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Design and Synthesis of Asymmetric Acyclic Phospholipid Bolaamphiphiles

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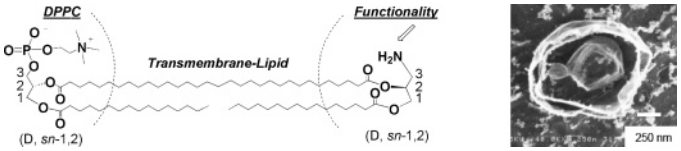
Received October 16, 2004



A synthetic route was devised for the generation of asymmetric lipid bolaamphiphiles through the sequential esterification of an alkyldioic acid, bearing distinct terminal protecting groups, with propanylamine and *lyso*-phosphatidylcholine headgroups. Bolaamphiphile self-assembly was investigated in solvent mixes of varying polarity by nuclear magnetic resonance (NMR) and Fourier transform-infrared (FT-IR) spectroscopy, as well as in water by cryo-high-resolution scanning electron microscopy (cryo-HRSEM). We anticipate that asymmetric lipid bolaamphiphiles will provide facile building blocks for engineering a variety of unique membrane-mimetic structures.

Introduction

The fabrication of supported bilayer lipid membranes (sBLMs) that mimic cell surfaces have attracted considerable attention due to their potential application as tools to probe cellular and molecular interactions and as bioactive coatings for biosensor or medical implant applications.[1,2] In most studies, phospholipids differing in chemical composition, degree of saturation, and size have been utilized as the primary building blocks of lipid membrane-based structures because of their capacity to self-assemble into lamellar systems of high packing density that exhibit limited nonspecific protein adsorption.[3−6] However, since the major driving forces for the assembly of lipid membranes or membrane mimics on solid supports are relatively weak hydrophobic interactions, film instability remains a major obstacle for many long-term applications of this technology. Prior studies from our group and others have demonstrated that the polymerization of a planar lipid assembly provides a feasible strategy for enhancing membrane stability.[7−17] Nonetheless, a limitation of polymerization approaches includes the potential of the initiating species or growing polymer chain to inactivate or otherwise alter membrane-

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10.1021/jo048167o CCC: $30.25
Published on Web 02/23/2005

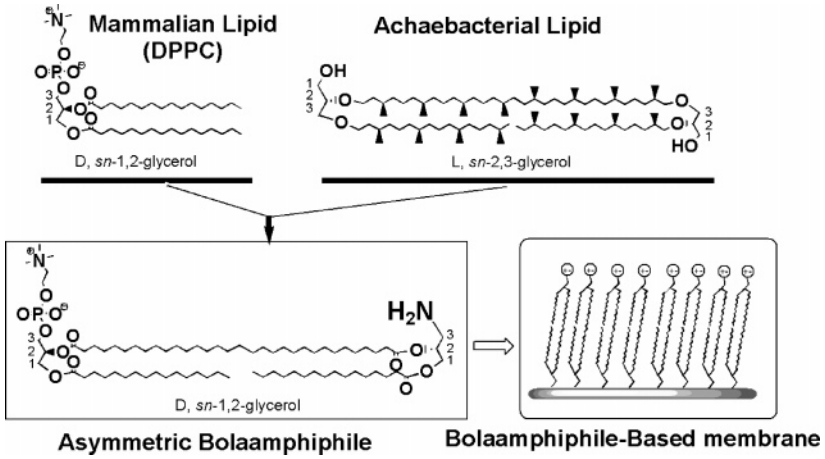

FIGURE 1. Design of phospholipid-based asymmetric bolaamphiphile.

associated bioactive proteins or carbohydrates. Further, the cost of an increase in membrane stability is a concomitant loss of local molecular mobility. That is, the creation of surface composed of either multiple lipid-based polymer chains or a cross-linked networks of chains restricts the movement of nonlipid components within the film.

Archaebacterial lipids are naturally occurring double-chain lipid bolaamphiphiles, which are members of a family of branched hydrocarbons that contain isoprenoid phytanol groups connected to glycerol backbones via an ether linkage. These lipid constituents allow archaebacteria and related synthetic systems to maintain membrane integrity at high temperature (~90 °C) and low pH (1−1.5).[18,19] Although several structural features of archaebacterial lipids are responsible for enhanced membrane stability, the principle of tail-to-tail lipid coupling has been recognized as a critical attribute.[20] As a result of these observations, we have postulated that membrane-spanning phospholipids bolaamphiphiles, either alone or as a constituent of a multicomponent lipid membrane would provide a starting point for generating membrane-mimetic films that exhibit long-term stability while facilitating lateral mobility of membrane associated guest species (Figure 1).

As an initial step toward this goal, we designed asymmetric bolaamphiphiles that contain ester linked phosphatidylcholine and amine functionalities at opposite chain ends. It is noteworthy that while a large variety of single- or double-chain lipid-based bolaamphiphiles have been synthesized, nearly all double-chain bolaamphiphiles are symmetric compounds with identical headgroups that are coupled to a lipophilic core through ether linkages.[21−31] Significantly, we believe that the presence of the amine group provides a convenient means for introducing a probe group or surface linker; the latter facilitating the formation of a substrate bound assembly of bolaamphiphiles. In this paper, we describe the synthesis of a new class of asymmetric phospholipid bolaamphiphiles and initial investigations of the self-assembly of these compounds in both organic and aqueous media.

Results and Discussion

A scheme was devised to synthesize asymmetric dialkyl bolaamphiphiles based upon the sequential esterification of an alkyldioic acid with propanylamine and *lyso*-phosphatidylcholine (PC) headgroups. The initial synthesis of an alkyl diester bearing unique terminal protecting groups provided a key intermediate for this strategy (Scheme 1).

The asymmetrically protected C32-diester **8a** was synthesized by C−C bond coupling of hexadecanoic acid to an ortho-protected iodoester (Scheme 2). Briefly, transformation of lactone **1a** using trimethylsilyl chloride and sodium iodide afforded 16-iodo acid **2a**, which was further converted to a benzyl protected iodo ester **3a** and an ortho-protected iodo ester **6a**. The benzyl ester can be cleaved either by using basic conditions or catalytic hydrogenation, while the ortho ester can be cleaved under acidic conditions. In this regard, the benzyl-protected iodo ester **3a** was oxidized to the aldehyde **4a** and the ortho-protected iodo ester **6** was converted to the phosphonium salt **7a**. The Wittig reaction of aldehyde **4a** and phosphonium salt **7a** was then performed to provide dotriacontan-16-ene dioic acid ester **8a** bearing distinct termi-

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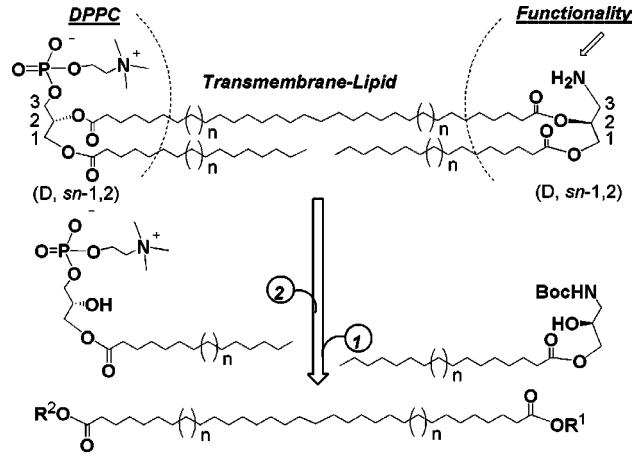
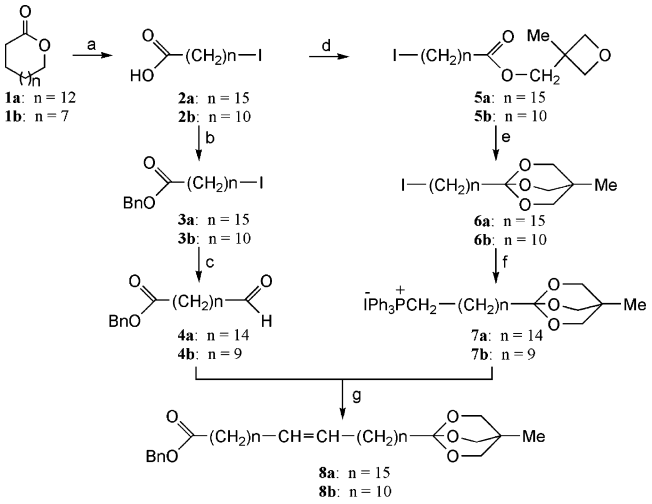
SCHEME 1. Retrosynthetic Analysis of Phospholipid-Based Bolaamphiphile



SCHEME 2. Synthesis of 32/22-Ene Dioic Acid Derivative 8[a]



[a] Reagents and conditions: (a) TMSCl, NaI/CH_3CN, reflux (**2a**: 96%, **2b**: 92%); (b) BnOH, DCC/Ch_2Cl_2 (**3a**: quant, **3b**: quant); (c) DMSO, $NaHCO_3$, 150 °C (**4a**: 72%, **4b**: 60%); (d) 3-methyl-3-oxetanemethanol, DCC/CH_2Cl_2 (**5a**: 91%, **5b**: 80%); (e) $BF_2{\cdot}Et_2O/CH_2Cl_2$, −15 to 0 °C (**6a**: 68%, **6b**: 60%); (f) Ph_3P/benzene−toluene, reflux (**7a**: quant, **7b**: 73%); (g) *n*-BuLi, THF, −78 °C (**8a**: 52%, **8b**: 61%).

nal protecting groups. C22-Diester **8b** was synthesized in a similar manner from lactone **1b** in good yield.

In performing the Wittig reaction, sodium methoxide was initially investigated as a base.[21] However, the desired product was not observed despite increasing base concentration (1.2−6.0 equiv), reaction temperature, or time. As a consequence, the effectiveness of *n*-butyllithium was evaluated in accord with prior reports.[32] The desired compound **8a** was obtained in 52% yield after detailed optimization of the reaction conditions (Table 1). ^{1}H NMR spectrum of **8a** demonstrated that the coupling constant of the two olefin protons was 9.2 Hz, which was consistent with a *cis*-isomer of the C=C double bond.

In evaluating optimal conditions for selective deprotection of **8a/8b**, it was noted that removal of the benzyl group by either catalytic hydrogenation or via use of potassium hydroxide led to the unintended decomposition of the ortho ester group (Scheme 3). However, selective deprotection of the ortho ester group could be achieved by adding pyridinium *p*-toluenesulfonate to a solution of **8a** in methanol followed by treatment with 1 equiv of lithium hydroxide to give **10a/b**.

The propanylamine intermediate **17a/17b** was synthesized from 1,2-dihydroxypropanylamine (**15**) by initial Boc protection of the amino group, followed by acylation of the primary hydroxyl group using 1 equiv of palmitic acid (Scheme 4). A trace byproduct of 1,2-diacylated propanylamine was noted. DCC-catalyzed esterification of compound **10a** with **17a** produced the *semi*-bolaamphiphile **18a** in 51% yield. Catalytic hydrogenation of **18a** afforded the carboxylic acid **19a** that was subsequently coupled with *lyso*-phosphatidylcholine to generate the Boc-protected asymmetric bolaamphiphile **20a** in 22% yield. Removal of the Boc group was performed quantitatively with trifluoroacetic acid to yield the desired compound **21a**. In a similar manner, a smaller bolaamphaphile **21b** was also synthesized from **10b**. All intermediates and the final compounds were characterized by ^{1}H NMR and mass spectroscopy. Of note, the presence of two distinctive multiplet peaks at 5.10 and 5.20 ppm in the spectra of bolaamphiphiles **20a** and **21a** indicate successful esterification of the C-2 hydroxyl groups of the two glycerol backbones.[33] In sonicated dispersions of **21a** and **21b** in pure water, endothermic transitions were observed at 82.8 and 55.5 °C, respectively, which are consistent with relative differences in bolaamphiphile size.

Solvent-Mediated Behavior of an Asymmetric Bolaamphiphile. ^{1}H NMR spectroscopy provided insight into the influence of solvent polarity on conformation and self-assembly of phospholipid bolaamphiphile **21a**. Characteristically, the motion of polar groups increases in solvents of increasing polarity, which is represented by relative line sharpening of the corresponding ^{1}H NMR signal.[34] While somewhat speculative, ^{1}H NMR spectroscopy suggests that micelle-like structures are formed in polar solvents, while reverse micelles are generated in apolar solvents. As shown in Figure 2, the downfield shift of the water peak δ = 1.65 ppm in $CDCl_3$, 4.00 ppm in $CDCl_3-CD_3OD$ (9:1), 4.12 ppm in $CDCl_3-CD_3OD$ (3:1), 4.62 ppm in $CDCl_3-CD_3OD$ (1:1) and 4.80 ppm in $CDCl_3-CD_3OD$ (1:3) referenced to TMS, is directly indicative of the increasing polarity of the solvent. Signals corresponding to N^+Me_3 protons (δ 3.20 ppm) and the C-2 protons of both glycerol backbones were quite broad in $CDCl_3$ but became sharper in solvent mixes of increasing polarity. In contrast, an opposite trend was noted for the signal attributed to the terminal methyl groups of both alkyl chains (δ 0.80 ppm). Of interest, a distinct resonance band at 4.50 ppm that is consistent with bound water was observed when the bolaamphiphile was dissolved in $CDCl_3-CD_3OD$ (1:3). Surprisingly, the high-field proton *Hb*-2 assigned to the propanyl moiety appeared downfield in $CDCl_3-CD_3OD$ (3:1−1:1) but shifted upfield with a lower integration

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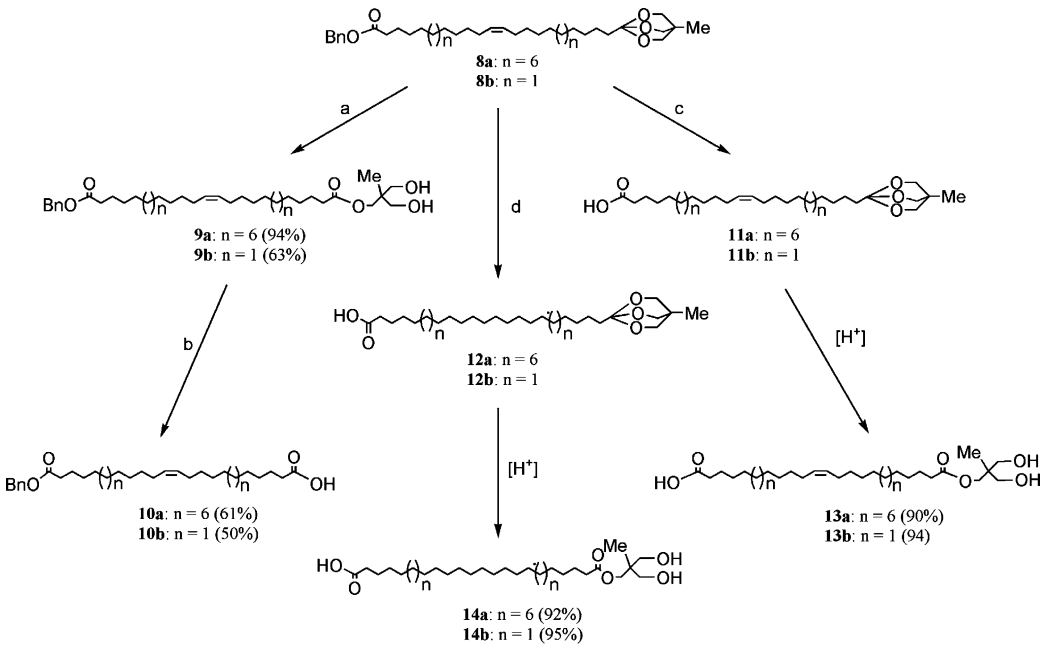
TABLE 1. Wittig Reaction of Aldehyde 4a and Phosphonium Salt 7a

			base				
run	aldehyde (equiv)	phosphonium salt (equiv)	MeONa (equiv)	*n*-BuLi (equiv)	solvent	time (h)	yield (%)
1	1.0	1.2−6.0	1.0−6.0		DMF	12−44	−[a]
2	1.0	6.0		7.2	THF	2	9
3	1.0	6.0		4.1	THF	2	21
4	1.0	3.0		3.6	THF	2	29
5	1.0	1.2		1.3	THF	2	52

[a] −, no reaction.

SCHEME 3. Selective Deprotection of 32/22-Ene Dioic Acid Derivative 8a/8b[a]



[a] Reagents and conditions: (a) PPTS/MeOH; (b) LiOH/THF; (c) KOH/MeOH−THF; (d) H_2, Pd−C/EtOH−AcOEt.

value in $CDCl_3$−CD_3OD (1:3). Hydration of the *sn*-2 ester carbonyl with small amounts of adventitious water provides a possible explanation for this observation. The different integration value indicates that the conformations of the two C-2 protons are different in the micellar domain in $CDCl_3$−CD_3OD (1:3), which is consistent with FTIR spectral data reported below. Further studies will need to be performed in order to address this phenomenon in greater detail.

It is noteworthy that both the trimethylammonium and terminal methyl groups of both alkyl chains present sharp signals in solvent mixes of intermediate polarity ($CDCl_3$−CD_3OD 3:1−1:1). Thus, under these conditions micelle formation does not occur with both hydrophilic and hydrophobic moieties of the bolaamphiphile having unrestricted motion.

FTIR Properties of Aymmetric Bolaamphiphiles. Fourier transform infrared spectroscopy provides a convenient tool for elucidating unique structural features of phospholipids in water or when in contact with ions and membrane proteins.[35,36] Infrared spectra for the asymmetric bolaamphiphile **21a** cast from cholorofom/methanol (1:1) or water were notable for adsorption bands consistent with the acyl chain region at 2900−2800 cm^{-1}, the interface between the hydrophobic and hydrophilic zones at 1780−1660 cm^{-1}, and the polar headgroup region at 1130−1150 cm^{-1} (Figure 3). Of note, two ester carbonyl stretching regions at 1770 and 1680 cm^{-1} were observed. The high-frequency component (1770 cm^{-1}) is mainly associated with free carbonyl groups, while the low frequency (1680 cm^{-1}) feature is typically assigned to carbonyl groups hydrogen-bonded to water. The intensity of the low-frequency carbonyl (1680 cm^{-1}) increased in the presence of water, which further supports this assignment.

To determine which ester carbonyl hydrogen bonded with water, FTIR studies were performed on two related building block components of bolaamphiphile **21a**, DPPC, and 1,2-dipalmitoylpropanylamine (**23**) (Figure 4). 1,2-Dipalmitoylpropanylamine (**23**) was synthesized, as shown in Scheme 5. A low-frequency carbonyl adsorption band (1680 cm^{-1}) was observed in the spectrum of **23**, the intensity of which increased when the compound was cast from water. Consistent with these data, prior reports indicate a greater preference of water to hydrogen bond to the C=O group of the *sn*-2 ester than that of the *sn*-1 ester.[35] Therefore, the low-frequency carbonyl band

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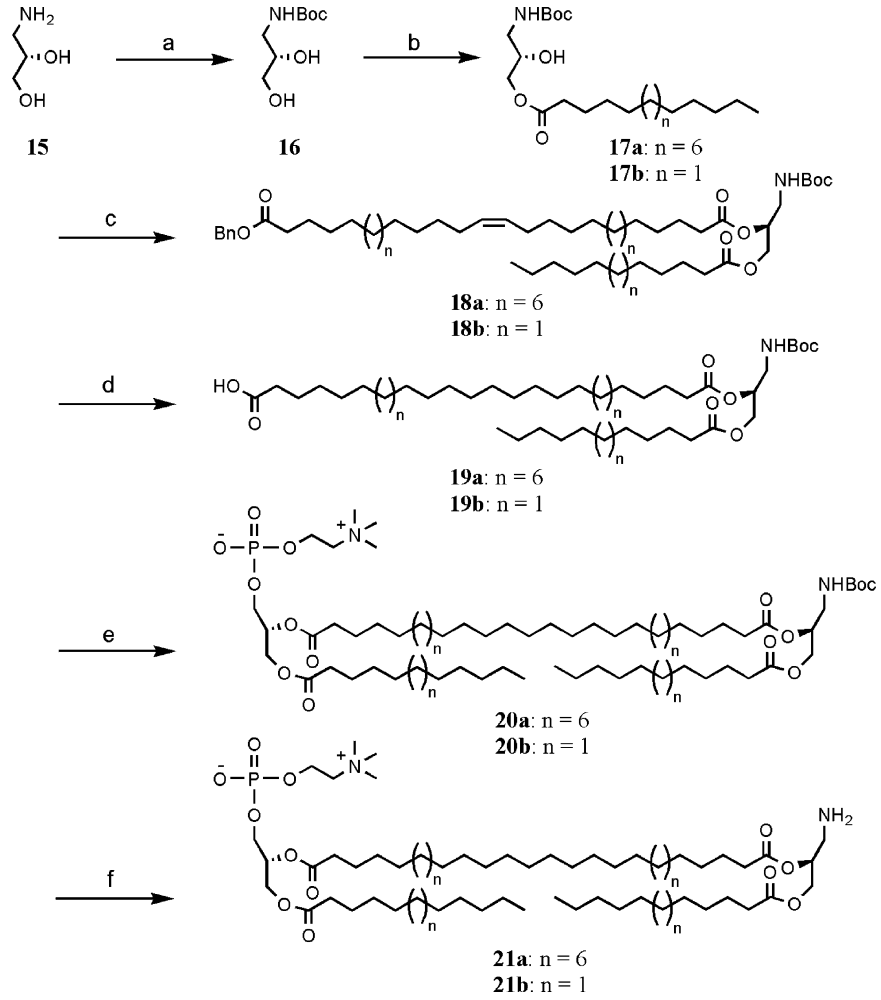
SCHEME 4. Synthesis of Asymmetric Bolaamphiphile 21[a]



[a] Reagents and conditions: (a) $(Boc)_2O$, DMAP/$CHCl_3$−MeOH (59%); (b) **a**: palmitic acid, **b**: undecanoic acid, DCC/CH_2Cl_2 (**17a**: 63%, **17b**: 63%); (c) 10, DCC/CH_2Cl_2 (**18a**: 70%, **18b**: 51%); (d) H_2, 10% Pd−C/EtOH (**19a**: 51%, **19b**: 81%); (e) **a**: 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine, **b**: 1-undecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine, DCC/CH_2Cl_2 (**20a**: 9%, **20b**: 22%); (f) TFA/CH_2Cl_2 (**21a**: quant, **21b**: quant).

(1680 cm^{-1}) of **21a** is likely attributable to the *sn*-2 ester carbonyl of the propanylamine backbone.

Cryo-High-Resolution SEM of Phospholipid Bolaamphiphiles in Water. The self-assembling properties of bolaamphiphilic lids **21a** and **21b** in water were evaluated using cryo-HRSEM, which provided direct visulization of the bolaamphiphile lipid membrane. As shown in Figure 5A,B, the C32 bolaamphiphile **21a** preferred to self-assembled as uniform microparticles of 1400−1500 nm diameter, which formed a homogeneous suspension in water and were stable for more than 3 months. Detailed imaging also revealed occasional formation of "onion" shape, giant multilamellar vesicles of 1500−2000 nm diameter (Figure 5C,D). The smaller C22 bolaamphiphile **21b** exhibited a preference for assembling as uniform, "onion" shape, giant multilamellar vesicles of 2500−3000 nm diameter (Figure 5E−H).

Conclusions

A synthetic route was devised for the generation of a new class of asymmetric phospholipid-based bolaamphiphiles through the sequential esterification of an alkyldioic acid, bearing distinct terminal protecting groups, with propanylamine and *lyso*-phosphatidylcholine headgroups. High thermal stability was verified by DSC and bolaamphiphile self-assembly investigated in solvent mixes of varying polarity by nuclear magnetic resonance (NMR) and Fourier transform-infrared (FT-IR) spectroscopy. Cryo-high-resolution scanning electron microscopy demonstrated that bolaamphiphiles in water could be processed to form multilamellar vesicles. We anticipate that asymmetric lipid bolaamphiphiles will provide facile building blocks for engineering a variety of unique membrane-mimetic structures with potential applications in drug delivery, biosensors, and biofunctional prosthetic devices.

Experimental Section

16-Iodohexadecanoic Acid (2a). 16-Hexadecanolide (20.0 g, 78.7 mmol), sodium iodide (35.4 g, 235.8 mmol), and acetonitrile (250 mL) were added to a 500 mL round-bottom flask. Chlorotrimethylsilane (29.9 mL, 235.8 mmol) in acetonitrile (20 mL) was subsequently added to this solution, and the reaction mixture refluxed under argon atmosphere for 16 h. The reaction mixture was then cooled to room temper-

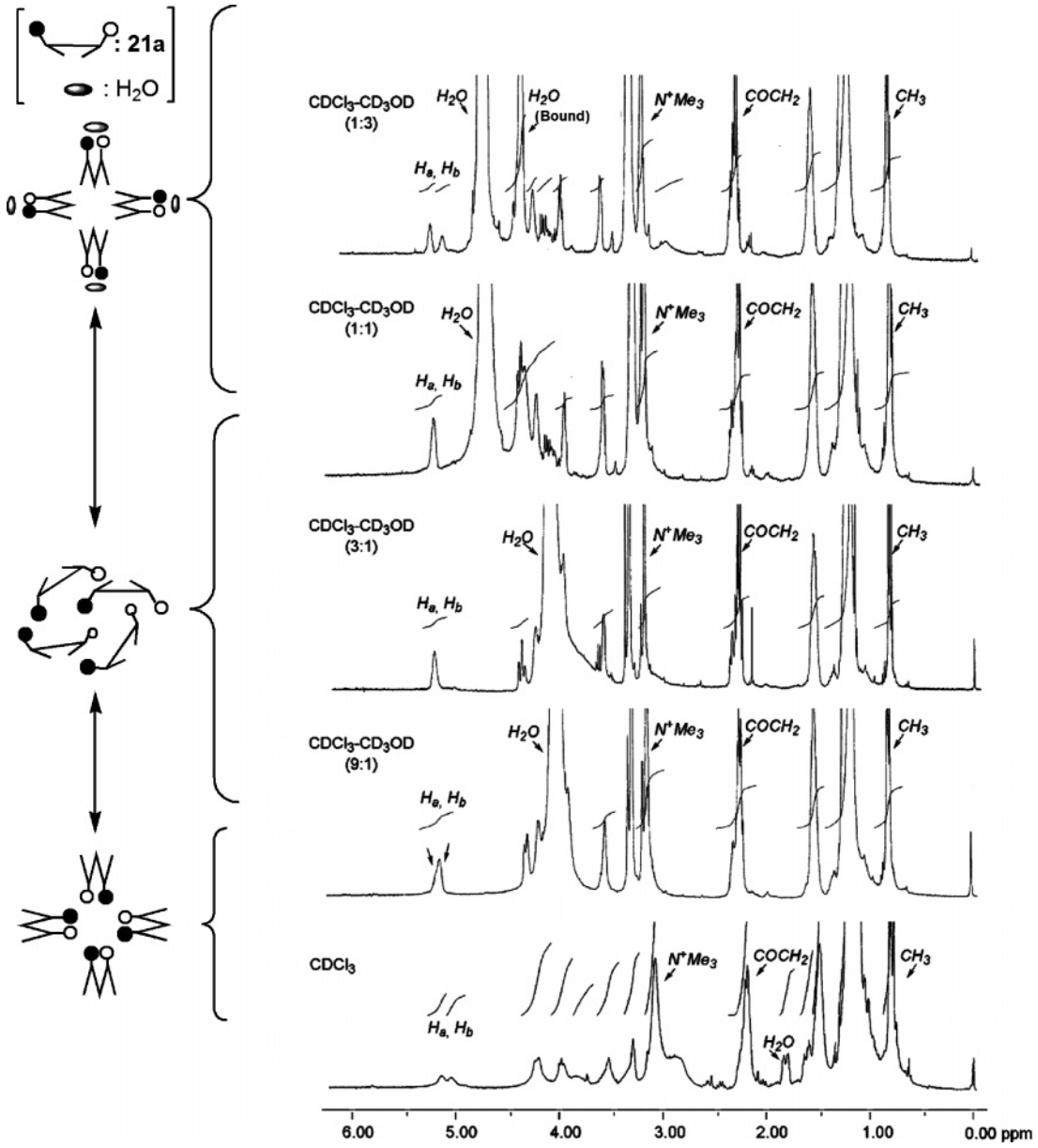


FIGURE 2. ^{1}H NMR spectra of the asymmetric bolaamphiphile in $CDCl_3-CD_3OD$ (9:1), (3:1), (1:1), and (1:3) mixtures, respectively.

ature, and the product silyl ester was hydrolyzed to the corresponding carboxylic acid by adding water (200 mL). The reaction mixture was taken up in ether (500 mL) and washed successively with water (200 mL × 3), 10% sodium thiosulfate solution (200 mL), and brine (200 mL) to remove inorganic salts and iodine. The ether layer was dried over sodium sulfate. Removal of ether afforded crude carboxylic acid **2a**, which was further purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 5:1) to obtain a colorless solid (27.71 g, 92%). Similarly, **2b** (16.3 g) was synthesized from **1b** (10.0 g, 54.3 mmmol) in 96% yield

2a: ^{1}H NMR ($CDCl_3$) δ 3.18 (t, 2H, J = 7.0), 2.34 (t, 2H, J = 7.4 Hz), 1.82 (q, 2H, J = 7.0 Hz), 1.63 (q, 2H, J = 7.4 Hz), 1.42−1.20 (br s, 22H); HR-MS (FAB) calcd for $C_{16}H_{31}O_2LiI$ 389.1529, obsd 389.1526 $[M + Li]^+$.

2b: ^{1}H NMR ($CDCl_3$) δ 3.18 (t, 2H, J = 7.1), 2.34 (t, 2H, J = 7.4 Hz), 1.81 (q, 2H, J = 7.1 Hz), 1.62 (q, 2H, J = 7.4 Hz), 1.39−1.20 (br s, 12H); HR-MS (FAB) calcd for $C_{11}H_{22}O_2I$ 313.0665, obsd 313.0660 $[M + H]^+$.

Benzyl 16-Iodohexadecanoate (3a). A solution of dicyclohexylcarbodiimide (22.6 g, 109.8 mmol) in dichloromethane (20 mL) was added dropwise at 0 °C to a solution of carboxylic acid **2a** (20.98 g, 54.9 mmol), benzyl alcohol (28.4 mL, 274.6 mmol), and 4-(dimethylamino)pyridine (0.67 g, 5.49 mmol) in dichloromethane (250 mL). The reaction mixture was stirred for 20 h at room temperature under argon atmosphere. Dicyclohexylurea was removed by filtering through Celite, and the filtrate was evaporated to provide a residue, which was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 10:1) to afford **3a** as a colorless solid (27.9 g, quant). Similarly, **3b** (7.73 g) was synthesized from **2b** (6.0 g, 19.2 mmol) quantitatively.

3a: ^{1}H NMR ($CDCl_3$) δ 7.36 (s, 5H), 5.11 (s, 2H), 3.19 (t, 2H, J = 7.7 Hz), 2.35 (t, 2H, J = 7.7 Hz), 1.82 (m, 2H), 1.63 (m, 2H), 1.40−1.20 (br s, 22H); HR-MS (FAB) calcd for $C_{23}H_{37}O_2LiI$ 479.1998, obsd 479.1982 $[M + Li]^+$.

3b: ^{1}H NMR ($CDCl_3$) δ 7.34 (s, 5H), 5.10 (s, 2H), 3.17 (t, 2H, J = 7.1 Hz), 2.34 (t, 2H, J = 7.4 Hz), 1.82 (m, 2H), 1.63 (m, 2H), 1.36−1.18 (br s, 12H); HR-MS (FAB) calcd for $C_{18}H_{28}O_2I$ 403.1134, obsd 403.1142 $[M + H]^+$.

Benzyl 16-Oxohexadecanoate (4a). A mixture of sodium hydrogen carbonate (4 g) and anhydrous methyl sulfoxide

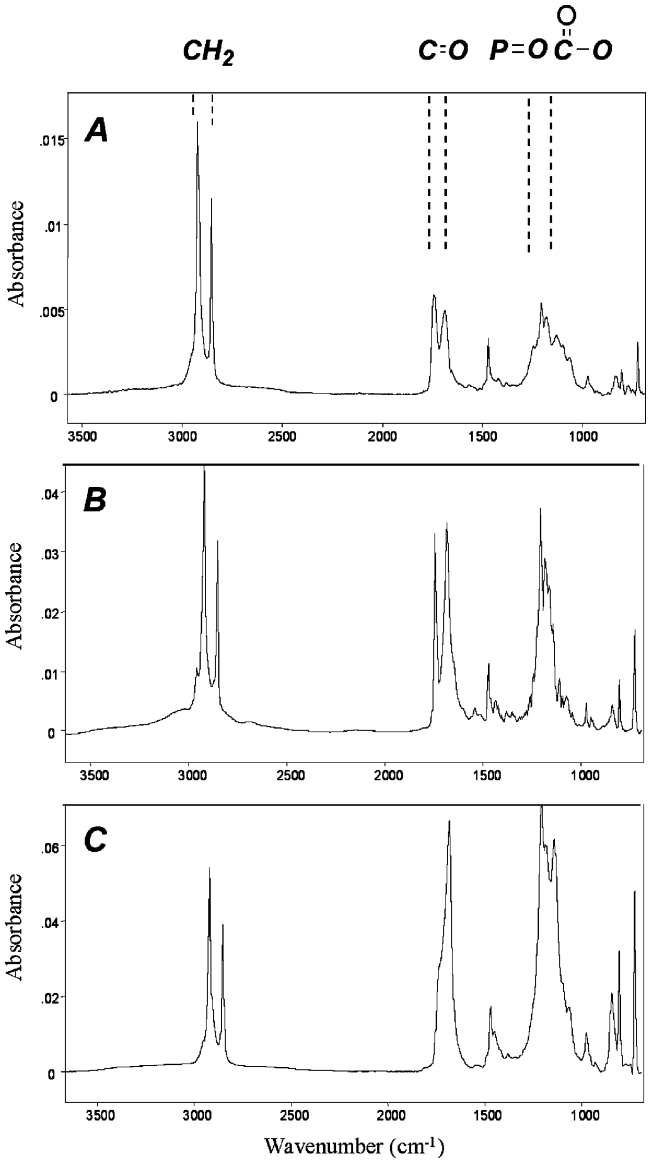


FIGURE 3. FTIR spectra of bolaamphiphile **21a** cast from (A) 1:1 chloroform−methanol, (B) water at 60 °C, and (C) water at 90 °C.

(100 mL) was heated to 150 °C under argon atmosphere. To this mixture was added compound **3a** (3.90 g, 8.262 mmol) in anhydrous methyl sulfoxide (10 mL), with continued heating and stirring for 10 min. The flask was cooled, and the mixture was poured into ice−water, which was stirred for another 1 h after TLC (*n*-hexane/ethyl acetate = 10:1) confirmation of the starting material disappearance. The white solid that formed was recovered by extracting with ether (100 mL × 4) and the ether layer dried over sodium sulfate. Removal of ether afforded crude aldehyde **4a**, which was further purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 8:1) to obtain a colorless solid (1.79 g, 60%). Similarly, **4b** (2.413 g) was synthesized from **3b** (4.63 g, 11.5 mmmol) in 96% yield.

4a: ^{1}H NMR ($CDCl_3$) δ 9.75 (t, 1H, J = 1.9 Hz), 7.36−7.33 (m, 5H), 5.10 (s, 2H), 2.41 (dt, 2H, J = 1.9, 7.2 Hz), 2.34 (t, 2H, J = 7.6 Hz), 1.66−1.59 (m, 4H), 1.34−1.22 (br s, 20H); HR-MS (FAB) calcd for $C_{23}H_{36}O_3Li$ 367.2824, obsd 367.2821 $[M + Li]^+$.

4b: ^{1}H NMR ($CDCl_3$) δ 9.75 (t, 1H, J = 2.2 Hz), 7.36−7.33 (m, 5H), 5.10 (s, 2H), 2.41 (dt, 2H, J = 2.2, 7.5 Hz), 2.35 (t, 2H, J = 7.5 Hz), 1.62 (q, 4H, J = 7.5 Hz), 1.34−1.22 (br s, 10H); HR-MS (FAB) calcd for $C_{18}H_{26}O_3Li$ 297.2042, obsd 297.2054 $[M + Li]^+$.

(3-Methyloxetan-3-yl)methyl 16-iodohexadecanoate (5a). A solution of dicyclohexylcarbodiimide (5.40 g, 26.2 mmol) in dichloromethane (20 mL) was added dropwise at 0 °C to a solution of carboxylic acid (**2a**) (5.00 g, 13.1 mmol), 3-methyl-3-oxetanemethanol (6.68 g, 65.4 mmol), and 4-(dimethylamino)pyridine (0.160 g, 1.31 mmol) in dichloromethane (200 mL). The reaction mixture was stirred for 20 h at room temperature under argon atmosphere. Dicyclohexylurea was removed by filtering through Celite, and the filtrate was evaporated to provide a residue, which was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 7:1) to afford **5a** as a colorless solid (4.83 g, 80%). Similarly, **5b** (9.38 g) was synthesized from **2b** (8.10 g, 25.9 mmmol) in 91% yield.

5a: ^{1}H NMR ($CDCl_3$) δ 4.49 (d, 2H, J = 5.5 Hz), 4.35 (d, 2H, J = 5.5 Hz), 4.13 (s, 3H), 3.15 (t, 2H, J = 6.8 Hz), 2.32 (t, 2H, J = 7.3 Hz), 1.96−1.54 (m, 6H), 1.35−1.22 (br s, 22H); HR-MS (FAB) calcd for $C_{21}H_{40}O_3I$ 467.2022, obsd 467.2027 $[M + H]^+$.

5b: ^{1}H NMR ($CDCl_3$) δ 4.50 (d, 2H, J = 5.4 Hz), 4.36 (d, 2H, J = 5.4 Hz), 4.13 (s, 3H), 3.17 (t, 2H, J = 6.7 Hz), 2.34 (t, 2H, J = 7.6 Hz), 1.80 (q, 2H, J = 6.8 Hz), 1.62 (q, 2H, J = 7.6 Hz), 1.38−1.26 (br s, 12H); HR-MS (FAB) calcd for $C_{16}H_{29}O_3ILi$ 403.1322, obsd 403.1323 $[M + Li]^+$.

1-(15-Iodopentadecanyl)-4-methyl-2,6,7-trioxabicyclo-[2.2.2]octane (6a). Boron trifluoride etherate (0.745 mL, 5.89 mmol) was added with continuous stirring to a solution of oxetane ester (10.97 g, 23.5 mmol) in anhydrous dichloromethane (23.5 mL) at −15 °C. After stirring at 0 °C under argon atmosphere for 16 h, the reaction mixture was quenched by the addition of triethylamine (3.28 mL, 23.5 mmol), diluted with ether (25 mL), and filtered to remove the amine-BF_3 complex. The filtrate was concentrated and the residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate/triethylamine = 60:10:1) to afford **6a** as a colorless solid (6.58 g, 60%). Similarly, **6b** (6.40 g) was synthesized from **5b** (9.38 g, 23.7 mmmol) in 68% yield.

6a: ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 6H), 3.36 (t, 2H, J = 7.0 Hz), 1.81 (q, 2H, J = 7.0 Hz), 1.64 (q, 2H, J = 7.0 Hz), 1.43−1.36 (m, 2H), 1.23 (br s, 22H), 0.79 (s, 3H); HR-MS (FAB) calcd for $C_{21}H_{40}O_3I$ 467.2022, obsd 467.2017 $[M + H]^+$.

6b: ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 6H), 3.17 (t, 2H, J = 6.8 Hz), 1.80 (q, 2H, J = 6.8 Hz), 1.67−1.62 (m, 2H), 1.40−1.33 (m, 2H), 1.30−1.25 (br s, 12H), 0.79 (s, 3H); HR-MS (FAB) calcd for $C_{16}H_{30}O_3I$ 397.1240, obsd 397.1225 $[M + H]^+$.

1-[(31-Benzyloxycarbonyl)hentriacont-15-en-1-yl]-4-methyl-2,6,7-trioxabicyclo[2.2.2] octane (8a). The ortho ester **6a** (5.02 g, 10.7 mmol) and triphenylphosphine (5.65 g, 21.5 mmol) were stirred in anhydrous benzene and anhydrous toluene (2:1, 75 mL) under refluxing conditions for 24 h in an argon atmosphere. After the solvent was removed, the residue was taken up in ether (100 mL) and the solution stirred vigorously for 1 h. The mixture was filtered to remove the triphenylphosphine in ether, and the white solid was collected and washed with ether to ensure that all excess triphenylphosphine was removed. The product (phosphonium salt) (**7a**) was dried with a vacuum pump for 24 h with a resultant yield of 5.68 g (73%). The salt (7) (5.68 g, 7.80 mmol) and anhydrous tetrahydrofuran (18.6 mL) were mixed in a round-bottom flask at −20 °C under argon atmosphere. *n*-Butyllithium (1.6 M) in hexane (5.28 mL, 8.45 mmol) was dropwise added to the solution. After 30 min at −20 °C, the mixture was cooled to −78 °C, followed by the addition of DMPU (3.93 mL, 32.5 mmol) and aldehyde **4a** (2.34 g, 6.50 mmol). After 1 h at −78 °C, the mixture was allowed to warm to room temperature. The mixture was extracted with ether (200 mL) and then washed with water (100 mL). The ether layer was dried over anhydrous sodium sulfate. Removal of ether afforded crude product **8a**, which was further purified by silica gel column chromatography (*n*-hexane/ethyl acetate/triethylamine = 80:

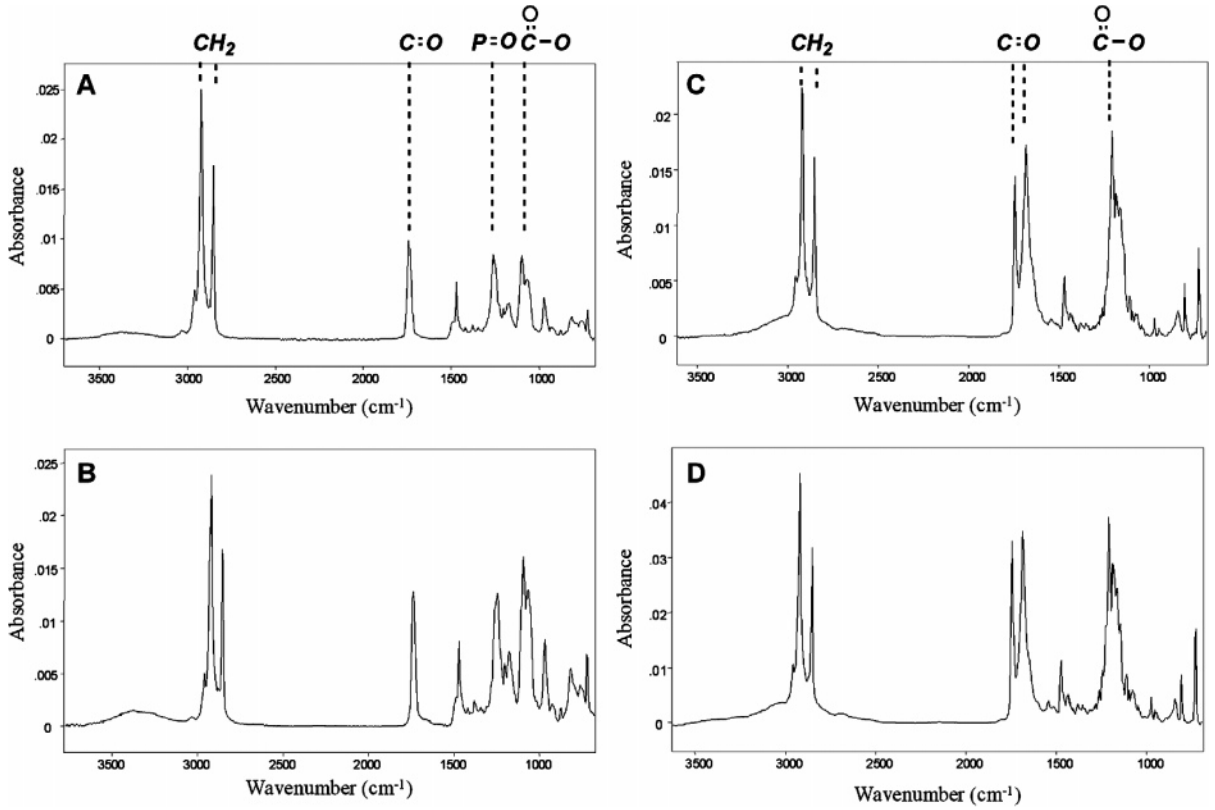
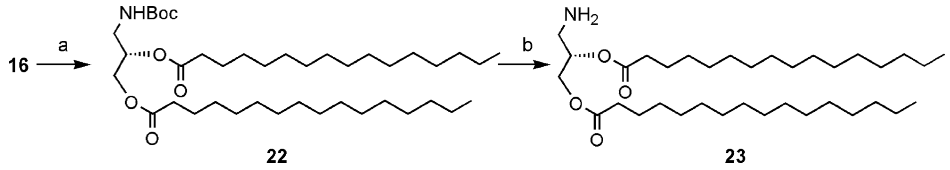


FIGURE 4. FTIR spectra of two structural building blocks of bolaamphiphile **21a**: (A) DPPC cast from 1:1 chloroform−methanol, (B) DPPC cast from water at 50 °C, (C) **23** cast from 1:1 chloroform−methanol, and (D) **23** cast from water at 50 °C.

SCHEME 5. Synthesis of 1,2-Dipalmitoylpropanyl Amine 23[a]



[a] Reagents and conditions: (a) palmitic acid, DCC/CH_2Cl_2 (95%); (b) TFA/CH_2Cl_2 (quant).

10:1) to obtain **8a** as a white solid (2.30 g, 52%). Similarly, **8b** (5.08 g) was synthesized from **6b** (6.12 g, 15.4 mmmol) and **4b** (10.1 g, 15.4 mmmol) in 61% yield.

8a: ^{1}H NMR ($CDCl_3$) δ 7.34−7.32 (m, 5H), 5.33 (dt, J = 4.6, 9.2 Hz), 5.09 (s, 2H), 3.87 (s, 6H), 2.33 (t, 2H, J = 7.7 Hz), 2.01−1.97 (m, 4H), 1.66−1.60 (m, 4H), 1.23 (br s, 46H), 0.77 (s, 3H); HR-MS (FAB) calcd for $C_{44}H_{74}O_5Li$ 689.5696, obsd 689.5696 $[M + Li]^+$.

8b: ^{1}H NMR ($CDCl_3$) δ 7.36−7.32 (m, 5H), 5.34 (dt, J = 4.8, 9.6 Hz), 5.11 (s, 2H), 3.89 (s, 6H), 2.35 (t, 2H, J = 7.6 Hz), 2.00 (q, 4H, J = 4.8 Hz), 1.68−1.61 (m, 4H), 1.44−1.39 (m, 2H), 1.32−1.24 (br s, 24H), 0.79 (s, 3H); HR-MS (FAB) calcd for $C_{34}H_{55}O_5$ 543.4050, obsd 543.4027 $[M + H]^+$.

Benzyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropyl-16-dotriacontenedioate (9a). *p*-Toluenesulfonate (18.0 mg, 0.0716 mmol) was added to a solution of **8a** (1.00 g, 1.47 mmol) in methanol and chloroform (2:1) (15 mL). The reaction mixture was stirred for 2 h at room temperature. Ethyl acetate (40 mL) was then added to the solution and the mixture was washed with saturated sodium hydrogen carbonate solution (20 mL). The organic layer was dried over anhydrous sodium sulfate. Removal of ether afforded crude product **9a**, which was further purified by silica gel column chromatography (chloroform/methanol = 50:1) to obtain **9a** as a colorless solid (0.972 g, 94%). Similarly, **9b** (1.62 g) was synthesized from **8b** (2.49 g, 4.59 mmmol) in 63% yield.

9a: ^{1}H NMR ($CDCl_3$) δ 7.36−7.33 (m, 5H), 5.34 (dt, J = 4.6, 9.2 Hz), 5.10 (s, 2H), 4.19 (s, 2H), 3.54 (q, 4H, J = 11.5 Hz), 2.72 (br s, 2H), 2.35 (t, 2H, J = 7.7 Hz), 2.34 (t, 2H, J = 7.7 Hz), 2.00 (q, 4H, J = 4.6 Hz), 1.63 (q, 4H, J = 7.4 Hz), 1.25 (s, 44H), 0.83 (s, 3H); HR-MS (FAB) calcd for $C_{44}H_{76}O_6Li$ 707.5802, obsd 707.5786 $[M + Li]^+$.

9b: ^{1}H NMR ($CDCl_3$) δ 7.36−7.32 (m, 5H), 5.34 (dt, J = 4.6, 9.2 Hz), 5.10 (s, 2H), 4.17 (s, 2H), 3.54 (q, 4H, J = 11.5 Hz), 2.93 (br s, 2H), 2.34 (t, 4H, J = 7.2 Hz), 2.00 (q, 4H, J = 4.7 Hz), 1.65−1.60 (m, 4H), 1.38−1.22 (br s, 24H), 0.83 (s, 3H); HR-MS (FAB) calcd for $C_{34}H_{56}O_6Li$ 567.4237, obsd 567.4248 $[M + Li]^+$.

Benzyl Hydrogen 16-Dotriacontenedioate (10a). Lithium hydroxide (33.3 mg, 1.39 mmol) was added to a solution of **9a** (0.972 g, 1.39 mmol) in tetrahydrofuran (15 mL) and water (5 mL). The mixture was stirred for 4 h at room temperature. Hydrochloric acid solution (1 M, 20 mL) was added to the solution, and the mixture was extracted with ethyl acetate (40 mL × 3). The ethyl acetate layer was dried over anhydrous sodium sulfate. Removal of ethyl acetate afforded crude product **10a**, which was further purified by silica gel column chromatography (chloroform/methanol = 50:1) to obtain **10a** as a colorless solid (0.508 g, 61%). Similarly, **10b** (164 mg) was synthesized from **9b** (400 mg, 0.715 mmmol) in 50% yield.

10a: ^{1}H NMR ($CDCl_3$) δ 7.34 (s, 5H), 5.34 (dt, J = 4.6, 9.2 Hz), 5.11 (s, 2H), 2.34 (t, 4H, J = 7.7 Hz), 2.02−1.96

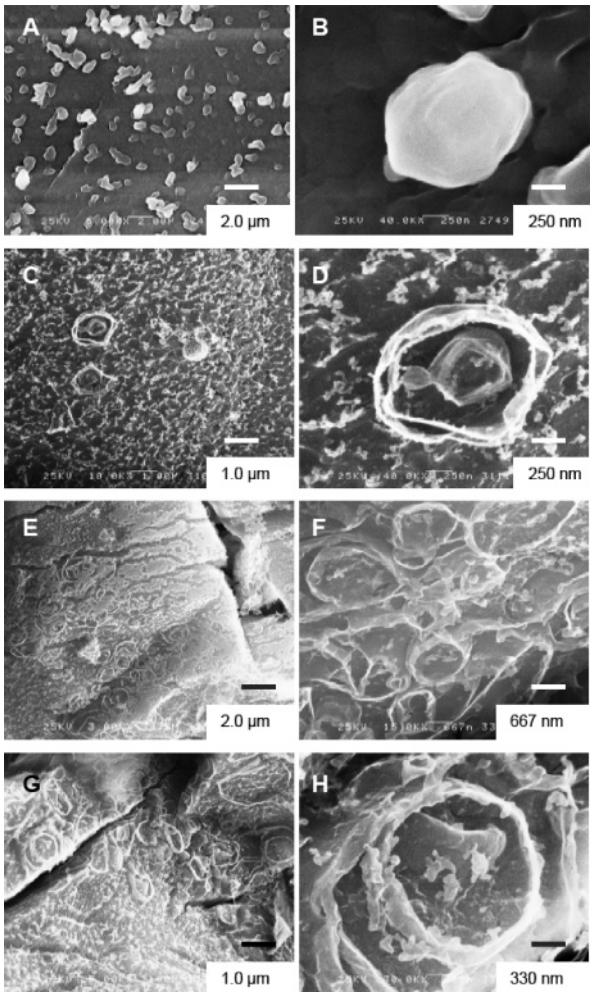


FIGURE 5. Cryo-high-resolution SEM of sonicated bola-amphiphile **21a** (A−D) and **21b** (E−H) in water (5 mg/mL).

(m, 4H), 1.66−1.60 (m, 4H), 1.25 (br s, 44H); HR-MS (FAB) calcd for $C_{39}H_{65}O_4$ 597.4883, obsd 597.4891 $[M]^+$.

10b: ^{1}H NMR ($CDCl_3$) δ 7.37−7.33 (m, 5H), 5.34 (dt, J = 4.6, 9.2 Hz), 5.11 (s, 2H), 2.35 (t, 2H, J = 7.5 Hz), 2.34 (t, 2H, J = 7.5 Hz), 2.00 (q, 4H, J = 4.8 Hz), 1.65−1.60 (m, 4H), 1.38−1.22 (br s, 24H); HR-MS (FAB) calcd for $C_{29}H_{46}O_4Li$ 465.3556, obsd 465.3535 $[M + Li]^+$.

(*S*)-3-*N*-(*tert*-Butoxycarbonyl)-amino-1,2-propanediol (16). Di-*tert*-butyl dicarbonate (7.18 g, 32.9 mmol), 4-(dimethylamino)pyridine (670 mg, 5.49 mmol), triethylamine (4.58 mL, 32.9 mmol) were added to a solution of (*S*)-3-amino-1,2-propanediol (**15**) (2.0 g, 22.0 mmol) in chloroform and methanol (1:1) (40 mL) at 0 °C under argon atmosphere. The reaction mixture was stirred for 16 h at room temperature. The solvent was evaporated and the residue was dissolved in ethyl acetate (100 mL), washed with 0.1 M hydrochloric acid solution (60 mL), saturated sodium hydrogen carbonate solution (60 mL), and brine (60 mL). The chloroform layer was dried over anhydrous sodium sulfate and the solvent was evaporated to give a residue, which was purified by silica gel column chromatography (chloroform/methanol = 15:1) to afford **16a** as an oil (2.46 g, 59%). ^{1}H NMR ($CDCl_3$) δ 5.51 (br s, 1H), 4.19 (br s, 2H), 3.69 (m, 1H), 3.58−3.45 (m, 2H), 3.23−3.14 (m, 2H), 1.39 (s, 9H); HR-MS (FAB) calcd for $C_8H_{17}O_4NLi$ 198.1318, obsd 198.1319 $[M + Li]^+$.

(*S*)-3-*N*-(*tert*-Butoxycarbonyl)-amino-1,2-propanediol 1-palmitate (17a). A solution of dicyclohexylcarbodiimide (0.647 g, 3.13 mmol) in dichloromethane (5 mL) was added dropwise at 0 °C to a solution of **16** (0.500 g, 2.61 mmol), palmitic acid (700 mg, 2.73 mmol), and 4-(dimethylamino)pyridine (31.9 mg, 0.261 mmol) in dichloromethane (10 mL). The reaction mixture was stirred for 20 h at room temperature under argon atmosphere. Dicyclohexylurea was removed by filtering through Celite and the filtrate was evaporated to give a residue, which was purified by silica gel column chromatography (chloroform/methanol = 50:1) to afford **17a** as a cololess solid (0.706 g, 63%). Similarly, **17b** (2.46 g) was synthesized from **16** (2.0 g, 0.715 mmmol) in 59% yield.

17a: ^{1}H NMR ($CDCl_3$) δ 5.00 (br s, 1H), 4.16−4.03 (m, 2H), 3.91 (m, 1H), 3.19−3.12 (m, 2H), 2.32 (t, 2H, J = 6.8 Hz), 1.60 (t, 2H, J = 6.8 Hz), 1.43 (s, 9H), 1.24 (br s, 24H), 0.86 (t, 3H, J = 6.2 Hz); HR-MS (FAB) calcd for $C_{24}H_{47}O_5NLi$ 436.3614, obsd 436.3598 $[M + Li]^+$.

17b: ^{1}H NMR ($CDCl_3$) δ 5.51 (br s, 1H), 4.19 (br s, 2H), 3.69 (m, 1H), 3.58−3.45 (m, 2H), 3.23−3.14 (m, 2H), 1.39 (s, 9H); HR-MS (FAB) calcd for $C_8H_{17}O_4NLi$ 198.1318, obsd 198.1319.

(S)-3-*N*-(*tert*-Butoxycarbonyl)amino-1,2-propanediol 1-Palmitate 2-(31-Benzyloxycarbonyl-16-hentriaconten)-oate (18a). A solution of dicyclohexylcarbodiimide (360 mg, 1.74 mmol) in dichloromethane (5 mL) was added dropwise at room temperature to a solution of **17a** (500 mg, 1.16 mmol), **10a** (836 mg, 1.39 mmol), and 4-(dimethylamino)pyridine (14.2 mg, 0.116 mmol) in dichloromethane (30 mL). The reaction mixture was stirred for 44 h at room temperature under argon atmosphere. Dicyclohexylurea was removed by filtering through Celite and the filtrate was evaporated to give a residue, which was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 4:1) to afford **18a** as a colorless solid (599 mg, 51%). Similarly, **18b** (2.46 g) was synthesized from **17b** (419 mg, 0.913 mmmol) and **10b** (492 mg, 1.369 mmmol) in 70% yield.

18a: ^{1}H NMR ($CDCl_3$) δ 7.34 (s, 5H), 5.33 (dt, 2H, J = 4.8, 9.6 Hz), 5.10 (s, 2H), 5.08 (q, 1H, J = 5.5 Hz), 4.75 (t, 1H, J = 5.5 Hz), 4.26 (dd, 1H, J = 5.5, 12.2 Hz), 4.03 (dd, 1H, J = 5.5, 12.2 Hz), 3.35 (dd, 2H, J = 5.5, 12.2 Hz), 2.36−2.28 (m, 6H), 2.00 (dd, 4H, J = 6.7, 12.5 Hz), 1.67−1.60 (m, 6H), 1.42 (s, 9H), 1.24 (br s, 68H), 0.87 (t, 3H, J = 6.7 Hz); HR-MS (FAB) calcd for $C_{63}H_{111}O_8NLi$ 1016.8470, obsd 1016.8485 $[M + Li]^+$.

18b: ^{1}H NMR ($CDCl_3$) δ 7.38−7.31 (m, 5H), 5.34 (dt, 2H, J = 4.6, 9.2 Hz), 5.11 (s, 2H), 5.08 (q, 1H, J = 5.3 Hz), 4.75 (t, 1H, J = 5.3 Hz), 4.26 (dd, 1H, J = 4.2, 12.0 Hz), 4.11 (dd, 1H, J = 5.3, 12.0 Hz), 3.35 (dd, 2H, J = 5.3, 12.0 Hz), 2.36−2.27 (m, 6H), 2.00 (dd, 4H, J = 6.4, 11.4 Hz), 1.65−1.58 (m, 6H), 1.43 (s, 9H), 1.36−1.19 (br s, 38H), 0.87 (t, 3H, J = 6.6 Hz); HR-MS (FAB) calcd for $C_{48}H_{81}O_8NLi$ 806.6122, obsd 806.6098 $[M + Li]^+$.

(S)-3-*N*-(*tert*-Butoxycarbonyl)amino-1,2-propanediol 1-palmitate 2-(Hentriacontane-31-carboxyl)ate (19a). To a solution of **18a** (392 mg, 0.387 mmol) in ethanol (20 mL) was added 10% palladium carbon (270 mg). Hydrogen gas was added to the solution, which was then stirred at room temperature for 16 h. The mixture was filtered, washed with chloroform, and evaporated to give a residue, which was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 4:1, and then 3:1) to obtain **19a** as a colorless solid (290 mg, 81%). Similarly, **19b** (95 mg) was synthesized from **18b** (209 mg, 0.261 mmmol) in 51% yield.

19a: ^{1}H NMR ($CDCl_3$) δ 5.08 (q, 1H, J = 5.7 Hz), 4.75 (t, 1H, J = 5.7 Hz), 4.26 (dd, 1H, J = 5.7, 12.5 Hz), 4.11 (dd, 1H, J = 5.7, 12.5 Hz), 3.33 (dd, 2H, J = 5.7, 12.5 Hz), 2.35−2.27 (m, 6H), 1.63−1.58 (m, 6H), 1.42 (s, 9H), 1.24 (br s, 76H), 0.87 (t, 3H, J = 6.7 Hz); HR-MS (FAB) calcd for $C_{56}H_{107}O_8NLi$ 928.8157, obsd 928.8176 $[M + Li]^+$.

19b: ^{1}H NMR ($CDCl_3$) δ 5.08 (q, 1H, J = 5.5 Hz), 4.76 (t, 1H, J = 5.5 Hz), 4.26 (dd, 1H, J = 4.1, 11.5 Hz), 4.11 (dd, 1H, J = 5.5, 11.5 Hz), 3.35 (dd, 2H, J = 5.5, 11.5 Hz), 2.35−2.27 (m, 6H), 1.63−1.58 (m, 6H), 1.43 (s, 9H), 1.36−1.20 (br s, 46H), 0.87 (t, 3H, J = 6.8 Hz); HR-MS (FAB) calcd for $C_{41}H_{77}O_8NLi$ 718.5809, obsd 718.5788 $[M + Li]^+$.

Boc-Protected Amine Bearing Phosphocholine Lipid (20a). A solution of dicyclohexylcarbodiimide (97.4 mg, 0.471 mmol) in dichloromethane (2 mL) was added dropwise at room temperature to a solution of **19a** (290 mg, 0.314 mmol), 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (312 mg, 0.629 mmol), and 4-(dimethylamino)pyridine (3.84 mg, 0.0314 mmol) in dichloromethane (20 mL). The reaction mixture was stirred for 7 d at room temperature under argon atmosphere. Dicyclohexylurea was removed by filtering through Celite and the filtrate was evaporated to give a residue, which was purified by silica gel column chromatography (chloroform/methanol/water = 65:25:3), and then freeze-dried to afford **20a** as a colorless powder (94.9 mg, 22%). Similarly, **20b** (12 mg) was synthesized from **19b** (95 mg, 0.134 mmmol) in 9% yield.

20a: ^{1}H NMR ($CDCl_3$) δ 5.19 (m, 1H), 5.08 (q, 1H, J = 5.2 Hz), 4.74 (t, 1H, J = 5.2 Hz), 4.26 (dd, 1H, J = 4.2, 12.2 Hz), 4.11 (dd, 1H, J = 5.8, 12.2 Hz), 3.93−3.83 (m, 2H), 3.35 (br s, 9H), 2.32−2.23 (m, 8H), 1.62−1.54 (m, 8H), 1.42 (s, 9H), 1.23 (br s, 100H), 0.86 (t, 6H, J = 6.5 Hz); HR-MS (FAB) calcd for $C_{80}H_{156}O_{14}N_2P$ 1400.1294, obsd 1400.1229 $[M + H]^+$.

20b: ^{1}H NMR ($CDCl_3$) δ 5.19 (br s, 1H), 5.09 (q, 1H, J = 5.2 Hz), 4.76 (t, 1H, J = 5.2 Hz), 4.26 (dd, 1H, J = 3.8, 11.7 Hz), 4.11 (dd, 1H, J = 5.2, 11.7 Hz), 3.37−3.32 (m, 2H), 3.34 br s, 9H), 2.32−2.24 (m, 8H), 1.62−1.54 (m, 8H), 1.43 (s, 9H), 1.36−1.20 (br s, 60H), 0.87 (t, 3H, J = 6.8 Hz); HR-MS (FAB) calcd for $C_{60}H_{116}O_{14}N_2P$ 1119.8164, obsd 1119.7532 $[M + H]^+$

Amine Bearing Phosphocholine Lipid (21a). Trifluoroacetic acid (3 mL) was added to a solution of **20** (94.9 mg, 0.0677 mmol) in dichloromethane (3 mL). This solution was stirred at room temperature for 16 h and the solvent was evaporated and coevaporated with ether to afford **21a** as a colorless solid (90 mg, quant.). Similarly, **21b** (10 mg) was synthesized from **20b** (12 mg, 0.011 mmmol) quantitatively.

21a: ^{1}H NMR ($CDCl_3$) δ 5.24 (m, 1H), 5.15 (m, 1H), 4.09−4.05 (m, 2H), 3.64−3.58 (m, 2H), 3.15 (br s, 9H), 2.32−2.23 (m, 8H), 1.60−1.52 (m, 8H), 1.21 (br s, 100H), 0.83 (t, 6H, J = 6.5 Hz HR-MS (FAB) calcd for $C_{75}H_{148}O_{12}N_2P$ 1301.9655, obsd 1301.7448 $[M + H]^+$.

21b: ^{1}H NMR ($CDCl_3$) δ 5.19 (br. m, 2H), 4.40−4.05 (m, 9H), 3.90 (m, 2H), 3.55 (m, 2H), 3.15 (br s, 9H), 2.38−2.23 (m, 8H), 1.60−1.52 (m, 8H), 1.21 (br s, 80H), 0.85 (t, 6H, J = 6.5 Hz, 6H); HR-MS (FAB) calcd for $C_{55}H_{108}O_{12}N_2P$ 1019.7640, obsd 1019.8171 $[M + H]^+$

(*S*)-3-*N*-(*tert*-Butoxycarbonyl)amino-1,2-propanediol 1,2-Dipalmitate (22). A solution of dicyclohexylcarbodiimide (0.807 g, 3.91 mmol) in dichloromethane (5 mL) was added dropwise at 0 °C to a solution of **16** (0.500 g, 2.61 mmol), palmitic acid (2.67 g, 10.4 mmol), and 4-(dimethylamino)-pyridine (3.91 mg, 0.261 mmol) in dichloromethane (10 mL). The reaction mixture was stirred for 20 h at room temperature under argon atmosphere. Dicyclohexylurea was removed by filtering through Celite and the filtrate evaporated to give a residue, which was purified by silica gel column chromatography (chloroform/methanol = 50:1) to afford **22** as a colorless solid (1.65 g, 95%): ^{1}H NMR ($CDCl_3$) δ 5.08 (q, 1H, J = 4.9 Hz), 4.73 (br. s, 1H), 4.26 (dd, 1H, J = 3.3, 12.4 Hz), 4.12 (dd, 1H, J = 5 0.5, 12.4 Hz), 3.55 (dd, 1H, J = 5.5, 12.4 Hz), 3.18 (dd, 1H, J = 3.3, 12.4 Hz), (m, 2H), 2.30 (t, 4H, J = 6.8 Hz), 1.60 (m, 4H), 1.24 (br s, 48H), 0.86 (t, 6H, J = 6.2 Hz).

(*S*)-1,2-Dipalmitoylpropanylamine (23). Trifluoroacetic acid (2 mL) was added to a solution of **22** (30 mg, 0.0449 mmol) in dichloromethane (2 mL). This solution was stirred at room temperature for 16 h, and the solvent was evaporated and coevaporated with ether to afford **23** as a colorless solid (90 mg, quant): ^{1}H NMR ($CDCl_3$) δ 5.26 (m, 1H,), 4.32 (m. s, 1H), 4.13 (m, 1H), 3.49 (dd, 1H, J = 5 0.4, 12.2 Hz), 2.32 (m, 4H), 1.23 (br. s, 48H), 0.87 (t, 6H, J = 6.8 Hz).

DSC, FTIR, and SEM Sample Preparation. Lipid sample **21a** (10 mg) was dissolved in 2 mL of 1:1 chloroform/methanol in a round-bottom flask and evaporated with a stream of nitrogen gas. Pure water (2 mL) was then added and the suspension hydrated by heating above the phase transition (T_m) with vortexing for 30 s. The resultant emulsion was sonicated for 30 min at 60 °C and remained stable for several months.

Differential Scanning Calorimetry (DSC). Phase-transition temperatures and enthalpies were determined by heating and cooling aqueous samples at a rate of 10 °C/h between 10 and 90 °C.

Cryo-High-Resolution SEM. Aliquots (∼10 μL) of the sonicated suspensions placed on gold planchets were plunge-frozen into liquid ethane at its melting point (−183.2 °C) and subsequently stored under liquid nitrogen. Ethane-encrusted samples were transferred into a precooled (−170 °C) cryo-preparation chamber under a positive flow of nitrogen gas (∼5 psi) and secured onto a cold stage. Specimens were fractured with the flick of a chilled razor blade and rinsed with liquid nitrogen. The shutters on the cold stage were closed to avoid frost contamination and the stage was quickly transferred into a Cr coater against a positive pressure of nitrogen gas. The Cr coater was evacuated to 2×10^{-7} Torr and then back-filled to 5×10^{-3} Torr with Ar gas. The shutters were opened and the frozen specimen, which was held below −150 °C, was sputter-coated with 1 nm of Cr after which the shutters were closed and the vacuum again brought to 2×10^{-7} Torr. The chamber of the Cr coater was flushed with dry nitrogen gas, returning it to atmospheric pressure, and the cold stage was removed and transferred to the upper stage of a field emission SEM. The temperature of the sample was increased from about −160 to −110 °C in order to allow any nanometer-sized frost, which had condensed on the surface of the Cr film to sublime in the microscope prior to imaging. The specimens were imaged at 25 kV accelerating voltage, digitally recorded, and processed.

Acknowledgment. This work was supported by grants from the NIH. We acknowledge the Emory University NMR and Mass Spectrometry Centers for their facilities.

Supporting Information Available: General methods and ^{1}H NMR spectra for key intermediates and final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO048167O