (br dd, 2H; 1/2C_1H_2), 3.95 (br s, 4H; C_3H_2), 3.80 (br s, 4H; CH_2N^+), 3.34 (s, 18H; $(CH_3)_3N^+$), 2.28 (m, 8H; C_1OCOCH_2, C_2OCOCH_2), 1.36 (m, 8H; $C_1OCOCH_2CH_2$, $C_2OCOCH_2CH_2$), 1.03 (s, 76H; CH_2), 0.64 (t, 6H; CH_3); ^{2}H NMR (76.73 MHz, $CDCl_3$/MeOD (2:1)): $\delta = 1.21$ (br s, 8D; CD_2); ^{31}P NMR (202.34 MHz, $CDCl_3$:MeOD (2:1)): $\delta = 0.72$ FAB-MS (NBA): *m/z*: 1384 $[M+Na]^+$, 1362 $[MH]^+$, 1179 $[M - C_5H_{13}NO_4P]^+$.

Partially perdeuterated 13-tetradecyn-l-ol: 1,3-Diaminopropane (ca. 90% N-deuterated, 25 mL) and lithium (720 mg, 103 mmol) were heated at 70–80 °C until the blue color discharged (6 h). The mixture was allowed to cool and potassium *tert*-butoxide (7.3 g, 66.4 mmol) was added. After 20 min, a solution of 3-tetradecyn-1-ol (4 g, 19.0 mmol) in *N*-deuterated 1,3-diaminopropane (10 mL) was added dropwise. The solution was stirred for 2.5 h and quenched in cool acidic water. The residue was extracted with hexanes (3 × 200 mL) and the combined organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried and evaporated. The crude product was chromatographed on silica (eluent: CH_2Cl_2 followed by hexane/EtOAc (8:2)) and recrystallized from hexanes at −18 °C to yield deuterated 13-tetradecyn-l-ol (2.9 g). ^{1}H NMR (499.84 MHz, $CDCl_3$): $\delta = 3.62$ (s, 2H; CH_2OH), 2.16 (br s, 0.7H; C≡CCH_2) 1.92 (s, 0.6H; XC≡C) 1.6–1.4 (m, 3H; C≡CCX$_2$CX_2, CX_2CX$_2$OH), 1.4–1.2 (m, 5H; CH_2); ^{2}H NMR (76.73 MHz, $CDCl_3$): $\delta = 2.18$ (br s, 1D; C≡CCX$_2$), 1.6–1.4 (m, 3D; C≡CCX$_2$CX_2, CX_2CX$_2$OH), 1.4–1.2 (m, 12D; CX$_2$). Approximately 60% deuteration in positions C2 to C12 based on ^{1}H NMR integration.

Partially pereuterated 13-tetradecyn-l-oic acid: Deuterated 13-tetradecyn-l-oic acid was prepared from the reaction of deuterated 13-tetradecyn-1-ol with Jones' reagent as reported previously.[35] ^{1}H NMR (499.84 MHz, $CDCl_3$): $\delta = 2.31$ (d, 0.5H; CX_2COOH), 2.15 (m, 0.8H; C≡CCX$_2$) 1.92 (s, 0.6H; XC≡C) 1.7–1.4 (m, 1.2H; C≡CCX$_2$CX_2, CX_2CX$_2$COOH), 1.4–1.1 (m, 4.3H; CX$_2$). Approximately 68% deuteration in positions C2 to C12 based on a comparison of the ^{1}H NMR integration with that of the starting alcohol.

Partially peuterated 13,15-octacosadiyn-1,28-dioic acid: The previously reported procedure for the preparation of 13,15-octacosadiyn-1,28-dioic acid was followed by using partially deuterated 13-tetradecyn-l-oic acid as starting material.[35] ^{1}H NMR (499.84 MHz, $CDCl_3$/MeOD (2:1)): $\delta = 2.08$ (br d, 1H; CX_2COOH), 2.02 (br d, 1.5H; C≡CX$_2$) 1.5–1.2 (m, 2H; C≡CCX$_2$CX_2, CX_2CX$_2$COOH), 1.2–0.9 (m, 7.5H; CX$_2$); ^{2}H NMR (76.73 MHz, $CDCl_3$/MeOD (2:1)): $\delta = 2.3$ (br s, 1D; C≡CCX$_2$, CX_2COOH), 1.45–1.7 (m, 1.5D; C≡CCX$_2$CX_2, CX_2CX$_2$COOH), 1.2–1.4 (m, 5D; CX_2). Approximately 72% deuteration in positions C2 to C12 based on a comparison of the ^{1}H NMR integration with that of the starting bis-fatty acid.

Partially peuterated octacosa-1,28-dioic acid ([H_8]-13,13,14,14,15,15,16,16): Deuterated octacosa-1,28-dioic acid ([H_8]-13,13,14,14,15,15,16,16) was prepared by using the same procedure as in the preparation of octacosa-1,28-dioic acid ([D_8]-13,13,14,14,15,15,16,16) described above except that H_2 gas was used. ^{1}H NMR (499.84 MHz, $CDCl_3$/MeOD (2:1)): $\delta = 2.09$ (br d, 1H; HOOCCX_2), 1.40 (m, 1H; HOOCCX$_2$CX_2), 1.08 (s, 21H; CX$_2$); ^{2}H NMR (76.73 MHz, $CDCl_3$/MeOD 2:1): $\delta = 2.1$ (br s, 1D; HOOCCX_2), 1.4 (br s, 1D; HOOCCX$_2$CX_2),1.05 (m, 10D; CX$_2$). Approximately 66% deuteration in positions C2 to C12 based on a comparison of the ^{1}H NMR integration with that of the starting bis-fatty acid.

2,2′-[Partially peuterated octacosa-1,28-dioyl ([H_8]-13,13,14,14,15,15,16,16)]-bis(1-myristoyl-*sn*-glycero-3-phosphatidylcholine) (III): Compound **III** was prepared with deuterated octacosa-1,28-dioic acid ([H_8]-13,13,14,14,15,15,16,16) using the same procedure as described above for the synthesis of **II**. R_f (silica, $CHCl_3$/MeOH/$H_2$0 70:26:4): 0.1; ^{1}H NMR (499.84 MHz, $CDCl_3$/MeOD 2:1): $\delta = 5.04$ (m, 2H; C_2H), 4.21 (br d, $J = 5.21$ Hz, 2H; 1/2C_1H_2), 4.05 (br s 4H; CH_2OP), 3.96 (br dd, 2H; 1/2C_1H_2), 3.81 (br s, 4H; C_3H_2), 3.40 (br s, 4H; CH_2N^+), 3.02 (s, 18H; $(CH_3)_3N^+$), 2.11 (m, 5H; C_1OCOCX_2, C_2OCOCX_2), 1.40 (m, 5H; $C_1OCOCX_2CX_2$, $C_2OCOCX_2CX_2$), 1.06 (s, 55H; CX_2), 0.68 (t, 6H; CX_3); ^{2}H NMR (76.73 MHz, $CDCl_3$/MeOD 2:1): $\delta = 2.12$ (br s, 1D; OOCCX$_2$) 1.41 (br s, 1D; OOCCX$_2$CX_2)) 1.08 (br s, 10D; CD_2) ^{31}P NMR (202.34 MHz, $CDCl_3$): $\delta = -0.63$; FAB-MS (NBA): *m/z*: $[MH]^+$ 1383 (range 1382–1389). Approximately 66% deuteration in positions C2 to C12 given that the molecular weight for nondeuterated **III** is 1354 g mol^{-1} and that the calculated molecular weight for 100% deuteration in the spanning *sn*-2 chain is 1398 g mol^{-1}.

2,2′-(Partially deuterated 13,15-octacosa-1,28-dioyl)bis(1-myristoyl-*sn*-glycero-3-phosphatidylcholine) bolaform phosphatidylcholine (IV): Compound **IV** was prepared with partially deuterated 13,15-octacosdiyn-1,28-dioic acid by using the same procedure as described above for the synthesis of **II**. TLC ($CHCl_3$/MeOH/H_2O 70:26:4): R_f 0.08; ^{1}H NMR (499.84 MHz, $CDCl_3$/MeOD 2:1): $\delta = 4.94$ (m, 2H; C_2H), 4.12 (br d, $J = 5.21$ Hz, 2H; 1/2C_1H_2), 3.96 (br s 4H; CH_2OP), 3.87 (br dd, 2H; 1/2C_1H_2), 3.70 (br t, 4H; C_3H_2), 3.32 (br s, 4H; CH_2N^+), 2.93 (s, 18H; $(CH_3)_3N^+$), 2.02 (m, 5H; C_1OCOCH_2, C_2OCOCH_2), 1.93 (t, 2H; CH_2C≡C) 1.30 (m, 5H; $C_1OCOCH_2CH_2$, $C_2OCOCX_2CH_2$), 1.18 (m, 2H; CH_2CX$_2$C≡C), 0.97 (s, 60H; CH_2), 0.59 (t, 6H; CH_3); ^{2}H NMR (76.73 MHz, $CDCl_3$): $\delta = 3.1$ (br s, 1D; C≡CCD_2, CD_2COOH), 2.0 (m, 1.6D; C≡CCX$_2$CD_2, CD_2CX$_2$COOH), 0.8–1.8 (m, 10D; CD_2); ^{31}P NMR (202.34 MHz, CDC1$_3$): $\delta = -0.26$; FAB-MS (NBA): *m/z*: $[MH]^+$ 1375 (range 1372–1386). Approximately 66% deuteration in positions C2 to C12 given that the molecular weight for nondeuterated **V** is 1345 g mol^{-1} and that the calculated molecular weight for 100% deuteration in the spanning *sn*-2 chain is 1390 g mol^{-1}.

1,2-(Octacosa-1,28-dioyl ([D_8]-13,13,14,14,15,15,16,16))-*sn*-glycero-3-phosphatidylcholine (V): 1,2-(13,15-Octacosadiyne-1,28-dioyl)-*sn*-glycero-3-phosphatidylcholine (20 mg, 0.030 mmol) was dissolved in freshly distilled, argon-saturated THF (7 mL) in a pressure reactor. Wilkinson's catalyst (20 mg, 0.02 mmol) was added and the reactor was evacuated and flushed with argon five times. The reactor was then flushed with deuterium gas and filled to a pressure of 10 psi. The mixture was stirred for 24 h at 35–40 °C. The solvent was then removed under vacuum and the residue purified by chromatography on silica ($CHCl_3$/MeOH/$H_2$0 70:26:4) to yield **V** (15 mg, 0.022 mmol; 73%). R_f (silica, $CHCl_3$/MeOH/$H_2$0 70:26:4): 0.4; ^{1}H NMR (499.84 MHz, $CDCl_3$): $\delta = 5.20$ (m, 1H; C_2H), 4.4 (br d, $J = 5.21$ Hz, 1H; 1/2C_1H_2), 4.32 (br s 2H; CH_2OP), 4.12 (br dd, 1H; 1/2C_1H_2), 3.95 (br s, 2H; C_3H_2), 3.80 (br s, 2H; CH_2N^+), 3.34 (s, 9H; $(CH_3)_3N^+$), 2.28 (m, 4H;

C_1OCOCH_2, C_2OCOCH_2), 1.58 (m, 4H; $C_1OCOCH_2CH_2$, $C_2OCOCH_2CH_2$), 1.25 (s, 36H; CH_2); ^{2}H NMR (76.73 MHz, $CDCl_3$): $\delta =$ 1.12 (brs; CD_2); ^{31}P NMR (202.34 MHz, $CDCl_3$:MeOD; 2:1): $\delta = -0.23$ FAB-MS (NBA): m/z: 706 $[M+Na]^+$, 684 $[MH]^+$, 501 $[M - C_5H_{13}NO_4P]^+$.

Differential scanning calorimetry (DSC): A Model MC-I Microcal Inc. (Amherst, MA) calorimeter was used for differential scanning calorimetry of lipids **I**, **II**, and **V**. Small amounts of lipid (4–8 mg) were suspended in Milli-Q water (1 mL; 18 MΩ) by heating at least 10 °C above the T_m of the gel to liquid-crystalline phase transition (to fully hydrate the lipid) and vortexing. The samples were allowed to cool slowly to room temperature in a water bath before being placed in a refrigerator (5 °C) overnight. The samples were degassed and 0.70 mL was injected into the liquid sample cell of the calorimeter and an equivalent amount of water was added to the reference cell. Scan rates of 12 °C h^{-1} (**I** and **V**) and 10 °C h^{-1} (**II**) were used. The phase transition temperature of the nondeuterated analogue of **IV** was 21.4 °C measured with a Perkin Elmer DSC7 calorimeter using Seiko liquid sample pans (1.4 mg lipid in 10 μL water with 10μL water in the reference pan; scan rate: 60 °C h^{-1}). The lipid sample was cycled below and above the phase transition temperature before recording the thermogram.

^{31}P NMR spectroscopy: The samples for ^{31}P NMR spectroscopy were prepared by mixing the nondeuterated analogues of **I**, **II**, and **V** with water (33 wt% lipid) and vortexing the slurry above the phase transition temperature of the lipid. Spectra were obtained for **I** (nondeuterated) at 40 °C, **II** (nondeuterated) at 70 °C, and **V** (nondeuterated) at 50 °C, using a Chemagnetics CMX-300 spectrometer operating at 121.279 MHz with proton decoupling. The 90° pulse width was 5 μs and 3×10^2 to 100×10^2 scans were acquired. The decoupler power was 50 kHz and the interpulse delays were 0.55 (**I**), 1.05 (**III**), and 1.05 (**V**).

^{2}H NMR spectroscopy: Samples of **I**–**V** for ^{2}H NMR spectroscopy were prepared by mixing the lipid with deuterium-depleted water (20 to 50 wt% lipid) and vortexing the slurry above the phase transition temperature of the lipid. The temperatures at which the spectra were obtained for **I** (40 °C), **II** (70 °C), **III** (70 °C), **IV** (50 °C), **V** (50 °C) and were all above the T_m values measured. ^{2}H NMR spectra were obtained by using a Chemagnetics CMX-300 spectrometer operating at 46.045 MHz using the quadrupole echo sequence with a recycle delay of 200 msec, 90° pulse width of 4 μs, and an inter-pulse spacing of 40 μs. The number of scans ranged from 11×10^3 to 150×10^3. A small amount of an isotropic phase is present in some of the spectra obtained. The slight asymmetry in some of the recorded ^{2}H NMR spectra may be attributable to temperature and field gradients across the samples. All the calculations presented were performed using an interactive dePakeing program developed by Sternin, Bloom, and MacKay.[24] For this work, a modified version of the program for use with a 486 PC was used. The method involves iteratively solving an equation representing the powder pattern spectrum as an integral of the normalized oriented lineshape. Powder pattern spectra containing 4096 data points were dePaked using ten iterations. This program is currently available through the internet at http://www.physics.brocku.ca/faculty/sternin/depake/.
The normalized segmental order parameter S_{mol} was calculated using the relationships $S_{mol} = -2S_{CD}$ for methylene groups and $S_{mol} = -6S_{CD}$ for a methyl group. S_{CD} is defined as the C–D bond orientational order parameter and is determined as per literature.

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