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Macrocyclic Eu^{3+} Chelates Show Selective Luminescence Responses to Anions

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A series of lanthanide-containing macrocycles, **Eu(2)**−**Eu(5)**, exhibited unique luminescent responses in the presence of strong hydrogen-bond-accepting anions (F^-, CH_3COO^-, and $H_2PO_4^-$) in dimethyl sulfoxide. The macrocycles examined herein were designed to include a lanthanide chelate, aromatic spacers that function as antennae, thiourea groups as anion-binding units, and an alkyl or aryl linker between the thioureas that tailors the size and rigidity of the macrocycle. The anion-induced change in the emission intensity (λ_{exc} = 272 nm; λ_{em} = 614 nm) varied across the series of macrocycles and was dependent on the basicity of the anion. The largest luminescence response was observed in **Eu(2)**, whereby the emission increased 77% upon the addition of 8 equiv of fluoride. A change in luminescence was not observed when exciting Eu^{3+} directly (λ_{exc} = 395 nm) over the course of anion titration experiments with all of the anions studied. These macrocycles contain only slight variations in structure, and insights into the mechanism of the anion interaction have been gained through monitoring of anion titrations via luminescence, absorbance, and luminescence lifetime measurements. In addition, model compounds (**2**–**5**) lacking the Eu^{3+} moiety were synthesized to study the binding pockets of **Eu(2)**−**Eu(5)** using absorbance and ^1H NMR spectroscopy. These studies indicate that the anions interact with the thiourea moiety of **Eu(2)**−**Eu(5)**, and the luminescent response is controlled by changes in the morphology of the macrocycle binding pocket.

Introduction

Because of the importance of anions in industrial and environmental applications, as well as their essential physiological roles, there has recently been an increased demand for selective anion sensors.[1] For example, the recent elevated consumption of fluoridated water has increased public concerns with the detrimental health effects of fluoride on the development of conditions such as dental and skeletal fluorosis.[2] Although efforts to discriminate fluoride from the other halides have been successful with a variety of designed receptors,[3,4] the selectivity in the presence of other common anions remains a challenge. In addition, the important role of carboxylates in many ligand-binding interactions with proteins[1] motivates fundamental studies of carboxylate recognition and sensing, ranging from the simple acetate anion[5] to more biologically relevant amino acids and di- and tricarboxylates.[6,7] Also, metabolic pathways driven by the hydrolysis of adenosine triphosphate involve the release of

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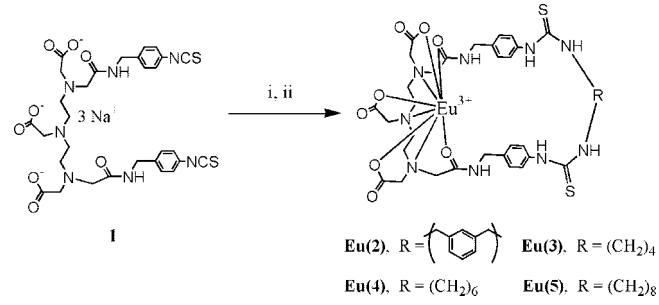
orthophosphate and pyrophosphate, and synthetic receptors selective for these anions have also been the objective of numerous studies.[8] Hence, the development of molecular systems combining both anion recognition components and signaling units for use as anion sensors is of high interest for probing biological systems.

In general, anions are large and carry a more diffuse charge relative to cations, which challenges chemists to design novel preorganized receptors to effectively compete with solvent molecules for anion binding of high affinity.[1] A variety of routes have been employed to design novel materials endowed with anion recognition properties. Many of the receptors used in recognition of the aforementioned anions employ neutral hydrogen-bond-donating groups such as amides, ureas, thioureas, and pyrroles. Thioureas are often used in receptor design because they offer directional hydrogen-bond donors (−NH), and the acidity of these moieties can be modulated by changing the electron-withdrawing nature of adjacent functional groups.[1h,4] Because it is common for hydrogen-bond-accepting anions (i.e., F^-, OAc^-, and $H_2PO_4^-$) to elicit a similar receptor affinity or sensor response with only moderate selectivities, research is generally focused on structurally tailoring many previous receptors to improve the selectivity for the anion of interest.[1,3,5–8] Toward this end, thiourea binding groups have been coupled with organic fluorescence signaling molecules where photoinduced electron transfer occurs from the sulfur atoms upon an anion-binding event, resulting in highly sensitive fluorescence quenching.[1,6b,c]

Scheme 1. Synthesis of **Eu(2)**−**Eu(5)**[a][11]



[a] (i) NH_2RNH_2, MeOH/H_2O (high dilution). (ii) $Eu(Cl)_3$, H_2O.

In the design of molecular systems for potential sensors in biological samples, it is important to anticipate the possibility of background fluorescence, which could interfere with measurements of target analytes. Lanthanides have found application in this area as signaling units because of their long luminescence lifetimes, which allow for time-resolved fluorescence (TRF) measurements to be obtained, which is particularly useful for biological systems with high autofluorescence.[9] Lanthanides have also been examined previously for anion sensing; however, direct coordination of the anion to the lanthanide is typically required, where in an aqueous solution, displacement of water molecules results in enhanced luminescence intensity and longer luminescence lifetimes.[7,10] Inspired by the properties of both systems, we have reported the synthesis of four macrocyclic systems, denoted **Eu(2)**−**Eu(5)** (Scheme 1).[11] These macrocycles were the first examples of receptors that incorporate two opposing thiourea groups that possibly serve as preorganized anion-binding sites and contain an *N*,*N*″-bis(amide) derivative of diethylenetriaminepentaacetic acid (DTPA) to chelate Eu^{3+} as a signaling unit.[11] This general motif has been designed to allow for the size, flexibility, and functionality of the macrocycle pocket to be synthetically varied with relative ease and to tune the system for enhanced selectivity. In addition, this series was designed to examine how structural changes affect anion response behavior. Our systems include functionalized aromatics to act as spacers between the binding and signaling moieties and serve as antennae to enhance the lanthanide emission intensity through energy transfer.[9] These macrocycles were designed with biological sensing in mind because of the possibility of performing time-resolved measurements.

Herein, we report the luminescence, anion recognition/sensing properties, and signaling mechanism studies of these

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novel macromolecules [**Eu(2)**−**Eu(5)**], and their responses are compared as a function of the linker moiety. It was generally found that the luminescent response to fluoride and acetate varies widely across the series of macrocycles studied and that the luminescence intensity changes observed during the anion titrations were not found to be correlated to changes in the absorption spectrum of the macrocycle in the solvent studied (dimethyl sulfoxide, DMSO). We have discovered that a particular system, **Eu(2)**, which contains a *m*-xylyl linker, shows a selective luminescence response to fluoride as compared to the other halide anions and is also selective for acetate over dihydrogen phosphate and nitrate in DMSO. To further examine this phenomenon, organic bis(thiourea) sensors **2**–**5** were synthesized and characterized as a model system to elucidate the sensing response of **Eu(2)**−**Eu(5)** to acetate and fluoride using absorption spectroscopy and ^{1}H NMR. These model studies, coupled with luminescence lifetime studies of Eu^{3+} emission in the original macrocycles, support our theory that thiourea-based (and not Eu^{3+}) anion binding is responsible for the recognition event with these materials. To the best of our knowledge, this is one of the first examples of lanthanide-based anion sensors that does not require direct coordination of anions to the metal.[8j,10] These macrocycles appear to exploit a unique signaling mechanism related to the efficiency of energy transfer and the distance of the ligand antennae to the lanthanide moiety.[10] Additionally, we reveal that **Eu(2)**−**Eu(5)** reversibly bind various anions and remain responsive to fluoride and acetate in DMSO solutions containing a high background of chloride ions. Because of the millisecond lifetimes of europium emission and our findings that anions do not directly coordinate to Eu^{3+}, these macrocycles show promise to be further developed as unique receptors that exploit the ratio in the emission intensity at the two different wavelengths of excitation [emission intensity at λ_{ex} = 272 (antennae)/ emission intensity at λ_{ex} = 395 (Eu^{3+})] for absolute concentration measurements. These findings are important scientific steps toward the design of selective, specific, and time-resolved sensors for analysis of biological samples where the physiological NaCl concentration can interfere with the measurement of target anions.

Experimental Section

General Procedures. All reagents used in the synthesis, if not specified, were obtained from either Aldrich Chemical Co. (Milwaukee, WI) or Acros Organics (Morris Plains, NJ) and were used without further purification. **Eu(2)**−**Eu(5)** were prepared as previously reported by our group, according to Scheme 1.[11] The mass spectra were obtained from an IonSpec HiResESI mass spectrometer in positive ion mode. NMR spectra were collected on a Bruker AV-400 MHz spectrometer. Titration experiments monitored via ^{1}H NMR were conducted according to the method of Snellink-Rüel et al.[8g] Ultraviolet absorption data were obtained using a Varian Cary 50 UV–visible spectrophotometer. Luminescence studies were performed using a Varian Cary Eclipse fluorescence spectrophotometer. Dimethyl sulfoxide (DMSO) used in the absorption and luminescence experiments was spectrophotometric grade, and CH_3CN was distilled over CaH_2 before use.

General Procedure for Model Receptor Syntheses. α,α′-Bis-(*N*′-*p*-tolylthioureylene)-*m*-xylene (2). *p*-Tolyl isothiocyanate (0.983 g, 6.60 mmol) was dissolved in CH_2Cl_2 (7 mL), and *m*-xylylenediamine (0.449 g, 0.330 mmol) in CH_2Cl_2 (3 mL) was added dropwise over 5 min. This mixture was stirred at room temperature for 14 h, during which a white solid precipitate formed. The precipitate was filtered, washed with CH_2Cl_2 (3 × 3 mL), and dried in vacuo, yielding a white solid. Yield: 1.22 g, 85.3%. ESI-MS. Calcd: *m*/*z* 435.168 [(**2**)H$^+$]. Found: *m*/*z* 435.158. UV–vis (DMSO): λ_{max}/nm (ϵ/M^{-1} cm^{-1}): 272 (24 700). UV–vis (CH_3CN) λ_{max}/nm (ϵ/M^{-1} cm^{-1}) 260 (26 900). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.54 (s, 2H, NH_{ar}), 8.07 (s, 2H, N*H*CH$_{2ar}$), 7.29–7.12 (m, 12H, H_{ar}), 4.73 (s, 4H, CH_2), 2.26 (s, 6H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 181.3 (*C*S), 139.7, 136.9, 134.1, 129.6, 128.7, 126.8, 126.4, 124.3, 47.7, 21.0.

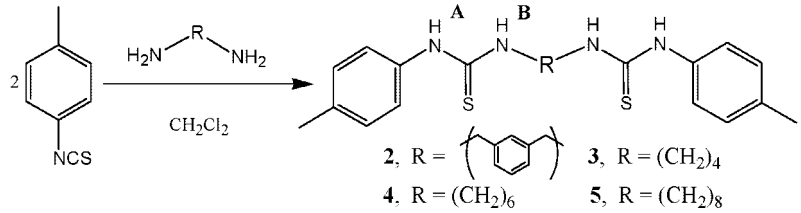
1,4-Bis(*N*′-*p*-tolylthioureylene)butane (3). *p*-Tolyl isothiocyanate (0.527 g, 3.54 mmol) and 1,4-diaminobutane (0.155 g, 1.76 mmol) were reacted as described above for **2**. Compound **3** was isolated as a white powder. Yield: 0.601 g, 88.5%. ESI-MS. Calcd: *m*/*z* 387.168 [(**3**)H$^+$]. Found: *m*/*z* 387.169. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.38 (s, 2H, NH_{ar}), 7.63 (s, 2H, N*H*CH$_2$), 7.23 (d, 4H, H_{ar}), 7.14 (d, 4H, H_{ar}), 3.47 (s, 4H, CH_2NH), 2.27 (s, 6H, CH_3), 1.54 (s, 4H, CH_2CH$_2$NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.8 (*C*S), 136.9, 133.9, 129.6, 124.0, 44.1, 26.6, 20.9.

1,6-Bis(*N*′-*p*-tolylthioureylene)hexane (4). *p*-Tolyl isothiocyanate (0.527 g, 3.54 mmol) and 1,6-diaminohexane (0.204 g, 1.76 mmol) were reacted as described above for **2**. Compound **4** was isolated as a white powder. Yield: 0.560 g, 76.8%. ESI-MS. Calcd: *m*/*z* 415.199 [(**4**)H$^+$]. Found: *m*/*z* 415.201. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.34 (s, 2H, NH_{ar}), 7.61 (s, 2H, N*H*CH$_2$), 7.25 (d, 4H, H_{ar}), 7.13 (d, 4H, H_{ar}), 3.44 (s, 4H, CH_2NH), 2.27 (s, 6H, CH_3), 1.53 (s, 4H, CH_2CH$_2$NH), 1.31 (s, 4H, CH_2CH$_2$CH$_2$NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.8 (*C*S), 136.9, 133.8, 129.5, 123.9, 44.3, 29.0, 26.7, 20.9.

1,8-Bis(*N*′-*p*-tolylthioureylene)octane (5). *p*-Tolyl isothiocyanate (0.523 g, 3.51 mmol) and 1,8-diaminooctane (0.250 g, 1.74 mmol) were reacted as described above for **2**. Compound **5**was isolated as a white powder. Yield: 0.605 g, 78.7%. ESI-MS. Calcd: *m*/*z* 443.230 [(**5**)H$^+$]. Found: *m*/*z* 443.228. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.33 (s, 2H, NH_{ar}), 7.59 (s, 2H, N*H*CH$_2$), 7.23 (d, 4H, H_{ar}), 7.13 (d, 4H, H_{ar}), 3.43 (s, 4H, CH_2NH), 2.27 (s, 6H, CH_3), 1.52 (s, 4H, CH_2CH$_2$NH), 1.29 (s, 8H, CH_2CH$_2$CH$_2$NH and CH_2CH$_2$CH$_2$CH$_2$NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.8 (*C*S), 137.1, 133.8, 129.5, 124.0, 44.3, 29.2, 29.0, 26.8, 20.9.

Luminescence Studies. Excitation and emission spectra of **Eu(2)**−**Eu(5)** (DMSO, 1.0 × 10^{-5} M) were obtained in phosphorescence mode with a total decay time of 0.01 s, a delay time of 0.10 ms, and a gate time of 5.0 ms. Excitation and emission slit widths were set at 5 and 10 nm, respectively. The excitation wavelengths (λ_{exc}) used in the emission studies were either 272 or 395 nm. The emission wavelength (λ_{em}) for the excitation studies was 614 nm. The anion titration studies were performed at the above instrument settings, and each aliquot of anion solution was made such that 10.0 μL = 1 equiv of anion, where "1 equiv" of anion means that the anion and receptor are present in equal molar amounts (a 1:1 molar ratio). The aliquots were added to a cuvette originally containing 3.00 mL of macrocycle solution in DMSO (1.0 × 10^{-5} M). Each data point was the average of three measurements, and the titrations were performed in duplicate (maximum error <5%). All anions were used as their tetrabutylammonium (TBA) salts. The stock solution of TBAOH was prepared using a 40% (w/w) TBAOH solution in water. The luminescence lifetime titrations of **Eu(2)**−**Eu(5)** were performed

Scheme 2. Synthesis of Compounds **2–5**, Where the Thiourea Protons Are Denoted as H_A and H_B



in DMSO (1.0×10^{-5} M) with a delay time of 0.05 ms, a gate time of 0.2 ms, $\lambda_{exc} = 272$ or 395 nm, and $\lambda_{em} = 614$ nm. Excitation and emission slit widths were 10 nm, and each data point was the average of five measurements.

Binding Constant Determinations. Binding constants were calculated from the ^{1}H NMR chemical shift data of the titration of **2–5** with TBAOAc (DMSO-d_6) using *WinEQNMR* software.[12] Binding constants for complexation of **Eu(2)** and **Eu(5)** with either acetate or dihydrogen phosphate (DHP) in DMSO were determined from the luminescence data using the method of Fery-Forgues et al.[13]

Results and Discussion

Synthesis. In an effort to design anion-selective sensors, previously, we reported the synthesis of compound **1**, along with **Eu(2)**−**Eu(5)** (Scheme 1).[11] These receptors were originally developed to include (i) a luminescent signal at the Eu^{3+} center, (ii) functionalized benzene spacers to act as antennae to enhance the Eu^{3+} emission, (iii) thiourea groups for anion binding, and (iv) a linker that connects the opposing thioureas and allows variation of the flexibility and size of the macrocycle cavity. This series of macrocycles was also developed to gain insight into how the size and flexibility of the macrocycle pocket, especially near the hydrogen-bond-donating thiourea moieties, affect anion binding and selectivity. As shown in Scheme 1, the macrocycles can be readily synthesized by the reaction of the bis(isothiocyanate) precursor, **1**, with an alkyl- or xylyl-diamine in dilute aqueous methanol. This synthetic pathway is advantageous in that a variety of macrocycles can be generated from one precursor, allowing for tailoring of the macrocycle pocket by the selection of the diamine used in this cyclization step. It should be noted that a slightly modified method of isolation and purification of the final ligands was utilized, involving precipitation of the macrocyclic ligand out of an aqueous solution using dilute HCl to remove bicarbonate impurities, prior to the addition of $EuCl_3$. In the present study, we sought to examine how the various structural changes within the series **Eu(2)**−**Eu(5)** affect the recognition and signaling properties in the presence of various anions through absorbance, luminescence, and ^{1}H NMR experiments.

To accompany the macrocycle-based studies, organic model compounds, **2–5**, similar in structure to **Eu(2)**−**Eu(5)** but lacking the DTPA-lanthanide unit, were synthesized to examine the role of the thiourea moiety in anion binding. Specifically, model compounds **2–5** were designed to assess the acidity of the thiourea protons in **Eu(2)**−**Eu(5)** and to determine if anions could be bound by the thiourea moieties separated by butyl, hexyl, octyl, and *m*-xylyl linkers utilized in these systems. Anion binding to these model compounds was monitored via absorbance and ^{1}H NMR to support our hypothesis that the lanthanide in the macrocycle is not involved in anion binding. This study was important because the Eu^{3+}-functionalized macrocycles cannot be structurally examined via NMR because of their paramagnetic nature and poor solubility properties and also crystallization has been unsuccessful. It is assumed that the acidities of the thiourea protons in the model compounds **2–5** and their macrocyclic analogues **Eu(2)**−**Eu(5)** should be similar. Compounds **2–5** were synthesized from *p*-tolyl isothiocyanate and the respective diamine in CH_2Cl_2 (Scheme 2), to model the binding pockets of **Eu(2)**−**Eu(5)**. During the course of each reaction, **2–5** precipitated as white solids and did not require further purification, prior to characterization via ESI-MS and ^{1}H and ^{13}C NMR. For compound **2**, resonances appeared at 8.07 and 9.54 ppm in the ^{1}H NMR spectrum, as well as at 181.3 ppm in the ^{13}C NMR spectrum, indicative of the thiourea protons and carbon atom, respectively. Similar resonances were observed in the spectra of **3–5**. In addition, the UV–vis absorbance profile was obtained for **2** ($\lambda_{max} = 272$ nm; $\epsilon = 24\,700$ M^{-1} cm^{-1}) and was quite similar to the spectrum previously reported for **Eu(2)** ($\lambda_{max} = 275$ nm; $\epsilon = 23\,000$ M^{-1} cm^{-1}).[11] These model compounds allowed for anion titration experiments to be performed and monitored via ^{1}H NMR (DMSO-d_6) and UV–vis (CH_3CN), providing insight into the binding mechanism of fluoride and acetate with **Eu(2)**−**Eu(5)** (vide infra). It should be noted that a family of bis(thioureas), structurally related to our model compound **2**, have been previously reported for use as anion binders.[14]

Model Studies. UV–vis absorbance was measured during titrations of **2** with either fluoride or acetate in both DMSO and CH_3CN (Figure 1). In DMSO, where there was a heavy solvent absorbance in the region of interest, the spectral changes were minimal (similar to those obtained for **Eu(2)**). However, in CH_3CN, where no interference from the solvent was present in the region of interest, a small decrease and red shift of the band at 260 nm was observed and was concurrent with the emergence and ensuing increase of a band at 248 nm (λ_{max}) over the course of fluoride or acetate addition (Figure 1). Isosbestic points were present at 258 and 272 nm. The changes observed in the absorbance spectra

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(13) Fery-Forgues, S.; Le Bris, M.; Guetté, J.; Valeur, B. *J. Phys. Chem.* **1988**, *92*, 6233–6237.
(14) (a) Bühlmann, P.; Nishizawa, S.; Xiao, K. P.; Umezawa, Y. *Tetrahedron* **1997**, *53*, 1647–1654. (b) Nishizawa, S.; Bühlmann, P.; Iwao, M.; Umezawa, Y. *Tetrahedron Lett.* **1995**, *36*, 6483–6486.

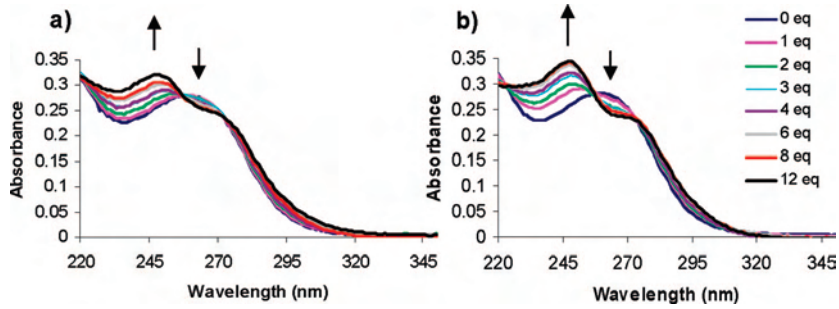

Figure 1. Absorbance titrations of the model compound **2** with (a) TBAF and (b) TBAOAc in CH_3CN (1.0×10^{-5} M).

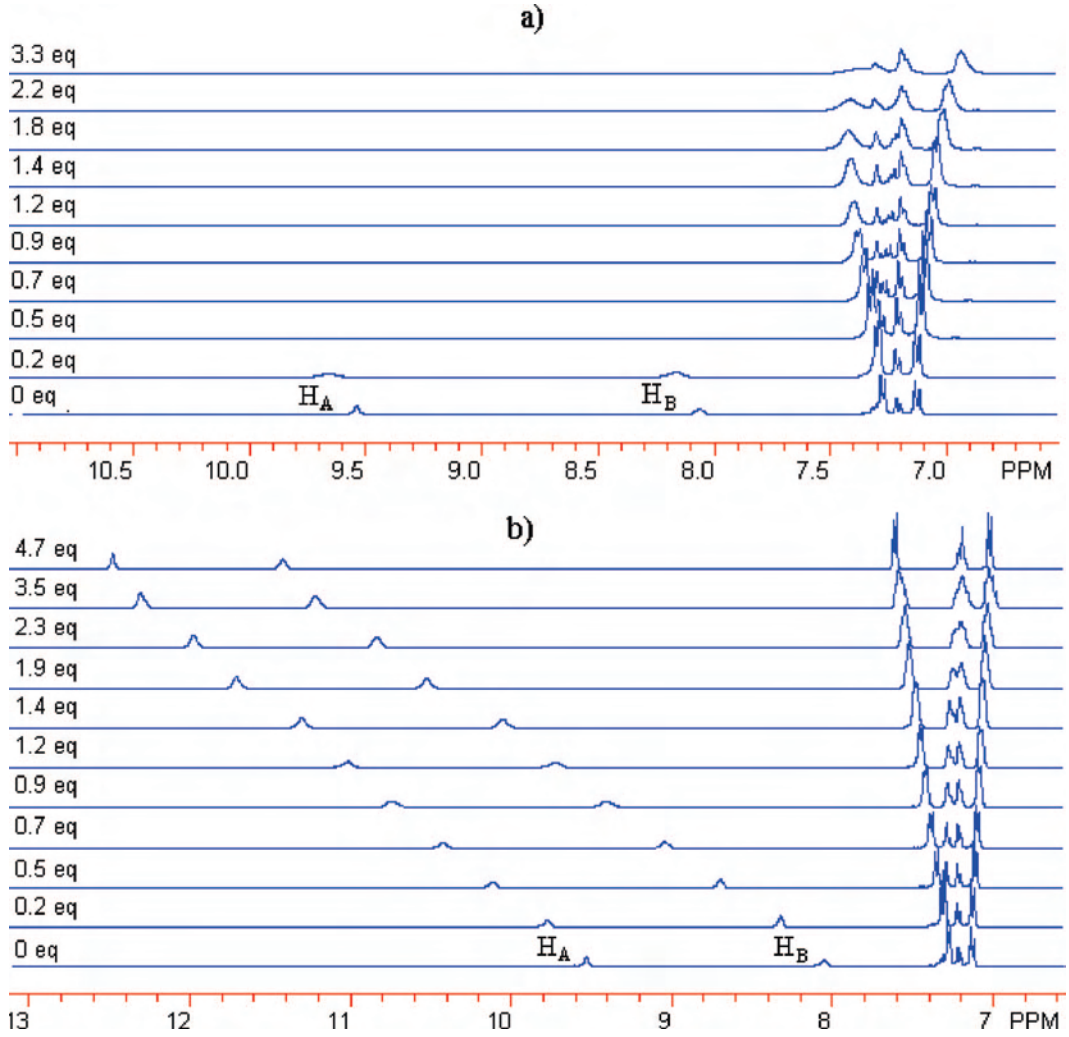

Figure 2. ^{1}H NMR of **2** upon titration with (a) TBAF and (b) TBAOAc in DMSO-d_6 (2.8×10^{-2} M).

were consistent with hydrogen-bond donation and/or deprotonation of the thiourea upon anion addition, affecting the nature of the benzene chromophore.[3f,g,4]

^{1}H NMR was used to more precisely study the interaction between the model compound thiourea protons and the anions by monitoring the change in chemical shift of the thiourea proton resonances of **2** during the titration experiments (Figure 2). Titration of **2** with fluoride in DMSO-d_6 (2.8×10^{-2} M) resulted in a downfield shift and broadening of both thiourea resonances, followed by the disappearance of the resonances entirely at 1.4 equiv. At 2.2 equiv of fluoride, a triplet appeared at 16.1 ppm, characterisitic of $[HF_2]^-$, indicating that deprotonation of the thiourea had occurred. Studies employing thiourea-based systems report similar findings upon titration with fluoride.[3d,4,8h] Titration of **2** with acetate yielded different results. The thiourea resonances were shifted downfield by 2.94 and 3.37 ppm (H_A and H_B, respectively) upon the addition of up to 4.7 equiv of acetate, and minimal broadening occurred, indicating the formation of a hydrogen-bonded [**2**•OAc]$^-$ complex. As shown in Figure 3, the binding stoichiometry was determined using the Job's plot method to be 1:2 (receptor−anion).[8g]

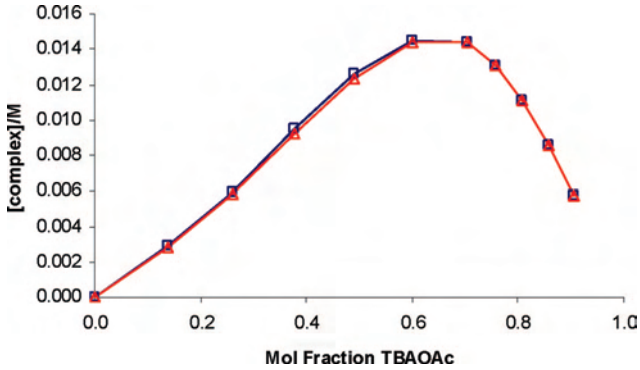

Figure 3. ^{1}H NMR Job's plot of **2** with TBAOAc in DMSO-d_6 using $\Delta\delta$ of H_A (blue squares) and H_B (red triangles).[8g]

Table 1. Association Constants of Compounds **2–5** (1.0×10^{-2} M, DMSO-d_6) with an Acetate Anion[12]

compound	log K_{11}	log K_{12}
2	3.33 ± 0.02	1.81 ± 0.03
3	3.29 ± 0.04	1.97 ± 0.05
4	3.21 ± 0.02	2.15 ± 0.03
5	3.19 ± 0.03	2.17 ± 0.04

However, the binding stoichiometry of the acyclic model **2** does not necessarily translate to **Eu(2)**, where there is a higher preorganization of the opposing thioureas. Job's plot analyses and anion titrations of **3–5** monitored using ^{1}H NMR were also performed and yielded results quite similar to those obtained with **2** for both acetate and fluoride. *WinEQNMR*[12] was then used to determine the association constants of compounds **2–5** with acetate, displayed in Table 1. The strongest association for 1:1 complex formation was observed for the most rigid and preorganized receptor, **2**, containing the xylyl spacer. In 1:1 complexes formed with receptors **3–5**, association was weaker as the spacer length increased. The formation of 1:2 (receptor−anion) complexes with acetate exhibited the opposite trend, as K_{12} was largest with **5**, where the thioureas are separated by the octyl chain. Under the assumption that each acetate anion is bound to only one thiourea group in the 1:2 complexes, the trend observed for K_{12} can be attributed to charge repulsion. As the spacer length increases, binding of a second acetate anion is facilitated. Overall, these binding constants are weak compared to similar receptors that employ electron-withdrawn thioureas[3] but are in the expected numerical range because of the relative electron-rich nature of the aromatic rings directly bonded to the thiourea in compound **2–5**, which has a large effect on the −NH acidity.[1h,4] Weaker binding from individual thiourea units due to lower −NH acidity may be advantageous in producing a reversible sensor molecule that does not irreversibly deprotonate. Strong binding may then be achieved through the preorganization of a number of anion-binding sites, as demonstrated in the enhanced binding affinities for acetate of macrocycles **Eu(2)** and **Eu(5)** (vide infra). The model studies with **2–5** suggest that deprotonation does not likely occur in the titration of macrocycles **Eu(2)**−**Eu(5)** with acetate but could possibly occur in the case of fluoride. In either case, however, there is a favorable interaction between the thioureas and anion(s) that affects

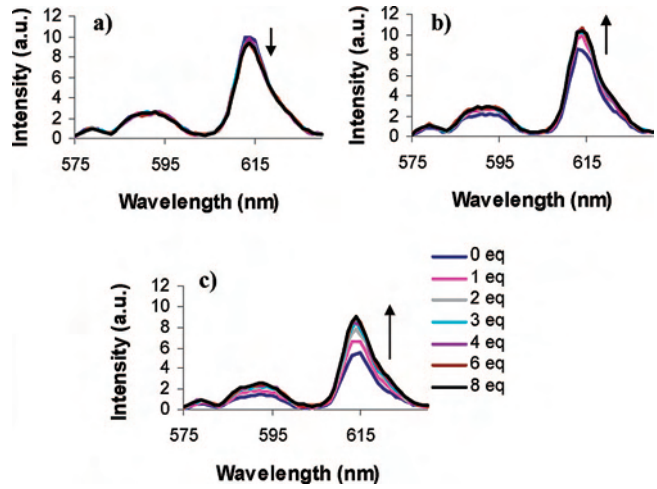

Figure 4. Titration of (a) **Eu(3)**, (b) **Eu(4)**, and (c) **Eu(5)** with TBAF in DMSO (1.0×10^{-5} M) as monitored via luminescence ($\lambda_{exc} = 272$ nm).

the chromophoric benzene antennae as revealed by the UV–vis absorption studies.

Anion Response of Eu^{3+} Macrocycles. All spectroscopic studies of **Eu(2)**−**Eu(5)** were carried out in DMSO because of a lack of solubility of these complexes in other organic solvents. The titration of **Eu(3)**−**Eu(5)** with increasing amounts of fluoride anion was monitored via Eu^{3+} luminescence at $\lambda_{exc} = 272$ nm (Figure 4). This wavelength was chosen for the most efficient sensitization of the Eu^{3+} luminescence through the antenna effect of the macrocyclic ligand (vide infra). In these experiments, the "anion equivalent" denotes the molar ratio of anion titrated into a fixed molar amount of dissolved macrocycle. Thus, "1 equiv of anion" means that the anion and receptor are present in equal molar amounts (a 1:1 molar ratio). It was observed that **Eu(3)** exhibits a slight *decrease* in the intensity of the emission at 614 nm ($^5D_0 \rightarrow {}^7F_2$) by up to 9% as F^- is added, whereas **Eu(4)** and **Eu(5)** show a maximum *increase* in the emission intensity of about 24% and 65%, respectively. The other emission bands ($^5D_0 \rightarrow {}^7F_0$, $^5D_0 \rightarrow {}^7F_1$, and $^5D_0 \rightarrow {}^7F_4$) are affected similarly by the addition of fluoride (the changes in luminescence are not f−f transition specific). These effects are observed over the course of fluoride addition up to 8 equiv (where the maximum change occurs) and appear to be dependent on the change in the pocket size of the macrocycle upon anion binding. A relatively small pocket, for example, in **Eu(3)**, leads to a decrease in the luminescence intensity upon interaction with fluoride, because the receptor pocket may increase in size, thus increasing the antenna−Eu^{3+} distance and decreasing the antenna effect. A larger pocket (i.e., **Eu(5)**) yields a significant increase in the intensity because the cavity size is significantly decreased upon anion binding, thus increasing the antenna effect. The anion titration experiments of **Eu(3)**−**Eu(5)** were also monitored at an excitation wavelength of 395 nm, where Eu^{3+} is excited directly (the ligand does not absorb). Interestingly, there is no change in the luminescence intensity (outside of what would be expected for dilution) over the course of the titration for **Eu(3)**−**Eu(5)** when the Eu^{3+} ion is directly excited ($\lambda_{exc} = 395$ nm), indicating that the lanthanide does

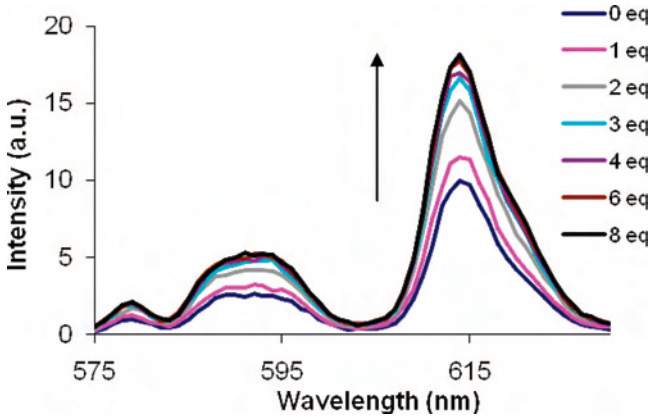

Figure 5. Titration of **Eu(2)** with TBAF in DMSO as monitored via luminescence (1.0×10^{-5} M; $\lambda_{exc} = 272$ nm).

not participate in anion binding. Luminescence lifetime studies to support this observation have been performed (vide infra). In general, these data suggest that a change in the conformation of the macrocycle upon anion binding alters the distance between Eu^{3+} and the antennae. On the basis of the model experiments with **3**–**5**, we predict anion binding to the thiourea groups in the pocket of the macrocycles **Eu(3)**−**Eu(5)**. Alternatively, anion binding and/or deprotonation of the thiourea (the −NH directly adjacent to the benzene moiety is expected to be most acidic)[4] results in more electron-rich antennae, which could also alter the efficiency of energy transfer to the Eu^{3+} center.

Titrations of **Eu(2)** with fluoride (TBAF) and acetate (TBAOAc) exhibited similar luminescence responses (Figures 5 and 6). The emission (272 nm excitation) of **Eu(2)** at 614 nm was greatly increased with fluoride and acetate (77% and 72%, respectively, with 8 equiv). This result was higher than expected for the cavity size (the thioureas are linked by a five-carbon spacer) because **Eu(5)** (eight-carbon spacer) revealed a lower intensity increase with the titration of fluoride. Similar to **Eu(3)**−**Eu(5)**, a change in the emission intensity was not observed for **Eu(2)** when Eu^{3+} was directly excited at a wavelength of 395 nm during these titrations.

The large emission enhancement in the presence of fluoride observed for **Eu(2)** and **Eu(5)** prompted further titration studies with an expanded series of anions to determine the potential selectivity of this macrocycle family. As shown in Figure 6, with all of the macrocycles, significant augmentation of emission occurred in the presence of fluoride and acetate. It should be pointed out that each bar in Figure 6 has an error of less than 5% between separate experiments. The addition of fluoride elicited a similar increase in the luminescence response in **Eu(2)** and **Eu(5)** (77% and 65%, respectively, at 8 equiv); however, in the presence of acetate, the luminescence response was significantly different when **Eu(2)** and **Eu(5)** are compared (increased by 72% and 37%, respectively, at 8 equiv). The response to DHP also varied between **Eu(2)** and **Eu(5)**, but unlike the observed responses to acetate, a weaker response to DHP was observed upon titration of **Eu(2)** as compared to the higher response of **Eu(5)** (increased by 3% and 33%, respectively, at 8 equiv). This may indicate that the luminescence signaling in these macrocycles is being affected via different mechanisms depending on the anion, the macrocycle, or both. All remaining anions (nitrate, chloride, bromide, and iodide) did not show a significant effect on the luminescence intensity of the macrocycle solutions. Such a trend in selectivity is common with molecules employing urea or thiourea groups as anion-binding sites, and the effect is associated with the hydrogen-bond-accepting abilities and basicities of the anions studied.[3,4] Here, we reveal that sensitized lanthanide luminescence can be exploited as a facile method of anion recognition and sensing.

Examination of the luminescent responses of macrocycles **Eu(3)**−**Eu(5)** to fluoride, acetate, and DHP indicates a selectivity trend based on the pocket size of the macrocycle (Figure 6). Macrocycle **Eu(3)**, with the shortest spacer between the two thiourea groups, showed a *decrease* in the luminescence intensity of increasing magnitude as the anions increased in size (fluoride < acetate < DHP), as shown in Figure 6b. These results are consistent with our hypothesis that larger anions are able to open the cavity by pushing the antennae farther away from Eu^{3+} in macrocycle **Eu(3)**, which is responsible for the decrease in the emission intensity. Similarly, both **Eu(4)** and **Eu(5)** (parts c and d of Figure 6, respectively) exhibited an *increase* in the luminescence intensity of decreasing magnitude as the anions increased in size. These results are consistent with our hypothesis that smaller anions are able to pull the antennae closer to Eu^{3+}, yielding an increase in the emission intensity. The luminescence intensity of macrocycle **Eu(2)** was observed to increase upon anion addition according to the same general trend as was observed with macrocycles **Eu(4)** and **Eu(5)** (fluoride > acetate > DHP). However, it was observed that the anion-induced luminescence enhancement of **Eu(2)** was in better agreement with the relative basicities of the anions than with their size, evident in the strong luminescence response to fluoride and acetate but only a slight response to DHP. The anion responses of **Eu(2)**−**Eu(5)** were also evaluated according to their I_{272}/I_{395} ratio, and a similar trend was observed. However, because of the large amount of noise in the relatively weak 614 nm emission when $\lambda_{exc} = 395$ nm, the error was substantially increased. Figure 7 illustrates two proposed mechanisms for the luminescence augmentation observed upon anion interaction for **Eu(2)**, **Eu(4)**, and **Eu(5)**: (1) interaction/binding between the anion(s) and the macrocyclic pocket induces a conformation change that shortens the Eu^{3+}−antennae distance or (2) hydrogen-bond donation or deprotonation of the thiourea group results in a change in the electronic structure of the antennae.

Association constants were determined for macrocycle−anion complexes for which both the luminescence enhancement was large (>30%) and a linear dependence of the luminescence enhancement on the concentration was observed, indicating 1:1 (macrocycle−anion) complex formation. Binding constants (log K) for **Eu(2)** and **Eu(5)** with acetate were 4.8 ± 0.1 and 4.5 ± 0.1, respectively, and that for **Eu(5)** with DHP was determined to be 4.4 ± 0.1 by the method of Fery-Forgues et al.[13] The more rigid and preorganized binding pocket of **Eu(2)** gave rise to the stronger

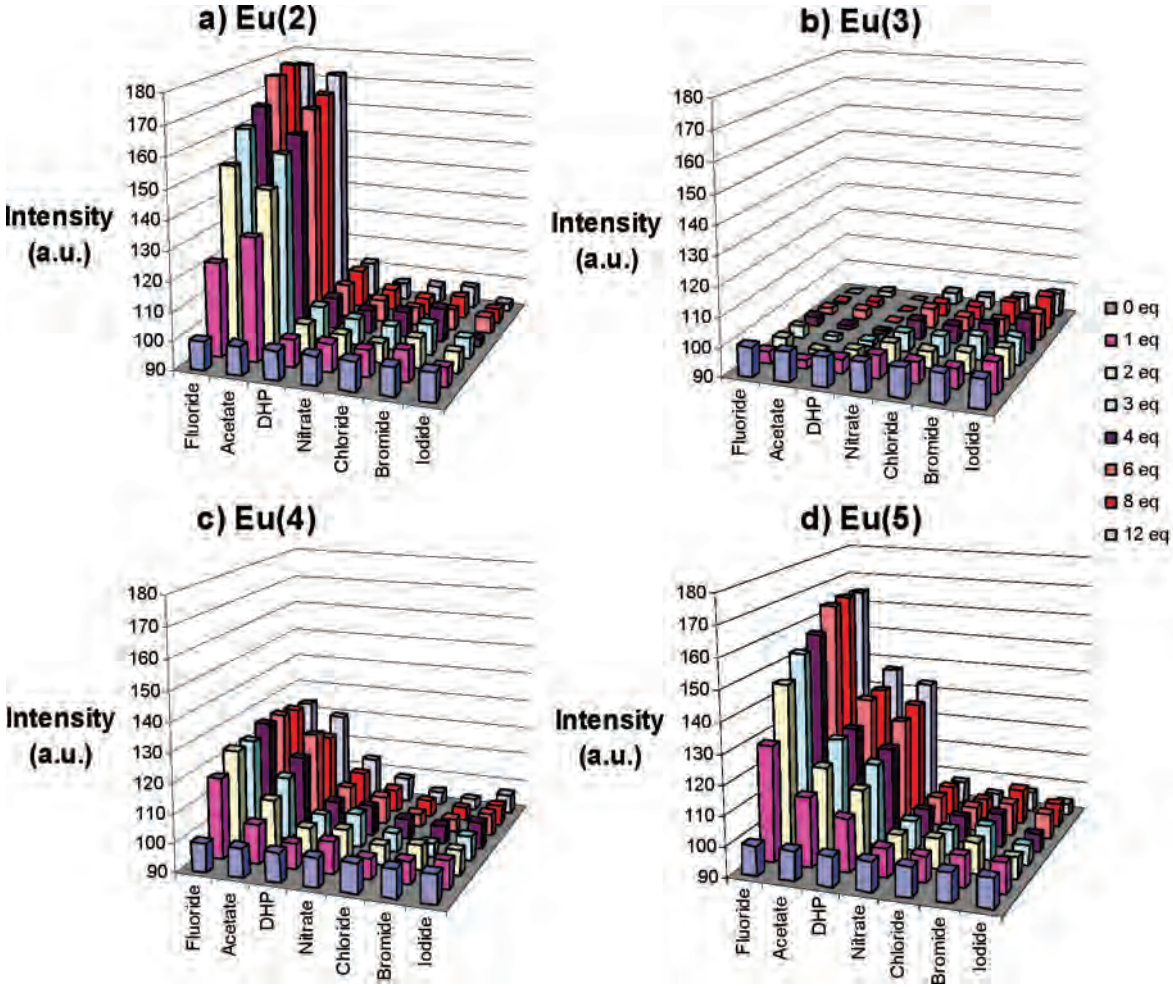

Figure 6. Effect of various anions on the luminescence emission intensity at 614 nm in DMSO of (a) **Eu(2)**, (b) **Eu(3)**, (c) **Eu(4)**, and (d) **Eu(5)** (λ_{exc} = 272 nm).

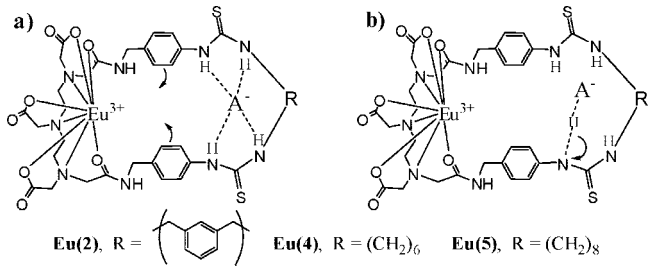

Figure 7. Proposed mechanisms for the luminescence enhancement in **Eu(2)**, **Eu(4)**, and **Eu(5)** upon the addition of anion (A^-): (a) anion binding shortens the antennae−Eu^{3+} distance; (b) deprotonation of the thiourea results in a more electron-rich antenna.

binding of acetate as compared to **Eu(5)**. Overall, **Eu(2)** and **Eu(5)** bind the above anions relatively strongly in DMSO, as compared to other neutral receptors,[5,6] and have *association constants greater than 1 order of magnitude stronger* than those of the acyclic models **2** and **5**. This augmented binding affinity is attributed to the macrocyclic effect in this preorganized system. The 1:1 complex formation was confirmed using the Job's plot method for **Eu(5)** and acetate. Binding constants for fluoride could not be determined because of the nonlinearity of the curve obtained when luminescence enhancement versus concentration was plotted. The analysis of the fluoride data indicated multiple processes (i.e., deprotonation) occurring during the titration.

To gain further insight into the luminescent response of **Eu(2)**−**Eu(5)** to fluoride and acetate in DMSO, UV–vis absorbance was measured during the titration, and a significant change in the absorption band at λ_{max} = 272 nm was not observed. Thiourea-containing systems have been shown to have dramatic changes in absorption upon anion binding and/or anion-induced deprotonation when the thiourea is appended to aromatic systems.[3,4] Unfortunately, as in the case of the model compounds **2**–**5**, the heavy solvent absorbance of DMSO below 260 nm interfered with absorbance changes in that region.

Selectivity and Reversibility. The selectivity of **Eu(2)** for fluoride and acetate in the presence of chloride ion (up to 120 equiv, where $[Cl^-] = 1.2 \times 10^{-3}$ M) was examined, and the luminescence response was affected only slightly (Figure 8). This feature is extremely valuable for monitoring of anion concentrations in environmental and biological samples where chloride is a common interference. Receptors **Eu(3)** and **Eu(5)** exhibited enhanced responses to 8 equiv of fluoride in the presence of 120 equiv of chloride, whereas the response of **Eu(4)** was slightly hindered under the same conditions. The acetate titration of macrocycles **Eu(3)**−**Eu(5)**

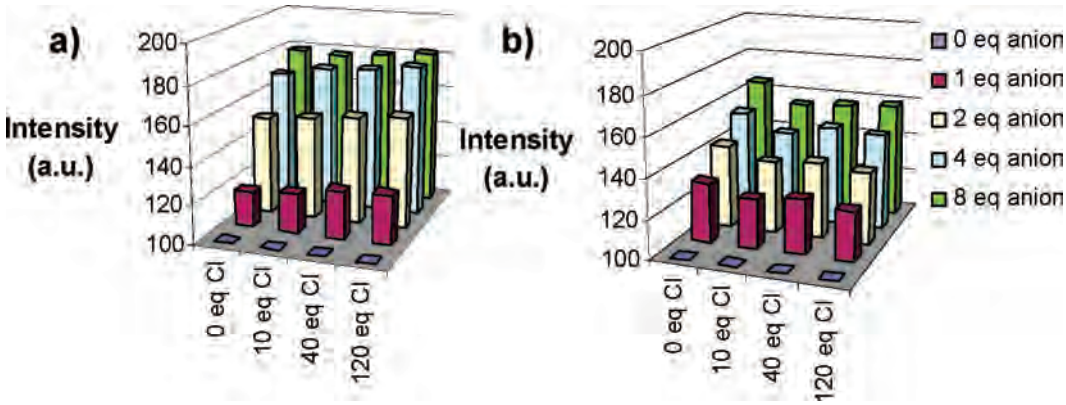

Figure 8. Titration of a 1.0×10^{-5} M solution of **Eu(2)** in DMSO with (a) TBAF and (b) TBAOAc as monitored via luminescence in the presence of increasing amounts of TBACl ($\lambda_{exc} = 272$ nm; $\lambda_{em} = 614$ nm; error <5%).

was also unaffected in a similar manner in the presence of 120 equiv of chloride. Thus, macrocycles **Eu(2)**−**Eu(5)** demonstrated a selectivity for either fluoride or acetate in the presence of a large excess of chloride, whereas **Eu(2)** was found to be the most selective, particularly for fluoride anions. The large difference in basicities between either fluoride or acetate and chloride is likely the cause of the observed selectivity.[3–5]

The effects of fluoride, acetate, and DHP on **Eu(2)**−**Eu(5)** were also found to be reversible upon the addition of NH_4Cl, where NH_4^+ functions as a stronger anion binder and pulls the anion out of the macrocycle. Reversibility is highly important in the design of molecules for sensing applications because it would be desirable for the receptor to be recycled, reused, or utilized in a continuous manner. Furthermore, consistent with all of the previous results, when the Eu^{3+} was directly excited at 395 nm, a change in the luminescence intensity was not observed ($\lambda_{em} = 614$ nm) during the titration experiments with the anions previously examined. This behavior is consistent with an interaction between the anion (fluoride, acetate, and DHP) and the thiourea moiety, resulting in an augmentation of the antenna effect through a change in the conformation and/or electronics of the macrocycle pocket. If the change in luminescence observed at $\lambda_{exc} = 272$ nm was brought on by interaction of the anion directly with the lanthanide, it is expected that a similar effect would be observed at $\lambda_{exc} = 395$ nm. An increase in luminescence is typically observed when water molecules directly bound to the Eu^{3+} ion are displaced by anions coordinating to the metal, and such an increase would be observed at all excitation wavelengths producing a Eu^{3+} emission.[7c] This effect does not appear to be the cause of the luminescence augmentation in the macromolecules presented here, where water is present in small quantities in the DMSO solvent.

Luminescence Emission and Lifetime Studies. Emission spectra were obtained at two different excitation wavelengths, 272 and 395 nm, to characterize the luminescence enhancement due to the antennae (I_{272}/I_{395}; Table 2) in the absence of anions. As shown in Figure 9, the Eu^{3+} ion is directly excited at 395 nm; however, the absorption maximum of the macrocycles was at 272 nm and is attributed primarily to the $\pi \rightarrow \pi^*$ transition of the thiourea-functionalized benzene moiety.[11] Thus, as shown in Figure 9, the antenna effect is evident, as emission from all macrocycles is enhanced via excitation at 272 nm (ligand absorption).[11] It was noticed that the emission intensity and enhancement increased according to a decrease in the macrocycle size, and these data correlated well with the length of the linker between the thioureas (Table 2). As shown in Scheme 1, **Eu(3)**−**Eu(5)** differ only in the number of carbons in the alkyl chain that links the two thiourea groups; thus, the cavity size and degree of flexibility increase with the linker length. However, **Eu(2)** is more rigid because of the *m*-xylyl linker, and the degree of luminescence enhancement (I_{272}/I_{395}) from the antenna effect is similar to that of the hexyl-linked **Eu(4)** (the spacer length is similar). As discussed previously, there did not appear to be a significant antenna effect from the *m*-xylyl group present in **Eu(2)**, which is likely due to the distance of this linker from the lanthanide ion.[11] In general, it was found that the emission intensity increased as the linker length decreased throughout the series **Eu(2)**−**Eu(5)**. This result is expected because the degree of energy transfer from the antenna to Eu^{3+} is dependent on the through-space distance between the aromatic antenna and the lanthanide ion.[9] A shorter linker may hold the antennae in a closer proximity to the Eu^{3+} center, which would subsequently increase luminescence enhancement through the antenna. Thus, the variation in the pocket size (and the Eu^{3+} to antennae distance) is shown to affect the Eu^{3+} emission intensity. Based on these observations, changes in the conformation induced by an anion-binding interaction that effect the pocket are expected to alter the emission intensity, as observed in the anion titrations of **Eu(2)**−**Eu(5)** with fluoride, acetate, or DHP.[15]

Table 2. Summary of the Emission Intensity Data at 614 nm for **Eu(2)**−**Eu(5)**, Where "*I*" is the Emission Peak Intensity at the Indicated Wavelength, "I_{272}/I_{395}" Denotes the Luminescence Enhancement due to the Antenna, "no. of C" Denotes the Number of Carbons That Link the Thiourea Moieties (Length of R in Scheme 1), and "type of linker" Describes Whether an Aryl or Alkyl R Group Is Present

compound	I_{272}	I_{272}/I_{395}	no. of C	type of linker
Eu(2)	8.55	7.44	5	aryl
Eu(3)	10.00	9.75	4	alkyl
Eu(4)	8.44	7.73	6	alkyl
Eu(5)	5.39	4.66	8	alkyl

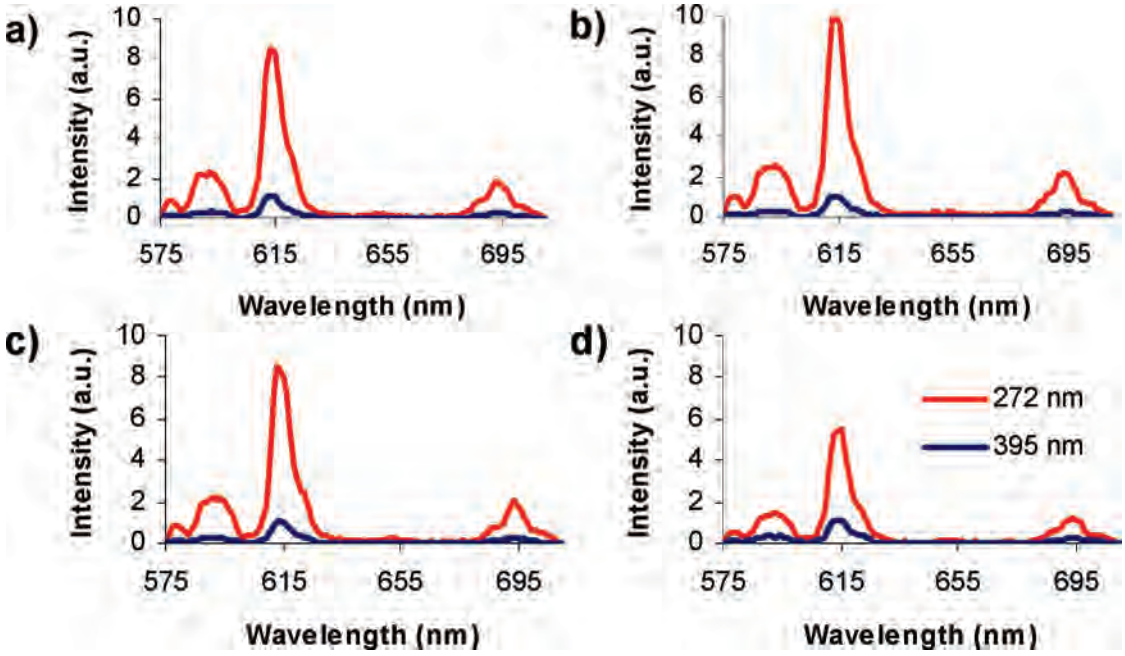

Figure 9. Emission spectra of (a) **Eu(2)**, (b) **Eu(3)**, (c) **Eu(4)**, and (d) **Eu(5)** in DMSO (1.0×10^{-5} M) at excitation wavelengths of 272 and 395 nm.

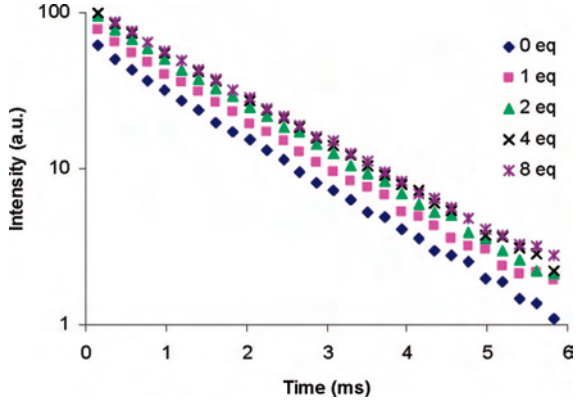

Figure 10. Titration of **Eu(2)** with TBAF in DMSO (1.0×10^{-5} M; λ_{exc} = 272 nm; λ_{em} = 614 nm) as monitored via luminescence lifetime.

The luminescence lifetime of **Eu(2)** was measured upon titration with fluoride (λ_{exc} = 272 and 395 nm) to determine whether the increase in luminescence was due to a longer luminescence lifetime (τ), where a luminescence quencher (such as a water molecule or hydroxide) would be removed upon anion binding (Figure 10 and Table 3). Although the luminescence lifetime did slightly increase (8–10%) with the addition of fluoride to **Eu(2)**, this small change does not account for the large increase in the luminescence intensity observed over the course of the titration, evident in the rise in the emission intensity at ~0 ms. Additionally, a significant change in the luminescence lifetime of **Eu(2)** was not observed throughout the addition of acetate when λ_{exc} = 272 nm (Table 3). The luminescence lifetime titration data are consistent with our two proposed mechanisms for the luminescence increase observed with **Eu(2)** and **Eu(5)**, as illustrated in Figure 7, in that neither of these events is expected to change τ, which is primarily dependent on the presence of nonradiative pathways for deactivation of the Eu^{3+} excited state.

Table 3. Luminescence Lifetime Data (τ/ms) over the Course of the Titration of **Eu(2)** with TBAF and TBAOAc in DMSO at λ_{exc} = 272 or 395 nm

no. of equiv of TBAX	τ_{272nm,F^-}	τ_{395nm,F^-}	τ_{272nm,OAc^-}	τ_{395nm,OAc^-}
0	1.36 ± 0.02	1.46 ± 0.03	1.30 ± 0.01	1.46 ± 0.02
1	1.41 ± 0.02	1.45 ± 0.03	1.32 ± 0.01	1.37 ± 0.02
2	1.44 ± 0.02	1.46 ± 0.03	1.34 ± 0.01	1.41 ± 0.02
4	1.48 ± 0.02	1.50 ± 0.03	1.34 ± 0.01	1.36 ± 0.02
8	1.49 ± 0.02	1.57 ± 0.04	1.34 ± 0.01	1.38 ± 0.02

Hydroxide Studies. The response of **Eu(2)**−**Eu(5)** to hydroxide was studied using luminescence titration experiments (Figure 11). Hydroxide was chosen to gain insights into whether fluoride was directly coordinating to Eu^{3+}. It

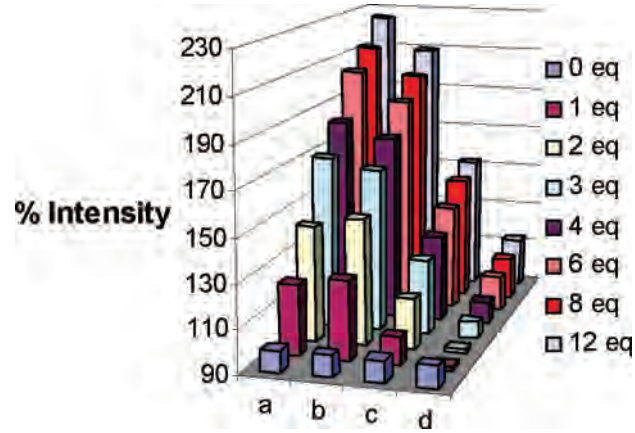

Figure 11. Percentage change (0 equiv ≡ 100%) in the luminescence intensity upon titration of (a) **Eu(5)**, (b) **Eu(2)**, (c) **Eu(4)**, and (d) **Eu(3)**, with TBAOH in DMSO (1.0×10^{-5} M; λ_{exc} = 272 nm; λ_{em} = 614 nm).

(15) It should be noted that our initial studies on **Eu(2)**−**Eu(5)** reported slightly different emission spectra than those presented here (they were all very similar), and this was attributed to the presence of bicarbonate in the final product, which likely bound to the macrocycle, altering the cavity size.[11] As previously stated, the macrocyclic receptors were carefully purified to remove bicarbonate prior to the luminescence measurements.

was assumed that hydroxide behaves similarly to fluoride because of its small size; however, it is a stronger base and deprotonation of the thiourea hydrogens is more likely. An augmentation of the emission intensity ($\lambda_{exc} = 272$ nm) was observed, exceeding the intensity enhancements observed during the fluoride titrations, and the emission intensity continued to increase until greater than 12 equiv of hydroxide was added. Despite an initial decrease in the emission intensity of **Eu(3)** (comparable to that observed with fluoride, acetate, and DHP), an increase in the emission intensity was observed after the addition of 6 equiv of hydroxide. Such behavior indicates that hydroxide affects the luminescence intensity of **Eu(3)** by a different mechanism than the other anions studied, especially after several equivalents. As observed in the response of **Eu(2)**−**Eu(5)** to all other anions studied, there was no change in the luminescence intensity after direct Eu^{3+} excitation ($\lambda_{exc} = 395$ nm). Furthermore, the luminescence lifetime of the 614 nm emission of **Eu(2)** was measured over the course of the hydroxide titration, and a significant change was not observed. If hydroxide coordinated directly to Eu^{3+}, a detectable decrease in the luminescence lifetime would be expected because of the quenching ability of the O−H bond.[7c] It does not appear that hydroxide interacts with Eu^{3+} directly, and the large luminescence enhancement is most likely induced by deprotonation of the thiourea in **Eu(2)**−**Eu(5)**.

Conclusion

Despite the similarities in structure among all of the macrocycles studied, **Eu(2)**−**Eu(5)** exhibit very different behavior in response to strong hydrogen-bond-accepting anions (F^-, CH_3COO^-, and $H_2PO_4^-$) when the luminescence is measured at 614 nm after excitation at 272 nm. At this excitation wavelength, the antenna absorbs light and transfers energy to Eu^{3+}, resulting in the observed luminescence of the macrocycles. A change in luminescence is *not* observed in the presence of anions when Eu^{3+} is excited directly at 395 nm, and thus the luminescent signaling is dependent on anion binding to the ligand pocket (antenna). The macrocyclic ligands differ only in the alkyl or aryl spacer between the opposing thiourea groups, and these spacers appear to control the anion response, possibly based on the pocket size and changes in conformation that allow the movement of the benzene-based antennae upon interaction with some anions. The strong response of **Eu(2)** to fluoride and acetate was not affected by a large excess of chloride anions, making this system promising for further anion sensing applications in biological systems. **Eu(3)**−**Eu(5)** also demonstrated a selectivity for either fluoride or acetate in the presence of excess chloride ions, but the luminescence response was only slightly affected.

The collective results presented herein suggest that the anions do not interact with Eu^{3+} directly because similar anion responses would be expected from **Eu(2)**−**Eu(5)** because of their practically identical DTPA-based chelates as the lanthanide-binding domain within the macrocycle. In addition, although it is well-known that O−H oscillators in the first or second coordination sphere (especially Eu^{3+} and Tb^{3+}) alter the lifetimes of Ln^{3+} emission because of nonradiative energy decay,[7,9] no significant changes in the luminescence lifetime occurred upon titration of the macrocycle solutions with fluoride, acetate, or hydroxide. Finally, although only slight changes occurred in the absorbance of **Eu(2)**−**Eu(5)** upon titration with fluoride or acetate in DMSO (due to possible interference in the absorption of DMSO), a clear shift in the absorbance band was observed with the structurally similar model compound **2** in CH_3CN. Titrations of **2**–**5** monitored via ^1H NMR also showed definitive evidence for interaction between the thiourea protons and either fluoride (hydrogen bonding followed by deprotonation) or acetate (hydrogen bonding). This evidence supports our hypothesis that a change in the conformation, possibly coupled with a change in the electronic structure of the antennae, is responsible for the changes in the luminescence of **Eu(2)**−**Eu(5)** upon selective anion interaction. The large binding constants ($> 10^4$) determined for acetate and DHP for **Eu(2)** and **Eu(5)** validate the design of the preorganized macrocyclic system in this work. However, the exact bound structure is unknown at this point because of the complexity and extreme difficulties in crystallizing these systems. The diamagnetic La^{3+} analogue to **Eu(2)** was also prepared to examine the anion interactions using ^1H NMR, but the extremely low solubility in DMSO and the presence of water dramatically reduced the accuracy of the experiment. In addition, the Tb^{3+} analogue to **Eu(2)** was also synthesized and exhibited a similar increase in luminescence upon titration with fluoride. Further experiments are underway to study the mechanism of the luminescent response to anions in these systems.

This unique series of macrocycles, **Eu(2)**−**Eu(5)**, exhibits differing responses to strong hydrogen-bond-accepting anions, especially fluoride and acetate. This is one of the first examples, to our knowledge, of a lanthanide-based system that yields a luminescent response upon anion interaction that does not require the direct coordination of anions to the lanthanide.[8j,10] Furthermore, because the luminescence signaling occurs only upon excitation through the antenna ($\lambda_{exc} = 272$ nm) and no change is observed at $\lambda_{exc} = 395$ nm, the signal intensity of the latter can function as an internal standard for determining the concentration of the macrocycle, for example, in biological samples. This trait, combined with the selectivities of these macrocycles in the presence of chloride and the TRF capabilities of the lanthanide metals, gives these systems great potential for further tailoring to be applied in biological studies that contain high background luminescence, such as the cellular environment.

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Supporting Information Available: Luminescence of **Eu(2)**−**Eu(5)** upon titration with TBAF ($\lambda_{exc} = 395$ nm) and upon the

addition of TBAF followed by NH_4Cl ($\lambda_{exc} = 272$ nm); luminescence response of **Eu(2)**−**Eu(5)** to TBAF and TBAOAc in the presence of 120 equiv of TBACl; UV–vis absorbance of **Eu(2)** and **2** upon titration with TBAF and TBAOAc in DMSO; luminescence lifetime of **Eu(2)** upon titration with TBAOH; anion response of **Eu(2)**−**Eu(5)** evaluated by I_{272}/I_{395}; Job's plot of **Eu(5)** and TBAOAc; and determination of association constants for **Eu(2)** and **Eu(5)**. This material is available free of charge via the Internet at http://pubs.acs.org.

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