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Synthesis of New, Pyrene-Containing, Metal-Chelating Lipids and Sensing of Cupric Ions

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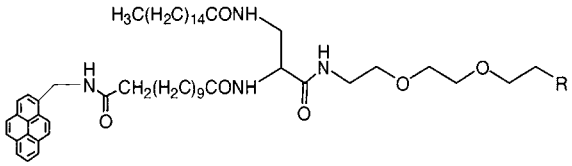
ABSTRACT



The syntheses of several saturated, pyrene-containing, metal-chelating lipids are described. These lipids are capable of strongly binding to transition metal ions employing the metal-chelating headgroup. The excimer-to-monomer ratio of the pyrene groups changes with addition of cupric ions to the liposomes. Three other transition metal ions (Zn^{2+}, Ni^{2+}, and Hg^{2+}) did not cause any appreciable changes in the excimer-to-monomer ratio.

Lipid bilayers (in particular, liposomes) have been used extensively in supramolecular chemistry as animal cell models, as separation agents, as catalysts, etc.[1] Due to their biocompatibility, liposomes are also widely used as technological tools for biomedical research, e.g., as drug carriers,[2] as diagnostic agents,[3] as gene delivery agents,[4] etc. The membrane surface of liposomes can be modified by incorporating functionalized lipids; the resultant liposomes have been used as biosensors and in molecular recognition.[5] In addition, polymerizable lipids have been incorporated into liposomes in order to fabricate stable lipid-based systems with controlled permeability.[5b,6]

Fluorescence spectroscopy (employing organic fluorophores) is a unique and useful technique for probing the binding of liposomes to proteins and other biomolecules.[7] Metal-chelating lipids with fluorescent tags have been used for molecular recognition of proteins[8] and for protein sensing.[9] One widely used fluorophore is the pyrene moiety.

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After excitation, pyrene emits fluorescence from the excited monomer and from the excimer (excited-state dimer).[10] In pyrene-containing lipids, the intensity of the excimer peak has been extensively used to study lipid redistribution in liposomes.[8b,9] When the lipids have metal-chelating headgroups, addition of metal ions to the resultant liposomes causes the lipids to disperse, leading to a decrease in the excimer emission intensity. The liposomes can be used as sensors for transition metal ions.[11]

The synthesis of various metal-chelating lipids are reported in the literature;[12] however, reports for the synthesis of pyrene-containing, metal-chelating lipids are relatively few.[13] For the reported lipids, the metal-chelating headgroups are usually iminodiacetate (IDA) or nitrilotriacetate (NTA). Herein, we describe the synthesis of several metal-chelating lipids with a variety of metal chelating headgroups and pyrene as the fluorophore. The EDTA and DTPA headgroups were selected for their ability to complex transition and lanthanide ions with high affinity.[14] The lipids were incorporated into liposomes, and the fluorescence property of the pyrene moiety (excimer-to-monomer ratio) was followed in the presence of various transition metal ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, and Hg^{2+}). The results indicated that the liposomes can be used to sense cupric ions.

The structures of pyrene-containing, metal-chelating lipids are shown in Figure 1. These lipids are based on racemic 2,3-diaminopropanoic acid. Lipid **1** has IDA as the metal-chelating headgroup. IDA has a strong affinity ($K > 10^{12}$ M^{-1}) for various transition metal ions (e.g., Cu^{2+}, Ni^{2+}, Co^{2+}, etc.).[15] Lipid **2** has two metal-chelating headgroups (IDA). The two IDA groups of this lipid can position two transition metal ions ~8 Å apart.[16] Lipid **3** contains the widely used EDTA as the metal-chelating headgroup. EDTA has a strong affinity ($K > 10^{15}$ M^{-1}) for lanthanide ions.[17] DPTA has been incorporated into lipid **4** as the metal-chelating headgroup. The ligand DTPA complexes lanthanide ions with high affinity ($K > 10^{20}$ M^{-1}).[18]

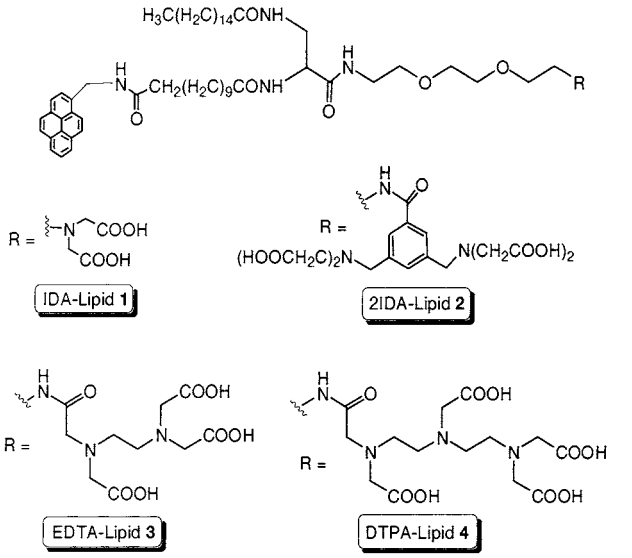

Figure 1. Structures of the lipids synthesized.

The synthesis of the lipids is depicted in Scheme 1. Commercially available diacid (1,10-decanedicarboxylic acid, **9**) was selectively converted into monoester **10**. Slow addition of a solution of ethanol (over a 10 h period, 5 mL/h, using a syringe pump) to a solution of acid chloride from **9** in the presence of pyridine afforded **10** in 49% yield after chromatographic purification. Acid-ester **10** was then coupled with 1-pyrenemethylamine using BOP reagent in excellent yield. Selective saponification with LiOH was used to generate acid **11**. Selective amidification of the primary amine over the secondary amine group in **12** (racemic) was performed with palmitoyl chloride (1 equiv) in the presence of triethylamine to give monoamide **13** in 43% yield after purification. Sequential amidification of monoamide **13** with **11** was carried out by BOP reagent. The ester groups were hydrolyzed by $LiOH{\cdot}H_2O$ to provide compound **14**. Compound **14** is the common synthon for the syntheses of lipids **1**−**4** (Scheme 1).

For the synthesis of IDA-based lipid **1**, acid **14** was combined with reported IDA−amine ester **15**[16] in the presence of BOP/Et_3N. The hydrolysis of ester group and subsequent precipitation by lowering the pH to 3.0 provided the lipid. Lipid **2** was synthesized by coupling of acid **14** with amine **16**[16] and subsequent ester hydrolysis. Acid **14** was combined with EDTA−amine **17**[13] in the presence of BOP reagent and followed by hydrolysis to afford lipid **3** in

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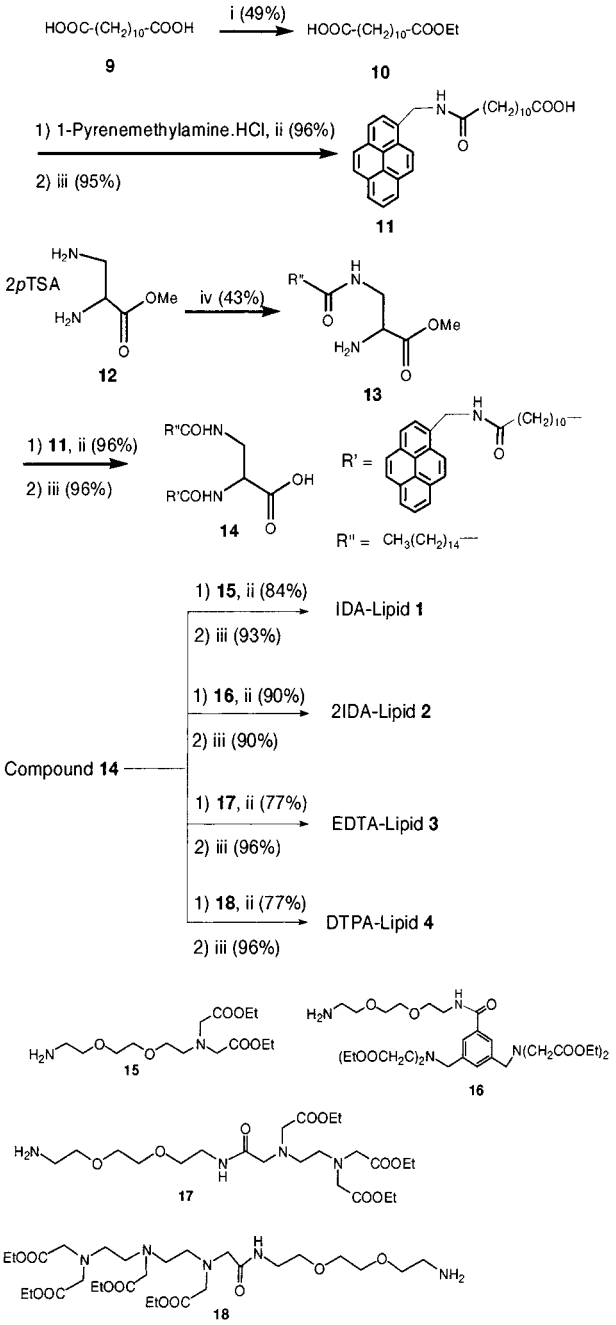
Scheme 1. Synthesis of the Lipids[a]



[a] Reagents and conditions: (i) $SOCl_2$, 5 h reflux, then EtOH (1.0 equiv), pyridine, CH_2Cl_2, 16 h, rt; (ii) BOP reagent, Et_3N, $CHCl_3$−DMF, rt; (iii) LiOH,H_2O, MeOH−THF, rt, then pH = 3.0 with 2 N HCl; (iv) palmitoylchloride (1.0 equiv), Et_3N, CH_2Cl_2, rt.

good yield. Finally, lipid **4** was prepared by coupling of acid **14** and DTPA−amine **18**[13] and subsequent hydrolysis with LiOH. Once isolated, the lipids were found to be pure (^{1}H NMR and elemental analysis, Supporting Information).

Fluorescence spectra of the lipids in DMSO solution did not indicate the presence of any excimers (data not shown). All of the lipids can form liposomes (concentration: 2.0 mg/mL) under standard liposome formation conditions.[11a] The liposomes gave intense pyrene excimer emission at 470 nm upon excitation at 342 nm. As expected, the excimer-to-monomer emission intensity ratio was insensitive to added transition metal ions for these single-component liposomes (data not shown).

For the fabrication of more useful systems, the lipids were incorporated into mixed liposomes (10% w/w, 90% distearoyl phosphocholine, 50 mM HEPES buffer, pH = 7.0, total lipid concentration = 2.0 mg/mL). Upon excitation of the pyrene moiety (342 nm), the emission spectrum showed that both pyrene monomers (395 nm) and excimers (470 nm) were present in the liposomes (Figure 2A). The excimer to

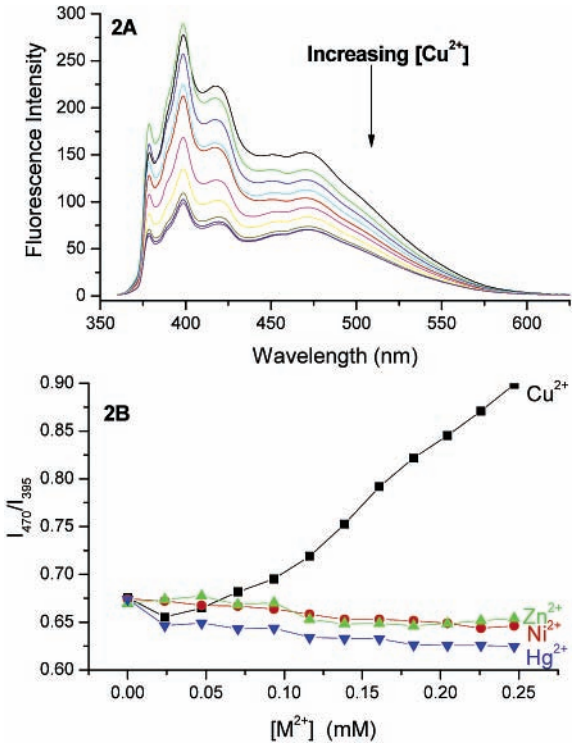


Figure 2. Titration of liposomes from lipid **1** (10% w/w) with cupric ions (2A) and the plot of the intensity ratios (I_{470}/I_{395}) as a function of the concentration of added metal ions (2B). The increase in cupric ion concentration is indicated in 2A.

monomer intensity ratio (I_{470}/I_{395} = 0.69) clearly indicated that lipid **1** was aggregated in the liposomes. The addition of cupric ions (aqueous $CuCl_2$ solution) caused the overall intensity of the emission spectra to decrease (Figure 2A), possibly indicating quenching by the cupric ions.[19] However, the ratio of the excimer to monomer emission intensity (I_{470}/I_{395}) increased (Figure 2B). Similar behaviors were also observed for liposomes from lipids **2**−**4** (Supporting Information) and liposomes with lower total lipid concentration (1 mg/mL). The increase of the pyrene excimer to monomer intensity ratio (I_{470}/I_{395}) for lipid **4** was not as pronounced as that for the other lipids (Supporting Information). TEM

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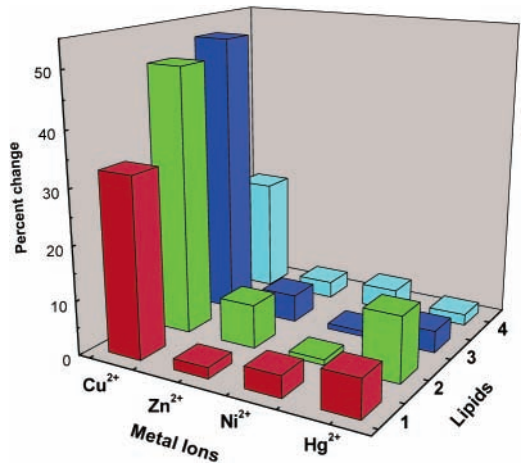

Figure 3. Relative increase of the I_{470}/I_{395} ratio with transition metal ions for the saturated lipids, [metal ions] = 250 μM.

studies after the titration experiments indicated that the liposomes were not aggregated by the transition metal ions.

Usually, metal-chelating lipids disperse when transition metal ions are added to liposomes.[9,11a−e] A few reports of lipid aggregation in liposomes mediated by cupric ions are known in the literature.[11f−h] In our case, since the total fluorescence intensity of the liposomes decreases with added cupric ions, the results indicate quenching by the cupric ions rather than lipid redistribution in the liposomes. It is possible that the cupric ions quench the excited states of pyrene monomer selectively compared to the pyrene excimer, leading to the increase in excimer-to-monomer ratio. Selective quenching of excited-state pyrene monomers by cupric ions has been reported in the literature by other groups also.[20]

Three other transition metal ions tested (Ni^{2+}, Hg^{2+}, and Zn^{2+}) did not cause any appreciable change in the excimer to monomer intensity ratio. The ions did not cause any significant decrease of the overall fluorescence intensity either (Supporting Information).

The relative increase in the I_{470}/I_{395} ratio for lipids **1**−**4** with the transition metal ions is graphically illustrated in Figure 3 ([metal ion] = 250 μM). The results indicate the ability of the liposomes from these lipids to sense cupric ions.

In conclusion, syntheses of four new, pyrene-containing lipids have been reported. The lipids aggregate in liposomes, and the pyrene monomer emission is selectively quenched in response to added cupric ions. Three other transition metal ions do not cause any changes to the pyrene excimer-to-monomer intensity ratio in the liposomes. The liposomes have the potential to be used as sensors for cupric ions.

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Supporting Information Available: Synthetic details for the reported lipids **1**−**4**, liposome formation and titration procedures, and fluorescence titration curves for lipids **2**−**4** for the transition metal ions Cu^{2+}, Ni^{2+}, Hg^{2+}, and Zn^{2+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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