Synthesis and Fluorescence Properties of New Fluorescent, Polymerizable, Metal-Chelating Lipids

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Liposomes incorporating fluorescent, metal-chelating lipids find applications in molecular recognition of peptides, 2D protein recystallization, protein targeting, and biological sensing. It would be advantageous to combine the usefulness of polymerizable, metal-chelating lipids and fluorescent lipids. Herein, we report the synthesis and fluorescence properties of several *fluorescent, polymerizable, metal-chelating lipids*. They have been successfully incorporated into liposomes and then polymerized. These lipids can be used as membrane probes to study the polymerizable liposomes in the unpolymerized state and to investigate lipid redistribution during polymerization. In addition, if a luminescent metal ion (e.g., Eu^{3+}, Tb^{3+}, etc.) is used to complex the headgroup, the lipids can probe the membrane interior and exterior simultaneously.

Liposomes are spherically closed lipid bilayers with aqueous interiors.[1] Due to the ease of preparation, they are widely used in supramolecular chemistry, e.g., as cell models, as separation agents, as catalysts, etc.[2] Liposomes are also widely used in pharmacology and medicine (as drug carriers, immunoadjuvants, and diagonistic agents) and in genetic engineering (for gene delivery).[3] For these applications, it is very important to study the interaction of the liposomes with their targets.

Fluorescence spectroscopy (employing fluorescent lipids) is an important technique to probe the binding of liposomes to proteins, DNA, and other biomolecules.[4] Fluorescent lipids with metal-chelating headgroups have been reported in the literature. The resultant liposomes find applications in molecular recognition of peptides,[5] 2D protein recystallization,[6] protein targeting,[7] and biological sensing.[8] Usually, for these applications, the fluorophore is positioned within the lipid bilayer of the liposomes. When a metal ion binds to the headgroups, fluorescence spectra change due to lipid redistribution in the bilayer. The resultant assembly can be imaged using epifluorescence microscopy.[6,7]

The need for increased stability and controlled permeability has led to the synthesis of polymerizable lipids and polymerizable liposomes.[9] Polymerized liposomes are appreciably more stable compared to the nonpolymerizable counterparts. Though a variety of polymerizable lipids have been reported in the literature,[9] reports for the synthesis of polymerizable metal-chelating lipids are relatively few.[10]

It would be advantageous to combine the usefulness of polymerizable, metal-chelating lipids and fluorescent lipids. Herein, we report the synthesis and fluorescence properties of several *fluorescent, polymerizable, metal-chelating lipids*. The metal-chelating lipids were prepared incorporating pyrene, rhodamine B, coumarin, (trifluoromethyl)coumarin, and disperse orange as the fluorescent groups. To our knowledge, only pyrene- and rhodamine B-based nonpolymerizable lipids have been used as membrane probes in the literature. The lipids were successfully incorporated into liposomes and then polymerized. Our studies demonstrate that the polymerizable, metal-chelating, pyrene-based lipid can be used as a membrane probe to study the polymerizable liposomes in the unpolymerized state and to investigate lipid redistribution during polymerization employing the organic fluorophore. In addition (not demonstrated in these studies), if a lanthanide ion[12] (e.g., Eu^{3+}, Tb^{3+}, etc.) is used to complex the headgroup, the luminescence property of the metal ions can be used to detect protein binding to the liposomes.

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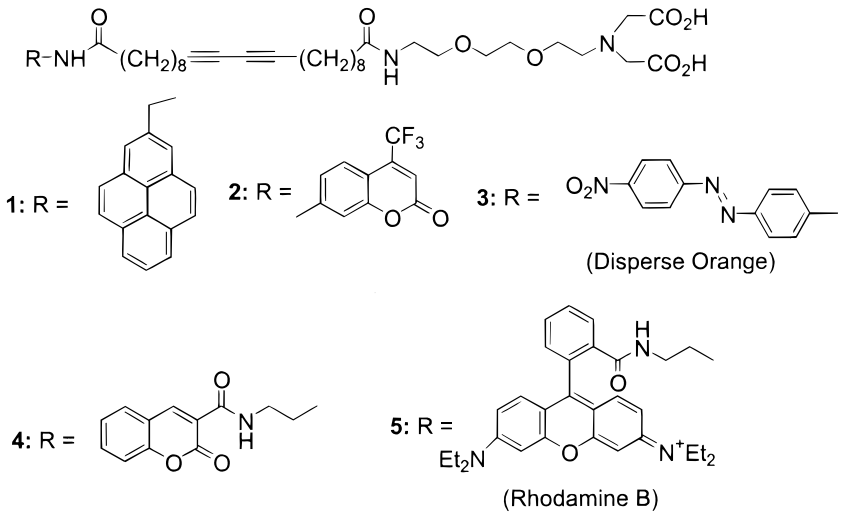

Figure 1. Structures of the fluorescent, polymerizable, metal-chelating lipids.

Results and Discussion

Synthesis of the Lipids. The structures of the lipids synthesized are shown in Figure 1. These lipids have the iminodiacetate (IDA) as the metal-chelating headgroup. IDA has strong affinity ($>10^{10}$ M^{-1}) for various transition-metal ions (e.g., Cu^{2+}, Ni^{2+}, Co^{2+}, etc.).[13] IDA has been successfully used to study metal-ion-mediated liposome−protein interactions for nonpolymerizable fluorescent lipids.[6−8] For the studies reported here, a conjugated dialkyne was chosen as the polymerizable group. Dialkyne lipids have been reported in the literature to form liposomes.[9] A diethylene glycol spacer was introduced between the polymerizable moiety and the metal-chelating headgroup. The liposome can be easily polymerized under UV irradiation (254 nm) to form stable structures.[14] Widely used fluorophores (pyrene, coumarin, rhodamine B, (trifluoromethyl)coumarin, and disperse orange)[15] have been used for our studies.

It is reported that pyrene and rhodamine, when attached to the hydrophobic alkyl groups of nonpolymerizable lipids, are embedded into lipid bilayers of liposomes.[7,8,16] Thus, the flourophores of lipids **1** and **5** should be in the bilayer region of the vesicles. In addition, the fluorophores of lipids **2**, **3**, and **4** have hydrophobic aromatic rings. The IDA headgroups of all of the lipids will be charged at pH 7.0. These lipids, when incorporated into liposomes, are expected to present the charged IDA groups on the surface and position the fluorophores inside the lipid bilayers.

Syntheses of the lipids (**1**, **2**, **3**, **4**, and **5**) are shown in Scheme 1. Commercially available polymerizable diacid **6** (available from GFS Chemicals Inc., Columbus, OH) was combined with the known amine **7**[10e] to afford **8**. Slow addition of a solution of **7** (over a 10 h period using a syringe pump) to a solution of the diacid **6** containing BOP reagent and triethylamine afforded **8** in 61% yield after chromatographic purification. For the synthesis of the lipids **1**, **2**, and **3**, compound **8** was reacted with the corresponding amines (pyrenemethylamine, amino(trifluoromethyl)coumarin, and disperse orange yellow, respectively). For lipids **4** and **5**, the fluorophores (carboxycoumarin and rhodamine B) contained carboxylic acids. The carboxylic acids were reacted with mono-*N*-Boc-ethylenediamine[11] followed by deprotection to generate amines **11a** and **11b**. These compounds were then coupled with **8**. After mild basic hydrolysis (LiOH), lowering the pH of the reaction medium with dilute HCl (pH $\sim$ 3.0) precipitated the lipids. Once isolated, the lipids were found to be pure (by ^{1}H and ^{13}C NMR and elemental analysis). The pure lipids were prepared in 140−280 mg quantity. They were stored at low temperature (−20 °C), under nitrogen, in the dark.

Vesicle Preparation. Vesicles were prepared (50 mM HEPES buffer, pH 8.0) following a literature procedure[17] incorporating polymerizable phosphocholine **12**[18] (85%), amine lipid **13**[19] (5%), cationic lipid **14**[20] (5%), and the fluorescent lipid (5%). The structures of lipids **12**, **13**, and **14** are shown in Figure 2. Experimental conditions (sonication time, power, rate of cooling, and temperature) were optimized (detailed procedures are given in the Experimental Section) for liposome formation. The resultant liposomes were polymerized by UV irradiation (450 W, 15 min) at 25 °C. Liposome formation was confirmed by transmission electron microscopy (TEM). When cupric chloride was added prior to probe sonication, the metal-chelating headgroups coordinated to Cu^{2+}. Representative transmission electron micrographs of polymerized liposomes incorporating the rhodamine lipid **5** (polymerized in the absence and presence of Cu^{2+}) are shown in Figure 2. All of the lipids synthesized were successfully incorporated into liposomes and then polymerized.

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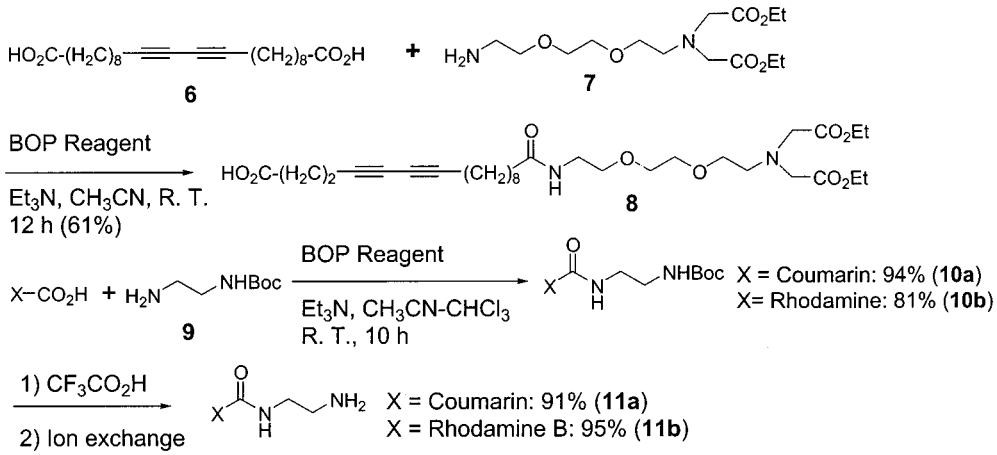
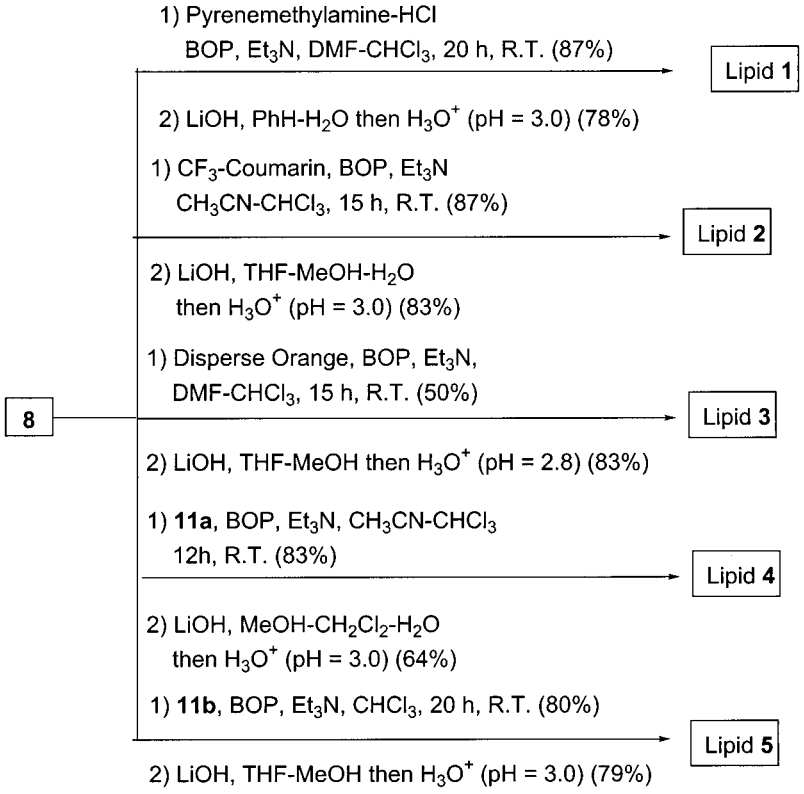
Scheme 1





Fluorescence Properties. Fluorescence spectra of the lipids and the polymerized liposomes were recorded with a continuous source (450 W xenon lamp) for sample excitation. The sample compartment of the spectrofluorimeter was constantly purged with dry nitrogen to minimize possible quenching by oxygen. Fluorescence spectra for pyrene-based polymerizable lipid **1** and the corresponding polymerized liposomes are shown in Figure 3 (spectra for other lipids are available as Supporting Information). Liposomes incorporating the other polymerizable lipids (**2**, **3**, **4**, and **5**) were found to be unsuitable as membrane probes. The fluorescence spectra in the absence and presence of cupric ions (during polymerization of the liposomes) did not show significant changes (fluorescence spectra are available as Supporting Information).

Pyrene-based nonpolymerizable lipids have been widely used as membrane probes.[21,15] When the lipids are aggregated, the excimer emission peak (480 nm) increases in intensity compared to the monomer peak (390 nm). The intensity ratio of the excimer and monomer peaks (I_{ex}/I_M) has been used for sensing metal ions and biological molecules.[8,22] Pyrene-based metal-chelating lipids also display similar properties.[8]

To investigate the ability of lipid **1** to detect lipid redistribution, liposomes were polymerized in the presence and absence of cupric ions. The Cu^{2+} ions coordinated to the IDA headgroup of lipid **1**. It has been reported (for nonpolymerizable lipids) that the coordination leads to the dispersion of IDA–Cu^{2+} lipids in

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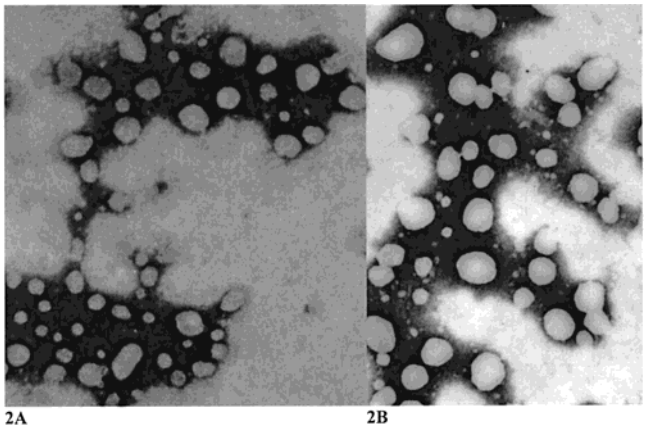

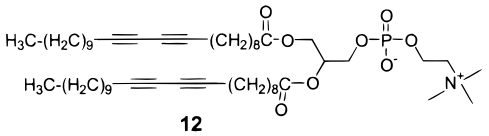

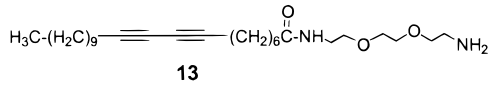

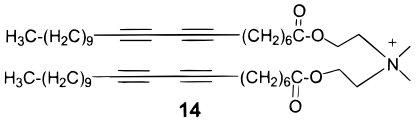

Figure 2. TEM pictures of polymerized liposomes (incorporating fluorescent lipid **5**) without (A) and with (B) Cu^{2+} ions. Structures of polymerizable lipids used (in addition to the lipids synthesized) are also shown.

liposomes.[22] The IDA−Cu^{2+} headgroup is negatively charged under the experimental conditions (pH 8.0) due to the deprotonation of the water molecules bound to the cupric ions.[23] The proximity of the negative charges is likely to contribute to the redistribution of lipid **1** during the polymerization process.

Figure 3 shows the fluorescence spectra of lipid **1** (B), and the polymerized liposomes with lipid **1** in the absence (C) and presence (D) of Cu^{2+}. The spectrum of pyrenemethylamine is included as a reference. Pyrenemethylamine in DMSO showed three excitation peaks at 280, 335, and 350 nm. Three fluorescence peaks were observed with maximum emissions at 380, 400, and 420 nm. The comparison of parts A and B of Figure 3 show the appearance of a relatively broad fluorescence band in the pyrene lipid **1** spectrum with maximum emission at 485 nm. Since the excitation spectrum followed at the emission maximum of this band corresponds to the excitation spectrum of pyrenemethylamine (data not shown), the emission at 485 nm can be attributed to the pyrene excimer. The comparison of parts B and C of Figure 3 shows significant changes in the spectral characteristics of lipid **1** after incorporation into liposomes and subsequent polymerization. Maximum excitation wavelengths were red-shifted by 10 nm (350 and 335 nm peaks) and 5 nm (280 nm peak). The two well-resolved emission peaks at 380 and 400 nm that are observed in Figure 3B are not present in the emission spectrum of the polymerized liposomes. Instead, a broad peak with maximum emission at 395 nm is observed. Since no significant modifications were observed in the spectral characteristics of pyrenemethylamine when an appropriate volume of its DMSO solution was mixed with the buffer solution, the spectral modifications shown in Figure 3 can be attributed to the different chemical environments surrounding the fluorescence probe.

Table 1 shows the fluorescence intensities and the intensity ratios of the pyrene excimer and monomer in different chemical environments. It is important to note that intercolumn comparison of fluorescence intensities is inappropriate because different concentrations were used to measure the reported values. However, the intensity ratios serve as an indication of the extent of excimer formation. The results indicate that when pyrenemethylamine is incorporated into the lipid (**1**), excimer formation is observed probably due to the proximity among pyrene moieties. When the liposomes incorporating lipid **1** are polymerized in the absence of Cu^{2+}, an increase in the excimer-to-monomer ratio is observed ($I_{ex}/I_M = 0.39$), and that can be attributed to a higher degree of aggregation. When Cu^{2+} ions are added before polymerization of the liposomes, the lipids are dispersed and the excimer-to-monomer ratio is reduced ($I_{ex}/I_M = 0.24$). The lipid **1** reports its aggregation state inside the lipid bilayer of the liposomes. Similar behavior has been reported for nonpolymerizable, metal-chelating lipids with the pyrene group as the fluorophore.[8,22]

In conclusion, several fluorescent, polymerizable, metal-chelating lipids have been synthesized. The lipids were successfully incorporated into liposomes and then polymerized by UV light. The pyrene-containing lipid **1** can be used as a membrane probe for lipid aggregation inside the bilayer of the liposomes. The fluorescence property of the lipid changes in response to cupric ions added during the polymerization process. Current studies in our laboratories include the potential application of this lipid for the detection of metal ion binding to the headgroup.

Experimental Details

Commercially available reagents were purchased from either Aldrich or Acros Chemical Co. and used as supplied unless stated otherwise. Polymerizable diacid (10,12-docosadiynedioic acid) was used as obtained from GFS Chemicals. Organic solvents were of spectroscopy grade from Fisher Scientific. All aqueous solutions were prepared from Nanopure water (Millipore). Experiments were conducted under an atmosphere of dry nitrogen. For workup, the organic layer was dried on anhydrous Na_2SO_4 and concentrated in vacuo. Melting points were recorded on a micro-melting-point apparatus, and all melting points are uncorrected. Elemental analyses were performed by an in-house materials characterization laboratory. TLC was performed with Absorsil Plus 1P, 20 × 20 cm plate, 0.25 μm (Alltech Associates, Inc.). Chromatography plates were visualized either with UV light or in an iodine chamber. ^{1}H, ^{13}C, and ^{19}F NMR spectra are recorded using 400 and 500 MHz spectrometers in one of the following solvents: $CDCl_3$, D_2O, CD_3OD, DMSO-d_6, C_6D_6, and CD_3COCD_3 with TMS as internal standard. ^{13}C NMR spectral data have been reported with two digits after the decimal to distinguish between close resonances. The polymerizable lipids were stored in the absence of light at −20 °C in chloroform solution under nitrogen. Melting points are reported for the compounds which have sharp melting points.

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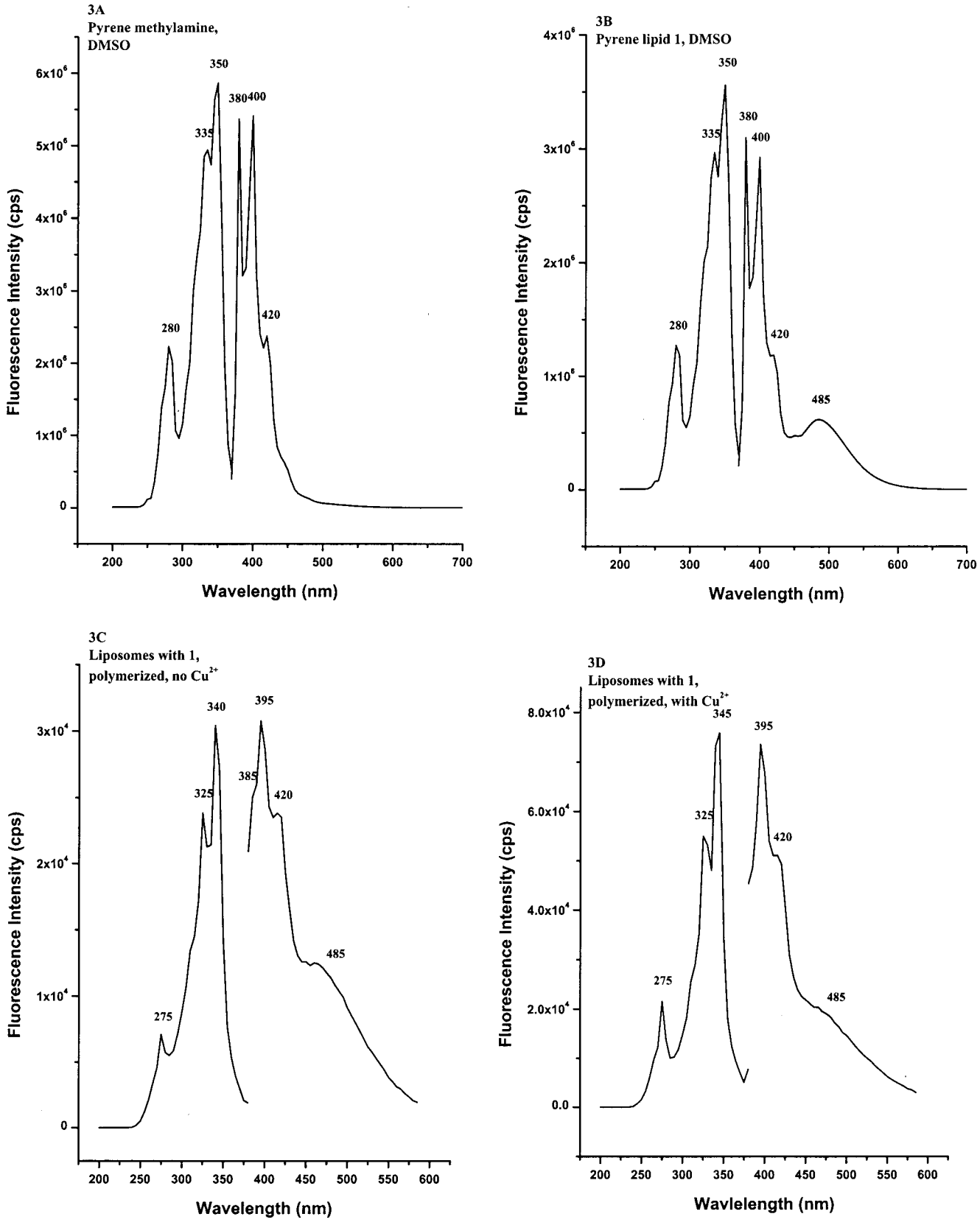

Figure 3. Fluorescence spectra of pyrenemethylamine (A), lipid **1** (B), and polymerized liposomes incorporating this lipid without (C) or with (D) Cu^{2+} added during liposome fabrication.

Synthesis. Polymerizable Acid 8. Amine ester **7**[10e] (1.3 g, 4.39 mmol) in 50 mL of acetonitrile was added to a solution of 10,12-docosadiynedioic acid (**6**; 1.59 g, 4.39 mmol), BOP reagent (1.94 g, 4.39 mmol), and Et_3N (2.1 mL, 3.5 equiv) in 70 mL of acetonitrile at room temperature over a period of 10 h (5 mL/h) by syringe pump. Thereafter, the reaction was continued for another 12 h at room temperature and then quenched with a saturated solution of NaCl. The compound was extracted by ethyl acetate. The combined organic layer was successively washed with 4% citric acid, water, 4%

Table 1. Fluorescence Intensities[a] and Intensity Ratios of Pyrene Monomer (380 nm) and Excimer (485 nm) Peaks

wavelength (nm)	pyrenemethylamine	pyrene lipid **1**	liposome with **1**, polymerized, no Cu^{2+}	liposome with **1**, polymerized, with Cu^{2+}
380	5212087	2939947	*b*	*b*
395	*b*	*b*	26994	69804
485	*c*	546699	10461	16900
485/380	*c*	0.186	*b*	*b*
485/395	*b*	*b*	0.388	0.242

[a] Fluorescence intensities correspond to background-subtracted intensities (net analyte signals). Net analyte signals are reported in counts per second (cps). The relative standard deviations for three measurements ($N = 3$) of fluorescence intensities and blank intensities are 3% and 5%, respectively. Deoxygenated solutions (N_2 bubbling) were used for fluorescence measurements. [b] No measurement was performed. [c] No net analyte signal was observed.

$NaHCO_3$ solution, and water. The organic layer was dried over anhydrous Na_2SO_4, and solvent was removed in vacuo at 40 °C to provide crude **8**. Purification was achieved by silica gel column chromatography with 3% methanol in $CHCl_3$ as the elutant ($R_f = 0.4$). Yield: 1.62 (61%). Semisolid. ^{1}H NMR (400 MHz, $CDCl_3-D_2O$): δ 1.19−1.35 (m, 22H), 1.44−1.51 (m, 4H), 1.56−1.62 (m, 4H), 2.14−2.23 (m, 6H), 2.28−2.33 (m, 2H), 2.96 (t, 2H, $J = 5.4$ Hz), 3.40−3.45 (m, 2H), 3.49−3.52 (m, 2H), 3.55 (s, 4H), 3.59−3.62 (m, 6H), 4.13 (q, 4H, $J = 7.1$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.34, 19.24, 24.76, 25.76, 28.28, 28.32, 28.34, 28.76, 28.83, 28.88, 28.91, 28.94, 29.03, 29.13, 29.28, 29.32, 29.35, 34.07, 36.71, 39.27, 53.52, 55.94, 60.64, 65.31, 65.35, 65.38, 70.03, 70.24, 70.36, 77.33, 77.55, 77.57, 173.74, 178.54, 179.01.

Compounds 10a and 10b. Coumarin or Rhodamine B (5.26 mmol) and BOP reagent (2.33 g, 5.26 mmol) were dissolved in CH_3CN (50 mL) followed by the addition of Et_3N (1.9 mL, 14 mmol). A solution of mono-*N*-Boc-ethylenediamine[11] (0.84 g, 5.26 mmol) in $CHCl_3$ (15 mL) was added, and the solution was stirred at room temperature for 10 h. The workup procedure was the same as described for **8**.

Compound 10a. 10a was purified by silica gel column chromatography with 2% MeOH in $CHCl_3$ as the eluant ($R_f = 0.4$) to yield a red solid. Yield: 1.65 g (94%). Mp: 142−143 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (s, 9H), 3.33−3.39 (q, 2H, $J = 9.0$ Hz), 3.46 (q, 2H, $J = 9.0$ Hz), 4.97 (br s, 1H), 7.33−7.40 (m, 2H), 7.63−7.69 (m, 2H), 8.88 (s, 1H), 8.97 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.60, 40.24, 40.79, 46.75, 116.86, 118.38, 118.70, 125.55, 130.06, 134.39, 148.71, 154.65, 161.49, 162.50.

Compound 10b. Product was purified by silica gel column chromatography with 2% methanol in $CHCl_3$ ($R_f = 0.4$) to afford the pure product as a pink solid. Yield: 2.64 g (81%). Mp: 62−64 °C. ^{1}H NMR (400 MHz, $CDCl_3-D_2O$): δ 1.15 (t, 12H, $J = 7.1$ Hz), 1.38 (s, 9H), 2.89−2.92 (m, 2H), 3.27−3.35 (m, 10H), 6.25 (dd, 2H, $J = 2.4$, 8.8 Hz), 6.36 (d, 2H, $J = 2.4$ Hz), 6.42 (s, 1H), 6.44 (s, 1H), 7.05 (dd. 1H, $J = 2.5$, 6.2 Hz), 7.42 (dd, 2H, $J = 3.0$, 5.9 Hz), 7.89 (dd, 1H, $J = 3.0$, 5.9 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.81, 28.65, 38.56, 40.64, 41.17, 44.56, 97.93, 105.18, 108.41, 123.05, 124.02, 128.27, 128.72, 130.73, 132.83, 149.02, 153.38, 154.11, 155.99, 169.63.

Amines 11a and 11b. The Boc-protected amine (1.18 mmol) was dissolved in cold trifluoroacetic acid (5 mL) and stirred at room temperature for 3 h. Excess TFA was removed in vacuo, and crude product was repeatedly treated with dry CH_2Cl_2; the CH_2Cl_2 was removed under vacuum to remove residual TFA. The free amine was regenerated from TFA salt by passing through the weakly basic ion exchange column (Dowex 66) and eluting with methanol. Eluants were collected until pH 7.0. The free amine was obtained by evaporating the solvent in vacuo.

Coumarin Amine 11a. Yellow solid. Yield: 0.38 g (91%). ^{1}H NMR (400 MHz, D_2O): δ 3.21 (t, 2H, $J = 8.7$ Hz), 3.69 (t, 2H, $J = 8.7$ Hz), 7.33−7.41 (m, 2H), 7.67−7.73 (m, 2H), 8.68 (s, 1H). ^{13}C NMR (125 MHz, CD_3OD-D_2O): δ 41.35, 43.41, 115.08, 118.81, 121.38, 124.68, 129.67, 130.86, 132.70, 133.66, 168.38, 170.04.

Rhodamine Amine 11b. Red solid. Yield: 0.9 g (95%). ^{1}H NMR (400 MHz, $CDCl_3-D_2O$): δ 1.15 (t, 12H, $J = 7.0$ Hz), 2.70 (t, 2H, $J = 5.4$ Hz), 3.26 (t, 2H, $J = 5.4$ Hz), 3.32 (q, 8H, $J = 7.2$ Hz), 6.24 (d, 1H, $J = 2.7$ Hz), 6.27 (dd, 1H, $J = 2.7$, 9.5 Hz), 6.34 (d, 2H, $J = 2.4$ Hz), 6.40 (s, 1H), 6.42 (s, 1H), 7.06−7.08 (m, 1H), 7.44−7.47 (m, 2H), 7.85−7.87 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.81, 40.81, 41.18, 44.57, 97.88, 104.24, 108.61, 123.23, 124.09, 128.51, 128.61, 130.32, 133.21, 149.18, 153.45, 153.88, 170.25.

Pyrene Lipid 1. 1-Pyrenemethylamine hydrochloride salt (0.22 g, 0.83 mmol) was suspended in 15 mL of DMF in the presence of Et_3N (0.5 mL, 3.2 mmol), and BOP reagent (0.366 g, 0.83 mmol) was added. Polymerizable acid **8** (0.55 g, 0.83 mmol) was dissolved in $CHCl_3$ (20 mL) and added dropwise at room temperature. Stirring was continued for 20 h. $CHCl_3$ was removed in vacuo. The DMF solution was added to a saturated aqueous NaCl solution. A white precipitate appeared. The precipitate was filtered and successively washed with water, 4% aqueous citric acid, 4% aqueous $NaHCO_3$ and water. It was dried under vacuum at room temperature to yield the product diester. Yield: 0.63 g (87%). ^{1}H NMR (400 MHz, $C_6D_6-CDCl_3$): δ 0.93−0.96 (m, 6H), 1.15−1.29 (m, 20H), 1.55−1.69 (m, 4H), 1.91−2.07 (m, 8H), 2.91 (q, 2H, $J = 5.1$ Hz), 3.20−3.28 (m, 6H), 3.36−3.39 (m, 2H), 3.40−3.45 (q, 2H, $J = 5.4$ Hz), 3.58 (s, 2H), 3.59 (s, 2H), 3.88−3.94 (m, 4H), 5.08 (d, 2H, $J = 5.1$ Hz), 6.25 (br s, 1H), 6.45 (br s, 1H), 7.75−7.83 (m, 4H), 7.90−7.96 (m, 4H), 8.32−8.35 (d, 1H, $J = 9.4$ Hz). ^{13}C NMR (100 MHz, C_6D_6-DMSO-d_6): δ 14.16, 18.95, 25.86, 25.99, 28.31, 28.69, 28.72, 28.94, 29.25, 29.30, 36.09, 39.16, 39.32, 39.53, 39.74, 39.95, 40.37, 40.57, 41.20, 53.70, 55.69, 59.95, 66.15, 70.04, 70.09, 70.16, 70.53, 77.76, 123.89, 124.83, 124.87, 124.97, 125.29, 129.03, 130.77, 130.99, 131.42, 133.75, 171.11, 172.38, 172.59.

The ester (0.25 g, 0.28 mmol) was dissolved in benzene (10 mL), and a solution of LiOH (0.05 g, 1.2 mmol) in water (5 mL) was added. The stirring was continued for 12 h at room temperature. Benzene was removed in vacuo, and insoluble starting ester (0.025 g) was filtered off. The aqueous solution was acidified by 1 N HCl (pH 3.0), and a white solid precipitated. The solid was filtered and washed with water to afford the gray white lipid **1**. Yield: 0.165 g (78%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15−1.24 (m, 14H), 1.33−146 (m, 6H), 1.52−1.56 (m, 2H), 2.04 (t, 2H, $J = 7.4$ Hz), 2.14−2.26 (m, 8H), 2.83 (t, 2H, $J = 5.6$ Hz), 3.18 (q, 2H, $J = 7.0$ Hz), 3.39 (t, 2H, $J = 5.9$ Hz), 3.46−3.51 (m, 10H), 5.01 (d, 2H, $J = 5.4$ Hz), 7.68 (t, 1H, $J = 5.4$ Hz), 8.01−8.09 (m, 2H), 8.14 (s, 1H), 8.15 (s, 1H), 8.21−8.31 (m, 4H), 8.36−8.41 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.83, 18.87, 25.76, 25.88, 28.27, 28.30, 28.70, 28.76, 28.89, 29.12, 29.14, 29.17, 35.89, 35.93, 39.04, 40.72, 40.95, 53.81, 55.91, 65.93, 69.77, 69.88, 70.06, 70.10, 78.54, 78.56, 123.86, 124.52, 124.63, 125.19, 125.64, 125.73, 126.74, 130.67, 130.88, 131.38, 133.76, 172.56, 172.76, 173.00. Anal. Calcd for $C_{49}H_{63}N_3O_8.C_6H_6$: C, 73.56; H, 7.52; N, 4.68. Found: C, 73.37; H, 7.61; N, 4.62.

(Trifluoromethyl)coumarin Lipid 2. The coupling of polymerizable acid **8** (0.5 g, 0.75 mmol) and 7-amino-4-(fluoromethyl)coumarin (0.17 g, 0.75 mmol) was achieved with BOP reagent (0.33 g, 0.75 mmol) and Et_3N (0.4 mL, 2.87 mmol) using $CH_3CN-CHCl_3$ as the solvent mixture (10 mL/10 mL). The reaction mixture was stirred at room temperature for 15 h. The workup procedure was the same as described for **8**. Crude product was purified by silica gel column chromatography ($R_f = 0.4$, 2% MeOH in $CHCl_3$) to give a yellow fluorescent solid. Yield: 0.58 g (87%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.14−1.31 (m, 20H), 1.41−1.46 (m, 10H), 2.04

(t, 2H, J = 7.6 Hz), 2.25−2.28 (m, 6H), 2.81 (t, 2H, J = 5.5 Hz), 3.16 (q, 2H, J = 5.9 Hz), 3.34−3.41 (m, 2H), 3.44−3.49 (m, 6H), 3.53 (s, 4H), 4.05 (q, 4H, J = 7.1 Hz), 6.45 (s, 1H), 6.50 (s, 1H), 6.54 (s, 1H), 6.65 (d, 1H, J = 7.6 Hz), 7.36 (d, 1H, J = 7.8 Hz), 7.82 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$−CD_3-OD): δ 13.59, 18.65, 24.75, 24.80, 25.76, 28.23, 28.47, 28.55, 28.59, 28.73, 28.77, 28.83, 28.86, 28.94, 29.02, 29.05, 33.69, 33.88, 35.94, 39.10, 50.99, 53.55, 55.60, 65.22, 69.46, 70.00, 70.02, 70.05, 76.73, 99.62, 103.09, 107.16 (q, J = 6.1 Hz), 123.35 (q, J = 273.6 Hz), 126.11 (q, J = 2.4 Hz), 142.09 (q, J = 31.7 Hz), 154.08, 157.03, 161.19, 171.72.

The diester (0.36 g, 0.21 mmol) was dissolved in THF (10 mL), and LiOH (0.06 g, 1.44 mmol) in MeOH/H_2O (10 mL/2 mL) was added (color changed from yellow to red; again with stirring, it turned back to yellow). The resulting mixture was stirred at room temperature for 15 h. After standard workup (same as lipid **1**), a yellow fluorescent solid of lipid **2** was obtained. Yield: 0.28 g (83%). ^{1}H NMR (400 MHz, DMSO-d_6-D_2O): δ 1.15−1.32 (m, 16 H), 1.38−1.51 (m, 8H), 2.05 (t, 2H, J = 7.4 Hz), 2.18 (t, 2H, J = 7.4 Hz), 2.24−2.30 (m, 4H), 3.01 (t, 2H, J = 4.8 Hz), 3.16 (t, 2H, J = 5.4 Hz), 3.38 (t, 2H, J = 5.8 Hz), 3.43−3.48 (m, 6H), 3.54 (s, 4H), 6.41(s, 1H), 6.51 (d, 1H, J = 1.6 Hz), 6.64 (d, 1H, J = 8.9 Hz), 7.34 (d, 1H, J = 8.9 Hz). ^{19}F NMR (376 MHz, DMSO-d_6): δ 200.15. ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD): δ 18.35, 24.66, 24.77, 25.67, 28.18, 28.50, 28.64, 28.69, 28.74, 28.78, 28.86, 28.89, 28.95, 33.44, 33.58, 35.69, 39.00, 50.64, 54.70, 65.05, 65.92, 69.38, 69.88, 70.03, 76.48, 76.51, 99.32, 103.20, 108.30 (q, J = 6.1 Hz), 123.48 (q, J = 264.0 Hz), 126.09 (q, J = 2.4 Hz), 140.93 (q, J = 31.7 Hz), 153.57, 157.12, 159.55, 172.34, 173.44. Anal. Calcd For $C_{42}H_{56}F_3N_3O_{10}$: C, 61.53; H, 6.88; N, 5.13. Found: C, 61.37; H, 6.89; N, 4.88.

Disperse Orange Lipid 3. Disperse orange (0.36 g, 1.49 mmol) and polymerizable acid **8** (0.8 g, 1.20 mmol) were dissolved in $CHCl_3$/DMF (20 mL/20 mL) (a portion of the dye was insoluble), and then BOP reagent (0.53 g, 1.20 mmol) and Et_3N (0.5 mL, 3.6 mmol) were added. The resulting deep red solution was stirred for 15 h at room temperature. The insoluble part was filtered off, and the workup procedure was the same as described for **8**. Pure product was obtained by silica gel column chromatography (2% MeOH/$CHCl_3$, R_f = 0.6) to afford the diester as a red semisolid. Yield: 0.46 g (50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22−1.38 (m, 22H), 1.45−1.54 (m, 6H), 1.58−1.62 (m, 2H), 2.16 (t, 2H, J = 7.8 Hz), 2.20−2.24 (m, 6H), 2.97 (t, 2H, J = 5.4 Hz), 3.41−3.46 (m, 2H), 3.52 (t, 2H, J = 4.7 Hz), 3.56 (s, 4H), 3.61−3.63 (m, 6H), 4.15 (q, 4H, J = 7.2 Hz), 6.73−6.76 (m, 2H), 7.84−7.86 (m, 2H), 7.92−7.95 (m, 2H), 8.05 (s, 1H), 8.07 (s, 1H), 8.31−8.35 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.41, 25.13, 25.95, 28.51, 28.53, 28.97, 29.02, 29.11, 29.19, 29.29, 29.45, 29.51, 34.30, 36.87, 39.35, 51.71, 51.86, 51.88, 53.87, 55.93, 55.97, 65.44, 65.46, 70.25, 70.38, 70.44, 70.51, 77.72, 77.45, 114.76, 123.04, 124.91, 126.42, 172.08, 173.58, 174.53.

Hydrolysis of the diester (0.18 g, 0.2 mmol) was carried out with LiOH (0.08 g, 1.9 mmol) in THF/MeOH (3 mL/6 mL) at room temperature for 12 h as described for lipid **1**. Lipid **3** was obtained as a deep red semisolid. Yield: 0.14 g (83%). ^{1}H NMR (400 MHz, $CDCl_3$−D_2O): δ 1.23−1.36 (m, 16H), 1.45−1.52 (m, 4H), 1.56−1.63 (m, 4H), 2.16−2.24 (m, 6H), 2.29 (t, 2H, J = 7.5 Hz), 2.97 (t, 2H, J = 5.2 Hz), 3.41−3.45 (m, 2H), 3.51−3.55 (m 2H), 3.60−3.65 (m, 6H), 3.69 (s, 4H), 6.76 (d, 2H, J = 8.6 Hz), 7.86 (d, 2H, J = 8.6 Hz), 7.94 (d, 2H, J = 8.8 Hz), 8.33 (d, 2 H, J = 9.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD): δ 18.91, 24.70, 25.67, 28.12, 28.14, 28.56, 28.61, 28.70, 28.81, 28.86, 28.89, 29.06, 29.09, 33.91, 36.17, 38.97, 39.00, 51.37, 51.74, 53.79, 55.32, 65.06, 69.64, 69.84, 70.01, 70.19, 77.42, 114.68, 119.67, 122.34, 123.10, 124.48, 124.58, 126.56, 144.59, 147.26, 174.04, 174.69. Anal. Calcd. For $C_{44}H_{60}N_6O_{10}$·HCl: C, 60.72; H, 7.02; N, 9.66. Found: C, 60.60; H, 6.85; N, 9.85.

Coumarin Lipid 4. To a solution of amine **11a** (0.43 g, 1.44 mmol) and polymerizable acid **8** (0.8 g, 1.22 mmol) in $CHCl_3$/CH_3CN (20 mL/20 mL) was added BOP reagent (0.53 g, 1.22 mmol) followed by the addition of Et_3N (0.8 mL, 5.7 mmol). The resulting clear red solution was stirred for 12 h at room temperature. Solvent was evaporated in vacuo, and 30 mL of saturated solution of NaCl was added. A red solid precipitated. The rest of the workup was the same as for **1**. The crude product was purified by silica gel column chromatography with 2% MeOH in $CHCl_3$ (R_f = 0.3). Yield: 0.87 g (83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.16−1.35 (m, 22H), 1.42−1.51 (m, 4H), 1.54−1.65 (m, 4H), 2.14−2.24 (m, 8H), 2.97 (t, 2H, J = 5.5 Hz), 3.41−3.45 (m, 2H), 3.47−3.53 (m, 4H), 3.57 (s, 4H), 3.59−3.63 (m, 8H), 4.15 (q, 4H, J = 7.0 Hz), 6.09 (br s, 1H), 6.24 (br s, 1H), 7.37−7.42 (m, 2H), 7.66−7.71 (m, 2H), 8.89 (s, 1H), 9.05 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.36, 19.26, 19.28, 25.79, 25.97, 28.33, 28.35, 28.38, 28.84, 28.86, 28.88, 29.00, 29.04, 29.28, 29.30, 29.31, 29.36, 36.74, 36.85, 39.22, 39.53, 40.49, 53.61, 56.04, 60.61, 65.33, 70.07, 70.28, 70.54, 77.34, 77.58, 77.60, 116.79, 118.15, 118.64, 125.51, 129.94, 134.46, 154.51, 161.39, 162.92, 171.53, 173.35, 173.72.

Selective hydrolysis of the ethyl esters (0.25 g, 0.28 mmol) was achieved in LiOH solution (0.04 g, 0.95 mmol) in a CH_2-Cl_2−MeOH−H_2O mixture (5 mL/5 mL/2 mL). Stirring was continued for 12 h at room temperature. The workup procedure was the same as that described for **1**. Crude product was dried in vacuo. Product was suspended in water and filtered off to provide a gray solid. Yield: 0.15 g (64%). ^{1}H NMR (400 MHz, $CDCl_3$−CD_3OD): δ 1.18−1.32 (m, 16 H), 1.36−1.50 (m, 4H), 1.54−1.60 (m, 4H), 2.11−2.21 (m, 8H), 3.35 (t, 2H, J = 5.5 Hz), 3.39−3.45 (m, 4H), 3.50−3.57 (m, 4H), 3.58−3.62 (m, 4H), 3.69 (t, 2H, J = 5.1 Hz), 3.78 (s, 4H), 7.40−7.44 (m, 2H), 7.69−7.74 (m, 1H), 7.78−7.81 (m, 1H), 8.84 (br s, 1H), 8,82 (s, 1H), 9.13 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD): δ 18.77. 25.75, 28.23, 28.25, 28.65, 28.81, 28.84, 29.03, 29.10, 36.02, 36.14, 38.78, 39.08, 39.17, 39.30, 54.78, 55.93, 65.27, 66.54, 69.60, 69.93, 70.21, 76.94, 76.98, 116.42, 118.07, 118.56, 125.42, 130.02, 134.46, 148.47, 154.50, 161.12, 162.78, 169.38, 175.16, 175.43. Anal. Calcd for $C_{44}H_{62}N_4O_{11}$: C, 64.21; H, 7.59; N, 6.81. Found: C, 64.06; H, 7.57; N, 6.61.

Rhodamine Lipid 5. The coupling reaction between amine **11b** (0.34 g, 0.65 mmol) and polymerizable acid **8** (0.43 g, 0.64 mmol) was carried out with BOP reagent (0.29 g, 0.65 mmol) using Et_3N (0.4 mL, 2.8 mmol) as a base and $CHCl_3$ (25 mL) as the solvent. The reaction mixture was stirred at room temperature for 20 h. The workup procedure was the same as that for **8**. The crude product was purified with column chromatography (silica gel, 4% MeOH/$CHCl_3$, R_f = 0.2) to afford the pure compound as a pink solid. Yield: 0.58 g (80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.16 (t, 12 H, J = 7.1 Hz), 1.23−1.37 (m, 22 H), 1.44−1.51 (m, 4H), 1.53−1.62 (m, 4H), 2.05 (t, 2H, J = 7.6 Hz), 2.14−2.24 (m, 6H), 2.97 (t, 2H, J = 5.4 Hz), 3.01−3.05 (m, 2H), 3.27−3.35 (m, 10 H), 3.41−3.45 (m, 2H), 3.52 (t, 2H, J = 5. 0 Hz), 3.57 (s, 4 H), 3.61−3.65 (m, 6H), 4.15 (q, 4H, J = 7.0 Hz), 4.71 (br s, 1H), 6.13 (br s, 1H), 6.25 (d, 1H, J = 2.4 Hz), 6.27 (d, 1H, J = 2.4 Hz), 6.36 (d, 2H, J = 2.4 Hz), 6.40 (s, 1H), 6.42 (s, 1H), 6.80 (br s, 1H), 7.05−7.08 (m, 1H), 7.43−7.46 (m, 2H), 7.88−7.90 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.67, 14.32, 19.26, 25.75, 25.79, 28.41, 28.88, 28.90, 29.03, 29.29, 29.36, 36.63, 36.68, 36.74, 36.77, 39.09, 39.22, 40.22, 40.51, 40.63, 44.41, 53.64, 55.76, 60.64, 65.32, 65.35, 65.74, 70.17, 70.21, 70.32, 77.30, 77.54, 77.60, 97.85, 104.81, 108.31, 122.84, 123.94, 128.24, 128.46, 130.49, 133.82, 149.00, 153.33, 154.15, 169.99, 171.94, 173.20, 173.42.

Selective hydrolysis of the ethyl esters (0.35 g, 0.31 mmol) was performed with LiOH (0.04 g, 0.95 mmol) in THF−MeOH (4 mL/8 mL). The reaction time and workup were the same as for lipid **1**. Lipid **5** was obtained as a pink solid. Yield: 0.26 g (79%). ^{1}H NMR (400 MHz, DMSO-d_6−D_2O): δ 1.05 (t, 12H, J = 7.0 Hz), 1.14−1.27 (m, 16H), 1.32−1.45 (m, 8H), 1.82 (t, 2H, J = 7.4 Hz), 2.03 (t, 2H, J = 7.3 Hz), 2.15−0.2.25 (m, 4H), 2.81−2.89 (m, 4H), 3.01 (t, 2H, J = 7.1 Hz), 3.13−3.3.17 (m, 2H), 3.26−3.32 (m, 8H), 3.36 (t, 2H, J = 5.7 Hz), 3.45 (s, 4H), 3.49−3.53 (m, 6H), 6.32−6.37 (m, 6H), 6.96−7.00 (m, 1H), 7.47−7.52 (m, 2H), 7.76−7.79 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.59, 18.39, 25.44, 25.69, 28.17, 28.22, 28.48, 28.55, 28.71, 28.77, 28.83, 28.91, 29.02, 33.46, 33.63, 35.70, 35.72, 38.16, 39.03, 39.56, 44.19, 44.24, 54.54, 56.23, 65.16, 65.82, 69.42, 69.88, 70.07, 76.58, 97.90, 97.98, 104.77, 104.86, 108.34, 108.41, 122.24, 123.80, 128.23, 128.30, 130.47, 132.91, 148.76,

148.82, 148.87, 153.37, 153.41, 153.96, 169.48, 174.67, 174.72, 175.14. Anal. Calcd for $C_{62}H_{87}N_6O_{10}$: C, 69.18; H, 8.15; N, 7.81. Found: C, 69.06; H, 7.98: N, 7.58.

Amine Lipid 13. A solution of the diamine 2,2-(ethylenedioxy)bis(ethylamine) (3.25 g, 21.98 mmol) and Et_3N (0.3 mL, 2.19 mmol) in $CHCl_3$ was cooled to −15 °C. Another solution of polymerizable acid 8,10-henecicosadiynedioic acid (0.7 g, 2.19 mmol) and BOP reagent (0.97 g, 2.19 mmol) in CH_3CN/$CHCl_3$ (30 mL/10 mL) was added dropwise over 5 h through a syringe pump under nitrogen. The resulting solution was stirred at −15 °C for 6 h and then at room temperature for 12 h. A white precipitate formed. The precipitate was filtered, and the workup procedure was the same as for lipid **1**. Crude product was purified by silica gel column chromatography (15% MeOH in $CHCl_3$, $R_f = 0.1$). Yield: 0.75 g (76%). Waxy white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84 (t, 3H, $J = 7.0$ Hz), 1.20−1.30 (m, 16H), 1.32−1.39 (m, 4H), 1.45−1.49 (m, 4H), 1.57−1.63 (m, 2H), 1.69 (bs, 2H), 2.14 (t, 2H, $J = 7.5$ Hz), 2.19−2.22 (m, 4H), 3.40−3.44 (m, 2H), 3.49−3.54 (m, 4H), 3.57−3.61 (m, 4H), 6.30 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.33, 19.33, 19.40, 22.88, 25.75, 28.32, 28.54, 28.71, 28.39, 29.06, 29.31, 29.51, 29.68, 29.77, 32.09, 36.76, 39.29, 41.84, 65.37, 65.58, 70.18, 70.29, 70.45, 73.43, 77.47, 77.84, 173.18. Anal. Calcd for $C_{22}H_{48}N_2O_3$: C, 72.28; H, 10.78; N, 6.24. Found: C, 72.30; H, 10.47; N, 6.16.

Vesicle Preparation. Solid phosphocholine **12** (42.5 mg, 85%), amine **13** (2.5 mg, 5%), cationic lipid **14** (2.5 mg, 5%), and fluorescent lipid (2.5 mg, 5%) were dissolved in 25 mL of HPLC grade chloroform in a clean 250 mL round-bottom flask. A thin lipid film was formed inside the flask by slowly evaporating the solvent in vacuo on the rotary evaporator. The film was then dried under vacuum for 20 h. This film was hydrated by 20 mL of HEPES buffer (50 mM, pH 8.0, filtered through a 0.1 μm filter). The suspension was warmed to 55 °C in a water bath and then frozen in a cooling bath (cooled with ethylene glycol/dry ice). This freeze−thaw cycle was repeated six times. For metal loading, after the suspension was warmed to room temperature, 130 μL of $CuCl_2$ solution (11.7 mM) was added dropwise in 10 mL of lipid suspension with continuous mixing in a vortex mixer (∼70% of the total Cu^{2+} compared to the metal-chelating lipids). This step was omitted for liposomes without any metal ions. It was sonicated by a probe sonicator (power 50 W) under nitrogen for 30 min at 55 °C, then cooled to 25 °C at a rate of ∼0.25 °C/min, and equilibrated at room temperature for 1 h. Liposomes were passed through a 0.5 μm polycarbonate filter (Nucleopore) at 55 °C using an extruder (Lipex Biomembranes Inc., Vancouver, British Columbia, Canada) under nitrogen pressure at 60 psi (six times). The temperature of the extruder was maintained at 55 °C with a circulating water bath, and the collector temperature was kept at 42 °C.

After extrusion, the clear liposome solution was cooled to 25 °C at 0.5 °C/min. After equilibrating at 25 °C, both solutions (liposomes with and without metal ions) were centrifuged for 10 min at 3200 rpm. The clear solution was decanted from sedimented solid and transferred into a quartz reaction chamber. The gently stirring vesicle solution was polymerized under a slow stream of nitrogen (15 min of UV irradiation, 450 W) and then stored at room temperature.

Fluorescence Spectra. Fluorescence measurements were performed with a Fluorolog-3 spectrofluorimeter (ISA, Jobin-Yvon-Spex, model FL3-11) equipped with a continuous source (450 W xenon) for sample excitation. Two single-grating (1200 grooves/mm) spectrometers were used for wavelength selection. The detector was a photomultiplier tube (Hamamatsu, model R928) operating at room temperature in a photon-counting mode. The appropriate software (dM 3000, Spex Industries, Inc.) was used for automated scanning and luminescence data acquisition. All luminescence spectra were uncorrected for instrumental response.

Quartz cuvettes, 10 mm × 10 mm × 45 mm (Fisher Scientific Co.), were used in all fluorescence measurements. No attempts were made to remove oxygen from the analyte solutions. A flow of dry nitrogen—previously passed through a $CaSO_4$ bed—was constantly purged through the sample compartment of the spectrofluorimeter to minimize possible oxygen quenching. Before measurement, each sample was exposed to nitrogen purging in the sample compartment for 2 min. Sample signals were collected at 90° using long pass filters to eliminate second-order emission from the excitation monochromator.

TEM Sample Preparation. One drop of the liposome solution was placed on a poly-L-lysine-coated (0.01%) grid and stained with phosphotungstic acid (negative staining). The liposomes were examined using a JEOL JEM 100CX transmission electron microscope and photographed with Kodak 4489 film (black and white).

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Supporting Information Available: Fluorescence spectra for the lipids **2**, **3**, **4**, and **5**, in solution and incorporated into liposomes, and then polymerized in the absence and presence of cupric ions. This material is available free of charge via the Internet at http://pubs.acs.org.

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