Synthesis of a Polymerizable Metal-Ion-Chelating Lipid for Fluid Bilayers

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Hydrated lipid structures, such as liposomes, that display tethered metal-ion-chelating groups have proven useful in peptide and protein binding, as well as 2D protein crystallization through molecular recognition of accessible histidine sites in proteins and peptides. Polymerizable metal-ion-chelating lipids bearing a reactive diacetylene group have been described. These interesting compounds can be polymerized in the solid-analogous phase. Here we describe the design of the first polymerizable metal-ion-chelating lipid that can be used in the fluid, i.e., liquid analogous, phase of lipid bilayers. The synthesis of 1-palmitoyl-2-[8-[(*E*,*E*)-2′,4′-hexadienoyloxy]octanoyl]-*sn*-glycero-3-*N*-[11-[*N*′,*N*′-bis[carboxymethyl]imino]-3,6,9-trioxaundecanoyl] phosphatidylethanolamine (**1**) is described. The chelator moiety, iminodiacetate (IDA), was linked to the polymerizable phosphatidylethanolamine (PE) with a terminal 2,4-hexadienoyl (sorbyl) group through an oligo(ethylene glycol)-based spacer. Lipid **1**−Cu complex is designed to be combined with the corresponding polymerizable matrix lipids (bis-SorbPC) to form functionalized liposomes that can be stabilized by various polymerization methods.

The hydration of lipids can yield various lamellar and nonlamellar lipid ensembles, including monolayers, supported bilayers, lipid bilayer vesicles (liposomes), and inverted hexagonal and bicontinuous cubic phases.[1−5] When the membrane surface of lipid layers is modified by incorporation of functionalized lipids, then these functional lipid structures offer many possible applications in biological and material sciences (e.g., catalysis, separation, surface modification, and sensors, among others). Lipid polymerization has been employed to stabilize the self-organized lipid assemblies that form from designed reactive lipids.[6] The combination of both functional and polymerizable groups in an appropriately designed lipid could ensure the motion of functional moieties tethered to fluid lipid layers prior to polymerization and provide for cross-linking stabilization of the supramolecular assemblies after binding of proteins or peptides.

Recently, metal-ion-chelating lipids have been synthesized and used for the formation of functional lipid surfaces where proteins can be targeted through coordination of chelated metal ion and surface histidine residues of a protein.[7−11] Immobilization and 2D crystallization of natural and histidine-tagged proteins have been accomplished on Langmuir monolayers or liposomes composed partially of chelating lipids.[12−14] The synthesis of some polymerizable diacetylenic chelating lipids has also been reported.[14−18] Diacetylenes can be readily polymerized in the solid-analogous phase but not in the more biologically relevant fluid, i.e., liquid-analogous, phase. In this paper, we describe the synthesis of a new polymerizable chelating lipid, 1-palmitoyl-2-[8-[(*E*,*E*)-2′,4′-hexadienoyloxy]octanoyl]-*sn*-glycero-3-*N*-[11-[*N*′,*N*′-bis[carboxymethyl]imino]-3,6,9-trioxaundecanoyl] phosphatidylethanolamine (**1**) (Figure 1). This lipid is based on phosphatidylethanolamine (PE) **9**, incorporating in the lipid tail the polymerizable group identical to that of the matrix lipid, bis-SorbPC$_{15,15}$. The liposomes of bis-SorbPC$_{15,15}$ show a main phase transition temperature (T_m) below room temperature.[19] In contrast to diacetylenic lipids, the sorbyl-substituted lipids can be polymerized either above or below the T_m. Consequently, these new

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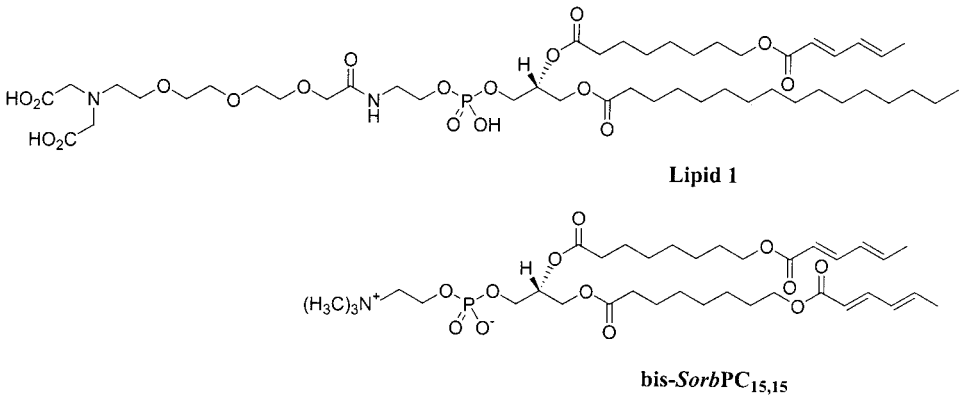


Figure 1.

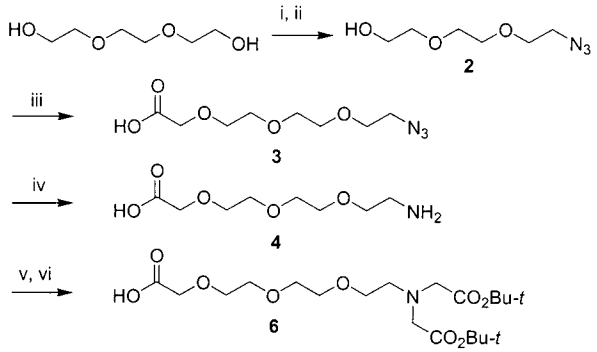
compounds can be used over a wider range of conditions than the previously reported diacetylene chelating lipids.

Results and Discussion

Three factors were considered in the design of lipid **1**: the polymerizable group, the metal-ion-chelator moiety, and a hydrophilic linker between the lipid and chelator. The compound was designed to be compatible with reactive lipids that are known to be polymerizable above or below the lipid T_m.[19,20] In particular, it is known that bis-SorbPC lipids can be photopolymerized at temperatures above or below the T_m. Therefore the PE **9**, which has a terminal 2,4-hexadienoyl (sorbyl) group, was chosen for derivatization. Among various chelator moieties, iminodiacetate (IDA) is proper for binding histidine residues on protein surfaces as a result of small size and high affinity of IDA-Cu for histidine.[13] Oligo(ethylene glycol) derivatives have been used as biocompatible, water soluble, flexible, and chemically inert spacers.[21−24] Because the spacer length can be readily varied by selection of the appropriate commercial precursors, this hydrophilic entity was used for the conjugation of IDA to the PE amino group.

IDA-spacer precursor **6** was prepared from heterobifunctional amino acid **4**, which should be useful for the conjugation of two different functional molecules by virtue of well-known protection−deprotection chemistry. We synthesized compound **4** with high yield by reduction of azido acid **3** which was prepared from triethylene glycol.[21,25] Although compound **4** was purified by column chromatography for analytical purposes, **4** isolated by extraction with diethyl ether to remove excess reducing agent 1,3-propane dithiol was pure enough for use in the next reaction. Thus obtained **4** was alkyated with *tert*-butyl bromoacetate in DMF in the presence of potassium carbonate and potassium iodide. Compound **5** was purified by flash column chromatography, and the ester was hydrolyzed by use of 1 N NaOH in ethanol to give compound **6** (Scheme 1).

Scheme 1[a]



[a] Reagents and conditions: **(**i) MsCl, Et_3N, THF; (ii) NaN_3, EtOH, reflux (i + ii, 75%); (iii) $BrCH_2CO_2H$, KOH, DMF (76%); (iv) $HS(CH_2)_3SH$, Et_3N, MeOH (89%); (v) $BrCH_2CO_2Bu$-*t*, K_2CO_3, KI, DMF (55%); (vi) 1 N NaOH, EtOH (62%).

Polymerizable PE **9** was prepared according to a known method with some modification.[26] The BOC group of compound **8** was hydrolyzed by the use of TFA in chloroform (1:2) because of poor solubility of **8** in 1,4-dioxane. Compound **9** and its possible positional isomer were used without further separation. Compound **10** was obtained by condensation of acid **6** with **9** in the presence of DCC and *N*-hydroxysuccinimide. Without the latter reagent, the yield was quite low, probably as a result of the formation of *N*-acylurea at the reaction temperature. Deprotection of **10** using TFA afforded the lipid **1**, whose structure was confirmed by TLC, high-resolution FAB-MS, and ^1H and ^{13}C NMR.

The lipid **1**−Cu(II) was prepared by adding a methanol solution of $CuCl_2$ to lipid **1** in chloroform, and large unilamellar liposomes of 100 nm diameter were made from the mixture of bis-SorbPC$_{15,15}$ and lipid **1**−Cu (2−5 mol %) by conventional extrusion procedures. Upon UV irradiation (a low-pressure Hg vapor pen lamp), the resulting metal-ion-chelating liposomes were polymerized at 25 °C with more than 90% conversion. Preliminary experiments showed that the proteins having surface histidine residues (lysozyme, cytochrome C, and myoglobin) were bound to the polymerized liposomes. The protein binding characteristics depending on polymerization of the metal-ion-chelating liposomes are currently under investigation.

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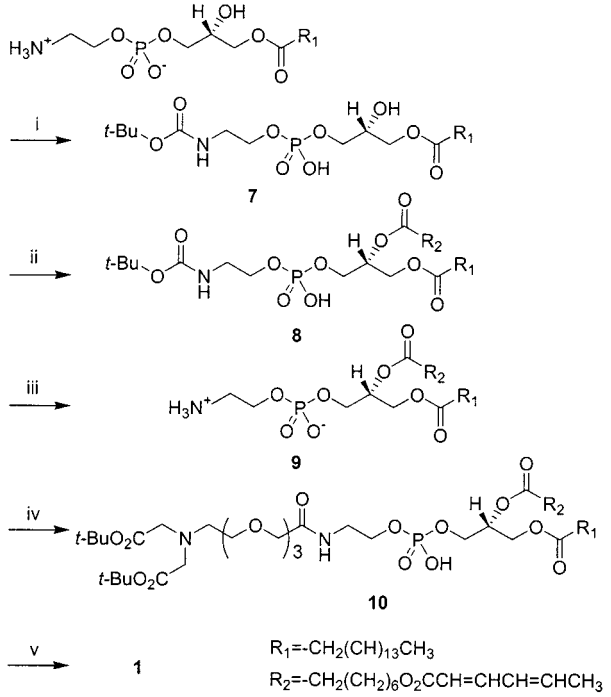
Scheme 2[a]



[a] Reagents and conditions: (i) *t*-Boc_2O, Et_3N, $CHCl_3$ (78%); (ii) R_2COOH, DCC, DMAP, THF (51%); (iii) TFA, $CHCl_3$, 0 °C to rt (95%); (iv) **6**, DCC, DMAP, NHS, Et_3N, $CHCl_3$, acetone (27%); (v) TFA, $CHCl_3$, 0 °C to rt, 1 h (65%).

Experimental Section

Instrumental Methods. ^1H and ^{13}C NMR spectra were recorded using 200 or 500 MHz spectrometers in chloroform-*d* with tetramethylsilane as an internal reference. Compounds containing the UV-sensitive groups were handled under yellow light. The reactions were monitored by TLC visualized by an UV lamp or iodine vapor. FAB-MS and high-resolution mass spectroscopy were performed by the mass spectroscopy facility at the University of Arizona.

Solvents and Reagents. Chemical reagents and starting materials were purchased from Aldrich Chemical Co. unless otherwise noted. Chloroform and triethylamine (Et_3N) were distilled from calcium hydride prior to use. Benzene and tetrahydrofuran were distilled from sodium−benzophenone ketyl. Dimethylformamide (DMF) was dried over 3 Å molecular sieves. 2,4-Hexadienoic acid was obtained from Merck, and 1,8-octanediol was obtained from Acros. 1-Palmitoyl-2-hydroxy-*sn*-glycero-3-phosphatidylethanolamine was purchased from Avanti Polar Lipids, Inc. 8-[(*E*,*E*)-2′,4′-Hexadienoyloxy]octanoic acid was prepared as described previously.[27] All other reagents were used as received without further purification. All reactions were performed under an argon atmosphere unless indicated otherwise.

1-Azido-8-hydroxy-3,6-dioxaoctane (2). A solution of triethylene glycol (21.0 g, 140 mmol), Et_3N (15 mL), and THF (100 mL) was cooled to 0 °C. To this was added dropwise methanesulfonyl chloride solution (3.87 mL, 50.0 mmol). The reaction mixture was then allowed to warm to room temperature and stirred vigorously overnight. The reaction contents were concentrated in vacuo. After 95% ethanol (100 mL) and sodium azide (6.50 g, 100 mmol) were added, the mixture was heated at reflux overnight, cooled to room temperature, and concentrated in vacuo. The residue was diluted with ether (250 mL), washed with brine (50 mL), and dried over $MgSO_4$. Solvent was removed on a rotovap to yield the crude product, which was purified by flash column chromatography, eluting with a gradient of hexane/ethyl acetate (1:1) to ethyl acetate to give 5.42 g of an oily **1** (75% yield): $R_f = 0.42-0.48$ in ethyl acetate; ^1H NMR ($CDCl_3$) δ 2.71 (br, 1H), 3.41 (t, $J = 5.0$ Hz, 2H), 3.70−3.76 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 50.47, 61.53, 69.86, 70.21, 70.48, 72.38; HRFAB (*m*/*z*) calcd for $C_6H_{14}N_3O_3$ 176.1035, found 176.1034.

11-Azido-3,6,9-trioxaundecanoic acid (3).[25] To a mixture of azido alcohol **2** (5.30 g, 30.3 mmol) and KOH (8.49 g, 151 mmol) in DMF (50 mL) was added dropwise a solution of bromoacetic acid (8.41 g, 60.5 mmol) in DMF (10 mL). The mixture was stirred at 45 °C for 12 h, and then KOH (1.87 g, 33.3 mmol) and bromoacetic acid (4.20 g, 30.3 mmol) were added again. After 12 h, H_2O (50 mL) was added and stirred overnight. The reaction mixture was partitioned between H_2O (200 mL) and CH_2Cl_2 (100 mL) and washed with CH_2Cl_2 (50 mL × 2). The aqueous solution was acidified to pH ∼2 with 1 N HCl and extracted with CH_2Cl_2 (100 mL × 3). The combined organic solution was dried over $MgSO_4$, and solvent was removed on a rotovap to yield the compound **2**, which was purified further by flash column chromatography, eluting with CH_2Cl_2/MeOH (1:1) to give 5.39 g of an oily **2** (76% yield): $R_f = 0.35$ in chloroform/methanol/water (65:25:4); ^1H NMR ($CDCl_3$) δ 3.40 (t, $J = 5.0$ Hz, 2H), 3.66−3.78 (m, 10H), 4.19 (s, 2H), 10.19 (br, 1H); ^{13}C NMR ($CDCl_3$) δ 50.59, 68.54, 70.01, 70.30, 70.43, 70.62, 71.29, 173.53; HRFAB (*m*/*z*) calcd for $C_8H_{16}N_3O_5$ 234.1090, found 234.1093.

11-Amino-3,6,9-trioxaundecanoic acid (4).[21] A solution of azido acid **3** (2.85 g, 12.2 mmol), Et_3N (10.23 mL, 73.4 mmol), and 1,3-propanedithiol (6.15 mL, 61.2 mmol) in absolute MeOH (20 mL) was prepared, stirred at room temperature for 48 h, and then concentrated in vacuo. The crude residue was diluted with water (150 mL) and washed with Et_2O (50 mL × 3). The aqueous solution was concentrated in vacuo to give a pale yellow oil. The residue was purified by flash column chromatography, eluting with a gradient of chloroform/methanol/water (65:25:1 to 65:25:4). Solvent was removed in vacuo. The resulting residue was dissolved in chloroform, filtered through a 0.22 μm PTFE filter, and reduced to give 2.25 g of a viscous oil (89% yield): $R_f = 0.23$ in chloroform/methanol/water (65:25:4); ^1H NMR ($CDCl_3$) δ 3.14 (t, $J = 4.8$ Hz. 2H), 3.61−3.72 (m, 8H), 3.81 (t, $J = 4.7$ Hz, 2H), 3.93 (s, 2H), 7.60 (br, 3H); ^{13}C NMR ($CDCl_3$) δ 38.97, 67.56, 69.27, 69.83, 70.14, 70.20, 71.11, 175.78; HRFAB (*m*/*z*) calcd for $C_8H_{18}NO_5$ 208.1185, found 208.1188.

11-[*N*,*N*-Bis[*tert*-butyloxycarbonylmethyl]imino]-3,6,9-trioxaundecanoic Acid (6). A mixture of amino acid **4** (2.25 g, 10.9 mmol), KI (1.80 g, 10.9 mmol), and K_2CO_3 (5.75 g, 54.3 mmol) in DMF (50 mL) was treated dropwise with *tert*-butyl bromoacetate (8.0 mL, 54.3 mmol). The reaction mixture was stirred at room temperature overnight, diluted with ethyl acetate (250 mL), and washed with saturated $NaHCO_3$ (50 mL × 3) and brine (50 mL × 3). The organic solution was dried over $MgSO_4$ and concentrated in vacuo to give the crude product. The oily residue was purified by flash column chromatography, eluting with a gradient of hexane/ethyl acetate (8:2 to 1:1) to give 3.27 g of a viscous oil **5** (55% yield): $R_f = 0.43$ in hexane/ethyl acetate (1:1); ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H), 1.47 (s, 9H), 2.94 (t, $J = 5.9$ Hz, 2H), 3.49 (s, 4H), 3.59−3.77 (m, 10H) 4.27 (s, 2H), 4.57 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 27.96, 28.12, 53.34, 56.63, 61.10, 68.31, 70.25, 70.30, 70.43, 70.53, 70.93, 80.75, 82.57, 166.35, 169.86, 170.75; HRFAB (*m*/*z*) calcd for $C_{26}H_{48}NO_{11}$ 550.3227, found 550.3221.

Compound **5** (3.02 g, 5.49 mmol) in 1 N NaOH (10 mL) and EtOH (50 mL) was stirred at room temperature for 1 h. The solution was neutralized with 1 N HCl and extracted with $CHCl_3$ (200 mL × 3). The organic solution was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash column chromatography, eluting with a gradient of CH_2Cl_2/MeOH (95:5 to 70:30) to give 1.48 g of a viscous oil (62% yield): $R_f = 0.39$ in chloroform/methanol (65:25); ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H), 2.92 (t, $J = 5.4$ Hz, 2H), 3.49 (s, 4H), 3.53−3.74 (m, 10H) 4.11 (s, 2H), 6.65 (br, 1H); ^{13}C NMR ($CDCl_3$) δ 28.09, 54.23, 56.80,

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69.05, 69.41, 69.81, 69.85, 70.24, 70.68, 81.44, 170.89, 172.81; HRFAB (*m*/*z*) calcd for $C_{20}H_{38}NO_9$ 436.2547, found 436.2543.

1-Palmitoyl-2-hydroxy-*sn*-glycero-3-(*N*-*tert*-butyloxycarbonyl) Phosphatidylethanolamine (7). 1-Palmitoyl-2-hydroxy-*sn*-glycero-3-phosphatidylethanolamine (500 mg, 1.10 mmol) was dissolved in chloroform (25 mL) and cooled in an ice bath, and then Et_3N (0.307 mL, 2.20 mmol) was added, followed by dropwise addition of *tert*-butyloxycarbonyl anhydride (0.304 mL, 1.32 mmol) in chloroform (10 mL). The reaction solution was stirred at room temperature overnight. The solvent was evaporated in vacuo, and the oily residue was separated by flash column chromatography, eluting with a gradient of chloroform/methanol (8:2) to chloroform/methanol/water (65:25:4) to give 479 mg of a viscous oil (78% yield): R_f = 0.63 in chloroform/methanol/water (65:25:4); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 1.16−1.35 (m, 24H), 1.43 (s, 9H), 1.54−1.63 (m, 2H), 2.31 (t, J = 7.5 Hz, 2H), 3.05 (br, 1H), 3.35 (br, 2H), 3.87−4.11 (m, 6H).

1-Palmitoyl-2-[8-[(*E*,*E*)-2′4′-hexadienoyloxy]octanoyl]-*sn*-glycero-3-[*N*-*tert*-butyloxycarbonyl] Phosphatidylethanolamine (8). A mixture of *t*-Boc-lysoPE (**7**) (479 mg, 865 μmol), 8-[(*E*,*E*)-2′,4′-hexadienoyloxy]octanoic acid (220 mg, 865 μmol), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (214 mg, 1.04 mmol), and (dimethylamino)pyridine (10.6 mg, 0.087 mmol) in THF (20 mL) was stirred at room temperature for 36 h. The white precipitate was filtered, and the filtrate was concentrated in vacuo. The crude product was separated by flash column chromatography, eluting with a gradient of chloroform/methanol (95:5 to 8:2) to give 347 mg of a white solid (51% yield): R_f = 0.18−0.27 in chloroform/methanol (9:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.2 Hz, 3H), 1.16−1.30 (m, 24H), 1.33 (m, 6H), 1.43 (s, 9H), 1.57−1.61 (m, 6H), 1.86 (d, J = 5.2 Hz, 3H), 2.28 (m, 4H), 3.35 (br, 2H), 3.87−4.05 (m, 4H), 4.12 (t, J = 6.7 Hz, 2H), 4.15−4.35 (m, 2H), 5.23 (br, 1H), 5.77 (d, J = 15.3 Hz, 1H), 6.15−6.19 (m, 2H), 7.15−7.27 (m, 1H); LRFAB (*m*/*z*) calcd for $C_{40}H_{73}NO_{12}P$ 790.5, found 790.7, 690.7 ($MH^+ - t$-BOC).

1-Palmitoyl-2-[8-[(*E*,*E*)-2′,4′-hexadienoyloxy]octanoyl]-*sn*-glycero-3-phosphatidylethanolamine (9). A solution of compound **8** (347 mg, 0.44 mmol) in chloroform (20 mL) was cooled to 0 °C and then slowly treated with TFA (10 mL). The reaction mixture was then allowed to warm to room temperature and stirred vigorously for 1 h. The reaction contents were concentrated in vacuo, and the crude product was purified by flash column chromatography, eluting with a gradient of chloroform/methanol (8:2) to chloroform/methanol/water (65:25:4). Solvent was removed in vacuo at room temperature. The resulting residue was dissolved in chloroform and filtered through a 0.22 μm PTFE filter and reduced to give 289 mg of a white solid (95% yield): R_f = 0.63 in chloroform/methanol/water (65:25:4); ^{1}H NMR ($CDCl_3$/CD_3OD (3:1)) δ 0.88 (t, J = 6.6 Hz, 3H), 1.20−1.30 (m, 24H), 1.34 (m, 6H), 1.53−1.69 (m, 6H), 1.87 (d, J = 5.3 Hz, 3H), 2.26−2.36 (m, 4H), 3.16 (m, 2H), 3.93−3.99 (m, 2H), 4.06−4.15 (m, 4H), 4.15−4.35 (m, 2H), 5.23 (m, 1H), 5.77 (d, J = 15.6 Hz, 1H), 6.17−6.20 (m, 2H), 7.15−7.27 (m, 1H).

1-Palmitoyl-2-[8-[(*E*,*E*)-2′,4′-hexadienoyloxy]octanoyl]-*sn*-glycero-3-*N*-[11-[*N*′,*N*′-bis[*tert*-butyloxycarbonylmethyl]imino]-3,6,9-trioxaundecanoyl] Phosphatidylethanolamine (10). A solution of (dimethylamino)pyridine (25.6 mg, 0.21 mmol), *N*-hydroxysuccinimide (62.4 mg, 0.54 mmol), and DCC (124 mg, 0.60 mmol) in acetone (10 mL) was cooled to 0 °C, and acid **6** (215 mg, 0.49 mmol) in chloroform (7 mL) was added dropwise. The reaction mixture was stirred at room temperature for 5 h and filtered. Compound **9** (283 mg, 0.41 mmol) and Et_3N (0.29 mL, 2.05 mmol) was added, and the mixture was stirred overnight at 40 °C. The solvent was removed on a rotovap to yield the crude product, which was purified by flash column chromatography, eluting with a gradient of chloroform/methanol (95:5 to 8:2) to give 123 mg of white solid (27% yield): R_f = 0.78 in chloroform/methanol (8:2); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.5 Hz, 3H), 1.20−1.30 (m, 24H), 1.34 (m, 6H), 1.43 (s, 18H), 1.56−1.64 (m, 6H), 1.86 (d, J = 5.4 Hz, 3H), 2.24−2.33 (m, 4H), 2.86 (t, J = 4.7 Hz, 2H), 3 0.40 (m, 8H), 3.56−3.84 (m, 8H), 3.93−4.03 (m, 4H), 4.09−4.12 (m, 4H), 4.18 (dd, $J1$ = 12.0 Hz, $J2$ = 6.3 Hz, 1H), 4.40 (dd, $J1$ = 12.0 Hz, $J2$ = 3.1 Hz, 1H), 5.22−5.27 (m, 1H), 5.77 (d, J = 15.7 Hz, 1H), 6.15−6.19 (m, 2H), 7.15−7.27 (m, 1H), 9.77 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.03, 18.55, 22.59, 24.71, 24.78, 25.72, 27.89, 28.58, 28.89, 29.05, 29.21, 29.25, 29.40, 29.59, 31.82, 34.00, 34.11, 42.41, 58.31, 62.64, 63.30, 63.42, 64.16, 69.43, 69.50, 69.56, 69.66, 70.43, 70.74, 71.45, 118.90, 129.69, 139.16, 144.82, 167.28, 169.78, 172.75, 173.29; HRFAB (*m*/*z*) calcd for $C_{55}H_{99}N_2O_{18}PNa$ 1129.6523, found 1129.6504.

1-Palmitoyl-2-[8-[(*E*,*E*)-2′,4′-hexadienoyloxy]octanoyl]-*sn*-glycero-3-*N*-[11-[*N*′,*N*′-bis[carboxymethyl]imino]-3,6,9-trioxaundecanoyl] Phosphatidylethanolamine (1). A solution of compound **10** (123 mg, 0.11 mmol) in chloroform (14 mL) was cooled to 0 °C and then treated with slowly added TFA (7 mL). The reaction mixture was then allowed to warm to room temperature and stirred vigorously for 1 h. The reaction contents were concentrated in vacuo, and the crude product was purified by flash column chromatography, eluting with a gradient of chloroform/methanol (8:2) to chloroform/methanol/water (65:25:4). Solvent was removed in vacuo at room temperature. The resulting residue was dissolved in chloroform, filtered through a 0.22 μm PTFE filter, and reduced to a white solid, which was lyophilized with *t*-BuOH and water (95:5) to give 74.2 mg of the compound **6** (65% yield): R_f = 0.16 in chloroform/methanol/water (65:25:4); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD (1:1)) δ 0.88 (t, J = 6.5 Hz, 3H), 1.20−1.34 (m, 30H), 1.56−1.64 (m, 6H), 1.86 (d, J = 5.4 Hz, 3H), 2.32 (br, 4H), 2.80 (br, 2H), 3.00−4.50 (br, 24H), 4.13 (t, J = 6.6 Hz, 2H), 5.21 (br, 1H), 5.76 (d, J = 15.7 Hz, 1H), 6.19 (m, 2H), 7.23 (dd, $J1$ = 17.5 Hz, $J2$ = 15.0 Hz, 1H); ^{13}C NMR (500 MHz, $CDCl_3$/CD_3OD (1:1)) δ 13.05, 17.49, 21.95, 24.13, 24.24, 25.13, 27.95, 28.27, 28.45, 28.66, 28.83, 28.96, 28.99, 29.69, 31.14, 33.34, 33.42, 39.53, 60.39, 62.01, 63.01, 63.40, 63.77, 67.88, 68.29, 68.43, 69.04, 69.34, 69.86, 117.92, 129.08, 139.18, 144.87, 161.33, 167.43, 172.81, 173.28; LRFAB (*m*/*z*) calcd for $C_{47}H_{83}N_2O_{18}PNa$ 1017.6, found 1017.6; for $C_{47}H_{83}N_2O_{18}PK$ 1033.6, found 1033.6; HRFAB (*m*/*z*) calcd for $C_{47}H_{84}N_2O_{18}P$ 995.5457, found 995.5446.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **3**−**6**, **10**, and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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