

Synthesis of Metal-Chelating Lipids to Sensitize Lanthanide Ions

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Sensitization of lanthanide ions is important for lanthanide ion-based assays and sensing. To the best of our knowledge, there are very few reports of lanthanide ion sensitization after it is incorporated into the liposome surface. This paper describes the syntheses of several saturated and polymerizable metal-chelating lipids based on chelidamic acid. The lipids are synthesized either from (S)-ornithine or racemic 2,3-diaminopropanoic acid. These lipids as well as polymerized liposomes incorporating these lipids sensitize lanthanide ions. Liposomes from the lipid **18**-Eu³⁺ provided a probe that relies not only on the emission wavelengths of Eu³⁺ but also on a reproducible lifetime that can be used for protein identification.

Detection of peptides and proteins is important for diagnosis of diseases¹ and sensing of toxins,² bacteria³ and viruses.⁴ Fluorescence spectroscopy enjoys a special role in protein detection because of its sensitivity and versatility arising from the availability of a wide range of fluorophores.⁵ Protein sensing also plays a crucial role in the rapidly expanding field of proteomics, the study of a large number of proteins simultaneously.⁵

Many fluorescence-based protein assays employ lanthanide ion complexes.⁷ The lanthanide complexes in solution exhibit many properties well-suited for protein

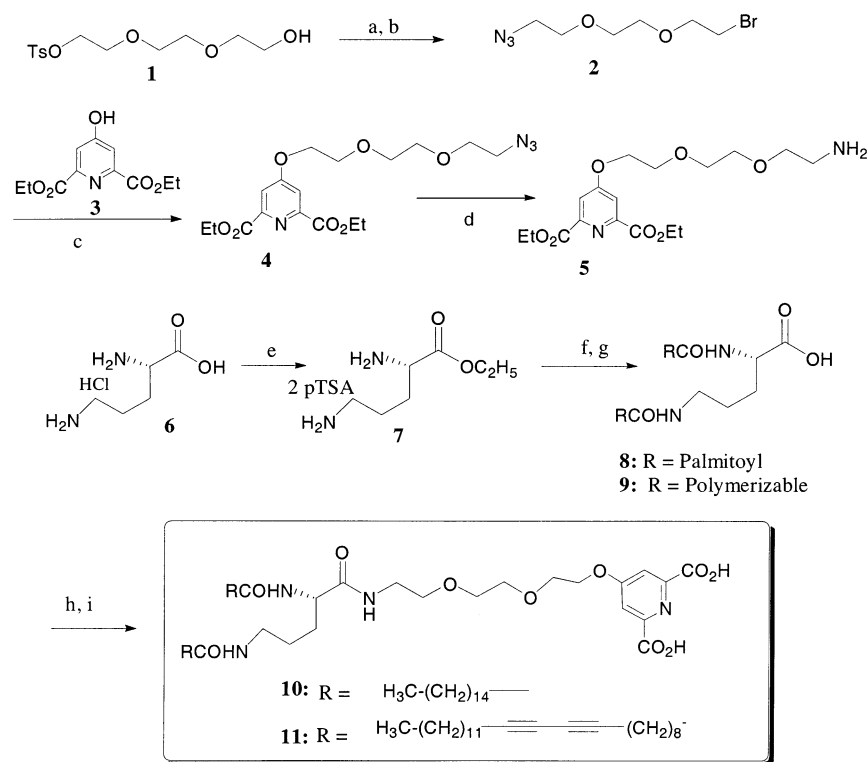
detection. The complexes usually have narrow emission bands, large Stokes' shifts, and long excited-state lifetimes.⁸ In addition, when the coordinating ligand is chiral, they can be used for chirality sensing of biological compounds.⁹

We are interested in employing polymerized liposome-bound lanthanide ions in protein detection. Liposome-based protein sensing systems often use nonpolymerizable liposomes² and rely on organic fluorophores. Polymerized liposomes with lanthanide ions have been extensively used as magnetic resonance contrast agents,¹⁰ but their potential to detect proteins remains unexplored. For this approach to be successful, two problems need to be addressed. Lanthanide ions have low molar extinction coefficients and the excited states are easily quenched by bound water molecules.⁸ To solve these problems, organic fluorophores have been extensively used to transfer excitation energy to the lanthanide ions.¹¹ These sensitizers are covalently attached to a polyvalent chelate for the lanthanide ions (to remove water molecules from the lanthanide ion coordination sphere). Noncovalent

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SCHEME 1. Structures and Syntheses of Lipids 10 and 11



Reagents and conditions: a) NaN_3 , DMF, 70 °C, 14 h, 94%; (b) PBr_3 , CHCl_3 , Reflux, 15 h, 74%; (c) Diethyl chelidamate (**3**), K_2CO_3 , DMF, 70 °C, 12 h, 90%; (d) H_2 , Pd/C (10%), MeOH, RT, 1 equiv HCl, 87%; (e) pTSA, EtOH, Reflux, 24 h, 93%; (f) Palmitoyl chloride (2 equiv), Et_3N , CH_2Cl_2 , RT, 8 h, 96%; or 10,12-pentacosadiynoic acid (2 equiv), BOP, Et_3N , CHCl_3 , RT, 10 h, 95%; (g) LiOH, THF-MeOH, 14 h, then pH = 3.0 with HCl; 99% (yield for **8**); 95% (yield for **9**); (h) **5**, BOP, Et_3N , CHCl_3 , RT, 10 h, 89% (for the saturated lipid); 95% (for the polymerizable lipid); (i) LiOH, THF-MeOH, RT, 12 h, then pH = 3.0 with HCl; 85% (for lipid **10**); 94% (for lipid **11**).

binding of sensitizers has been reported also to transfer energy to EDTA-bound Eu^{3+} and Tb^{3+} .¹²

We previously reported the photophysical properties of Tb^{3+} ions bound on polymerized liposomes by the EDTA headgroup.¹³ The conjugated dialkyne of the lipid or the ene-yne backbone of the polymerized liposomes were found to sensitize Tb^{3+} ions. However, due to the poor spectral overlap, energy transfer was modest.¹³

Herein, we report the synthesis and luminescence properties of several lipids (saturated as well as polymerizable) with lanthanide-chelating headgroups based on chelidamic acid.¹⁴ The lipids in solution, as well as when incorporated into liposomes, sensitize Eu^{3+} and Tb^{3+} ions efficiently. Due to the similarity of the results, only the Eu^{3+} system is described in detail here.

Results and Discussion

The structures of the lipids synthesized are shown in Schemes 1 and 2. The lipids are based on (*S*)-ornithine (Scheme 1) and racemic 2,3-diaminopropanoic acid (Scheme 2). For the saturated lipids (**10** and **17**), palmitic acid was used as the hydrophobic moiety; for the polymerizable lipids (**11** and **18**), a conjugated diacetylene was used in the fatty acid (10,12-pentacosadiynoic acid). The fatty acid and lipids from this acid form liposomes and can be polymerized by UV light.¹³ A diethylene glycol spacer separated the metal-chelating headgroup from the fatty acids conjugated to ornithine and 2,3-diaminopropanoic acid.

Chelidamic acid and iminodiacetic acid functionalized chelidamic acid (**15**) were used as the metal-chelating headgroup in these lipids. Chelidamic acid efficiently sensitizes Eu^{3+} and Tb^{3+} emissions.¹⁵ Chelidamic acid and other structurally related complexes have been used to label proteins.¹⁶ However, the ligand has only three

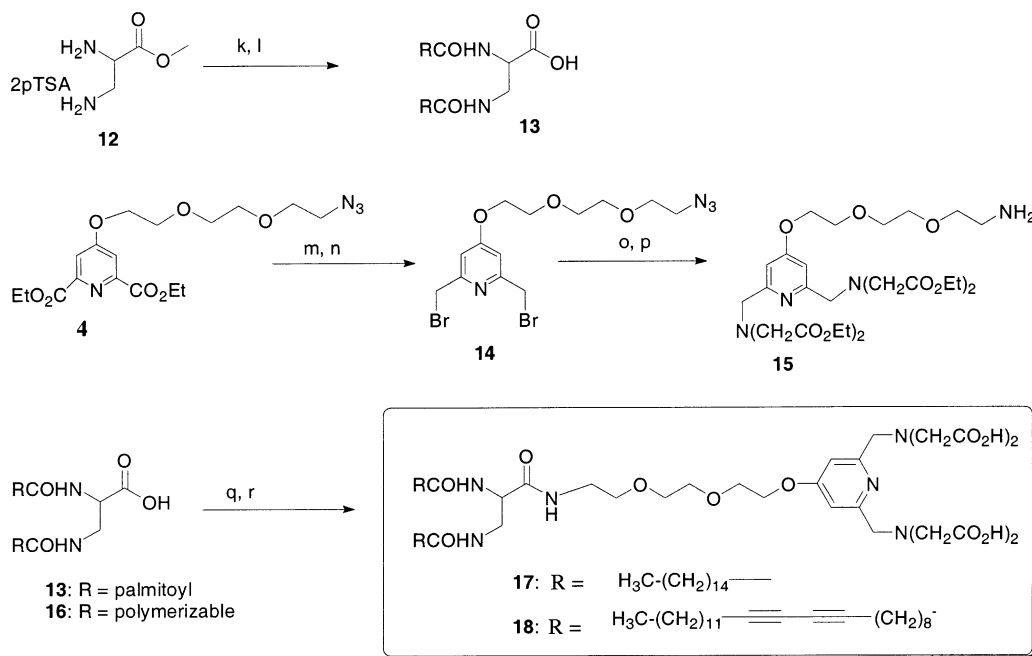
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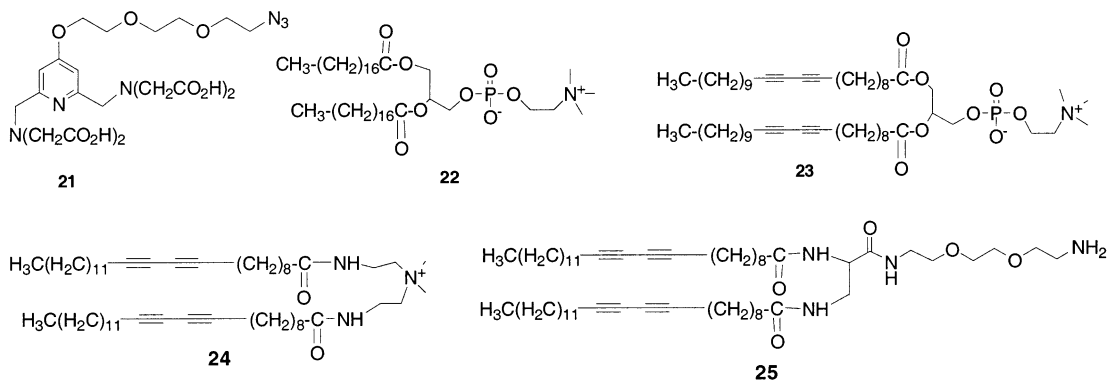
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SCHEME 2. Structures and Syntheses of the Diaminopropanoic Derived Lipids **17** and **18** and Structures of the Saturated (**22**) and Polymerizable (**23**) Phosphocholines, the Head Group **21**, Cationic Lipid (**24**), and Amine Lipid (**25**) Used in This Study^a

Reagents and conditions: (k) Palmitoyl chloride, Et_3N , CH_2Cl_2 , 5°C , 10 h, 99%; (l) LiOH , CH_2Cl_2 -THF-MeOH, 40°C , 40 h, 98%; (m) NaBH_4 , CaCl_2 , 25°C , 3 h, 68%; (n) PBr_3 , CH_2Cl_2 , reflux, 18 h, 88%; (o) $\text{HN}(\text{CH}_2\text{CO}_2\text{Et})_2$, K_2CO_3 , CH_3CN , reflux, 15 h, 97%; (p) H_2 , Pd/C (10%), MeOH, 25°C , 1 equiv of HCl , 20 h, 98%; (q) **15**, BOP, Et_3N , CHCl_3 , 25°C , 12 h, 72% for palmitoyl, 94% for the polymerizable; (r) LiOH , CH_2Cl_2 -THF-MeOH, 12 h, 25°C , 71% for palmitoyl, 85% for the polymerizable.



^a The syntheses of **21** and **25** are included in the Supporting Information. The synthesis of cationic lipid **24** has been reported previously.¹³

coordinating atoms for the lanthanide ions; so it is possible that two or more ligands bind to one lanthanide ion. On the other hand, ligand **15** (Scheme 2) has seven coordination sites that can bind lanthanide metal ions. The syntheses of the lipids **10**, **11** (ornithine derived) and **17**, **18** (diaminopropanoic acid derived) are shown in Scheme 1 and Scheme 2, respectively.

For the synthesis of ornithine-based lipids, selectively protected diethylene glycol **1**¹⁷ was elaborated to produce the bromo-azide **2**. The bromide was then displaced by the diethyl ester of chelidamic acid (**3**).¹⁸ The azide was

hydrogenated in the presence of 1 equiv of acid to produce the amine (the acid protonated the amine and prevented it from reacting with ester groups). This amine was then coupled with diacyl ornithine, using BOP as the coupling agent. Hydrolysis of the ester groups afforded the final lipids as white solids.

For the synthesis of diaminopropanoic acid derived lipids, the reduction of ester **4** to alcohol was carried out according to a literature procedure.¹⁸ The hydroxyl groups were then converted into the corresponding bromides with PBr_3 ; subsequently the bromides were replaced with

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diethyliminodiacetate. The azide was reduced to amine under neutral conditions to afford compound **15**. This compound was used as the common metal-chelating headgroup for saturated and polymerizable metal-chelating lipids. To synthesize the saturated lipid **17**, compound **15** was coupled with the dipalmityl amide of 2,3-diaminopropanoic acid (**13**). The ethyl ester groups were hydrolyzed to yield lipid **17**. Similarly, coupling of **15** with the unsaturated amide **16** followed by ester hydrolysis produced the polymerizable lipid **18**.

For luminescence studies with the headgroup only, compound **21** (structure shown in Scheme 2) was synthesized (Supporting Information). For this synthesis, di-*tert*-butyliminodiacetate **20** was synthesized from benzylamine **19** with excellent yields; after reaction with dibromide **14**, the *tert*-butyl groups were removed by HCl.

The saturated lipids can be stored at room temperature without any loss of purity. The polymerizable lipids were stored in the dark at -20°C .

Mixed liposomes (polymerizable) were prepared by using lipid **11** (or **18**) and 90% of polymerizable phosphocholine **23** by following a standard procedure¹³ (25 mM HEPES buffer, pH 7.0). Transmission electron microscopic studies indicated that the structures were closed vesicles (TEM pictures of the liposomes are included in the Supporting Information).

The resultant liposomes were incubated with a solution of EuCl_3 to form the complex. In this procedure, the Eu^{3+} ions are expected to be chelated to lipids on the outside surface of the liposomes. Alternatively, the preformed Eu^{3+} complexes of lipid **11** (or **18**) were used to form the liposomes. In these liposomes, the metal ions will be on the outside as well as on the inside surface of the liposomes. The diameter of the outer lipid layer is larger compared to the diameter of the inner lipid layer. On the basis of literature reports,¹⁹ it is estimated that about 60% of these metal ions will be on the outside surface of the liposomes.

A considerable amount of precipitation was observed during the liposome formation and polymerization when the chelidamic acid headgroup was used (lipid **11**). Transmission electron microscopic pictures also indicated liposome aggregation in this case (data not shown). Chelidamic acid has only three binding sites for the lanthanide ion. It is possible that one lanthanide ion may bind two chelidamic acid groups on two liposomes, causing liposome aggregation and precipitation. For the lipids with the modified chelidamic acid headgroup (**18**), no precipitation was observed during the liposome formation process and electron microscopic studies did not indicate any liposome aggregation. Similarly, liposomes were fabricated from the saturated lipids **10** or **17** (10% w/w) with distearoyl phosphocholine (**19**) and the headgroups were capable of sensitizing the emission from Eu^{3+} ions. However, polymerized liposomes are considerably more stable compared to liposomes from saturated lipids.^{10e} The increased stability is advantageous for protein sensing. Therefore, the focus of this paper is this property of the polymerized liposomes, and the saturated liposomes are not discussed in detail.

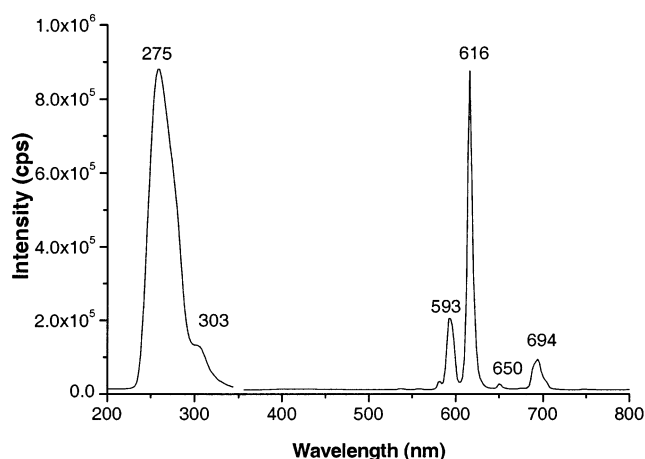


FIGURE 1. Excitation ($\lambda_{\text{em}} = 616\text{ nm}$) and steady-state emission ($\lambda_{\text{ex}} = 275\text{ nm}$) spectra of chelidamic acid and EuCl_3 mixture at the 1:1 molar ratio (final concentrations were 10^{-4} M , 25 mM HEPES buffer, pH 7.0). Spectra were recorded with the following parameters: excitation band-pass = 6 nm, emission band-pass = 4 nm, cut-off filter at 350 nm.

Spectral Characteristics of the Chelidamic Acid– Eu^{3+} Complex. Since the fluorescence emission of chelidamic acid partially overlaps with one excitation peak of Eu^{3+} (maximum wavelength at 396 nm), the possibility of energy transfer from the chelating agent (donor) to the lanthanide ion (acceptor) exists (the excitation and emission spectra of Eu^{3+} and chelidamic acid in HEPES buffer, 25 mM, pH 7.0, are shown in the Supporting Information). Figure 1 shows the excitation and emission spectra of the chelidamic acid– Eu^{3+} complex. The luminescence bands are characteristic of Eu^{3+} and correspond to the various transitions that occur from the $^5\text{D}_0$ to the ^7F manifold. The two intense peaks at 593 and 616 nm result from the transitions $^5\text{D}_0 \rightarrow ^7\text{F}_1$ and $^5\text{D}_0 \rightarrow ^7\text{F}_2$, respectively. The other peaks result from the transitions $^5\text{D}_0 \rightarrow ^7\text{F}_3$ (650 nm) and $^5\text{D}_0 \rightarrow ^7\text{F}_4$ (694 nm).⁸ The profile of the excitation spectrum resembles the absorption spectrum of the mixture (maximum absorption wavelength at 273 nm). This fact strongly suggests energy transfer from chelidamic acid to Eu^{3+} .

Another indication of energy transfer is the luminescence enhancement that occurs when the mixture is excited at 275 nm. When compared to the signal intensity of Eu^{3+} (see Supporting Information), the luminescence of the mixture is approximately $3\times$ higher. This is a significant enhancement considering that the concentration of Eu^{3+} in the mixture was 10 times lower than that in the standard. In aqueous solutions, water molecules coordinate to Eu^{3+} ions and cause efficient nonradiative deactivation of the excited $^5\text{D}_0$ level.⁸ The binding between chelidamic acid and Eu^{3+} removes water molecules from the coordination sphere of the lanthanide ion and sensitizes its luminescence. It is also interesting to note the change in the relative intensities of the 593- and 616-nm peaks. The $^5\text{D}_0 \rightarrow ^7\text{F}_2$ transition (616 nm) exhibits hypersensitivity to the chemical environment and certainly reflects the binding of chelidamic acid to Eu^{3+} .

Spectral Characteristics of the Lipid **11– Eu^{3+} Complex and the Corresponding Polymerized Liposomes.** Parts A and B in Figure 2 show the excitation and emission spectra of the lipid **11**– Eu^{3+} complex and

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the polymerized liposomes incorporating this complex (10% w/w), respectively. Comparison of spectrum A in Figure 2 to that of chelidamic acid (Supporting Information) shows that binding to the lipid does not affect the luminescence properties of the chelate. The comparison of spectra A and B in Figure 2 shows the appearance of a broad band (350–550 nm) in the emission spectrum of the liposome. The broad band results from the conjugation of diacetylene units after polymerization and cross-linking of the lipids.¹³ The broad band appears in the steady-state spectrum of the mixture but disappears in the time-resolved spectrum (Figure 2C). Because of its relatively short lifetime, a 30- μ s delay after the excitation pulse completely removes its contribution to the luminescence spectrum and provides a probe that relies only on the emission wavelengths of Eu^{3+} .

Luminescence Lifetime of Eu^{3+} in the Polymerized Liposomes from Lipid 11· Eu^{3+} . Table 1 shows the luminescence lifetime of Eu^{3+} in the polymerized liposomes with the lipid 11· Eu^{3+} complex. In all cases, single exponential decays were observed. Fitted decay curves ($y = y_0 + a_1 \exp^{-(x-x_0)/\tau_1}$) were obtained by fixing y_0 and x_0 at a value of zero. The residuals between the calculated and the observed points were less than 1% and showed no systematic errors. However, the sample-to-sample reproducibility (relative standard deviation ~12%) was unsatisfactory for achieving accurate protein sensing, our ultimate goal. The poor reproducibility was attributed to inter-liposome binding through Eu^{3+} ions and consequent liposome aggregation, a phenomenon later confirmed by TEM analysis (data not shown).

Luminescence Properties of the Lipid 18· Eu^{3+} Complex. The poor sample-to-sample reproducibility of liposome-bound Eu^{3+} lifetimes led us to modify the chemical structure of the chelate to bind Eu^{3+} (lipid 18). The spectral characteristics of this headgroup (compound 21, Scheme 2) and the Eu^{3+} complex (Supporting Information) are very similar to those from chelidamic acid. Liposomes were prepared from lipid 18 (10% w/w) and polymerizable phosphocholine 23 (total lipid concentration: 2 mg/mL, 50 mM HEPES buffer, pH 7.0) and polymerized; unlike liposomes from lipid 11, no precipitation of these liposomes was observed.

To determine the number of sites on the europium ion in 18· Eu^{3+} available for coordination with amino acids, the complex 21· Eu^{3+} was used. This number was determined as the number of water molecules (q) associated with the Eu^{3+} ion, using the equation $q = A_{\text{LN}}(\tau_{\text{H}_2\text{O}}^{-1} - \tau_{\text{D}_2\text{O}}^{-1})$, where A_{LN} is the proportionality constant (1.05) for europium, and $\tau_{\text{H}_2\text{O}}$ and $\tau_{\text{D}_2\text{O}}$ are the luminescence lifetimes of the complex in H_2O and D_2O , respectively.¹⁹ The lifetime values ($\tau_{\text{H}_2\text{O}} = 347 \pm 9 \mu\text{s}$ and $\tau_{\text{D}_2\text{O}} = 1102.5 \pm 114 \mu\text{s}$) used in the equation represent the average of six independent measurements. The standard deviations include the uncertainties of the statistical fittings and the random errors associated with sample-to-sample measurements.

The number of free coordination sites ($q = 2.07$) is in excellent agreement with stoichiometry studies. The stoichiometry of the 21· Eu^{3+} complex was determined by titrating 10^{-5} M solutions of 21 with Eu^{3+} while monitoring complex formation at the excitation wavelength (262

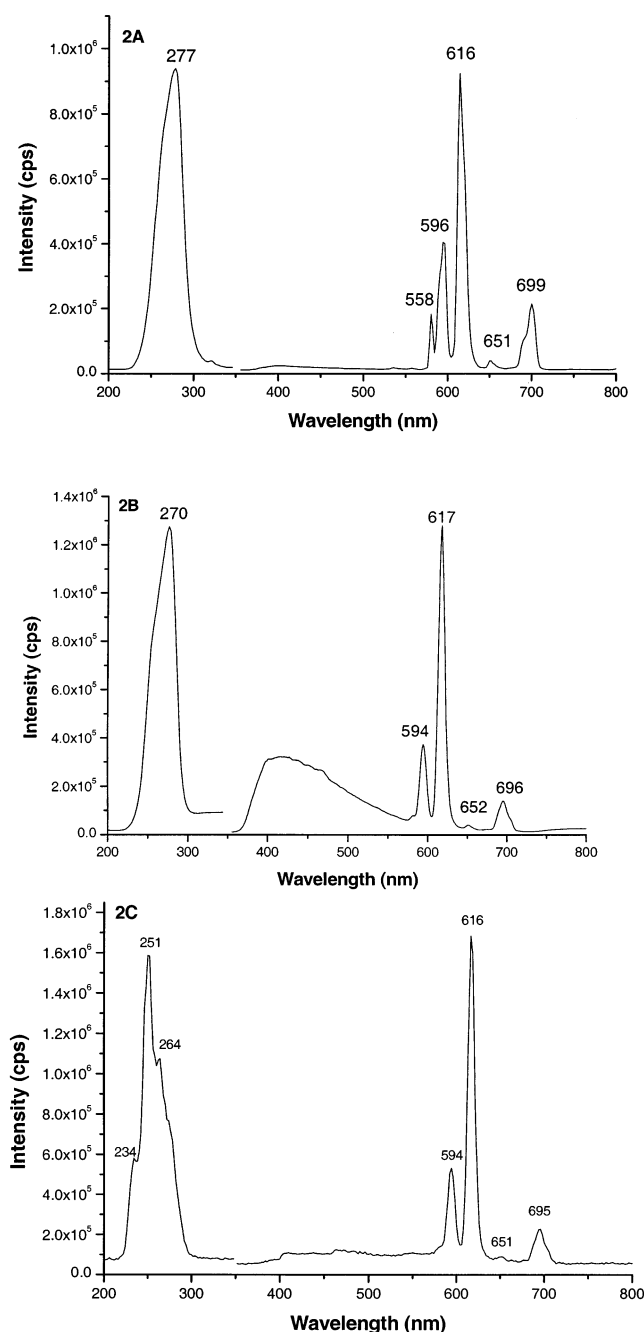


FIGURE 2. Excitation and steady-state emission spectra of the lipid 11· Eu^{3+} complex (A); the polymerized liposomes incorporating this complex (B) and time-resolved excitation and emission spectra of the polymerized liposomes (C). Solvents were methanol and HEPES buffer (25 mM, pH 7.0) for the lipid and the liposome, respectively. All spectra are blank subtracted. Spectra were recorded with the following parameters: (A) excitation band-pass = 4 nm, emission band-pass = 4 nm, cut-off filter at 350 nm; (B) excitation band-pass = 8 nm, emission band-pass = 6 nm, cut-off filter at 350 nm; (C) excitation band-pass = 16 nm, emission band-pass = 12 nm, cut-off filter at 350 nm, delay time = 30 μs , gate time = 9 ns, 100 pulses per data point. All excitation spectra were recorded at the maximum emission wavelength; all emission spectra were recorded at the maximum excitation wavelength.

nm) of energy transfer and one of the emission wavelengths (616) of the complex. It is clear from Figure 3 that a 1:1 complex is formed.

TABLE 1. Luminescence Lifetimes^a of Eu³⁺ in Liposomes from Lipid 11·Eu³⁺

sample	lifetime (μ s)
1	628
2	814
3	594
4	707
5	625
6	617
7	600
average	655 ± 79^b

^a Lifetime measurements were made at 266 (excitation) and 616 nm (emission) wavelengths. ^b Sample-to-sample standard deviation.

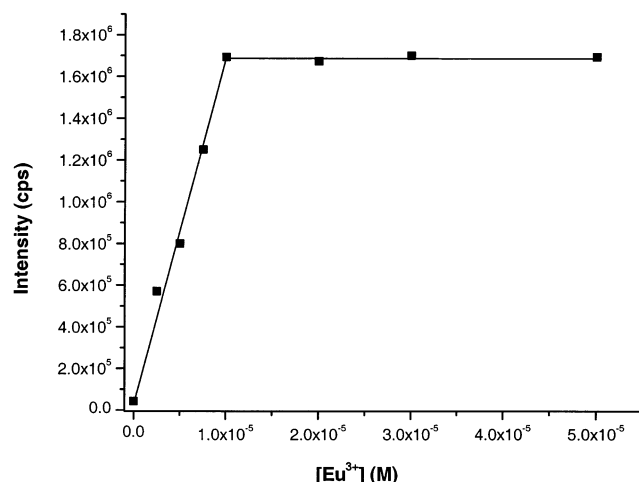
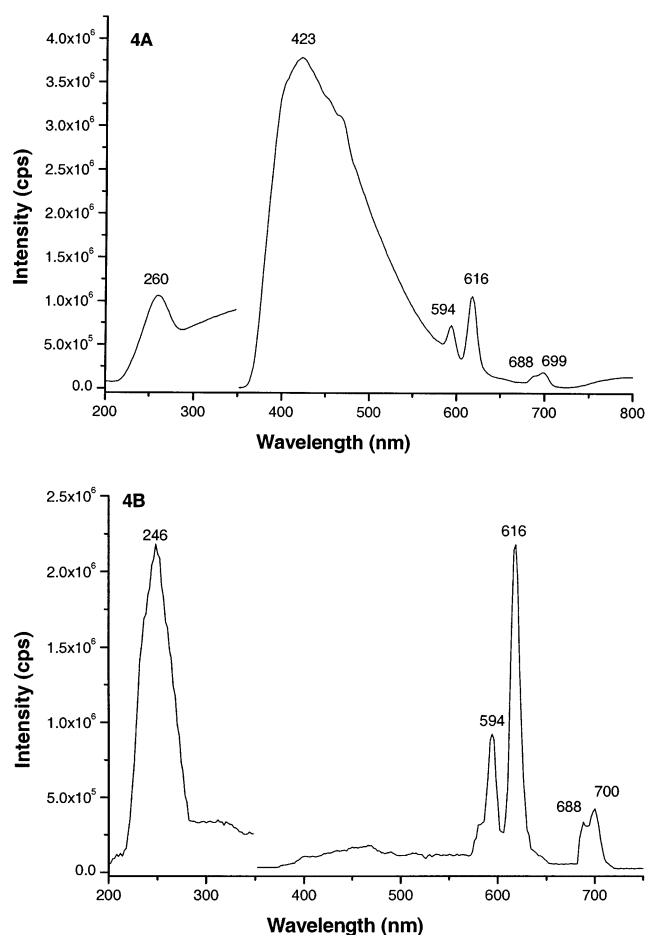
**FIGURE 3.** Luminescence intensity of **21** as a function of Eu³⁺ concentration. Measurements were taken from a 10^{−5} M **21**/0.3 M NaClO₄ prepared in HEPES buffer (25 mM, pH 7.0). Laser excitation and luminescence collection were at 268 and 616 nm, respectively.

Figure 4 shows the excitation and emission spectra of the polymerized liposomes incorporating lipid **18**·Eu³⁺ under steady-state (Figure 4A) and time-resolved (Figure 4B) conditions. Similarly to the lipid **11**·Eu³⁺ liposome, a 30- μ s delay after the excitation pulse completely removed the fluorescence contribution to the luminescence spectrum. Unlike the polymerized liposomes from lipid **11**·Eu³⁺, the lifetime of the Eu³⁺ for these liposomes can be determined reliably. The relative standard deviation of six lifetime measurements taken from six aliquots of the same liposome batch was 3.7% (Table 2).

In conclusion, the syntheses of four lipids capable of sensitizing Eu³⁺ emission are described. Liposomes from lipid **18**·Eu³⁺ provided a probe that relies not only on the emission wavelengths of Eu³⁺ but also on a reproducible lifetime that can be used for protein identification.

Experimental Details

Commercially available reagents were used as supplied unless stated otherwise. All aqueous solutions were prepared from Nanopure water. Experiments were conducted under an atmosphere of dry nitrogen. For workup, the organic layer was dried on anhydrous Na₂SO₄ and concentrated in vacuo. Melting points were recorded on a micro-melting-point apparatus, and all melting points are uncorrected. Melting points are reported for the compounds which have sharp melting points. Elemental analyses were performed by an in-house materials character-

**FIGURE 4.** Excitation and luminescence spectra of polymerized liposomes incorporating **18**·Eu³⁺ (10% w/w) recorded under steady-state (A) and time-resolved (B) conditions. Both spectra are blank subtracted (25 mM HEPES buffer, pH 7.0). Steady-state spectra were recorded with 20 and 8 nm excitation and emission band-pass, respectively. Time-resolved spectra were recorded with 0.03 ms and 0.1 ms delay and gate times, respectively. Excitation and emission band-pass were 11 and 4 nm, respectively. For part A, the excitation and emission spectra were recorded with λ_{em} = 616 nm and λ_{ex} = 260 nm; for part B, the spectra were recorded with λ_{em} = 616 nm and λ_{ex} = 246 nm.**TABLE 2. Luminescence Lifetimes^a of Eu³⁺ in Polymerized Liposomes from Lipid 18·Eu³⁺**

sample	lifetime (μ s)
1	356
2	328
3	334
4	349
5	354
6	359
average	347 ± 12^c

^a Lifetime measurements were made at 265.2 (laser excitation) and 616 nm (emission) wavelengths. Slit width: 500 μ m. Gate delay: 50 μ s. Gate width: 5 ms. Gate step: 50 μ s. ^b Standard deviation of statistical fitting. ^c Sample-to-sample standard deviation.

ization laboratory. TLC was performed with Absorsil Plus 1P, 20 $\times$ 20 cm plate, 0.25 μ m. Chromatography plates were visualized either with UV light or in an iodine chamber. ¹H, ¹³C NMR spectra are recorded with 300, 400, and 500 MHz spectrometers in one of the following solvents: CDCl₃, D₂O,

DMSO- d_6 , CD_3OD with TMS as internal standard. ^{13}C NMR spectra data have been reported with two digits after the decimal to distinguish between close resonances. ^{13}C NMR spectra of lipids **10** and **17** could not be recorded due to the poor solubility of these two compounds in common organic solvents.

Compound 2: The alcohol-tosylate **17** (13.0 g, 42.70 mmol) was dissolved in dry DMF and NaN_3 (3.70 g, 56.76 mmol) was added. It was stirred at 70 °C for 14 h and the solvent was then removed under vacuo. The solid was triturated with CH_2Cl_2 . The combined CH_2Cl_2 layer was removed under vacuo and it was pure enough to use for the next step without any purification. Yield: 7.8 g (94%). 1H NMR (500 MHz, $CDCl_3$) δ 3.58–3.62 (m, 4H), 3.64–3.68 (m, 4H), 3.71–3.76 (m, 4H), 4.21–4.24 (m, 1H).

The azide-alcohol (3.0 g, 17.14 mmol) was dissolved in dry $CHCl_3$ (20 mL) and PBr_3 (1.9 mL, 20.57 mmol) was added and refluxed for 15 h. It was then cooled to 5 °C and the organic layer was washed with aqueous 4% $NaHCO_3$ solution. The mixture was purified by silica gel column chromatography with 2% MeOH in $CHCl_3$ (R_f 0.8) to provide compound **2** as a liquid (3.02 g, 74%). 1H NMR (300 MHz, $CDCl_3$) δ 3.40 (t, 2H, J = 5.0 Hz), 3.48 (t, 2H, J = 6.2 Hz), 3.65–3.70 (m, 6H), 3.83 (t, 2H, J = 6.2 Hz).

Compound 4: The diethyl ester of chelidamic acid¹⁸ (**3**, 1.0 g, 4.18 mmol) was dissolved in dry DMF and then the bromo-azide **2** (1.2 g, 5.04 mmol) and anhydrous K_2CO_3 (0.7 g, 5.0 mmol) were added. The mixture was stirred for 12 h at 70 °C. The solid was filtered and compound was extracted by ethyl acetate. The purification was carried out by silica gel column chromatography with 2% MeOH in $CHCl_3$ (R_f 0.4) to obtain a viscous liquid. Yield: 1.49 g (90%). 1H NMR (500 MHz, $CDCl_3$) δ 1.42 (t, 6H, J = 7.1 Hz), 3.36 (t, 2H, J = 5.0 Hz), 3.63–3.68 (m, 4H), 3.70–3.73 (m, 2H), 3.90 (t, 2H, J = 4.5 Hz), 4.29 (t, 2H, J = 4.5 Hz), 4.43 (q, 4H, J = 7.1 Hz), 7.79 (s, 2H).

The azide-diester (0.95 g, 2.4 mmol) was dissolved in dry methanol (30 mL) and concentrated hydrochloric acid (0.25 mL, 2.4 mmol) was added. A small portion of 10% Pd/C was added and hydrogen was bubbled through the solution with stirring until the reaction was complete (checked via TLC). The solid Pd/C was filtered off and solvent was removed under vacuo to afford compound **4** as a viscous oil (0.85 g, 87%). 1H NMR (500 MHz, $CDCl_3$) δ 1.39 (br s, 6H), 3.20–3.95 (m, 12H), 4.31–4.42 (m, 4H), 7.75 (s, 2H), 8.01–8.08 (br s, 3H).

Compound 7: L-Ornithine hydrochloride (**6**, 4.0 g, 25.56 mmol) was dissolved in dry ethanol (50 mL) and $pTSA$ (15.7 g, 81.77 mmol) was added. The mixture was refluxed for 24 h. Solvent was removed under vacuo. The crude viscous liquid was recrystallized from ether. Yield: 12 g (93%). 1H NMR (500 MHz, DMSO- d_6) δ 1.20–1.24 (m, 3H), 1.54–1.62 (m, 1H), 1.64–1.69 (m, 1H), 1.72–1.83 (m, 2H), 2.29 (s, 6H), 2.76–2.84 (br s, 2H), 4.04–4.07 (m, 1H), 4.19–4.22 (m, 2H), 7.12 (d, 4H, J = 7.9 Hz), 7.47–7.50 (m, 4H), 7.75 (br s, 3H), 8.37 (br s, 3H).

Compound 8: L-Ornithine ethyl ester **7** (1.16 g, 2.32 mmol) was dissolved in dry $CHCl_3$ (25 mL) in the presence of Et_3N (1.9 mL, 13.92 mmol). Catalytic DMAP (20 mg) was added and the reaction mixture was cooled to 5 °C. Palmitoyl chloride (1.28 g, 4.65 mmol) was added dropwise and the mixture was stirred at room temperature for another 7 h. The reaction was quenched with water. The organic solvent was removed under vacuo at 45 °C and then cooled to 5 °C. The white solid was filtered and washed with water. Yield: 1.4 g (96%). 1H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, 6H, J = 7.0 Hz), 1.22–1.34 (m, 51H), 1.56–1.74 (m, 7H), 1.84–1.91 (m, 1H), 2.18 (t, 2H, J = 7.5 Hz), 2.27 (t, 2H, J = 7.6 Hz), 3.27–3.36 (m, 2H), 4.22 (q, 2H, J = 7.0 Hz), 4.51–4.62 (m, 1H), 5.95 (br s, 1H), 6.28 (d, 1H, J = 7.8 Hz). ^{13}C NMR (100 MHz, $CHCl_3$ - CD_3OD) δ 13.89, 22.60, 25.56, 25.68, 25.86, 29.13, 29.21, 29.29, 29.31, 29.37, 29.47, 29.58, 29.62, 31.85, 36.07, 36.41, 36.47, 38.72, 38.84, 52.09, 61.44, 172.83, 174.63, 174.83.

The ester (0.65 g, 1.02 mmol) was dissolved in CH_2Cl_2 /THF/MeOH (5/5/10 mL), solid $LiOH \cdot H_2O$ (86 mg, 2.05 mmol) was added, and the mixture was stirred for 14 h at room temper-

ature. The pH of the solution was adjusted to 3.0 by dilute HCl. The organic solvents were removed under vacuo, and solid was then filtered and washed with water to afford the compound **7**. Yield: 0.615 g (99%). 1H NMR (400 MHz, $CDCl_3$ - CD_3OD - D_2O) δ 0.819–0.938 (m, 6H), 1.22–1.35 (m, 48H), 1.50–1.74 (m, 7H), 1.80–1.91 (m, 1H), 2.12–2.19 (m, 2H), 2.21–2.26 (m, 2H), 3.15–3.26 (m, 2H), 4.38–4.45 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$ - CD_3OD) δ 13.86, 22.58, 25.25, 25.65, 25.84, 29.19, 29.24, 29.27, 29.30, 29.35, 29.45, 29.56, 29.61, 31.83, 36.18, 36.39, 38.72, 52.09, 52.18, 61.44, 174.48, 174.66, 174.83.

Compound 9: L-Ornithine ethyl ester **7** (2.0 g, 3.96 mmol) and 10,12-pentacosadiynoic acid (2.96 g, 7.92 mmol) were dissolved in dry $CHCl_3$ (30 mL), followed by the addition of BOP reagent (3.51 g, 7.92 mmol) and Et_3N (3.16 mmol, 22.71 mmol). Stirring was continued for 12 h at room temperature. The reaction mixture was quenched by addition of saturated NaCl solution. The solvent was removed under vacuo at room temperature. A white solid precipitated, which was filtered and washed with water. Yield: 3.3 g (95%). 1H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, 6H, J = 7.1 Hz), 1.20–1.36 (m, 51H), 1.43–1.53 (m, 8H), 1.56–1.65 (m, 7H), 1.82–1.86 (m, 1H), 2.13–2.17 (m, 4H), 2.22 (t, 12H, J = 6.9 Hz), 3.22–3.30 (m, 2H), 4.18 (q, 2H, J = 7.2 Hz), 4.56 (m, 1H), 5.96 (br s, 1H), 6.28 (d, 1H, J = 7.7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.38, 14.43, 18.62, 19.42, 19.44, 22.94, 25.75, 25.82, 26.01, 28.55, 28.59, 29.02, 29.11, 29.18, 19.35, 29.43, 29.49, 29.60, 29.73, 29.86, 29.87, 29.89, 30.36, 32.16, 36.73, 36.93, 39.08, 51.98, 61.85, 65.44, 65.53, 68.38, 77.66, 77.83, 84.93, 172.60, 173.51, 173.82.

The polymerizable ester (1.0 g, 1.15 mmol) was dissolved in THF/MeOH (10/10 mL) mixture and solid $LiOH \cdot H_2O$ (80 mg, 1.9 mmol) was added. The reaction mixture was stirred at room temperature. The work-up procedure was the same as described for **8**. Yield: 0.88 g (95%). 1H NMR (500 MHz, $CDCl_3$) δ 1.02 (t, 6H, J = 7.2 Hz), 1.33–1.55 (m, 48H), 1.62–1.69 (m, 8H), 1.72–1.80 (m, 6H), 1.84–1.89 (m, 1H), 2.03–2.07 (m, 1H), 2.30–2.42 (m, 16H), 3.44–3.51 (m, 2H), 4.70–4.73 (m, 1H), 6.51 (br s, 1H), 6.93 (d, 1H, J = 7.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.41, 18.64, 19.47, 22.96, 25.86, 26.04, 28.59, 28.63, 28.68, 29.07, 29.14, 29.19, 29.22, 29.38, 29.44, 29.49, 29.62, 29.75, 29.88, 29.90, 29.92, 32.18, 36.69, 36.94, 39.36, 52.30, 65.46, 65.53, 68.44, 77.70, 77.89, 84.97, 174.22, 174.58, 175.14.

Lipid 10: Compound **8** (0.37 g, 0.614 mmol) was dissolved in $CHCl_3$ (20 mL), followed by addition of Et_3N (0.4 mL, 2.87 mmol) and BOP reagent (0.275 g, 0.614 mmol). Compound **5** (0.25 g, 0.614 mmol) was dissolved in $CHCl_3$ (5 mL) and the solution was added dropwise to this reaction mixture. The reaction mixture was stirred at room temperature for 10 h. The work-up procedure was the same as described for lipid **8** ester. The crude product was purified by silica gel column chromatography with 8% MeOH in $CHCl_3$ (R_f 0.5) to provide 0.52 g (89%) of white powder. 1H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, 6H, J = 7.1 Hz), 1.20–1.29 (m, 48H), 1.47 (t, 6H, J = 7.1 Hz), 1.52–1.63 (m, 7H), 1.78–1.81 (m, 1H), 2.17 (t, 2H, J = 7.6 Hz), 2.22 (t, 2H, J = 7.6 Hz), 3.39–3.48 (m, 4H), 3.55–3.58 (m, 2H), 3.64–3.66 (m, 2H), 3.72–3.74 (m, 2H), 3.93 (t, 2H, J = 4.5 Hz), 4.34 (t, 2H, J = 5.0 Hz), 4.46–4.50 (m, 4H), 4.51–4.55 (m, 1H), 6.01 (br s, 1H), 6.54 (d, 1H, J = 7.9 Hz), 7.29 (br s, 1H), 7.84 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.34, 14.42, 22.90, 25.88, 26.02, 26.21, 29.52, 29.57, 29.74, 29.87, 29.91, 30.12, 32.12, 36.84, 37.05, 38.69, 39.45, 52.20, 62.62, 68.49, 69.40, 69.87, 70.54, 71.12, 114.65, 114.90, 164.90, 166.96, 172.11, 173.59, 174.09.

The nonpolymerizable lipid ester (0.25 g, 0.26 mmol) was dissolved in THF/MeOH (5/5 mL) and solid $LiOH \cdot H_2O$ (22 mg, 0.52 mmol) was added. The resulting mixture was stirred at room temperature for 12 h. The work-up procedure was the same as described for compound **7**, providing the lipid **10** as a white solid. Yield: 85% (0.20 g). $[\alpha]_D^{25}$ -5.2° (c 0.5, $CHCl_3$). 1H NMR (400 MHz, $CDCl_3$ - CD_3OD - D_2O) δ 0.89 (t, 6H, J = 7.1 Hz), 1.21–1.37 (m, 48H), 1.46–1.52 (m, 2H), 1.55–1.67 (m, 5H), 1.73–1.82 (m, 1H), 2.18 (t, 2H, J = 7.5 Hz), 2.24 (t, 2H, J = 7.5 Hz), 3.14–3.23 (m, 2H), 3.39–3.43 (m, 2H), 3.53–3.57

(m, 2H), 3.63–3.69 (m, 2H), 3.73–3.77 (m, 2H), 3.92–3.98 (m, 2H), 4.43–4.38 (m, 2H), 4.40–4.42 (m, 1H), 7.90 (s, 2H). Anal. Calcd for $C_{50}H_{88}N_4O_{10}$: C, 66.34; H, 9.80; N, 6.19. Found: C, 66.15; H, 9.68; N, 6.06.

Lipid 11: The coupling of acid **9** (0.46 g, 0.545 mmol) and amine **5** (0.225 g, 0.545 mmol) was achieved in the presence of BOP (0.245 g, 0.545 mmol) and Et_3N (0.3 mL, 2.15 mmol) in CH_2Cl_2 (25 mL). The reaction was carried out for 10 h at room temperature. The work-up procedure was the same as mentioned for **9** ester. The lipid was precipitated from water, filtered, and washed with water to afford lipid ester as a white solid (0.61 g, 95%). 1H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 6H, $J = 7.1$ Hz), 1.25–1.39 (m, 50H), 1.42–1.67 (m, 18H), 1.70–1.79 (m, 1H), 1.89–2.05 (m, 1H), 2.10–2.28 (m, 16H), 3.35–3.48 (m, 4H), 3.49–3.56 (m, 2H), 3.59–3.67 (m, 2H), 3.69–3.73 (m, 2H), 3.88–3.94 (m, 2H), 4.29–4.37 (m, 2H), 4.42–4.55 (m, 5H), 5.94 (br s, 1H), 6.49 (d, 1H, $J = 8.0$ Hz), 6.87 (br s, 1H), 7.82 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.29, 14.42, 18.64, 19.43, 22.90, 25.81, 25.94, 26.10, 28.57, 28.62, 28.67, 28.89, 29.02, 29.10, 29.15, 29.17, 29.32, 29.37, 29.40, 29.46, 29.51, 29.56, 29.70, 29.82, 29.85, 29.87, 30.68, 32.14, 36.77, 36.98, 38.71, 39.45, 52.19, 62.64, 65.53, 65.67, 68.52, 69.44, 69.91, 70.50, 71.16, 77.65, 77.85, 114.69, 150.33, 165.09, 166.99, 172.11, 173.57, 174.15.

The polymerizable lipid ester (0.5 g, 0.418 mmol) was dissolved in CH_2Cl_2 /THF/MeOH (4/2/6 mL) and solid $LiOH \cdot H_2O$ (61 mg, 1.45 mmol) was added. It resulting mixture was stirred at room temperature for 12 h. The work-up procedure was the same as described for lipid **10**. Yield: 0.385 g (81%). $[\alpha]_D^{25} -3.4^\circ$ (c 0.5, $CHCl_3$). 1H NMR (300 MHz, $CDCl_3$ - CD_3 -OD- D_2O) δ 0.92 (t, 6H, $J = 7.1$ Hz), 1.27–1.47 (m, 48H), 1.47–1.56 (m, 8H), 1.58–1.67 (m, 7H), 1.72–1.80 (m, 1H), 2.13–2.21 (m, 4H), 2.24–2.30 (m, 12H), 3.14–3.25 (m, 2H), 3.35–3.42 (m, 2H), 3.52–3.58 (m, 2H), 3.63–3.68 (m, 2H), 3.70–3.76 (m, 2H), 3.92–3.98 (m, 2H), 4.30–4.43 (m, 1H), 4.42–4.49 (m, 2H), 7.91 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.40, 18.65, 19.50, 22.98, 25.81, 25.97, 26.13, 28.67, 28.96, 29.11, 29.16, 29.25, 29.39, 29.47, 29.64, 29.77, 29.91, 29.94, 29.98, 32.21, 36.80, 36.90, 39.26, 39.70, 52.73, 65.54, 65.61, 68.51, 69.13, 69.93, 70.55, 71.21, 77.64, 77.71, 114.39, 148.62, 165.31, 168.83, 172.45, 174.37, 174.97. Anal. Calcd for $C_{68}H_{108}N_4O_{10} \cdot H_2O$: C, 70.36; H, 9.48; N, 4.82. Found: C, 70.85; H, 9.53; N, 4.61.

Lipid 11-Eu³⁺ complex: The polymerizable acid **11** (50 mg, 0.043 mmol) was dissolved in $CHCl_3$ /MeOH (2/2 mL) and solid $EuCl_3$ (17 mg, 0.043 mmol) was added with stirring at room temperature for 30 h. The solvent was removed under vacuo to afford a white solid as the Eu^{3+} complex. Yield: 55 mg (98%). Anal. Calcd for $C_{68}H_{112}N_4O_{13}EuCl_3 \cdot 3H_2O$: C, 58.96; H, 8.17; N, 4.06. Found: C, 59.14; H, 7.98; N, 3.68.

Compound 13: The racemic 2,3-diaminopropanoic ester (5.0 g, 10.82 mmol) was suspended in dry CH_2Cl_2 (60 mL), followed by the addition of DMAP (50 mg, 0.41 mmol) and Et_3N (8.5 mL, 61.1 mmol). The resulting solution was cooled to 5 °C, then palmitoyl chloride (5.8 g, 21.0 mmol) was added dropwise. Stirring was continued for another 8 h at room temperature. The reaction was quenched with ice cold water (80 mL). The organic solvent was removed under vacuo and white solid precipitated out. The precipitate was then filtered and washed with water successively. Yield: 6.37 g (99%). 1H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, 6H, $J = 7.1$ Hz), 1.19–1.33 (m, 48H), 1.56–1.63 (m, 4H), 2.16 (t, 2H, $J = 7.7$ Hz), 2.22 (t, 2H, $J = 7.7$ Hz), 3.61–3.64 (m, 2H), 3.75 (s, 3H), 4.57–4.61 (m, 1H), 6.15 (t, 1H, $J = 5.8$ Hz), 6.8 (d, 1H, $J = 5.3$ Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.35, 22.92, 25.77, 29.48, 29.61, 29.63, 29.75, 29.88, 29.90, 29.94, 32.16, 32.69, 36.81, 41.87, 52.98, 53.73, 171.00, 174.15, 174.99.

The ester (2.0 g, 3.36 mmol) was hydrolyzed with $LiOH$ (0.215 g, 5.12 mmol) in CH_2Cl_2 -THF-MeOH (10/10/20 mL). The work-up procedure was the same as described for lipid **8**. Yield: 1.92 g (98%). 1H NMR (400 MHz, $DMSO-d_6$) δ 0.86 (t, 6H, $J = 7.1$ Hz), 1.16–1.32 (m, 48H), 1.47–1.58 (m, 4H), 2.09 (t, 2H, $J = 7.5$ Hz), 2.14 (t, 2H, $J = 7.5$ Hz), 3.43–3.53 (m, 2H), 4.32–4.41 (m, 1H), 7.64 (br s, 1H), 7.72 (br s, 1H).

Compound 14: Compound **4** (1.39 g, 3.51 mmol) was dissolved in absolute ethanol (50 mL) and $NaBH_4$ (0.40 g, 10.25 mmol) was added. After hydrogen evolution ceased, $CaCl_2$ (1.16 g, 10.45 mmol) was added batch-wise with stirring. Stirring was continued for 3 h and reaction was quenched with water. Solvent was evaporated under reduced pressure and the white solid was triturated with CH_2Cl_2 . The combined CH_2Cl_2 layer was removed under reduced pressure. Yield: 0.95 g (86%). 1H NMR (400 MHz, $CDCl_3$ - D_2O) δ 3.38 (t, 2H, $J = 5.0$ Hz), 3.64–3.74 (m, 6H), 3.87 (t, 2H, 5.0 Hz), 4.18 (t, 2H, $J = 5.01$ Hz), 4.66 (s, 4H), 6.73 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 50.87, 64.41, 67.71, 69.58, 70.30, 70.90, 71.07, 105.76, 160.77, 166.46.

To a solution of the above alcohol (0.89 g, 2.25 mmol) in CH_2Cl_2 (20 mL) was added PBr_3 (1.22 g, 4.51 mmol). The resulting solution was refluxed with stirring for 18 h. The solution was neutralized with an aqueous solution of $NaHCO_3$ (5%) in ice cold condition. The organic solvent was washed with water and was dried on anhydrous Na_2SO_4 . Yield: 1.1 g (88%). 1H NMR (300 MHz, $CDCl_3$) δ 3.43 (t, 2H, $J = 5.0$ Hz), 3.70–3.97 (m, 6H), 3.91–3.95 (m, 2H), 4.26 (t, 2H, $J = 4.5$ Hz), 4.52 (s, 4H), 6.96 (s, 2H).

Compound 15: Dibromo compound **14** (0.71 g, 1.62 mmol) and diethyliminodiacetate (0.62 g, 3.28 mmol) were dissolved in CH_3CN and anhydrous K_2CO_3 (1.8 g, 12.9 mmol) was added. The mixture was refluxed for 15 h. The solid was filtered and solvent was removed under vacuo. The crude product was purified by silica gel column chromatography with 10% MeOH in $CHCl_3$ (R_f 0.4) to afford a yellowish viscous liquid. Yield: 1.03 g (97%). 1H NMR (400 MHz, $CDCl_3$) δ 1.27 (t, 12H, $J = 7.1$ Hz), 3.41 (t, 2H, $J = 4.9$ Hz), 3.49 (t, 2H, $J = 4.9$ Hz), 3.60 (s, 8H), 3.69–3.72 (m, 4H), 3.73–3.76 (m, 2H), 3.88 (t, 2H, $J = 4.8$ Hz), 3.99 (s, 4H), 4.14–4.21 (m, 8H), 7.10 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.81, 50.87, 67.89, 69.54, 70.33, 70.92, 71.14, 109.64, 158.34, 166.64, 171.21.

To a solution of the above azide-ester (0.66 g, 1.01 mmol) was added a small portion of Pd-black and the solution was stirred at room temperature for 20 h while H_2 gas was bubbled through (the completion of the reaction was confirmed by TLC). The Pd-black was filtered off and solvent was removed to afford compound **15** as a viscous oil. Yield: 0.625 g (98%). 1H NMR (300 MHz, $CDCl_3$ - D_2O) δ 1.29 (t, 12H, $J = 7.1$ Hz), 3.46–3.50 (m, 2H), 3.59–3.64 (m, 10H), 3.71–3.76 (m, 6H), 3.86–3.89 (m, 2H), 4.00 (s, 4H), 4.18 (q, 8H, $J = 7.1$ Hz), 7.11 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.09, 60.13, 60.72, 67.52, 69.60, 70.64, 71.01, 107.87, 160.33, 166.54, 171.40, 171.53.

Lipid 17: The coupling of nonpolymerizable acid **13** (0.2 g, 0.425 mmol) with amine **15** (0.35 g, 0.55 mmol) was carried out in the presence of BOP (0.19 g, 0.43 mmol) and Et_3N (0.2 mL, 1.43 mmol) in $CHCl_3$. The work-up procedure was the same as mentioned for lipid **9** (ester). The crude product was purified by silica gel column chromatography with 8% MeOH in $CHCl_3$. Yield: 0.43 g (72%). 1H NMR (500 MHz, $DMSO-d_6$) δ 0.90–0.95 (m, 6H), 1.22–1.26 (m, 12H), 1.28–1.39 (m, 48H), 1.49–1.58 (m, 4H), 2.09 (t, 2H, $J = 7.7$ Hz), 2.15 (t, 2H, $J = 7.5$ Hz), 3.57–3.62 (m, 12H), 3.64–3.69 (m, 6H), 3.89–3.85 (m, 4H), 3.91 (s, 4H), 4.13 (q, 8H, $J = 7.1$ Hz), 4.19–4.24 (m, 1H), 7.05 (s, 2H), 7.71 (br s, 1H), 7.75 (br s, 1H), 7.95 (br s, 1H).

The above ester (0.26 g, 0.24 mmol) was dissolved in CH_2Cl_2 -THF-MeOH (2/2/4 mL) and solid $LiOH$ (60 mg, 1.43 mmol) was added. The mixture was then stirred at room temperature for 15 h. The work-up procedure was the same as described for lipid **8**. Yield: 0.19 g (71%). 1H NMR (500 MHz, $DMSO-d_6$) δ 0.86–0.90 (m, 6H), 1.20–1.32 (m, 48H), 1.44–1.52 (m, 4H), 2.03–2.08 (m, 2H), 2.10–2.14 (m, 2H), 3.19–3.32 (m, 8H), 3.45 (s, 8H), 3.51–3.55 (m, 2H), 3.59–3.64 (m, 2H), 3.75–3.78 (m, 2H), 3.91 (s, 4H), 4.13–4.19 (m, 1H), 7.00 (s, 2H), 7.69 (br s, 2H), 7.78 (br s, 1H). Anal. Calcd for $C_{56}H_{98}N_6O_{14}$: C, 62.31; H, 9.15; N, 7.79. Found: C, 62.65; H, 9.30; N, 7.52.

Lipid 18: Polymerizable acid **16**¹³ (0.31 g, 0.38 mmol) was coupled with amine **15** (0.33 g, 0.525 mmol) in the presence of BOP reagent (0.17 g, 0.38 mmol) and Et_3N (0.1 mL, 0.72 mmol) in $CHCl_3$. The rest of the procedure was the same as mentioned for lipid **9** (ester). The crude product was purified by silica gel

column chromatography with 10% MeOH in CHCl₃. Yield: 510 mg (94%). ¹H NMR (500 MHz, CDCl₃) δ 0.89 (t, 6H, *J* = 7.1 Hz), 1.25–1.30 (m, 52H), 1.34–3.39 (m, 8H), 1.49–1.55 (m, 8H), 1.60–1.65 (m, 4H), 2.16–2.20 (m, 2H), 2.22–2.28 (m, 14H), 3.43–3.48 (m, 2H), 3.56–3.61 (m, 2H), 3.63–3.67 (m, 2H), 3.70–3.75 (m, 4H), 3.85–3.89 (m, 2H), 3.99 (s, 4H), 4.16–4.20 (m, 8H), 4.57–4.61 (m, 1H), 6.12 (br s, 1H), 6.72 (br s, 1H), 7.11 (s, 2H), 7.29 (br s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 14.40, 19.47, 22.96, 25.62, 25.81, 26.02, 28.59, 28.63, 28.67, 29.06, 29.14, 29.18, 29.37, 29.62, 29.76, 29.91, 32.19, 36.41, 36.51, 41.47, 56.03, 65.40, 65.45, 65.49, 65.54, 67.28, 69.15, 69.52, 70.29, 70.84, 77.63, 77.66, 77.81, 77.88, 106.02, 138.17, 157.92, 171.55, 176.43.

The polymerizable ester (0.192 g, 0.134 mmol) was hydrolyzed with LiOH (40 mg, 0.95 mmol). The work-up procedure was the same as mentioned before for compound **9**. Yield: 150 mg (85%). ¹H NMR (500 MHz, CDCl₃) δ 0.91 (t, 6H, *J* = 7.1 Hz), 1.25–1.34 (m, 40H), 1.36–1.41 (m, 8H), 1.50–1.58 (m, 8H), 1.60–1.65 (m, 4H), 2.25–2.32 (m, 16H), 3.32–3.54 (m, 4H), 3.57–3.68 (m, 12H), 3.81–3.90 (m, 4H), 4.23–4.42 (m, 6H), 4.46–4.55 (m, 1H), 6.66 (br s, 1H), 7.16 (s, 2H), 7.53 (br s, 1H), 7.92 (br s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 14.36, 19.44, 22.93, 25.58, 25.77, 25.98, 28.55, 28.59, 28.63, 29.02, 29.10, 29.14, 29.34, 29.36, 29.58, 29.72, 29.87, 32.15, 36.37, 36.47, 36.51, 41.35, 55.88, 55.92, 65.43, 65.48, 65.52, 65.56, 67.30, 77.62, 77.84, 105.58, 138.06, 157.57, 171.50, 176.46. Anal. Calcd for C₇₄H₁₁₈N₆O₁₄: C, 67.55; H, 9.04; N, 6.39. Found: C, 67.83; H, 9.13; N, 6.25.

Lipid 18-Eu³⁺ complex: The lipid **18** (50 mg, 0.03 mmol) was dissolved in CHCl₃/MeOH (1 mL/2 mL) and solid EuCl₃·6H₂O (14 mg, 0.03 mmol) was added with stirring at room temperature for 8 h. Solvent was removed under vacuo and a white solid was obtained. Yield: 57 mg (98%).

Vesicle Preparation. Solid phosphocholine **23** (30 mg, 81.5%), cationic lipid **24**¹³ (2.3 mg, 6.2%), lipid **18**-Eu³⁺ (2.25 mg, 6.1%) and amine **25** (synthesis shown in Supporting Information, 2.25 mg, 6.1%) were dissolved in a mixture of HPLC grade CHCl₃/MeOH (18 mL/2 mL) in a clean 250-mL round-bottomed flask. A thin lipid film was formed inside the flask by slowly evaporating the solvent under vacuo on the rotary evaporator at 40 °C. The film was then dried under vacuum for 20 h at room temperature. This film was hydrated by 15 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). This freeze-thaw cycle was repeated 10 times. The mixture was then sonicated by a probe sonicator (power 50 W) under nitrogen for 30 min at 55 °C, centrifuged (1500 rpm) for 5 min and 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 0.05-μm polycarbonate filter (Nucleopore) at 55 °C, using an extruder under nitrogen pressure at 80 psi (10 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, the solution was centrifuged for 5 min at 3200 rpm. The clear solution was decanted from sedimented solid and transferred into a quartz reaction chamber. The gently stirring solution was then polymerized (only polymerizable lipids) under a slow stream of nitrogen (15 min of UV irradiation, 450 W) and finally stored at room temperature.

Luminescence Spectra. Luminescence spectra were recorded with a commercial spectrofluorimeter with a continuous source (450 W xenon lamp) for sample excitation of steady state spectra. Time-resolved spectra were recorded with a pulsed-lamp phosphorimeter attachment, which provided signal-gating circuitry so that only a selected window of sample emission (gate time), after excitation (delay time), was allowed to reach the detector. The pulsed source consisted of a UV-vis Xenon lamp with adjustable pulse rate between 0.05 and 33 flashes/s. The full-width at half-maximum (fwhm) of each pulse was 3 μs. Two single-grating (1200 grooves/mm) spec-

trometers were used for wavelength selection. Their excitation and emission blaze wavelengths are 250 and 500 nm, respectively. The reciprocal linear dispersion of both monochromators was 4.2 mm⁻¹. The detector was a photomultiplier tube operating at room temperature in a photon-counting mode. Computer control was used for automated scanning and luminescence data acquisition. Sample signals and luminescence spectra were collected at 90°. Luminescence spectra were corrected for instrumental response, using the radiometric correction factors included in the XCORRECT and MCORRECT files of the spectrofluorimeter's software. When needed, long pass filters discriminated against second-order emission.

Quartz cuvettes, 10 mm × 10 mm × 45 mm, were used in all luminescence measurements. No attempts were made to remove oxygen from the analytical samples. The best reproducibility of measurements from chelidamic acid and Eu³⁺ mixtures was obtained after approximately 24 h of preparation time. During this period of time, the mixtures were kept in the dark at room temperature.

Luminescence Lifetimes. The luminescence lifetimes were determined via a three-step procedure: (1) full sample and background wavelength-time matrices collection; (2) the background decay curve was subtracted from the luminescence decay curve at the wavelength of maximum sample emission (616 nm); and (3) the background corrected data were fit to single exponentials. The excellent accuracy of this procedure was previously evaluated.²¹

Wavelength-time matrices were collected with a laser system previously developed for time-resolved luminescence spectroscopy.²² Ultraviolet output was generated by directing the output of a tunable dye laser through a KDP frequency-doubling crystal. An output wavelength range from 277 to 290 nm was generated with Rhodamine 6G dye (Exciton). When pumped with approximately 30 mJ from the 2nd harmonic generator of a Nd:YAG Q-switched laser, the dye laser produced more than 5 mJ at Rhodamine 6G peak wavelength in a spectral bandwidth less than 0.03 nm. The intensifier of the ICCD had a minimum gate time (full width at half-maximum) of 2 ns and the CCD chip had an active area of 690 × 256 pixels (26 μm² pixel size at photocathode). The ICCD was positioned at the exit focal plane of the spectrograph, which was equipped with a 1200 grooves/mm grating blazed at 500 nm. The pulse delay generator controlled the acquisition parameters (gate delay, gate width, and the gate step) of the ICCD. These parameters were introduced in the controlling computer and sent to the pulse delay generator via a GPIB interface. The acquired data were transferred from the detector head to the controller card (32-bit Intelligent Bus-Mastering PCI card) in the computer. A single optical fiber was used as the excitation probe to guide excitation energy from the dye laser to the sample. Luminescence was collected with an emission probe that consisted of six fibers arranged in a circle and a slit configuration at the analysis and measurement ends, respectively. At both ends, the fibers were bundled with Torr-Seal vacuum epoxy. All fibers were silica clad-silica, 500 μm core diameter, and 0.6 m long.

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Supporting Information Available: Synthetic details for di-*tert*-butyl iminodiacetate, compound **21**, and amine lipid **25**; fluorescence spectra for EuCl₃, chelidamic acid, compound **21**, and **21**-Eu³⁺ complex; TEM pictures of polymerized liposomes incorporating **11**-Eu³⁺ and **18**-Eu³⁺. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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