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Polymerized Fluorescent Liposomes Incorporating Lanthanide Ions

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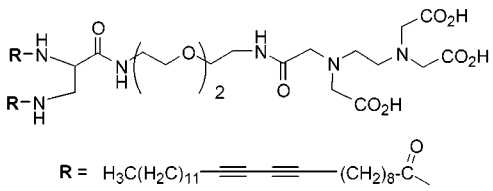
ABSTRACT



This paper describes the design and synthesis of a polymerizable lipid capable of complexing lanthanide ions. The lipid has been successfully incorporated into liposomes and then polymerized. Fluorescence studies indicate that the diacetylene (unpolymerized lipid) and the conjugated alkenes (after polymerization) can be used as sensitizers for the lanthanide ion.

Liposomes are spherical lipid bilayers with aqueous interiors. They can be prepared in variety of sizes in a well-controlled fashion.[1] This has led to many applications for liposomes. In basic science research, they are used as animal cell models, as separation agents, as catalysts, as microreactors, etc.[2] Due to similarity with animal cells, liposomes find wide use in medical and pharmacological research, e.g., as drug carriers,[3] diagnostic agents,[4] gene delivery agents,[5,6] etc.

Fluorescence spectroscopy is a powerful technique for protein sensor construction.[7] The changes in fluorescence spectra of an organic fluorophore[8] or lanthanide metal ions (Eu^{3+}, Tb^{3+}, etc.)[9] are usually monitored (in the presence of the analyte protein). Fluorometric protein detection and assay are preferred to methods using radioisotopes due to the associated health risks of the latter procedure. Fluorometric detection also has a higher sensitivity.[9c] In addition, the lanthanide ions have large stokes shifts, long lifetimes, and narrow bands in the emission spectra.[9b]

A major disadvantage of the lanthanide ions is low molar extinction coefficients. This results in low emission intensity in the luminescence spectra. To solve this problem, sensitizers have been attached to the ligands chelating the lanthanide ions.[9] The sensitizers are usually organic molecules with

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strong absorbance. They absorb the excitation light and transfer the energy to the lanthanide ions, thereby increasing the intensity of luminescence spectra of the lanthanide ions.

For sensing purposes, the binding of peptides, proteins, DNA, and RNA to liposomes has been studied by fluorescence spectroscopy employing organic fluorophores[10] or lanthanide ions.[11] In addition, fluorescent lipids with a metal-chelating headgroup have been used in molecular recognition of peptides,[12] 2D protein recystallization,[13] protein targeting,[14] and biological sensing.[15]

The need for enhanced stability and controlled permeability has led to the synthesis of polymerizable lipids and the corresponding liposomes.[6b,16] Polymerized liposomes have been used for protein sensing employing colorimetric[17] or fluorescence[18] detection methods. Though the syntheses of various polymerizable lipids are reported in the literature,[6b] reports of the synthesis of polymerizable metal-chelating lipids are relatively few.[19,20]

We are interested in the synthesis of polymerizable lipids capable of coordinating to lanthanide ions strongly ($K > 10^{15}$ M^{-1}). The resultant polymerized liposomes can be used to detect peptides, proteins, and other biomolecules by monitoring the changes of luminescence property of the lanthanide ions (intensity and lifetime) in the presence of the analyte. The stability of the polymerized liposomes also makes them suited for use in relatively harsh environments (high salt concentration, low pH, etc.).

Except for one report (to chelate Gd^{3+}),[19d] the metal-chelating headgroups of the polymerizable metal-chealting lipids were designed to complex to transition metal ions (Cu^{2+}, Ni^{2+}). These headgroups (iminodiacetate or nitrilotriacetate) do not have strong affinity for the lanthanide ions ($K < 10^8$ M^{-1}).[21] Herein, we report the synthesis (racemic) of a polymerizable lipid (**1**, Figure 1) with strong affinity

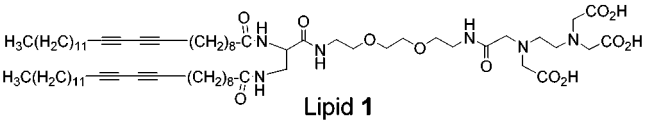

Figure 1. Structure for the polymerizable, metal-chelating lipid capable of binding to a lanthanide ion strongly.

for lanthanide ions. We also report that this lipid−terbium complex (**1**·Tb^{3+}) can be successfully incorporated into liposomes and polymerized. In addition, the diacetylene moiety of the lipid (before polymerization) or the conjugated alkenes (after polymerization of the liposomes) can be used as sensitizers for the lanthanide emission. Because of the presence of Tb^{3+}, the polymerized liposomes are paramagnetic. They have the potential[19d] to be used as magnetic resonance contrast agents.

Lipid **1** incorporates the widely used[22] ethylenediaminetetraacetic acid (EDTA) as the metal-chelating moiety. EDTA complexes lanthanide ions with high affinity ($K > 10^{15}$ M^{-1}),[23] and the resultant complexes have defined structures.[24] EDTA−Tb^{3+} complexes have been used to label proteins[11c,d] and to detect proteins by fluorescence spectroscopy.[11b] Lipid **1** has a diacetylene as the polymerizable unit. This group can be efficiently polymerized in liposomes by UV irradiation.[16,17,19] A triethylene glycol spacer[13,25] separates the metal-chelating headgroups from the polymerizable unit.

Synthesis of the lipid **1** is depicted in Scheme 1.[26] EDTA−triethyl ester **3**[27] was combined with the selectively protected diamine **4**[20b] and subsequently hydrogenated to afford the amine **5**. Without HCl, the free amine **5** was found to react with the ester groups to give a complex mixture of products. Next, the methyl ester of 2,3-diaminopropanoic acid (racemic) was converted to the intermediate **7**. Coupling of **5** with **7** and subsequent ester hydrolysis afforded the lipid **1** as a waxy solid. The lipid was prepared in a 150 mg quantity and stored as a solid at −20 °C under a nitrogen atmosphere. The Tb^{3+} complex of **1** was prepared by the addition of a

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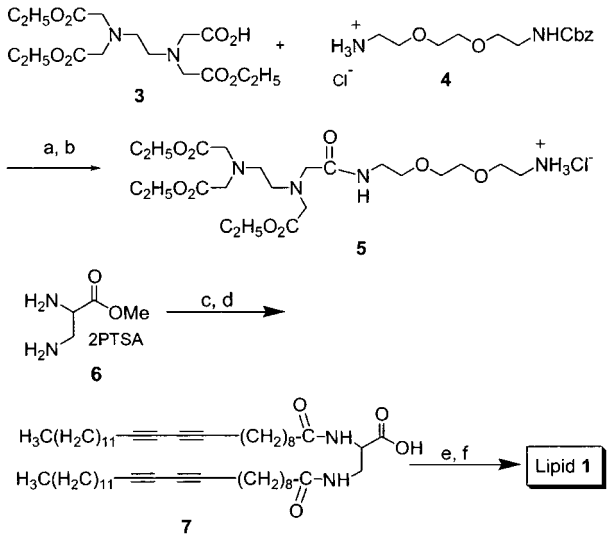
Scheme 1. Synthesis of Lipid **1**



Reagents:

(a) BOP, Et_3N, CH_3CN, 25 °C, 12 h (92%); (b) Pd-black, H_2, MeOH, HCl (2 eq.), 25 °C, 10 h (93%); (c) $H_3C(H_2C)_{11}$—≡—≡—$(CH_2)_8$-COOH, (2eq.), BOP, Et_3N, $CHCl_3$-DMF (2:1), 25 °C, 20 h (80%); (d) LiOH (1.5 eq.), MeOH-THF-CH_2Cl_2-H_2O (10:10:3:1), 25 °C, 18 h (84%); (e) **5**, BOP, Et_3N, $CHCl_3$-DMF (3:1), 25 °C, 24 h (88%); (f) LiOH (4 eq.), THF-MeOH (2:5), 25 °C, 20 h (77%).

solution of $TbCl_3 \cdot 6H_2O$ (in methanol−chloroform) to a methanolic solution of the lipid.[26]

Liposomes were prepared (total lipid concentration: 2.0 mg/mL), incorporating lipids **1**·Tb^{3+} (5%) with polymerizable phosphocholine **8**[28] (90%) and cationic lipid **9**[29] (5%)

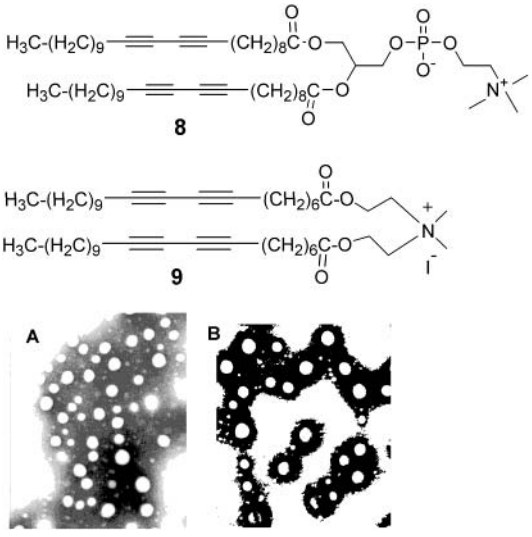


Figure 2. Structures for the lipids used along with **1** to fabricate the liposomes. The TEM pictures of the polymerized liposomes without **1**·Tb^{3+} (A) and with **1**·Tb^{3+} (B) are also shown. The average diameter of the liposomes was found to be 600 Å.

in HEPES buffer (50 mM, pH = 8.0, structures of **8** and **9** are indicated in Figure 2) following a literature procedure.[20b] After extrusion through polycarbonate membranes (pore size 500 Å), the liposomes were polymerized by UV irradiation (254 nm) at 25 °C (450 W, 15 min). Formation of the liposomes was confirmed by transmission electron microscopy (Figure 2).

Figure 3A shows the excitation and luminescence spectra

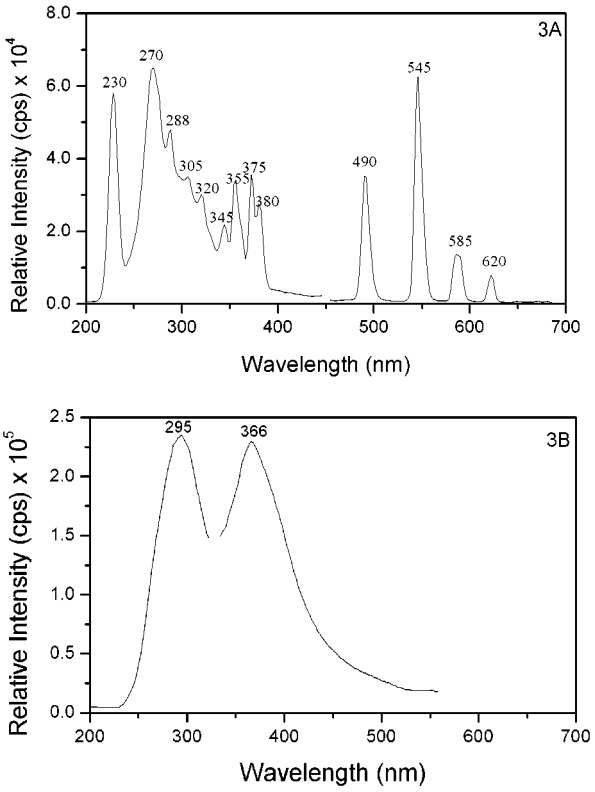


Figure 3. (A) Excitation and emission spectra of $TbCl_3$ in HEPES buffer solution (pH = 8). Lanthanide concentration was 7.9 mg/mL. (B) Excitation and emission spectra of lipid **1** solution prepared in $CHCl_3$:CH_3OH (0.5:9.5 v/v) at the 0.19 mg/mL concentration. In all cases, blank contributions are subtracted from spectra.

of $TbCl_3$ in HEPES buffer solution (50 mM, pH = 8.0). The excitation spectrum was recorded by monitoring the fluorescence emission at 545 nm as a function of excitation wavelength (200−450 nm). The emission spectrum (450−700 nm) was recorded upon sample excitation at 230 nm. The fluorescence bands are characteristic of free Tb(III) ions in solution and correspond to the electronic transitions $^5D_4$−$^7F_6$ (490 nm), $^5D_4$−$^7F_5$ (545 nm), $^5D_4$−$^7F_4$ (585 nm), and $^5D_4$−$^7F_3$ (620 nm).[30] Figure 3B shows the excitation (200−320 nm) and fluorescence emission (330−550 nm) spectra of lipid **1**. The fluorescence emission results from the diacetylene unit. These spectra were recorded at the maxi-

(28) Commercially available from Avanti Polar Lipids, Alabaster, AL.
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mum excitation (295 nm) and emission (366 nm) wavelengths of the lipid.

The excitation (200−450 nm) and emission (400−700 nm) spectra of the preformed **1**·Tb^{3+} complex are shown in Figure 4. The emission spectra were recorded upon sample excitation at 247 nm (A, exciting Tb^{3+}) and 306 nm (B, exciting the lipid). Comparing these spectra with the luminescence spectrum of free Tb^{3+} (Figure 3A), it was noted that, with the exception of one peak at 620 nm (for which maximum wavelength was shifted 5 nm to the red), all the other wavelengths remained constant. This was expected, since Tb(III) electronic transitions involve the promotion of 4f electrons, which are shielded from chemical perturbation by the presence of electrons in the outer 5th shell. Energy transfer from the lipid to the metal ion is suggested by the enhancement of luminescence emission observed upon excitation of **1**·Tb^{3+} at 306 nm (I_{306}) when compared to the signal intensity upon excitation at 247 nm (I_{247}). I_{306} is approximately 50 times higher than I_{247}. It is important to note that this enhancement should not be attributed to chelation between EDTA and Tb^{3+}. The fluorescence enhancement due to chelation is only 1.7. In addition, the excitation spectrum of the EDTA.Tb^{3+} complex shows minor modifications when compared to the excitation spectrum of free Tb^{3+} in solution (Supporting Information).

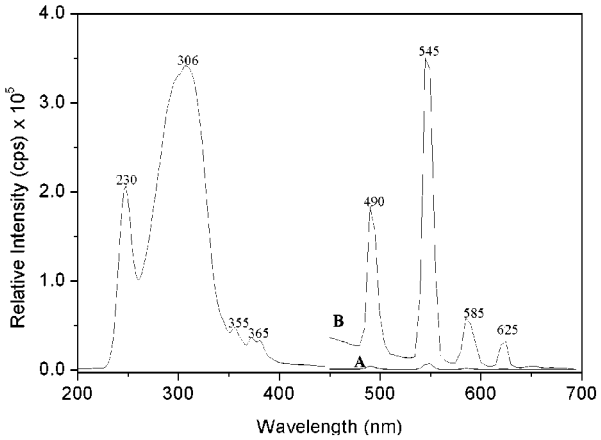

Figure 4. Excitation and emission spectra of lipid **1**:Tb^{3+} complex in $CHCl_3$:CH_3OH solution (1:9 v/v). Complex concentration was 0.17 mg/mL. Emission spectra were recorded upon sample excitation at 247 nm (A) and 306 nm (B). In all cases, blank contributions are subtracted from spectra.

Upon polymerization, the excitation and emission spectra of the liposome showed maximum wavelengths at 375 and 450 nm, respectively (Figure 5A). When compared to the lipid's maximum wavelengths, a considerable red shift (~80 nm) was noted. This shift can be attributed to the conjugation of fluorophore units after polymerization and cross-linking of the lipid.[6b] Figure 5B shows the emission spectrum of the Tb(III)-polymerized liposomes (i.e., **1**·Tb^{3+} incorporated into liposomes and then polymerized) at four excitation wavelengths. The effect of energy transfer is noted by comparing the spectral features of Tb(III). Shifting the excitation wavelength from 288 to 311 nm increases Tb(III) luminescence intensity. Sample excitation beyond this wavelength causes an opposite effect. At 311 nm, however, the efficiency of the energy transfer process from the liposome to Tb(III) is lower than the one observed from the lipid to the metal. Two possibilities can be pointed out for this observation: (1) the 80 nm shift observed in the fluorescence spectrum of the donor, which reduces overlapping with the metal absorption; (2) inner filter effects caused by liposome. Further studies are in progress to completely understand this phenomenon.

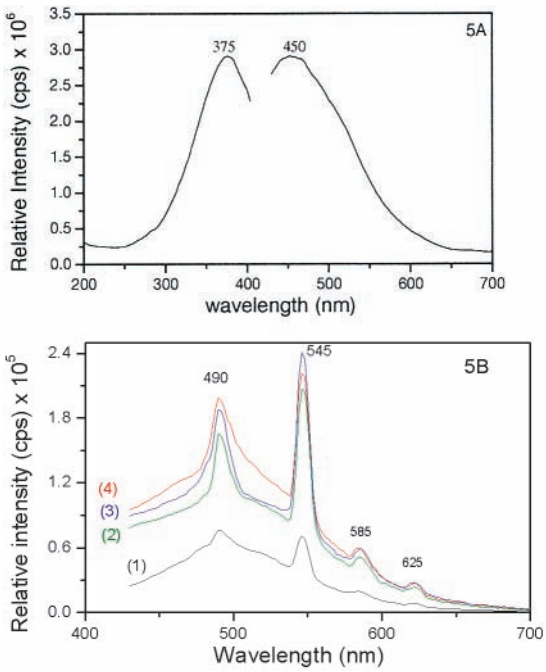

Figure 5. (A) Excitation and emission spectra of liposome in HEPES solution (pH = 8). Total lipid concentration was 2 mg/mL. (B) Emission spectra of the liposome:Tb^{3+} complex in HEPES solution (pH = 8) at the 2 mg/mL concentration. Sample excitation was performed at (1) 288 nm; (2) 305 nm; (3) 311 nm; (4) 320 nm. In all cases, blank contributions are subtracted from spectra.

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Supporting Information Available: Characterization data for lipid **1** and the complex **1**·Tb^{3+} and luminescence spectra for EDTA−Tb^{3+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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