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pH-Responsive Lanthanide Complexes Based on Reversible Ligation of a Diphenylphosphinamide

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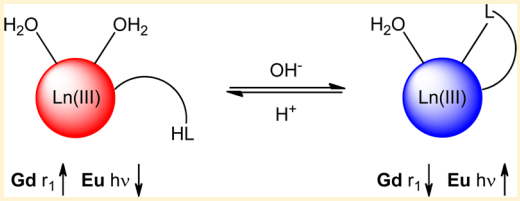
Supporting Information

ABSTRACT: Ln-dpp-DO3A-based complexes [dpp is a pendant diphenylphosphinamide moiety and DO3A is 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane] exhibit pH-responsive reversible ligation of the phosphinamide for both the gadolinium(III) and europium(III) analogues. pK_a values were 8.1 (±0.1) and 7.8 (±0.1) for **Gd.1** and **Gd.2**, respectively. The relaxivities (20 MHz, 298 K) of the gadolinium(III) analogues were r_1 = 7.9 mM^{-1} s^{-1} (**Gd.1**) and r_1 = 8.2 mM^{-1} s^{-1} (**Gd.2**) in acidic media, corresponding to a hydration state q = 2; in basic media, deprotonation and coordination of the phosphinamide occurs, with r_1 = 5.4 mM^{-1} s^{-1} (**Gd.1**) and r_1 = 4.4 mM^{-1} s^{-1} (**Gd.2**) corresponding to q = 1. Sensitized luminescent emission was observed from the europium(III) analogues following excitation at λ_{ex} = 270 nm. The hydration state values of the europium(III) analogues were consistent with those of the gadolinium(III) complexes, i.e., q = 1 and 2 in basic and acidic media, respectively. The ratio of the emission intensities of the ΔJ = 1 and 2 bands enables concentration-independent reporting of the pH. Excited-state pK_a values were 8.3 (±0.1) and 8.5 (±0.1) for **Eu.1** and **Eu.2**, respectively.



INTRODUCTION

The application of gadolinium(III)-based contrast agents in magnetic resonance imaging (MRI) is widespread.[1] Over recent years, one aspect of contrast agent research has focused on the design of probes that respond to an external stimulus, so-called "responsive", "activated", or "smart" contrast agents.[2] Such agents rely on modulation of the water-proton relaxation rate in response to a change in the in vivo environment, typically changes in the pH,[3] redox potential,[4] and presence of enzymes and proteins,[5] metal ions,[6] and endogenous anions.[7] Many approaches in this area focus on the development of q-modulated contrast agents, where q refers to the number of inner-sphere water molecules on the gadolinium(III) complex.[7,8] In such examples, the number of inner-sphere water molecules, i.e., q, is changed by, e.g., cleavage of a masking group, as a result of the enzyme activity or reversible binding of a protonated ligand donor.

The efficacy of an MRI contrast agent is defined by its relaxivity (r_1), the total paramagnetic relaxation rate enhancement of the water protons per unit concentration of the contrast agent (mM^{-1} s^{-1}). The design of contrast agent ligands is such that one or two coordination sites of the usually nine-coordinated gadolinium(III) ion are reserved for water molecules to reversibly bind, exchanging with bulk water. The most readily controlled relaxation contribution with regard to contrast agent design comes from the inner sphere. The longitudinal inner-sphere relaxation rate is expressed by

$$R_{1,p}^{IS} = \frac{Cq}{55.6}\frac{1}{T_{1,M} + \tau_m} \quad (1)$$

where C is the concentration of the paramagnetic ion, q is the number of coordinated water molecules, $T_{1,M}$ is the longitudinal relaxation time of the inner-sphere water molecules, and τ_m is the water-exchange lifetime. Relaxivity enhancement can be achieved by lengthening the rotational correlation time, τ_R (decreasing $T_{1,M}$), as well as shortening τ_m; however, it is clear from eq 1 that increasing the hydration state (q) of the complex increases the relaxivity. The displacement of inner-sphere water molecules and, hence, modulation of r_1 can be achieved by both *inter-* and *intra*molecular reversible anion binding to the gadolinium(III) center of 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A)-based complexes.[3f,9]

There has been increasing interest in developing contrast agents that respond to changes in the in vivo pH.[3] One reason for this is born out of the knowledge that tumors are acidic (around pH 6.8−6.9) compared to healthy tissue (pH 7.4).[10] Changes or abnormalities in the tissue pH can be a marker of disease, aiding diagnosis, but can also be used to monitor treatment.[11]

Diphenylphosphinyl has been used to protect amines in peptide synthesis as diphenylphospinamide (dpp).[12] *N*-Diphenylphospinamide aziridines provide a convenient method of introducing an aminoethyl functionality into a molecule, by ring opening of the corresponding aziridine.[13,14] This protecting group can be readily removed under acidic conditions (e.g., 95% trifluoroacetic acid). We have used this methodology to introduce aminoethyl groups onto DO3A-

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based ligands; however, we discovered that there is more to this unit than simply a protecting group. We recently reported the synthesis of some DO3A-based chelates bearing a pendant dpp moiety.[14] These $q = 2$ complexes were shown to interact with human serum albumin (HSA), exhibiting binding constants in the region 20000−30000 M^{-1}; however, they only exhibited modest increases in relaxivity in the presence of HSA. Significant increases were expected based on slowing of the rotational correlation time; the lower than expected values were attributed to changes in inner-sphere hydration when bound to the protein [demonstrated by a luminescence study of the corresponding europium(III) complexes], i.e., a change in q giving a lower r_1. Such observations are often a result of intermolecular anion binding that is typically observed for $q = 2$ species (from, e.g., carboxylate residues on the protein).[5b,c,7,14,15]

In order to assume that the disappointing relaxivities observed for the HSA-bound complexes were a result of water displacement by anionic residues on the protein, it is important to know whether the phosphinamide is protonated [and, hence, not coordinated to gadolinium(III)] at physiological pH. Herein we report that these complexes are prone to the displacement of inner-sphere water molecules by not only *inter*molecular anion binding[14] but also *intra*molecular anion binding. The hydration state, q, varies with the pH; the dpp can undergo reversible, intramolecular coordination to lanthanides when appended to a Ln-DO3A complex. This is manifested as a change in the hydration state that affects the water-proton relaxivity (Gd) and the luminescence intensity and lifetime (Eu) of these complexes. The luminescence properties of the europium(III) analogues of these complexes enable hydration state determination as well as demonstrate the potential use of such complexes as concentration-independent pH-responsive luminescent probes (following sensitization via the dpp moiety at $\lambda_{ex} = 270$ nm).

EXPERIMENTAL SECTION

The syntheses of **Ln.1** and **Ln.2** were reported in a prior publication.[14]

^{1}H Relaxation Data. The observed longitudinal water-proton relaxation times ($T_{1,obs}$) were measured on a Stelar Spinmaster spectrometer [Stelar, Mede (PV), Italy], operating at 20 MHz, by means of the standard inversion−recovery technique (16 experiments, 2 scans). A typical 90° pulse width was 3.5 μs, and the reproducibility of the $T_{1,obs}$ data was ±0.5%. The paramagnetic water-proton relaxation rate, $R_{1,p}$, and relaxivity, r_1, were determined by following eqs 2−4, where 0.38 is the diamagnetic contribution of the bulk water molecules.[16] Complex concentrations were determined via mineralization with nitric acid or by inductively coupled plasma mass spectrometry.

$$R_{1,obs} = \frac{1}{T_{1,obs}} \quad (2)$$

$$R_{1,p} = R_{1,obs} - 0.38 \quad (3)$$

$$r_1 = R_{1,p}/[Gd] \quad (4)$$

Luminescence Spectroscopy. Luminescence emission spectra were recorded using a Jobin Yvon Horiba FluoroMax-P spectrometer (using *DataMax* for *Windows v2.2*). Samples were held in a 10 × 10 mm or 10 × 2 mm quartz Hellma cuvette, and a cutoff filter (550 nm) was used to avoid second-order diffraction effects. Europium(III) excitation was either direct ($\lambda_{ex} = 395$ nm) or indirect ($\lambda_{ex} = 270$ nm).

Hydration State, q, Determination. Lifetimes were measured by direct (395 nm) excitation of the sample with time per flash = 40 ms (500 flashes per point) followed by monitoring of the integrated intensity of light ($\Delta J = 2$) emitted during a fixed gate time of 0.1 ms, at a delay time later. Delay times were set at 0.1 ms intervals, covering four or more lifetimes. Excitation and emission slits were set to 5 nm. The data are applied to the standard first-order decay (eq 5), minimized in terms of k by iterative least-squares fitting operation using Microsoft Excel, where I_{obs} is the observed intensity, I_0 is the initial intensity, and t is the time (ms). The calculated k values are then applied to eq 6 to calculate the hydration state, q.[17]

$$I_{obs} = I_0 e^{-kt} + \text{offset} \quad (5)$$

$$q = 1.2[(k_{H_2O} - k_{D_2O}) - 0.25] \quad (6)$$

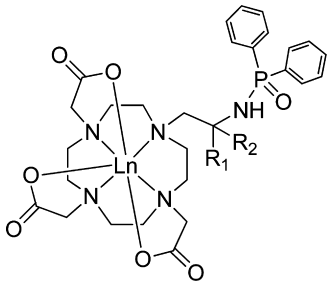
pH Titrations. pH measurements were recorded using a Jenway 3510 pH meter with a BDH probe, model 309-1025-02, calibrated at pH 4, 7, and 10. Luminescence versus pH titrations of **Eu.1/2** (1.0 mM) were carried out in a background of constant ionic strength (I = 0.1 NaCl, 298 K). Solutions were made basic by the addition of 1 M NaOH and titrated to acidic pH by the addition of small aliquots of 1 or 0.1 M HCl. Corrected emission spectra were recorded with an excitation wavelength of 270 nm using a 550 nm cutoff filter. Excitation and emission slits were set to 10 and 1 nm bandpass, respectively. Data analysis was performed using an iterative least-squares fitting procedure, operating in Microsoft Excel. Equation 7 was minimized in terms of K_a, where I_{obs} is the observed ratio $\Delta J = 2/\Delta J = 1$ of emission intensities and I_{HA} and I_{A^-} are the $\Delta J = 2/\Delta J = 1$ intensity ratios of the fully protonated and fully deprotonated phosphonamide.

$$\frac{[H^+]I_{HA} + K_a I_{A^-}}{[H^+] + K_a} = I_{obs} \quad (7)$$

pH titrations of **Gd.1/2** were conducted in a similar manner, substituting I_{obs}, I_{HA}, and I_{A^-} for $r_{1,obs}$, $r_{1,HA}$, and r_{1,A^-}, respectively.

RESULTS AND DISCUSSION

The syntheses of complexes **Ln.1** (racemic) and **Ln.2** were reported in an earlier publication.[14]



Ln.1 R_1 = H, R_2 = Me
Ln.2 R_1 = R_2 = Me
Ln = Gd, Eu

Relaxivity Study. The relaxivities of **Gd.1** and **Gd.2** were measured and vary with the solution pH (Table 1). From titration of the observed relaxivity versus pH, the pK_a of each complex was obtained; this protonation event is ascribed to pH-dependent ligation of the phosphinamide (Figure 1 and Scheme 1).

Table 1. Calculated pK_a Values for Gd.1 and Gd.2 and Relaxivity (20 MHz) Values, r_1, at pH 5.0, 7.4, and 10.0 (298 K, 0.1 M NaCl)

		r_1 (mM^{-1} s^{-1})		
	pK_a	pH 5.0	pH 7.4	pH 10.0
Gd.1	8.1 (±0.1)	7.9	7.6	5.4
Gd.2	7.8 (±0.1)	8.2	7.3	4.4

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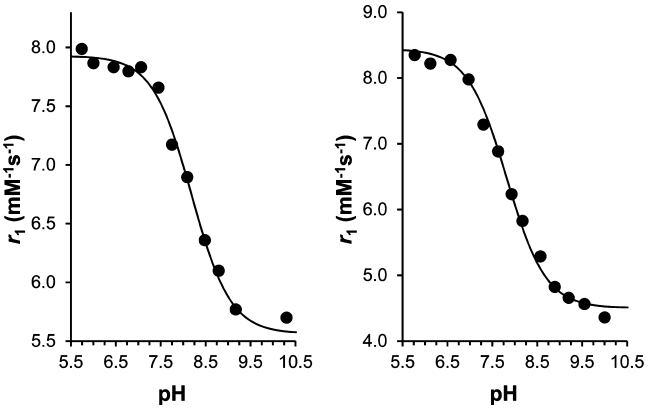
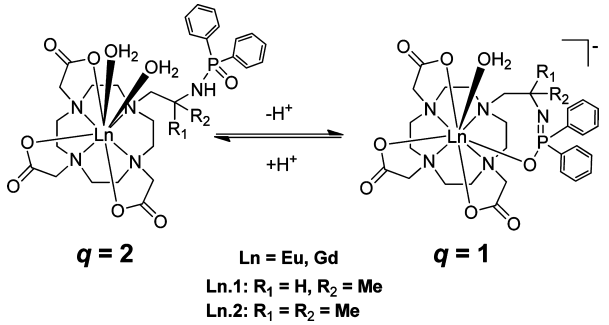


Figure 1. Relaxivity (r_1) versus pH for **Gd.1** (left) and **Gd.2** (right) showing the experimental (dots) and calculated data (line). Conditions: 1.0 mM **Gd.1**/**2**, 0.1 M NaCl, 20 MHz, 298 K.

Scheme 1. Possible Nature of the pH-Dependent Reversible Binding of dpp



It is clear that there is a change in the complex hydration state as the relaxivity increases in acidic media; the relaxivity values suggest movement from one to two inner-sphere water molecules as the pH is lowered. The r_1 values of 4.4 and 5.4 mM^{-1} s^{-1} observed at pH 10.0 are typical of a q = 1 gadolinium species, while 7.9 and 8.2 mM^{-1} s^{-1} observed at pH 5.0 are typical for q = 2.[1] This suggests that there is displacement of a bound water molecule as a result of changes of the pH. The pK_a values determined suggest that, at physiological pH, these complexes are predominantly in the protonated, i.e., q = 2, form. With respect to the previous publication concerned with the noncovalent binding of these complexes to HSA,[14] this would suggest that the lower than expected relaxivities observed in the presence of HSA were a result of the majority of the gadolinium(III) complex being in a q = 2 state at pH 7.4; therefore, as a neutral complex, it is susceptible to the displacement of inner-sphere water molecules by coordination of, e.g., carboxylate residues on the protein.

The pH-responsive nature of the complexes depicted in Scheme 1 suggests reversible binding of the phosphinamide. The scheme shows coordination of the phosphinamide oxygen to the gadolinium center following deprotonation of the pendant nitrogen group at basic pH, generating the pendant negatively charged phosphinamide. Lanthanide ions have a high affinity for both nitrogen and oxygen coordination. Binding via the nitrogen atom would result in the formation of a five-membered chelate ring; however, because of the oxophilicity of the lanthanide ions, coordination to oxygen was considered to be more likely and so this coordination mode is tentatively assigned. The r_1 values are typical of a q = 1 species when the solution is basic, suggesting ligation from the oxygen, which results in the formation of a seven-membered chelate ring. Less steric crowding of the gadolinium(III) center is expected than for the five-membered chelate ring, which would be formed following ligation to nitrogen; this allows coordination of one water molecule. A further discussion of the hydration state and evidence for reversible phosphinamide coordination is reported in the luminescence studies of the europium(III) analogues. Reversible phosphinamide ligation was also suggested by the pH dependence of the ^{1}H NMR spectra of the europium(III) analogues. Spectra recorded at pD 10 (400 MHz, 278 K) are expected to have dpp coordinated; thus, there is a slowing of the fluxional processes such as arm rotation; the ^{1}H NMR spectra exhibit resolved resonances for the cyclen ring hydrogen atoms because coordination of europium(III) is in the more rigid eight-coordinate manner (with respect to ligand **1**/**2**).[14] This is typical of an eight-coordinate unsymmetrical europium(III) complex exhibiting a broad range of resonances from −19 to 35.6 ppm and is indicative of phosphinamide coordination to europium(III). The restricted arm rotation in eight-coordinate (cf. seven-coordinate) complexes slows the exchange between the various stereoisomers, resulting in resolution of the axial and equatorial cyclen ring hydrogen atoms. For the spectra recorded at pD 10, even at 278 K the spectra are not fully resolved; i.e., exchange between isomeric forms has still not been completely frozen out. This provides another piece of evidence to suggest coordination via the phosphinamide oxygen (seven-membered chelate ring); an even more rigid structure, i.e., slower exchange, would be expected for phosphinamide nitrogen coordination (five-membered chelate ring) akin to that seen for related sulfonamide complexes.[3,21] The major resonances observed at δ 25.7, 33.0, 34.7, and 34.9 (**Eu.1**) and δ 24.8, 26.2, 33.6, and 35.6 (**Eu.2**) are typical of the square-antiprismatic geometry about the europium(III) center.[18] The equivalent ^{1}H NMR spectra recorded at pD 5 were more broad and featureless, typical of less rigid q = 2 europium(III) complexes (seven-coordinate with respect to the ligand).[5b,c,19]

Luminescence Study. Europium(III) analogues of the complexes were prepared and their luminescent properties studied. Because of the similar size and charge of the ions, conclusions drawn from luminescence studies on europium(III) can, with caution, be inferred for the gadolinium(III) analogue. In contrast to the gadolinium(III) relaxivity, the europium emission intensity decreases as the hydration state increases because of water molecules' ability to quench the europium(III) ^{5}D$_0$ excited state. The radiationless energy-transfer processes that occur between the vibrational energy levels of H_2O and D_2O with the excited states of europium(III) can be exploited in order to determine q.[17]

The calculated q values in acidic media (pH 5.5) are q = 1.7 for both **Eu.1** and **Eu.2**, indicating two inner-sphere water molecules (Table 2). This value is typical of a complex containing two inner-sphere water molecules.[5b,c,19,20] In basic media (pH 10.5), the q values are q = 0.7 and 0.6 for **Eu.1** and **Eu.2**, respectively. At such high pH, deprotonation of bound water molecules occurs; i.e., at pH 10, it is likely that a portion of OH^- rather than H_2O is bound to europium(III). The pK_a of bound water for such complexes is typically ∼9−10.[19,20] The complexes can be said to be q = 1 as pH 9 is approached and then change to q = 0.5 as the pH is raised further and the bound water is deprotonated (although a hydration equilibrium cannot be excluded). This q = 1 value further suggests

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Table 2. Radiative Rate Constants (k) and Measured Hydration States (q) for Eu.1 and Eu.2[a]

	$k(H_2O)$ (ms^{-1})	$k(D_2O)$ (ms^{-1})	q (±0.2)
Eu.1 at pH 5.5	2.34	0.65	1.7
Eu.1 at pH 10.5	1.46	0.65	0.7
Eu.2 at pH 5.5	2.84	1.19	1.7
Eu.2 at pH 10.5	1.56	0.82	0.6

[a]Conditions: 1.0 mM **Eu.1/2**, 0.1 M NaCl, 298 K, λ_{ex} = 395 nm, and λ_{em} = 614 nm.

coordination via the dpp oxygen in basic media, resulting in the formation of a seven-membered chelate ring. Reduced steric crowding of the europium(III) center would arise through oxygen binding compared to the five-membered chelate ring, which would be formed if binding was to occur through the nitrogen atom. Lowe and Parker observed the reversible binding of the sulfonamide groups of europium(III) charge-neutral complexes bearing an arylsulfonamide moiety occurred via the sulfonamide nitrogen, resulting in the formation of a five-membered chelate ring.[3,21] The hydration state of the resulting complex in basic media was calculated as q = 0 because of the increased steric crowding about the europium-(III) center. The same could be assumed here should the dpp moiety bind to the europium(III) center via the nitrogen atom, forming a five-membered ring. As q = 0 is not observed, oxygen coordination is tentatively assigned.

Europium emission was observed following indirect excitation via the dpp chromophore. Increased emission intensity was observed when the complexes were excited via the chromophore at λ_{ex} = 270 nm compared to direct excitation at 395 nm. Emission spectra at pH 5.5, 8.4, and 10.0 are shown in Figure 2. The form of the spectrum at pH 5 is typical of a

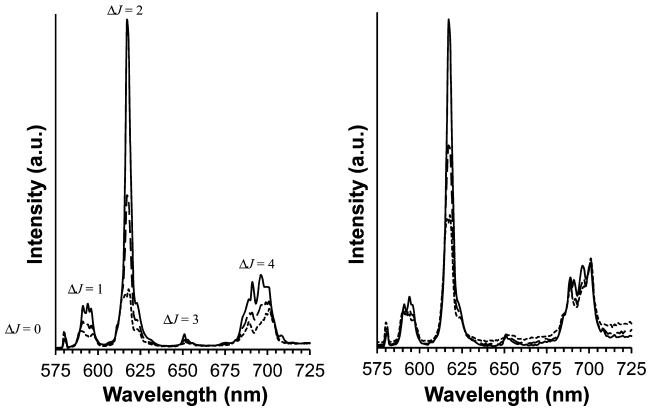

Figure 2. Europim(III) emission spectra for **Eu.1** (left) and **Eu.2** (right) at pH 10.0 (solid line), 8.4 (dashed line), and 5.5 (dotted line). Conditions: 1.0 mM **Eu1/2**, 0.1 M NaCl, λ_{ex} = 270 nm, 298 K.

seven-coordinate q = 2 EuDO3A-based complex; the ΔJ = 0 to 4 transitions ($^5D_0 \rightarrow {}^7F_J$) are shown. The change in the spectral form as the pH is increased is marked: there is a change in the spectral form of ΔJ = 1 and a significant increase in the intensity of the hypersensitive ΔJ = 2 transition. This transition is particularly sensitive to the polarizability of the axial donor atom.[22] A similar increase in the intensity of this transition relative to ΔJ = 1 is seen in related europium(III) complexes, where q is switched from q = 2 to 1/0.[3f,5b,c,21] Luminescence versus pH titrations (analogous to relaxivity vs pH for gadolinium) were carried out, revealing an increase in the europium(III) emission intensity in basic pH that can be clearly seen in Figures 2 and 3. The results mirror those of the

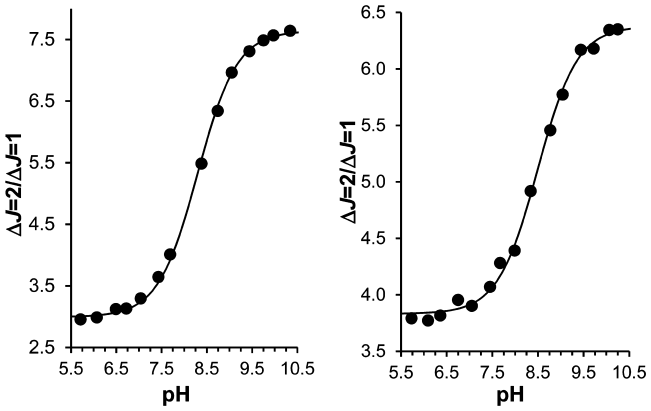

Figure 3. Europium(III) emission intensity ΔJ = 2/ΔJ = 1 versus pH for **Eu.1** (left) and **Eu.2** (right) showing the experimental (dots) and calculated (line) data. Conditions: 0.1 mM **Eu.1/2**, 0.1 M NaCl, λ_{ex} = 270 nm, 298 K.

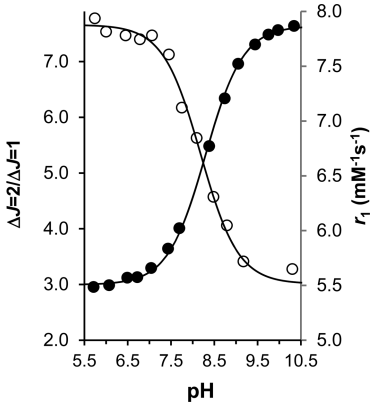

Figure 4. Europium(III) emission intensity ΔJ = 2/ΔJ = 1 (closed circles) and gadolinium(III) relaxivity r_1 (open circles) versus pH for **Ln.1**. Conditions: 0.1 mM **Ln.1**, 0.1 M NaCl, λ_{ex} = 270 nm, 298 K.

gadolinium(III) relaxivity versus pH curves (Figure 4). This is expected because of the increase in the hydration state q in acidic media; europium(III) emission is quenched, giving rise to less intense spectra in acidic media (the sensitizing dpp chromophore will also be more remote when not coordinated), while the gadolinium(III) relaxivity is enhanced under the same conditions because higher r_1 values are achieved with increased q. The opposite is true in basic media, where q = 1; i.e., the europium(III) luminescence intensity is increased, whereas the gadolinium(III) relaxivity is decreased.

The efficiency of energy transfer is enhanced by minimizing the lanthanide(III)−chromophore distance. The rate of energy transfer is $1/r^6$ distance-dependent, where r is the distance between the metal and chromophore center.[23] The increased europium emission intensity observed in basic media can be attributed to the closer proximity of the chromophore to the europium(III) center, making energy transfer more efficient; furthermore, dpp coordination in basic media displaces a quenching water molecule, which further increases the europium(III) emission intensity. Although there is an overall

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increase in the intensity of the spectra as the pH moves toward more basic media, the most interesting change is the increase of the hypersensitive $\Delta J = 2$ band at around 617 nm, which is greatly affected by the coordination geometry about the europium(III) center. The plots in Figure 3 show variation of the ratio of intensities of the $\Delta J = 2$ and 1 bands versus pH. Ratiometric comparisons between the $\Delta J = 2$ and 1 bands allow for concentration-independent determination of the pH within the range ~7−9, with excited-state pK_a values determined as 8.3 (±0.1) and 8.5 (±0.1) for **Eu.1** and **Eu.2**, respectively.

CONCLUSIONS

It has been shown that dpp's undergo pH-dependent ligation when incorporated into Eu^{III}- and Gd^{III}-DO3A chelates. Upon excitation of the dpp antenna at λ_{ex} = 270 nm, sensitized emission of europium(III) was observed. pH-responsive behavior was exhibited as changes in europium(III) luminescence, from which hydration state values have been measured at $q = 1$ and 2 in basic and acidic media, respectively. Furthermore, variation in the emission intensities between the $\Delta J = 1$ and 2 bands demonstrates the potential application of such compounds a ratiometric probes for concentration determination of the pH. Relaxometric studies demonstrated the reversible binding nature of the pendant dpp moiety with the gadolinium(III) analogue. Relaxivities were measured as r_1 = 7.9 mM^{-1} s^{-1} (**Gd.1**) and r_1 = 8.2 mM^{-1} s^{-1} (**Gd.2**) in acidic media with the complex in its $q = 2$ form. Because deprotonation and ligation of the dpp oxygen occurs in basic media and the complex hydration state is $q = 1$, the measured relaxivity values are r_1 = 5.4 mM^{-1} s^{-1} (**Gd.1**) and r_1 = 4.4 mM^{-1} s^{-1} (**Gd.2**). These observed relaxivity values are consistent with a complex with a change in the hydration state from $q = 2$ to 1. The pK_a values are 8.1 (±0.1) and 7.8 (±0.1) for **Gd.1** and **Gd.2**, respectively, calculated from relaxivity versus pH studies; unfortunately, this lies somewhat higher than the physiological pH conditions (pH 7.4). At physiological pH, these complexes exist as ~95% $q = 2$. This suggests that these may be useful contrast agents if this hydration state can be maintained under physiological conditions, i.e., if displacement of these water molecules by endogenous anions can be prevented. Incorporation of negatively charged residues in the α position on the carboxylate arm is expected to suppress competition from endogenous anions.[5b,c,24] It has been shown that adding substituents to DO3A-based chelates confers additional stability, e.g., as observed for Gd-DO3MA, which contains a methyl in these positions (log K = 25.3); this modification is also expected to increase the thermodynamic stability of the seven-coordinate complexes in the $q = 2$ form.[25]

ASSOCIATED CONTENT

Supporting Information

Emission spectra from pH titrations of **Eu.1**/**2**, excited-state lifetime determination, and ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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