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Solid-Supported Synthesis of Polymerizable Lanthanide-Ion Chelating Lipids for Protein Detection

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Usually, lipids are synthesized employing solution-phase organic synthesis techniques. Though successful, the purifications can be difficult to accomplish due to the amphiphilic nature of the molecules. Herein, we demonstrate the advantages of a solid-phase approach for preparing a variety of metal-chelating lipids. A number of saturated and polymerizable metal-chelating lipids were prepared using this methodology. This approach requires one chromatographic purification after cleaving the lipids from the solid support. We also demonstrate that the resulting polymerized liposomes (containing Eu^{3+}) possess the appropriate luminescence properties for the qualitative and quantitative determination of proteins.

Introduction

Liposomes are spherical, bilayer assemblies of lipids with aqueous interiors and exteriors.[1] They can be prepared in a variety of sizes, and compounds can be encapsulated in the aqueous interior. Because of the ease of preparation and biocompatibility, liposomes have found many medical and nonmedical applications.[2,3] Most of the medical applications are in drug delivery, especially when active targeting and triggered release are needed.[2,4] Liposomes from cationic lipids are widely used in gene delivery.[5]

Polymerized liposomes are considerably more stable and less permeable compared to the liposomes prepared from saturated lipids.[6] Polymerized liposomes from conjugated diacetylenes have found numerous applications in colorimetric sensing of a variety of analytes.[7] However, there are not many examples of fluorescence sensing of analytes employing these liposomes.[8]

We are interested in the detection of proteins using the luminescence property of lanthanide ions on the surface of polymerized liposomes from conjugated diacetylene lipids.[9] The lanthanide ions have several advantages as probes over organic fluorophores. The emission bands are sharp and show large Stokes' shifts.[10] The excited-state lifetimes are longer compared to the organic fluorophores; this allows time-

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resolved detection of the lanthanide emission.[10] The inherently low molar extinction coefficients of these ions have led to the development of a large number of sensitizers.[10,11]

We have reported the syntheses of several polymerizable lipids capable of chelating lanthanide ions.[9] The synthetic approach we employed involved standard, solution-phase organic synthesis techniques. Unfortunately, the purification of intermediate compounds after each reaction step required lengthy chromatographic procedures. In addition, a number of the purifications were difficult to accomplish due to the amphiphilic nature of the molecules. In these studies, we propose a much simpler synthetic route based on a solid-phase approach.

Solid-phase synthesis enjoys several advantages compared to solution-phase methods: purification of the products from unreacted reagents and byproducts by filtration, possibility of automation, and synthesis of libraries of compounds. It has been widely used in the synthesis of peptides, nucleotides, carbohydrates, small organic molecules, and various combinatorial libraries.[12−16] The availability of a variety of polymeric supports and linkers makes this a very useful approach for organic synthesis.[17,18] Though there are many reported examples of solid-supported fatty acid conjugation to peptides and other biomolecules, examples of solid-supported lipid synthesis are relatively few in the literature.[19,20]

Herein, we report the solid-supported synthesis of several metal-chelating lipids containing the EDTA as the headgroup. These lipids are then used to chelate a lanthanide ion, Eu^{3+}. Using this methodology, we have varied the hydrophobic tails (saturated and polymerizable), the backbone (2,3-diaminopropanoic acid, ornithine, and lysine), and the spacer (oligoethylene glycols of two different sizes). The solid-supported protocol allowed us to rapidly synthesize these lipids with only one chromatographic purification step after cleavage of the lipid from the solid support. We also demonstrate that the resulting polymerized liposomes (containing Eu^{3+}) possess the appropriate luminescence properties for the qualitative and quantitative determination of proteins.

Results and Discussions

Solid-Phase Synthesis of the Lipids. The structures of the lipids synthesized are depicted in Figure 1. The saturated lipids (**3** and **5**) have stearic acid as the hydrophobic moiety; the polymerizable lipids (**1**, **2**, **4**, and **6**) have 10,12-pentacosadiyonic acid as the polymerizable group. Lipids **1** and **2** have the racemic 2,3-diaminopropanoic acid as the backbone. Lipids **3** and **4** incorporate (*S*)-ornithine; lipids **5** and **6** have (*S*)-lysine as the backbone. All of these lipids have oligoethylene glycols as spacers and EDTA as the metal-chelating headgroup. EDTA has very high affinity ($K > 10^{20}$ M^{-1}) for the lanthanide ions.[21]

The syntheses of the lipids incorporating the triethyleneglycol spacer (i.e., **2**−**6**) are shown in Scheme 1. Lipid **1** was synthesized in an analogous manner using **10** as the spacer, and this is not included in the scheme (Supporting Information). Detailed synthetic procedures for all of the reported lipids are included in the Experimental Procedures.

4-Methoxytrityl chloride resin was used as the solid support. This allowed us to cleave the lipid (after the synthesis was completed) by dilute trifluoroacetic acid and to recycle the resin. We observed that the resin can be used up to 3 times without any loss of lipid yield.

For the synthesis of suitably protected spacers, the amine-terminated oligoethylene glycol spacers **7** and **10** were reacted with 1 equiv of *tert*-butyldiphenylsilyl chloride (TBDPS-Cl). This generated the monoprotected and diprotected products (**8** and **9** from **7**; **11** and **12** from **10**, Scheme 1), with the monoprotected amines **8** and **11** as the major products. This mixture was reacted with the resin **13** without separation of the monoprotected amine from the diprotected product. The silyl protecting group was removed by tetra-*n*-butylammonium fluoride (TBAF) to generate the free amine functionality (**15**) on the polymer bound linker.

The diamino acids were coupled to this resin-bound amine linker using *O*-benzotriazol-1-yl-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxy benzotriazole (HOBT) as the coupling reagents. These reagents have been widely used in peptide synthesis[12] and were found to be the reagents of choice. After removal of the fluorenylmenthyl (Fmoc) groups, the fatty acids (saturated or polymerizable) were coupled to the resin-bound amine groups using the same coupling reagents. The lipid was then cleaved from the resin by dilute trifluoroacetic acid, the product was purified by

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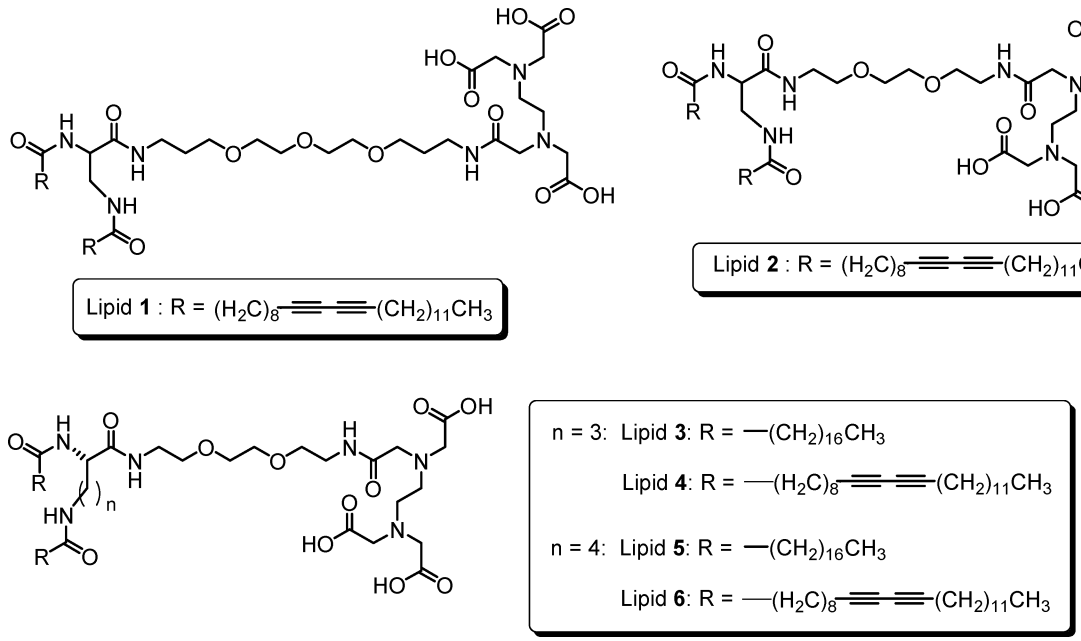

Figure 1. Structures of the metal-chelating lipids synthesized by solid-phase methodology are shown.

column chromatography, and the EDTA (ethylenediamine tetraacetate) headgroup was coupled following literature procedures.[9] The metal-chelating lipids were isolated by hydrolyzing the ester groups with LiOH, followed by lowering the pH to 3.0. The lipids were complexed to Eu^{3+} by adding solid $EuCl_3 \cdot 6H_2O$ to methanolic solutions of the lipids.[9] 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.

Preparation of Liposomes. Liposomes were prepared from the Eu^{3+} complexes of the synthesized lipids (10 wt %) and the commercially available polymerizable phosphocholine **PC1** (90 wt %, the structure is shown in Scheme 1) in 25 mM HEPES buffer, pH of 7.0. The liposomes were polymerized at 0 °C with UV light (254 nm), and the polymerization was followed by UV−vis spectrometry. The absorption for the dialkyne (240 nm) was found to be reduced to 90% of the original value after 15 min of irradiation. Transmission electron microscopic studies indicated that the liposome structures are retained after polymerization (Scheme 1). The polymerized liposomes were found to be stable at room temperature for more than a month. However, the unpolymerized liposomes were found to precipitate within 2 h; hence, no electron microscopic studies were performed with the unpolymerized liposomes.

In this manuscript, detailed photophysical data are provided for the polymerized liposomes from lipid **2**−Eu^{3+}. Liposomes from the other polymerizable lipids behaved similarly and these are not discussed in detail.

Spectral Characterization of Polymerized Liposomes. The long-lived luminescence lifetimes of Eu^{3+} and Tb^{3+} make them desirable probes for protein sensing.[10,22] Their long-lived decays are a good match to time-resolved luminescence techniques, which discriminate against short-lived background fluorescence and scattered excitation light. Because lanthanide's luminescence is typically weak in aqueous solvents, most sensing schemes typically employ a sensitizer (or antenna).[11] Sensitizers are usually organic molecules that strongly absorb and transfer excitation energy to the metal ion providing significant luminescence enhancements for analytical use. An additional benefit to chemically attaching a sensitizer to the lanthanide ion is the possibility to tune the excitation wavelength to a region of minimum protein absorption. Strong protein absorption usually occurs below 300 nm. Spectral bands are fairly broad, and typical full-widths at half-maximum may vary from 45 to 65 nm. This spectral region is undesirable for bioanalytical work as the assay may be prone to primary inner filter effects.

Our approach does not use a sensitizer. We take advantage of the backbone of the polymerized liposome to enhance the signal of the lanthanide ion and provide a tunable excitation wavelength range above 300 nm. Figure 2 shows the time-resolved excitation and luminescence spectra of EDTA−Eu^{3+} in 25 mM HEPES buffer (pH = 7.0). The luminescence bands are characteristic of Eu^{3+} and correspond to the various transitions that occur from the $^0D_5$ to the ^7F manifold.[9] Their maximum excitation is obtained at 266 nm, a wavelength region prone to strong protein absorption and other types of potential interferents. Luminescence excitation at wavelengths higher than 320 nm reduces potential protein inner filter effects. Sample excitation at 397 nm, that is, the maximum wavelength of the most intense excitation peak above 320 nm, reduces the luminescence intensity of the

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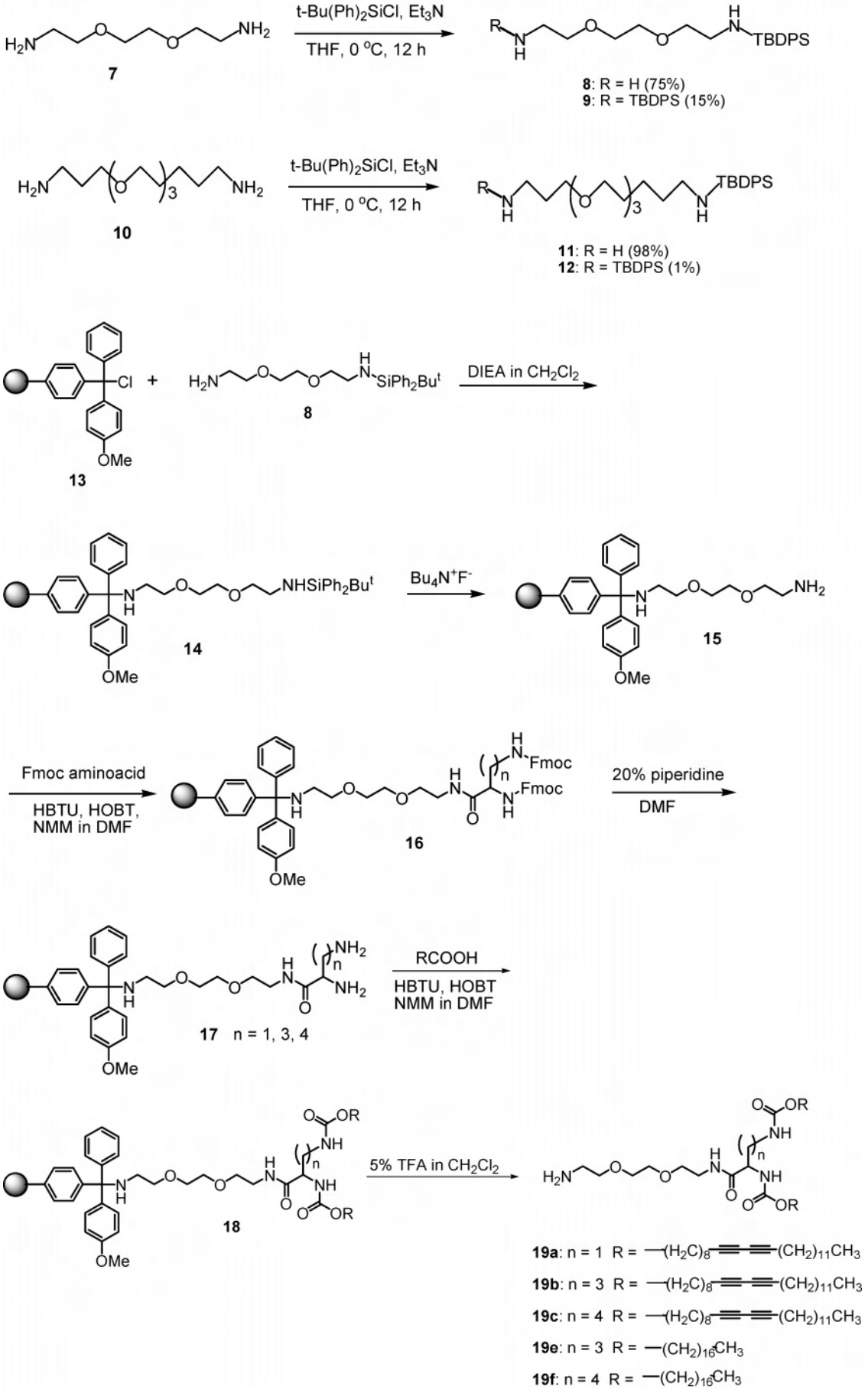
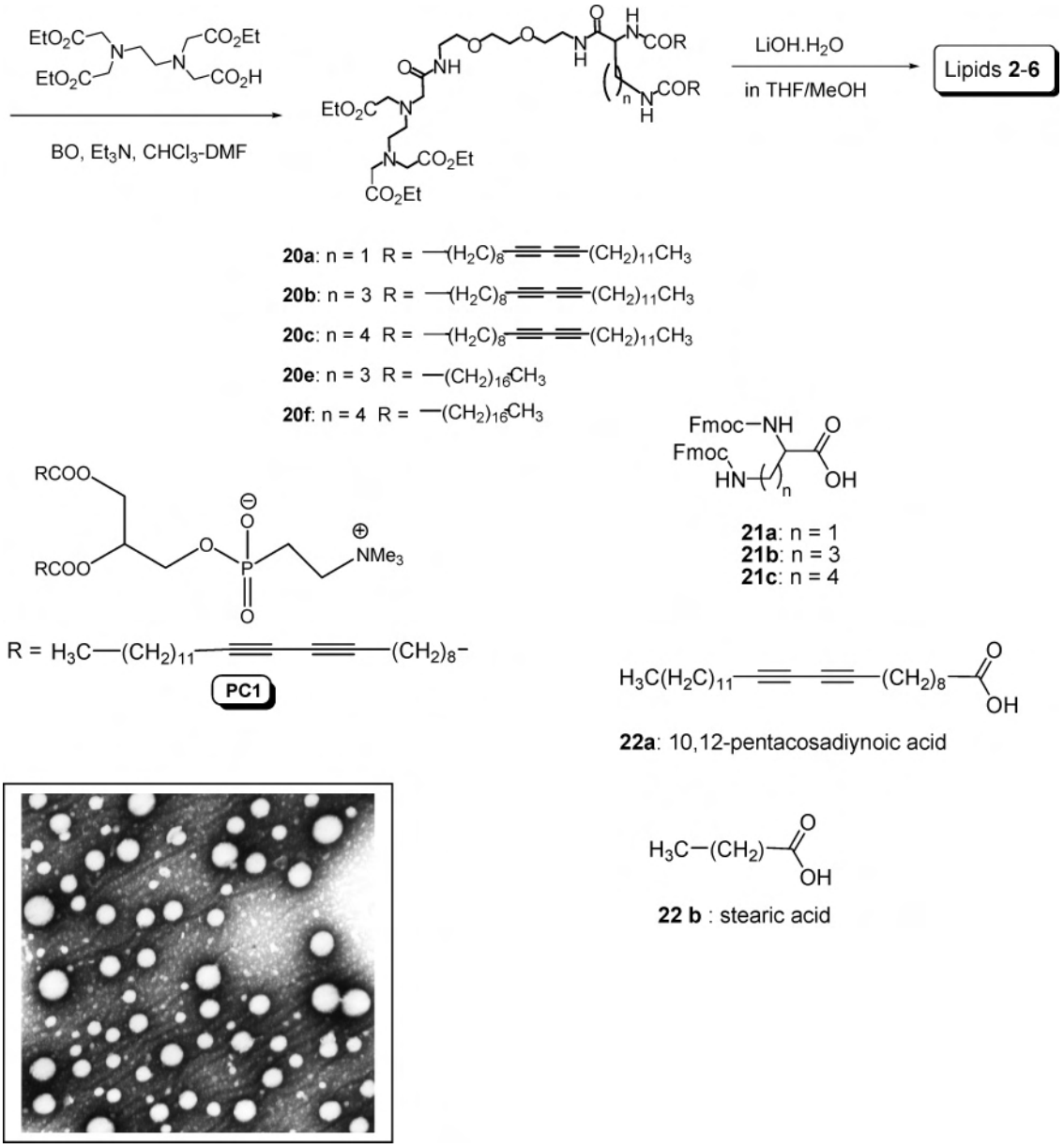
Scheme 1. Solid-Phase Syntheses of Lipids[a]



Scheme 1. (Continued)



[a]The structure of the commercially available polymerizable phosphocholine (**PC1**) used in this study to form the liposomes is also indicated. Transmission electron micorgraph of the polymerized liposomes incorporating 10% (by weight) of lipid **2**−Eu^{3+} and 90% of **PC1** is also shown (magnification, 46 400; scale, 1 mm in the picture corresponds to 21 nm). The average diameter was found to be ∼1000 Å.

lanthanide ion by approximately 33% but still provides a strong reference signal for protein analysis.

Figure 3A depicts the steady-state excitation and emission spectrum of the polymerized liposomes incorporating lipid **2**−Eu^{3+}. The broad excitation and emission bands correspond to the fluorescence of the liposome, which results from the conjugated eneynes. The luminescence contribution of Eu^{3+} appears in the form of a shoulder (592 nm) and a small peak (616 nm). The time-resolved excitation and emission spectrum of the liposomes (Figure 3B) confirms the presence of the lanthanide ion. A 150 μs delay removes the strong fluorescence from the liposome backbone and reveals the luminescence from Eu^{3+}.

Concentration of EDTA−Eu^{3+} in Polymerized Liposomes. The concentration of Eu^{3+} in the liposomes was estimated with the method of standard additions. This approach was the method of choice to compensate for potential matrix interference in the quantitation of EDTA−Eu^{3+}. Figure 4 shows the least-squares fit of the luminescence intensity as a function of effective analyte standard concentration $[nC_sV_s/(V_x + V_s)]$, where C_s is the concentration of standard, V_s is the volume of standard addition, V_x is the volume of aliquot sample, and n is the number of standard additions ($n = 0-5$). The luminescence intensities plotted in the graph were subtracted from the blank intensity, which corresponded to the average intensity of six measurements taken from a 25 mM HEPES buffer solution (pH = 7.0). Similarly, each point in the calibration graph corresponds to the average of six intensity measurements taken from six individual aliquots of standard solution. The correlation

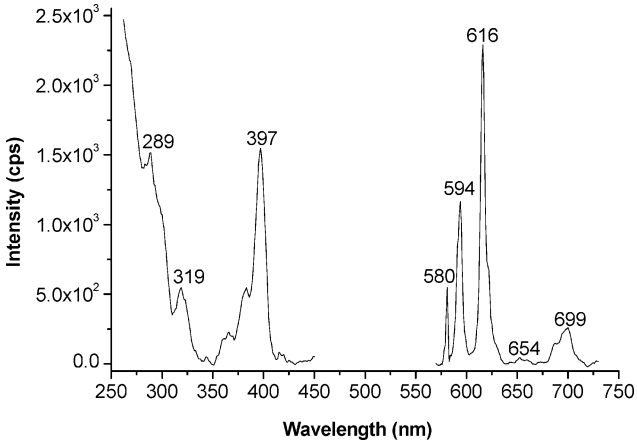


Figure 2. Timed-resolved excitation (250−450 nm) and emission (575−725 nm) spectra of 4.6×10^{-5} M EDTA−Eu^{3+} in 25 mM HEPES buffer (pH = 7.0). Spectra were blank subtracted and recorded with the following parameters: excitation band-pass = 10 nm, emission band-pass = 1.5 nm, cutoff filter at 450 nm, delay time = 150 μs, gate time = 1 ms, 50 pulses per data point. Emission spectrum was recorded upon excitation at 266 nm and excitation spectrum upon emission at 616 nm.

coefficient close to unity (0.9992) demonstrates the linear relationship between luminescence intensity and lanthanide ion concentration. The extrapolation of the linear plot to $y = 0$ provides the concentration of Eu^{3+} in the polymerized liposome (4.6×10^{-5} M). Because the liposome solution was diluted 100 times, the concentration of lanthanide ion in the original liposome sample was 4.6×10^{-3} M. It is interesting to note that a 4.6×10^{-5} M concentration of EDTA−Eu^{3+} in 25 mM HEPES buffer provides a luminescence signal approximately 10 times higher than the signal observed from the liposomes. The signal attenuation is attributed to the liposome absorption in the excitation region of the lanthanide ion and reinforces the use of the standard addition method.

Energy Transfer from the Conjugated Alkenes to the Lanthanide Ions. The comparison of the luminescence profile of lipid **2**−Eu^{3+} incorporated into the liposomes (Figure 3B) to the emission spectrum of the free EDTA−Eu^{3+} complex (Figure 2) shows no significant differences. This was expected for the following two reasons: (i) the chelating agent bound to Eu^{3+} was the same in both cases (EDTA) and (ii) the electronic transitions leading to the luminescence of Eu^{3+} are shielded from chemical perturbation by the presence of electrons in the outer shell of the lanthanide ion. On the other end, the time-resolved excitation spectra of the free complex (Figure 2) and the liposome (Figure 3B) are considerably different. The time-resolved excitation spectrum of the liposome is similar to its absorption spectrum (data not shown). This is an indication of energy transfer from the backbone of the liposome to Eu^{3+}. Another indication of energy transfer is the appearance of strong lanthanide luminescence upon sample excitation at the maximum fluorescence excitation wavelength (355 nm) of the liposome (see Figure 3). Liposome excitation at this wavelength provides a reproducible lanthanide signal with a signal-to-background ratio equal to 8 ($S/B = 8$). On the other end, the excitation of EDTA−Eu^{3+} at 355 nm produces

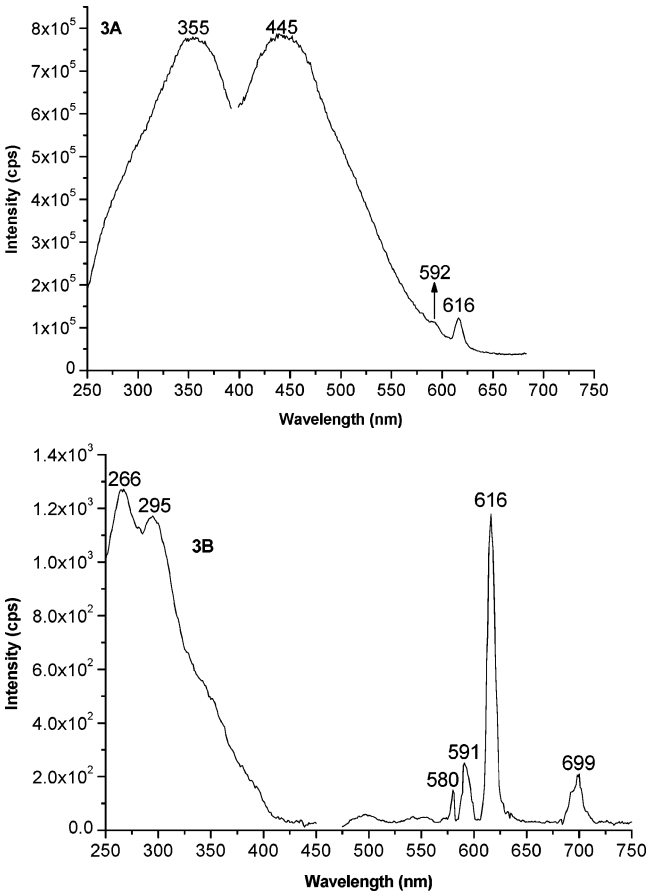


Figure 3. Excitation and emission spectra of polymerized liposomes incorporating lipid **2**−Eu^{3+} complex recorded under steady-state (A) and time-resolved (B) conditions. In (A), the excitation spectrum (250−400 nm) was recorded upon emission at 445 nm and the emission spectrum (400−675 nm) was recorded upon excitation at 355 nm. In (B), the excitation spectrum (250−450 nm) was recorded upon emission at 616 nm and the emission spectrum (475−750 nm) was recorded upon excitation at 266 nm. All spectra were blank subtracted (25 mM HEPES buffer, pH = 7.0) and recorded from a 92.3 mg L^{-1} liposome solution. Steady-state spectra were recorded with 10 and 2 nm band-pass, respectively. Time-resolved spectra were recorded with 0.15 and 1 ms delay and gate times, respectively, and 50 pulses per data point. Excitation and emission band-pass were 10 and 3 nm, respectively. A cutoff filter at 450 nm was used in all cases.

no lanthanide's luminescence (see residual excitation around 355 nm in Figure 2).

Quantitative Potential for Protein Analysis. The quantitative potential of the polymerized liposomes (incorporating **2**−Eu^{3+}) for protein detection was demonstrated with the protein carbonic anhydrase (bovine erythrocyte, E.C. 4.2.1.1). This enzyme is responsible for reversible hydration of carbon dioxide to bicarbonate and is implicated in a variety of human diseases including cancer.[28] Inhibitors for CA are clinically

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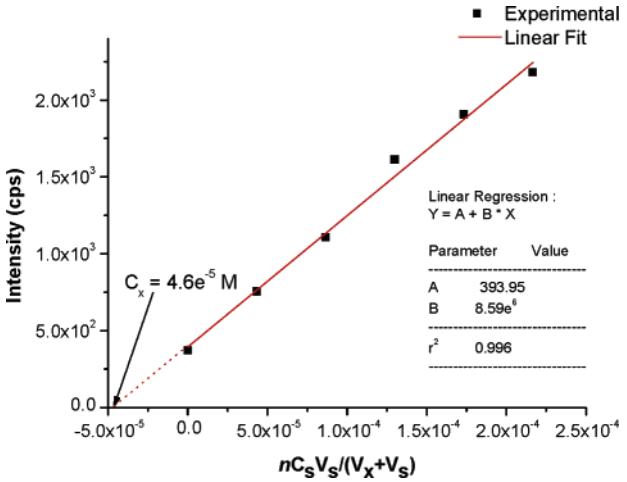

Figure 4. Luminescence intensity of polymerized liposomes incorporating **2**−Eu^{3+} as a function of standard addition concentration. All intensities were blank subtracted (25 mM HEPES buffer). Intensities were recorded at $\lambda_{exc}/\lambda_{em}$ = 266/616 nm with 0.15 and 1 ms delay and gate times, respectively, and 50 pulses per data point. Excitation and emission bandpass were 20 and 2 nm, respectively. A cutoff filter at 450 nm was used.

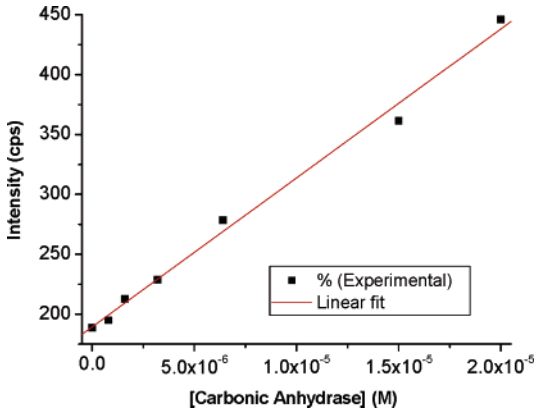

Figure 5. Calibration curve for carbonic anhydrase obtained with a solution of polymerized liposomes incorporating lipid **2**−Eu^{3+} at the 4.6 × 10^{-5} M concentration. Polymerized liposomes and carbonic anhydrase were mixed in 25 mM HEPES buffer, pH = 7.0, and measurements were performed after 15 min of mixing. Instrumental parameters were the following: $\lambda_{exc}/\lambda_{em}$ = 355/615 nm, time delay = 1 μs, gate width = 500 μs, number of accumulations per spectrum = 100 laser pulses, slit-width of spectrograph = 1 mm.

used to treat glaucoma and we have recently developed a novel "two-prong" strategy for designing highly potent inhibitors for the enzyme.[26] The enzyme has several histidines and acidic residues on the surface (determined using the molecular modeling software BioMed CAChe 6.1, Fujitsu Scientific, from the pdb file 1can.pdb) which can bind to the lanthanide ions. In addition, the protein is known to interact with EDTA−Eu^{3+} under neutral pH.[9a]

Quantitative protein analysis is based on the enhancement of luminescence intensity upon protein interaction with the liposome. The luminescence enhancement results from the replacement of O−H oscillators by lower frequency oscillators in the first coordination sphere of Eu^{3+}.[9a,b] Figure 5 shows the calibration curve of carbonic anhydrase (CA) obtained with a solution of polymerized liposomes containing lipid **2**−Eu^{3+} at the 4.6 × 10^{-5} M concentration level. The experiments were performed in batch (25 mM HEPES buffer), and signal intensities were measured after 15 min of protein mixing. The luminescence intensities plotted in the calibration graph are the averages of individual measurements taken from three aliquots of the same working solution. Clearly, there is a direct correlation between the luminescence intensity of the liposome and the protein concentration. The correlation coefficient (0.9965) is close to unity, demonstrating a linear relationship between protein concentration and signal intensity. The limit of detection (LOD) was calculated[27] with the equation LOD = $3s_R/m$, where m is the slope of the calibration curve ($I = 1.24 \times 10^7$ [CA] + 189.5) and s_R is the standard deviation from 16 measurements of the reference signal, that is, the luminescence of the liposome in the absence of protein. With the aid of an appropriate delay (150 μs) after the excitation pulse, the fluorescence background from the protein is eliminated, and blank measurements only detect instrumental noise. Because the magnitude of the reference signal was significantly above the one of the instrumental noise (S/B = 8), the reference signal was highly reproducible (s_R = 0.11), and the resulting LOD was 26.6 nM (or approximately 0.8 μg mL^{-1}).

Qualitative Potential of Luminescence Lifetime. The luminescence lifetimes of the lanthanide ions are usually sensitive to the microenvironment of the luminophor.[10] Hence, we investigated the feasibility of using this parameter for qualitative analysis of CA. The experiments were carried out in batch (25 mM HEPES buffer, pH = 7.0) with a fixed concentration of liposomes. The exponential decays were collected at $\lambda_{exc}/\lambda_{em}$ = 355/615 nm after 15 min of protein mixing. Protein concentration (1.6 × 10^{-5} M) in the final mixture was in excess with respect to liposome concentration. Figure 6 shows typical exponential decays in the absence and the presence of CA. The agreement between the calculated and observed points over the first two lifetimes of the decays agreed to within about 1%, and the residuals showed no systematic trends. Because the time resolution of our system is equivalent to the minimum gate of the ICCD (2 ns), we can confidently state that we are instrumentally capable of distinguishing two lifetimes that are microseconds apart. For a confidence level of 95% (α = 0.05; $N_1 = N_2$ = 6),[23] the observed lifetime in the absence of CA (231.78 ± 13.21 μs) was statistically different from the lifetime in the presence of protein (283.73 ± 18.5 μs), demonstrating that the lifetime of the liposome-bound Eu^{3+} is sufficiently sensitive to probe the presence of a target protein on the bases of lifetime analysis.

Conclusions

We have demonstrated that saturated and polymerizable lipids can be efficiently synthesized employing solid-phase strategy. The described method requires one chromatographic purification after the cleavage of the lipids from the solid support. The generality of the method is demonstrated by

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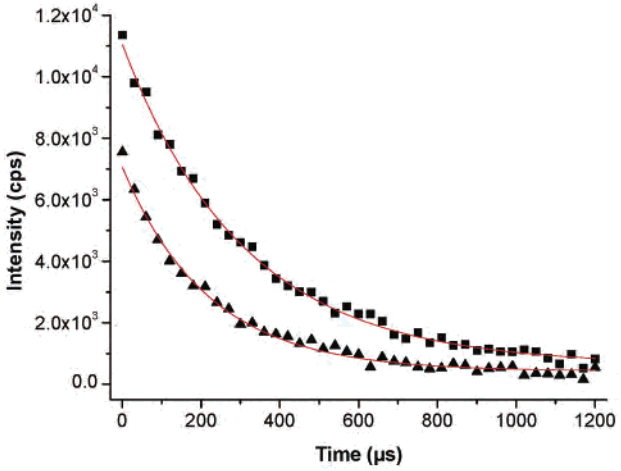

Figure 6. Fitted luminescence decay curves for polymerized liposome in the absence (▲) and the presence (■) of carbonic anhydrase. Experimental parameters for wavelength−time matrix collection were the following: $\lambda_{exc}/\lambda_{em}$ = 355/615 nm, time delay = 1 μs, gate width = 500 μs, gate step = 40 μs, number of accumulations per spectrum = 100 laser pulses, number of kinetic series per wavelength time matrix = 40, slit-width of spectrograph = 1 mm. Polymerized liposome and carbonic anhydrase were mixed in 25 mM HEPES buffer, pH = 7.0, and measurements were performed after 15 min of mixing.

varying the fatty acids, the backbone, and the oligoethylene glycol spacer of the lipids. The method can be easily adapted for rapid and combinatorial synthesis of lipids.

The reported metal-chelating lipids contain EDTA as the headgroup. The luminescence intensity of the complexed Eu^{3+} ion was sufficient for the detection of the enzyme carbonic anhydrase in 27 nM concentration. However, if higher sensitivity is needed, an organic fluorophore can be attached to the EDTA headgroup by small modification of the synthetic scheme. We have already demonstrated that 5-aminosalicylic acid and coumarin-3-carboxylic acid can efficiently sensitize the emissions from Eu^{3+} and Dy^{3+} ions, respectively.[9a] Solid-phase syntheses of lipids incorporating these fluorophores are in progress and will be reported in the future.

The excited-state lifetime of **2**−Eu^{3+} can be used to detect the presence of CA, and the intensity of the emission can be used to quantify the concentration of the protein. We are currently testing the lifetime measurement studies with other proteins, especially with recombinant human carbonic anhydrases-IX and -XII in microliter plates. These two isozymes of CA are involved in hypoxia and in a variety of cancers.[28] Results from these studies will be reported elsewhere.

Experimental Procedures

Materials. All the materials were used as supplied unless stated otherwise. TLC was performed with 20 cm × 20 cm plates, 0.25 μm. Chromatography plates were visualized either with UV light or with an iodine chamber. ^{1}H and ^{13}C NMR spectra were recorded using 300 and 400 MHz spectrometers in one of the following solvents: $CDCl_3$ and CD_3OD with TMS as an internal standard. 2,3-Bis(pentacosa-10,12-diynamido)propanoic acid was obtained by a previously reported procedure.[9]

N-Protection of 2,2′-(Ethylenedioxy)bis(ethylamine). *tert*-Butylchloridediphenylsilane (4.64 g, 16.9 mmol) in 10 mL of dry THF was added to a solution of the diamine **7** (50.57 g, 338 mmol) and triethylamine (3.41 g, 33.8 mmol) in 200 mL of dry THF at 0 °C over a period of 4 h (3.6 mL/h) by a syringe pump. The reaction was continued for another 12 h at room temperature, and the solvent was removed under reduced pressure. The remaining oily material was diluted with 150 mL of water and extracted with dichloromethane. Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. A yellow oily product (a mixture of **8** and **9)** was obtained (4.8 g, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.04 (s, 9H), 2.2−2.6 (br), 3.02 (t, 2H, J = 5.4 Hz), 3.58 (t, 2H, J = 5.1 Hz), 3.64 (t, 2H, J = 5.4 Hz), 3.69−3.73 (m, 2H), 3.81 (t, 2H, J = 5.1 Hz), 7.41 (m, 6H), 7.72 (m, 4H).

N-Protection of 4,7,10-Trioxa-1,13-tridecanediamine. *tert*-Butylchloridediphenylsilane (3.05 g, 11.1 mmol) in 12 mL of dry THF was added to a solution of the diamine **10** (49 g, 222 mmol) and triethylamine (1.23 g, 12.2 mmol) in 200 mL of dry THF at 0 °C over a period of 4 h (3.75 mL/h) by a syringe pump. The reaction was continued for another 12 h at room temperature, and the solvent was removed under reduced pressure. The remaining oily material was diluted with 150 mL of water and extracted with dichloromethane. Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. A yellow oily product (a mixture of **11** and **12)** was obtained (5.05 g, 98%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.04 (s, 9H), 1.71 (m, 4H), 2.05 (br), 2.77 (t, 4H, J = 6.6 Hz), 3.51−3.64 (m, 12H), 7.35 (m, 6H), 7.73 (m, 4H).

Solid-Phase Synthesis. General Method: Amine-Grafted Resin. A sample of 4-methoxytrityl chloride resin beads **5** (1 equiv) was poured into a reaction vessel and washed with dichloromethane. Protected diamine mixture **8** and **9** (2 equiv) and diisorpopylethylamine (6.8 equiv) in 30 mL of dichloromethane were added to the resin. The suspension was shaken for 4 h at room temperature. The resin was filtered and washed with DCM/MeOH/DIEA (8.5:1:0.5), dichloromethane, DMF, dichloromethane, and MeOH.

Deprotection of Silyl Group. The resin **14** (1 equiv) was suspended in 30 mL of THF, and 1 M TBAF (2 equiv) was added to the reaction vessel. The suspension was shaken for 5 h, and the vessel was drained. The resin was washed with THF, dichloromethane, and MeOH.

N-Acylation of Amine with Acid 23. A solution of acid **23** (0.25 equiv), HBTU (0.25 equiv), HOBT (0.25 equiv), and NMM (1.5 equiv) in 45 mL of DMF was added to the resin (1 equiv). The suspension was shaken for 12 h at room temperature. The vessel was drained, and the resin beads were washed with DMF, MeOH, and dichloromethane.

Deprotection of Fmoc Group. The resin was suspended in 20% piperidine in DMF (20 mL) and shaken for 1 h at room temperature. The solvents were filtered, and the resin was washed with dichloromethane, DMF, and dichloromethane.

N-Acylation with Acid 25. A solution of 10,12-pentacosadiynoic acid **25** (0.5 equiv), HBTU (0.5 equiv), HOBT (0.5 equiv), and NMM (2.5 equiv) in 25 mL of DMF was added to the resin (1 equiv). The suspension was shaken for 12 h. The vessel was drained, and the resin beads were washed with DMF, MeOH, and dichloromethane.

Cleavage from the Resin. The resin was suspended in 5% CF_3CO_2H in dichloromethane (20 mL) for 1.5 h. The solution was filtered, and the resin was washed with dichloromethane, MeOH, and dichloromethane. The organic fractions were combined and concentrated. The crude product was purified by silica gel column chromatography and eluted with 5% MeOH in $CHCl_3$. A pinkish solid was recovered.

Compound 19a. Yield: 85%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, 6H, J = 6.9 Hz), 1.25−1.35 (m, 52H), 1.46−1.53 (m,

10H), 2.23 (m, 12H), 3.3 (m, 2H), 3.4 (m, 2H), 3.6 (m, 8H), 3.69 (m, 2H), 3,81 (m, 2H), 4.41 (m, 1H), 7.02 (m, 1H), 7.41 (m, 1H), 7.7 (m, 1H), 7.83 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.11, 19.21, 22.76, 25.43, 25.65, 25.96, 28.35−29.62, 31.89, 36.02, 36.38, 41.06, 41.59, 55.02, 65.34, 67.95, 69.27, 69.39, 69.50, 70.30, 71.12, 77.54, 171.17, 174.84, 176.93.

Compound 19b. Yield: 37%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, 6H, J = 6.6 Hz), 1.22−1.36 (m, 44H), 1.44−1.55 (m, 16H), 1.62−1.72 (m, 2H), 1.73−1.88 (m, 2H), 2.11 (t, 2H, J = 8 Hz), 2.2−2.25 (m, 10H), 3.14−3.29 (m, 6H), 3.51−3.85 (m, 12H), 4.41−4.49 (m, 1H), 6.77 (m, 1H), 7.97 (m, 1H), 7.59 (m, 1H), 7.76 (m, 1H), 8.24 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.34, 19.42, 22.9, 25.91−26.11, 28.58−29.86, 32.13, 36.53, 36.79, 40.11, 40.16, 52.92, 65.46, 65.49, 66.88, 69.69, 70.12, 70.43, 77.81, 111.14, 118.55, 124.66, 125.35, 173.13, 174.49, 174.87.

Compound 19c. Yield: 23%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 6H, J = 8 Hz), 1.25 (m, 46H), 1.33−1.36 (m, 10H), 1.48−1.56 (m, 8H), 1.74−1.78 (m, 2H), 2.15 (t, 2H, J = 8 Hz), 2.23 (t, 10H, J = 8 Hz), 3.16−3.21 (m, 4H), 3.30−3.38 (m, 1H), 3.52−3.66 (m, 8H), 3.76 (t, 2H, J = 4 Hz), 4.28 (m, 1H), 6.22 (m, 1H), 6.9 (m, 1H), 7.61 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.17, 22.40, 22.65, 25.6, 25.8, 28.30−29.61, 31.42, 32.05, 36.27, 36.81, 38.62, 39.44, 40.13, 53.45, 65.22, 65.28, 66.8, 69.97, 70.15, 77.14−77.56, 172.95, 174.14, 174.58.

Compound 19d. Yield: 89%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 6H, J = 8 Hz), 1.25−1.36 (m, 48H), 1.49−1.51 (m, 10H), 1.59 (m, 4H), 2.02 (br, 2H), 2.22−2.25 (m, 14H), 3.19 (m, 2H), 3.31 (m, 2H), 3.44−3.49 (m, 4H), 3.57−3.63 (m, 8H), 3.67 (m, 2H), 3.77 (t, 2H, 4 Hz), 4.39 (m, 1H), 6.51 (m, 1H), 6.87 (m, 1H), 7.21 (m, 2H), 7.84 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.11, 19.19, 22.67, 25.43, 25.65, 25.96, 28.35−29.62, 31.89, 36.02, 36.38, 41.56, 41.59, 55.92, 65.24, 67.95, 69.27, 69.39, 69.50, 70.39, 71.02, 77.54, 171.17, 174.84, 176.93.

Compound 19e. Yield: 71%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, 6H, J = 9 Hz), 1.24 (m, 50H), 1.56−1.58 (m, 8H), 2.12−2.23 (m, 8H), 3.12−3.20 (m, 6H), 3.33 (m, 2H), 3.49−3.64 (m, 8H), 3.17 (m, 2H), 4.36 (m, 1H).

Compound 19f. Yield: 47%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, 6H, J = 7 Hz), 1.24 (m, 50H), 1.46 (m, 2H), 1.58 (m, 4H), 2.19 (m, 4H), 3.16 (m, 4H), 3.38 (m, 10H), 3.59 (m, 6H), 3.66 (m, 2H), 3.74 (m, 2H), 4.32 (m, 1H).

Coupling of 19a with EDTA Triester. EDTA triester[9] (0.05 g, 0.12 mmol), HBTU (0.07 g, 0.19 mmol), HOBT (0.026 g, 0.19 mmol), and triethylamine (0.026 g, 0.25 mmol) were dissolved in 10 mL of chloroform and 5 mL of DMF, then added to a solution of **19a** (0.11 g, 0.11 mmol) in 8 mL of chloroform. The reaction mixture was stirred at room temperature overnight. Brine solution was added to the mixture, and solvents were concentrated under reduced pressure. The precipitate was filtered and washed several times with water, then dried in vacuo overnight. The crude product was purified by silica gel column chromatography and eluted with 5% MeOH in $CHCl_3$ to yield a pinkish solid (R_f = 0.3, 0.12 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 6H, J = 8 Hz), 1.24−1.29 (m, 62H), 1.33−1.38 (m, 6H), 1.48−1.53 (m, 8H), 1.59−1.66 (m, 4H), 1.88 (m, 1H), 2.16−2.26 (m, 12H), 2.78−2.82 (m, 4H), 3.32 (s, 2H), 3.43 (s, 4H), 3.49−3.51 (m, 2H), 3.51−3.56 (m, 8H), 3.58−3.64 (m, 4H), 4,14 (q, 6H, J = 8 Hz), 4.42−4.45 (m, 1H), 6.7 (m, 1H), 7.32 (m, 1H), 8.2 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.07, 14.21, 19.16, 22.64, 25.47, 25.62, 28.33−29.58, 31.88, 36.49, 38.82, 39.27, 42.28, 52.23, 52.81, 54.82, 55.72, 58.57, 60.55, 60.65, 65.19, 65.27, 69.54, 69.75, 70.41, 77.39, 77.55, 170.13, 171.13, 171.22, 171.82, 174.26, 175.12.

Coupling of 19b with EDTA Triester. EDTA triester[9] (0.036 g, 0.096 mmol), HBTU (0.163 g, 0.096 mmol), HOBT (0.01 g, 0.096 mmol), and triethylamine (0.009 g, 0.096 mmol) were dissolved in 8 mL of chloroform and 4 mL of DMF, then added to a solution of **19b** (0.08 g, 0.08 mmol) in 8 mL of chloroform. The reaction mixture was stirred at room temperature overnight. Brine was added to the mixture, and solvents were concentrated under reduced pressure. The precipitate was filtered and washed several times with water and dried in vacuo overnight. The crude product was purified by silica gel column chromatography and eluted with 5% MeOH in $CHCl_3$ to yield a pinkish solid (R_f = 0.2, 0.078 g, 76%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85 (t, 6H, J = 6 Hz), 1.22−1.27 (m, 56H), 1.47−1.49 (m, 16H), 1.82 (m, 1H), 1.91 (s, 2H), 2.16 (t, 2H, J = 5.7 Hz), 2.2 (t, 10H, J = 5 Hz), 2.78 (m, 4H), 3.21 (m, 2H), 3.28 (s, 2H), 3.38 (m, 2H), 3.40 (s, 2H), 3.50−3.56 (m, 12H), 4.11 (q, 6H, J = 6 Hz), 4.54 (m, 1H), 6.19 (m, 1H), 6.62 (m, 1H), 7.15 (m, 1H), 8.21 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.16, 14.21, 19.17, 22.83, 25.81−25.86, 28.36−28.39, 28.76, 28.83, 28.92, 29.06, 29.18−29.26, 29.31, 29.44, 29.59, 30.62, 31.88, 36.57, 36.71, 38.46, 38.86, 39.30, 52.23, 52.79, 55.68, 58.60, 60.58, 60.69, 65.19, 65.26, 69.71, 70.11, 70.48, 77.41, 77.59, 171.14, 171.25, 171.97, 172.02, 173.14, 173.56.

Coupling of 19c with EDTA Triester. EDTA triester[9] (0.111 g, 0.29 mmol), HBTU (0.112 g, 0.29 mmol), HOBT (0.04 g, 0.29 mmol), and triethylamine (0.06 g, 0.58 mmol) were dissolved in 5 mL of chloroform and 3 mL of DMF and added to a solution of **19c** (0.244 g, 0.24 mmol) in 4 mL of chloroform. The reaction mixture was stirred at room temperature overnight. Brine was added to the mixture, and solvents were evaporated under reduced pressure. The precipitate was filtered and washed several times with water, then dried in vacuo overnight. The crude product was purified by silica gel column chromatography and eluted with 5% MeOH in $CHCl_3$ to yield a pinkish solid (R_f = 0.2, 0.04 g, 12%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 6H, J = 8 Hz), 1.26−1.29 (m, 62H), 1.32 (m, 8H), 1.51−1.61 (m, 16H), 2.20 (m, 2H), 2.24 (t, 10H, J = 8 Hz), 2.82 (m, 4H), 3.23 (m, 2H), 3.32 (s, 2H), 3.44 (m, 4H), 3.53−3.54 (m, 6H), 3.59 (m, 6H), 4.17 (q, 6H, J = 8 Hz), 4.44 (m, 1H), 5.94 (m, 1H), 6.58 (m, 1H), 6.89 (m, 1H), 8.21 (1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.78, 13.93, 18.86, 22.12, 22.34, 25.31, 25.45, 27.99, 27.02, 28.52−29.30, 31.57, 32.01, 36.22, 36.43, 38.46, 38.57, 38.97, 51.92, 52.30, 52.50, 54.51, 55.42, 58.30, 60.28, 60.38, 64.90, 64.96, 69.35, 69.42, 69.77, 70.23, 170.85, 171.3, 171.68, 173.01, 173.02.

Coupling of 19d with EDTA Triester. EDTA triester[9] (0.05 g, 0.12 mmol), HBTU (0.05 g, 0.12 mmol), HOBT (0.017 g, 0.12 mmol), and triethylamine (0.026 g, 0.25 mmol) were dissolved in 8 mL of chloroform and 4 mL of DMF, then added to a solution of **19d** (0.1 g, 0.11 mmol) in 8 mL of chloroform. The reaction mixture was stirred at room temperature overnight. Brine was added to the mixture, and solvents were removed under reduced pressure. The precipitate was filtered and washed several times with water, then dried in vacuo overnight. The crude product was purified by silica gel column chromatography and eluted with 5% MeOH in $CHCl_3$ to yield a white-bluish solid (R_f = 0.2, 0.04 g, 31%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, 6H, J = 7 Hz), 1.25−1.39 (m, 60H), 1.42−1.49 (m, 10H), 1.58−1.62 (m, 4H), 1.63−1.78 (m, 2H), 1.79−182 (m, 4H), 2.2−2.25 (m, 12H), 2.79−2.83 (m, 4H), 3.29 (s, 2H), 3.32−3.41 (m, 4H), 3.42 (s, 2H), 3.49−3.56 (m, 8H), 3.58−3.63 (m, 4H), 3.60−3.65 (m, 4H), 4.14 (q, 6H, J = 6 Hz), 4.32−4.39 (m, 1H), 6.62 (m, 1H), 7.23 (m, 1H), 7.27 (m, 1H), 8.07 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.09, 14.22, 19.17, 22.65, 25.47, 25.63, 28.32−29.70, 31.89, 36.49, 37.46, 42.43, 52.24, 52.92, 54.83, 54.89, 55.89, 58.62, 60.57,

60.69, 65.27, 65.29, 69.05, 69.24, 70.17, 70.48, 77.58, 169.92, 171.05, 171.23, 171.25, 171.43, 174.34, 175.18.

Coupling of 19e with EDTA Triester. EDTA triester[9] (0.09 g, 0.24 mmol), HBTU (0.11 g, 0.24 mmol), HOBT (0.04 g, 0.24 mmol), and triethylamine (0.048 g, 0.48 mmol) were dissolved in 8 mL of chloroform and 4 mL of DMF, then added to a solution of **19e** (0.19 g, 0.24 mmol) in 10 mL of chloroform. The reaction mixture was stirred at room temperature overnight. Brine was added to the mixture, and solvents were evaporated under reduced pressure. The precipitate was filtered and washed several times with water, then dried in vacuo overnight. The crude product was purified by silica gel column chromatography and eluted with 5% MeOH in $CHCl_3$ to yield a white solid ($R_f = 0.3$, 0.147 g, 55%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 6H, $J = 8$ Hz), 1.25−1.28 (m, 58H), 1.62 (m, 8H), 1.83−1.85 (m, 6H), 2.18−2.25 (m, 4H), 2.79−2.83 (m, 4H), 3.32−3.41 (m, 6H), 3.32−3.47 (m, 6H), 3.49−3.64 (m, 14H), 4.17 (q, 6H, $J = 8$ Hz), 4.41 (m, 1H), 6.12 (m, 1H), 6.63 (m, 1H), 7.01 (m, 1H), 7.87 (m, 1H).

Coupling of 19f with EDTA Triester. EDTA triester[9] (0.16 g, 0.44 mmol), HBTU (0.167 g, 0.44 mmol), HOBT (0.06 g, 0.44 mmol), and triethylamine (0.09 g, 0.88 mmol) were dissolved in 15 mL of chloroform and 5 mL of DMF, then added to a solution of **19f** (0.3 g, 0.37 mmol) in 15 mL of chloroform. The reaction mixture was stirred at room temperature overnight. Brine was added to the mixture, and solvents were removed under reduced pressure. The precipitate was filtered and washed several times with water, then dried in vacuo overnight. The crude product was purified by silica gel column chromatography and eluted with 5% MeOH in $CHCl_3$ to yield a white solid ($R_f = 0.2$, 0.205 g, 48%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 6H, $J = 8$ Hz), 1.25−1.29 (m, 58H), 1.62 (m, 4H), 2.19 (m, 4H), 2.78 (m, 4H), 3.31 (s, 4H), 3.41−3.61 (m, 26H), 3.71 (m, 2H), 4.17 (q, 6H, $J = 8$ Hz).

Lipid 2. To a solution of lithium hydroxide monohydrate (0.034 g, 0.81 mmol) in methanol (4 mL), **20a** (0.177 g, 0.13 mmol) in THF/dichloromethane (3/0.5 mL) was added, and the solution was stirred at room temperature overnight. The reaction mixture was acidified (to pH = 3.0 with 2 N HCl), and the solvents were removed under reduced pressure to form a precipitate in the aqueous solution. The precipitate was filtered, washed with water, and dried to yield lipid **2** as a white solid (152 mg, 91%). ^{1}H NMR ($CDCl_3$ + CD_3OD, 400 MHz): δ 0.88 (t, 6H, $J = 7.2$ Hz), 1.23−1.28 (m, 42H), 1.49−1.57 (m, 10H), 2.18 (m, 2H), 2.24 (t, 2H, $J = 4$ Hz), 3.11 (m, 2H), 3.19 (m, 2H), 3.38−3.41 (m, 2H), 3.45 (m, 2H), 3.51 (m, 2H), 3.54−3.57 (m, 4H), 3.58 (s, 4H), 3.60−3.63 (m, 4H), 3.73 (m, 4H), 4.48 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.92, 19.02, 22.53, 25.41, 25.60, 28.22, 28.69, 28.72, 28.84, 28.95, 29.09, 29.19, 29.33, 29.48, 31.77, 36.11−36.14, 39.05, 48.67−45.53, 54.01, 65.06, 65.15, 69.31, 69.32, 77.46, 169.21, 170.56, 171.14, 172.06, 174.93, 175.62. Anal. Calcd for $C_{67}H_{114}N_6O_{12}\cdot H_2O$: C, 66.41; H, 9.48; N, 6.94. Found: C, 66.64; H, 9.21; N, 6.79.

Lipid 3. To a solution of lithium hydroxide monohydrate (0.011 g, 0.26 mmol) in methanol (3 mL), **20e** (0.05 g, 0.04 mmol) in THF/dichloromethane (2/1 mL) was added, and the solution was stirred at room temperature overnight. The reaction mixture was acidified to pH = 3.0 with 2 N HCl, and the solvents were removed under reduced pressure to form a precipitate in the aqueous solution. The precipitate was filtered, washed with water, and dried to yield lipid **3** as a white solid (37 mg, 87%). ^{1}H NMR ($CDCl_3$ + CD_3OD, 300 MHz): δ 0.88 (t, 6H, $J = 9$ Hz), 1.25 (m, 60H), 1.59 (m, 6H), 1.79 (m, 4H), 2.18 (m, 4H), 2.79 (m, 1H), 2.88 (m, 2H), 3.19 (m, 2H), 3.33 (m, 4H), 3.38 (m, 2H), 3.52 (m, 8H), 3.57−3.71 (m, 12H), 3.71 (m, 4H), 3.78 (m, 6H), 4.34 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.89, 22.51, 25.21, 25.55, 25.73, 29.19−29.54, 31.75, 39.16, 36.37, 39.04, 48.48−49.51, 51.52, 51.71, 52.37, 69.37, 70.05, 169.87, 171.34, 172.47, 174.29, 174.59. MS (ESI−TOF) *m*/*z* calcd for $C_{57}H_{108}N_6O_{12}$, 1068.80; found, 1070.11 $[M + 2H]^+$. $[\alpha]^{20}_D$ +2.0° (*c* 1, $CHCl_3$).

Lipid 4. To a solution of lithium hydroxide monohydrate (0.03 g, 0.72 mmol) in methanol (10 mL), **20b** (0.16 g, 0.12 mmol) in THF/dichloromethane (4/2 mL) was added, and the solution was stirred at room temperature overnight. The reaction mixture was acidified to pH = 3.0 with 2 N HCl, and the solvents were removed under reduced pressure to form a precipitate in the aqueous solution. The precipitate was filtered, washed with water, and dried to yield lipid **4** as a white solid (142 mg, 98%). ^{1}H NMR ($CDCl_3$ + CD_3OD, 300 MHz): δ 0.87 (t, 6H, $J = 6$ Hz), 1.21−1.26 (m, 54H), 1.45−1.48 (m, 8H), 1.49−1.54 (m, 10H), 1.61 (m, 4H), 2.62 (t, 2H, $J = 6.2$ Hz), 2.42 (t, 10H, $J = 5.9$ Hz), 2.96−3.45 (m, 2H), 3.12−3.24 (m, 4H), 3.36 (m, 2H), 3.40 (m, 2H), 3.46 (m, 2H), 3.54−3.57 (m, 4H), 3.59−3.62 (m, 4H), 3.67 (s, 1H), 3.71 (s, 2H), 3.73 (s, 2H), 4.38 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.12, 19.17, 22.67, 22.64−25.81, 28.34, 28.36, 38.85−29.61, 31.90, 36.59, 39.28, 51.92, 65.17−65.24, 70.10, 77.60, 171.17, 172.19, 173.99, 174.08, 174.42, 174.50. Anal. Calcd for $C_{71}H_{120}N_6O_{12}$: C, 68.23; H, 9.68; N, 6.72. Found: C, 68.54; H, 9.93; N, 6.41. $[\alpha]^{20}_D$ +0.3° (*c* 1, MeOH/$CHCl_3$).

Lipid 5. To a solution of lithium hydroxide monohydrate (0.025 g, 0.6 mmol) in methanol (8 mL), **20f** (0.13 g, 0.1 mmol) in THF (2 mL) was added, and the solution was stirred at room temperature overnight. The reaction mixture was acidified to pH = 3.0 with 2 N HCl, and the solvents were removed under reduced pressure to form a precipitate in the aqueous solution. The precipitate was filtered, washed with water, and dried to yield lipid **5** as a white solid (84 mg, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 6H, $J = 8$ Hz), 1.26 (m, 60H), 1.59 (m, 6H), 1.79 (m, 4H), 2.18 (m, 4H), 2.79 (m, 1H), 2.88 (m, 2H), 3.19 (m, 2H), 3.33 (m, 4H), 3.38 (m, 2H), 3.52 (m, 8H), 3.57−3.71 (m, 12H), 3.71 (m, 4H), 3.78 (m, 6H), 4.34 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.99, 22.58, 25.70, 29.26−29.61, 31.82, 36.40, 38.95, 48.71−49.99, 51.89, 55.75, 69.79, 70.02, 171.22, 173.10, 174.27, 174.43, 174.51. MS (ESI−TOF) *m*/*z* calcd for $C_{58}H_{110}N_6O_{12}$, 1082.81; found, 1084.82 $[M + 2H]^+$. $[\alpha]^{20}_D$ +10.7° (*c* 1, $CHCl_3$).

Lipid 6. To a solution of lithium hydroxide monohydrate (0.008 g, 0.2 mmol) in methanol (6 mL), **20c** (0.04 g, 0.03 mmol) in THF/dichloromethane (2/1 mL) was added, and the solution was stirred at room temperature overnight. The reaction mixture was acidified to pH = 3.0 with 2 N HCl, and the solvents were removed under reduced pressure to form a precipitate in the aqueous solution. The precipitate was filtered, washed with water, and dried in vacuo overnight to give lipid **6** as a white solid (31 mg, 81%). ^{1}H NMR ($CDCl_3$ + CD_3OD, 400 MHz): δ 0.88 (t, 6H, $J = 7$ Hz), 1.22−1.39 (m, 56H), 1.47−1.54 (m, 12H), 1.56−1.60 (m, 4H), 2.15 (m, 2H), 2.23 (t, 8H, $J = 7$ Hz), 2.91−2.98 (m, 2H), 3.21 (m, 2H), 3.48 (m, 2H), 3.54 (m, 2H), 3.59 (m, 10H), 3.69−3.78 (m, 10H), 4.47 (m, 1H), 7.3 (m, 2H), 7.95 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.10, 19.20, 22.68, 28.36−29.60, 31.90, 65.22, 65.24, 87.89, 171.17, 172.19, 173.99, 174.08, 174.42, 174.50. Anal. Calcd for $C_{72}H_{122}N_6O_{12}$: C, 68.43; H, 9.73; N, 6.65. Found: C, 68.29; H, 10.07; N, 6.35. $[\alpha]^{20}_D$ − 0.7° (*c* 1, MeOH/$CHCl_3$).

Liposome Formation and Polymerization. Solid polymerizable phosphocholine [1,2-bis(10,12)-tricosadiyniyl-*sn*-glycerol-3-phosphocholine, 22.5 mg] and lipid **2**−Eu^{3+} complex (2.5 mg) were dissolved in 12 mL of chloroform and 1 mL of MeOH (both HPLC grade) in a clean 250 mL round-bottom flask. A thin film of the lipids was formed by slow evaporation of the solvent under vacuo

on a rotary evaporator. The film was then dried under high vacuum for 20 h. This film was hydrated by 10 mL of HEPES buffer (50 mM, pH = 7.0). The suspension was warmed at 60 °C with continuous stirring on rotary for a period of 2 h. It was then sonicated by a probe sonicator (power 50 W) under nitrogen for a period of 1.5 h at 60 °C and cooled to room temperature slowly. Liposomes were passed through a 0.1 μm polycarbonate filter (nucleopore) at 60 °C using an extruder (Lipex Biomembranes Inc., Vancouver, British Columbia, Canada) under nitrogen pressure at 60 psi (6 times). The temperature of the extruder was maintained at 60 °C with a circulating water bath, and the collector temperature was also kept at 60 °C. After extrusion, the clear liposome solution was slowly cooled to room temperature. A portion of it was taken for polymerization under UV irradiation (450 W) for 8 min at 0 °C. The solution became slightly pinkish after polymerization with no precipitation. The polymerized vesicle solution was stored at room temperature. Transmission electron micrographs were recorded at the on campus TEM facility (for details, see ref 9d).

Luminescence Measurements. Excitation and emission spectra were collected with a commercial spectrofluorimeter using standard quartz cuvettes (1 cm × 1 cm). No sample deoxygenation was attempted. For steady-state measurements, the excitation source was a continuous wave 75 W xenon lamp with broadband illumination from 200 to 2000 nm. Detection was made with a photomultiplier tube with wavelength range from 185 to 650 nm. The method of detection was analog for high-signal levels or photon counting for low signal levels. In analog mode, the inherent peak-to-peak noise was 50×10^{-12} A with 0.05 ms time-constant. In photon counting mode, the maximum count rate was 4 MHz, pulse pair resolution 250 ns, rise time 20 ns, and fall time 100 ns with a 220 ns pulse width. For time-resolved measurements, the excitation source was a pulsed 75 W xenon lamp (wavelength range from 200 to 2000 nm), variable repetition rate from 0 to 100 pulses per second, and a pulse width of approximately 3 μs. Detection was by means of a gated analog PMT with extended wavelength range from 185 to 900 nm. SS and TR spectra were collected with excitation and emission monochromators having the same reciprocal linear dispersion (4 nm mm^{-1}) and accuracy (±1 nm with 0.25 nm resolution). Their 1200 grooves/mm gratings were blazed at 300 and 400 nm, respectively. The instrument was computer-controlled using commercial software specifically designed for the system.

Luminescence lifetimes were measured with the aid of a fiber optic probe and a laser system mounted in our laboratory.[24] The probe assembly consisted of one excitation and six collection fibers fed into a 1.25 m long section of copper tubing that provided mechanical support. All the fibers were 3 m long and 500 μm core diameter silica-clad silica with polyimide buffer coating. At the analysis end, the excitation and emission fibers were arranged in a conventional six-around-one configuration, bundled with vacuum epoxy, and fed into a metal sleeve for mechanical support. The copper tubing was flared stopping a swage nut tapped to allow for the threading of a 0.75 mL polypropylene sample vial. At the instrument end, the excitation fiber was positioned in an ST connection and aligned with the beam of the tunable dye laser while the emission fibers were bundled with vacuum epoxy in a slit configuration, fed into a metal sleeve, and aligned with the entrance slit of the spectrometer.

Samples were excited directing the output of a tunable dye laser through a KDP frequency-doubling crystal. The dye laser was operated on LDS 698 (Exiton), and it was pumped with the second harmonic of a 10 Hz Nd:YAG Q-switched solid-state laser. Luminescence was detected with a multichannel detector consisting of a front-illuminated intensified charge fiber-coupled device (ICCD). The minimum gate time (full width at half-maximum) of the intensifier was 2 ns. The CCD had the following specifications: active area = 690 × 256 pixels (26 mm^2 pixel size photocathode), dark current = 0.002 electrons/(pixel s), and readout noise = 4 electrons at 20 kHz. The ICCD was mounted at the exit focal plane of a spectrograph equipped with a 1200 grooves/mm grating blazed at 500 nm. The system was used in the external trigger mode. The gating parameters (gate delay, gate width, and the gate step) were controlled with a digital delay generator via a GPIB interface. Custom software was developed in-house for complete instrumental control and data collection.

Lifetime determination followed a three-step procedure:[25] (1) collection of full sample and background wavelength−time matrices, (2) subtraction of background decay curve from the luminescence decay curve at the target wavelengths of the sensor, and (3) fitting the background corrected data to single exponential decays. The decay curve data were collected with a minimum 150 μs interval between opening of the ICCD gate and the rising edge of the laser pulse, which was sufficient to avoid the need to consider convolution of the laser pulse with the analyte signal (laser pulse width = 5 ns). In addition, the 150 μs delay completely removed the fluorescence of the sample matrix from the measurement. Fitted decay curves ($y = y_0 + A_1 \exp^{-(x-x_0)t_1}$) were obtained with a commercial software by fixing y_0 and x_0 at a value of zero.

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Supporting Information Available: Full experimental details for the synthesis of lipid **1** and NMR spectra of the reported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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