pH-Sensitive Modulation of the Second Hydration Sphere in Lanthanide(III) Tetraamide–DOTA Complexes: A Novel Approach to Smart MR Contrast Media

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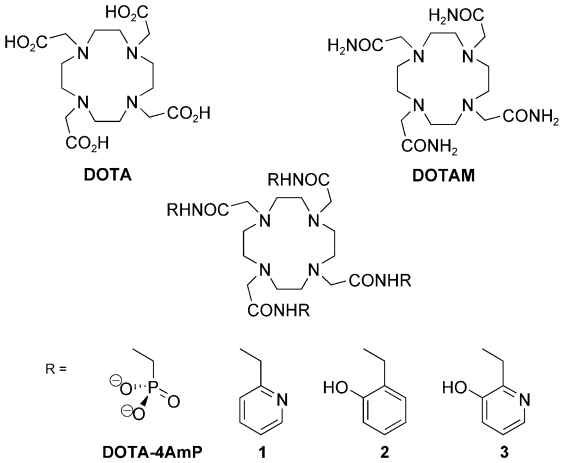
Abstract: The lanthanide(III) complexes of three tetraamide DOTA bearing pyridyl, phenolic and hydroxypyridyl substituents have been studied by NMR, luminescence and cyclic voltammetry. The relaxivity profiles of the gadolinium complexes of the pyridyl and phenolic ligands were flat and essentially the same between pH 2 and 8. The hydroxypyridyl ligand, however, exhibited two regions of enhanced relaxivity. The small relaxivity enhancement (25%) at lower pH (pH 2–4) has been attributed to an increase in the prototropic exchange of the coordinated water molecule while the slightly larger enhancement (84%) at higher pH (pH 6–9) reflects deprotonation of the ligand amide protons. Deprotonation of the amides results in the formation of an intramolecular acid–base pair interaction with the phenolic protons and this, in turn, causes a highly organized second hydration sphere to come into effect, thereby increasing the relaxivity. The water relaxivity of the Gd^{3+}-hydroxypyridyl complex is further enhanced upon binding to serum albumin.

Keywords: imaging agents · lanthanides · macrocyclic ligands · responsive probes · second-sphere hydration

Introduction

Since the introduction of contrast media for MR imaging in 1983, three primary approaches have been taken toward development of improved agents. The first is higher relaxivity agents that would enable administration of lower doses of contrast agent.[1] The second is to conjugate the agent to a targeting vector designed to highlight certain tissue types.[2] The third approach is a responsive or "smart" agent that switches on (and sometimes off also) in response to some biological stimulus.[3] Agents that report the presence of the metal cations,[4, 5] anions,[6, 7] enzymes,[8] proteins[9] and pH[10, 11] have now been reported.

We recently reported a Gd^{3+} complex of a DOTA tetraamide derivative, GdDOTA-4AmP^{5-},[11] that has unusual water relaxation characteristics. Although water exchange in this complex is quite slow compared to more typical contrast agents, the bulk water relaxivity of GdDOTA-4AmP^{5-} was found to increase from 3.8 mM^{-1}s^{-1} to 9.8 mM^{-1}s^{-1} as the pH was changed from ≈8 to ≈6, the same pH range over which the phosphonate groups of GdDOTA-4AmP^{5-} are protonated in four successive steps. This led to the hypothesis that the increased relaxivity was due to the formation of a hydrogen-bonding network between the bulk solvent, the phosphonates and the slowly exchanging, Gd^{3+}-bound water molecule. The rate of proton exchange in this system appears to vary as a function of phosphonate protonation. As the pH approaches 6, the system appears to be in rapid exchange even though ^{17}O



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NMR studies showed that this is not the case. Furthermore, the four phosphonates are predominantly monoprotonated at pH 6, which suggests that proton exchange is most rapid when the amide-appended side chains contain monoprotonated functionalities. To test this hypothesis, three additional DOTA-tetraamide ligands (designated **1**, **2** and **3**) having extended amide substituents capable of forming hydrogen bonds with a Ln^{3+}-coordinated cation have been prepared. The synthetic route to these ligands was reported elsewhere.[12] Here, a physical-chemical characterization of several lanthanide(III) complexes of these ligands are reported.

Results

^{1}H and ^{17}O NMR Studies: A necessary requirement for prototopic exchange to dominate water relaxivity in Gd^{3+} complexes is for water molecule exchange to be relatively slow. Although the rate of water exchange in lanthanide(III) tetraamide complexes is comparatively slow ($10^3-10^5\ s^{-1}$), these complexes often exist as a mixture of two coordination isomers that are in dynamic equilibrium.[13–15] In a number of studies, the rate of water exchange in these two isomers was found to differ by about two orders of magnitude.[14, 16, 17] For example, the rate of water exchange in the twisted square antiprismatic isomer (TSAP) of EuDOTAM^{3+} is $3.3\times10^5\ s^{-1}$ but only about $8.3\times10^3\ s^{-1}$ in the corresponding square antiprismatic isomer (SAP).[17] Thus, one may assume that the SAP geometry is preferred for slow water exchange complexes. The highly shifted resonances of the axial ring protons are a convenient handle for identifying which coordination isomers are present in solution.[15] The population of these two coordination isomers is easily identified by the highly shifted H_4 axial ring proton resonances in the high resolution ^{1}H NMR spectra of the Eu^{3+} complexes (Figure 1).[15] The ^{1}H NMR spectra of Eu(**1**)$^{3+}$, Eu(**2**)$^{3+}$ and Eu(**3**)$^{3+}$ each have H_4 resonances near 25–30 ppm, typical of SAP structures. One can estimate from these spectra that the TSAP isomer is present in solution at no more than 1–2%.

This observation suggests that the rate of water exchange in these complexes might be sufficiently slow to allow prototropic exchange to dominate the water relaxivity. Given that Ln^{3+} cations of smaller ionic radii have an even greater preference for the SAP geometry, one can reasonably assume that Gd(**1**)$^{3+}$, Gd(**2**)$^{3+}$ and Gd(**3**)$^{3+}$ are also largely SAP. A variable temperature ^{17}O NMR linewidth of solvent water was used to estimate water molecule exchange in these complexes. A fitting of those data to standard theory gave water residence lifetimes (τ_M) of 38, 56 and 46 μs for Gd(**1**)$^{3+}$, Gd(**2**)$^{3+}$ and Gd(**3**)$^{3+}$, respectively (see Supporting Information). Although somewhat longer than the water residence lifetimes of EuDOTAM^{3+} ($\tau_M=19$ μs)[15] and DyDOTA-4AmP^{5-} ($\tau_M=21$ μs),[11] these values are nonetheless consistent with SAP structures.

Bulk water relaxation: The bulk water relaxivity of Gd(**1**)$^{3+}$, Gd(**2**)$^{3+}$ and Gd(**3**)$^{3+}$ are shown as a function of pH in Figure 2. The profile of Gd(**1**)$^{3+}$ is typical of that of tetraamide

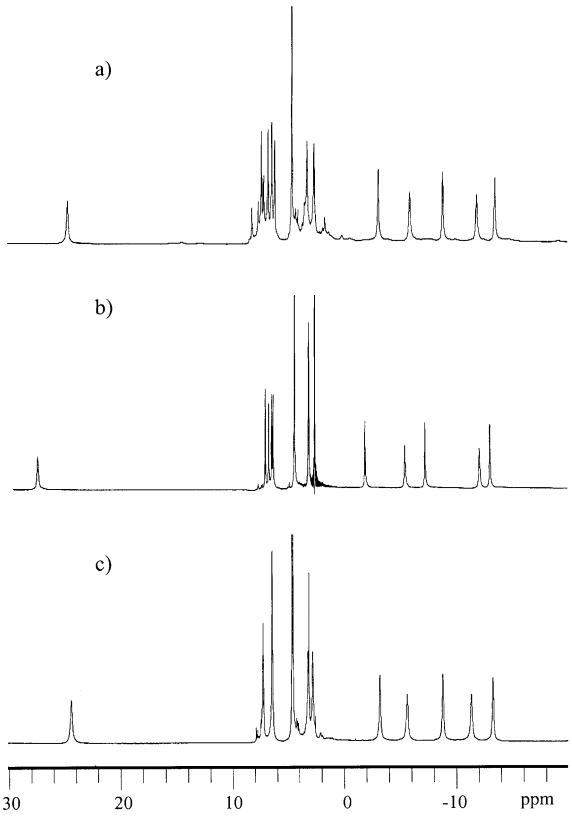


Figure 1. The extended sweep width high resolution ^{1}H NMR of a) Eu(**1**)$^{3+}$, b) Eu(**2**)$^{3+}$ and c) Eu(**3**)$^{3+}$ recorded in D_2O at 500 MHz and pH 4.5.

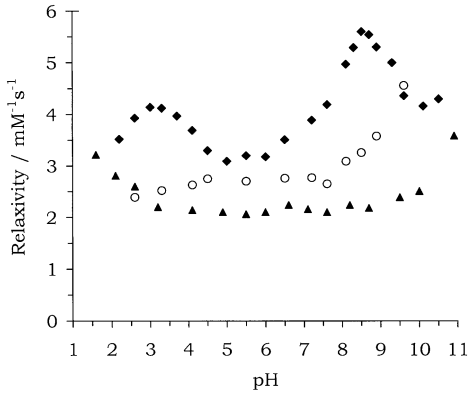


Figure 2. The relaxivity pH profiles of Gd(**1**)$^{3+}$ (▲), Gd(**2**)$^{3+}$ (○) and Gd(**3**)$^{3+}$ (◆) recorded at 20 MHz and 25 °C.

derivatives of DOTA, essentially flat across the pH range 2–9 with an increase in relaxivity at the extremes of pH.[15] The enhanced relaxivity at high pH has been ascribed to an increase in the rate of prototropic exchange between the protons of the bound water and the coordinating amides. Likewise, the profile of Gd(**2**)$^{3+}$ is flat from pH 2–8. In this case the increase in relaxivity due to prototropic exchange occurs at slightly lower pH, presumably the result of an increase in contributions to relaxivity from amide proton exchange. In contrast, the relaxivity profile of Gd(**3**)$^{3+}$ exhibits two regions of enhanced relaxivity between pH 2 and 9. One an enhancement of ≈25% near pH 3 and the

other, an enhancement of ≈85% near pH 8.5 which falls away again until about pH 10 at which point relaxivity begins to rise again. To test whether these responses reflect increases in prototropic exchange, the pK_a of the pyridyl moieties in Eu(**3**)$^{3+}$ were estimated by fluorescence spectroscopy. A pK_a of 2.9 was estimated from the pH dependence of the excitation intensity at 328 nm (excitation of the protonated pyridine) ($\lambda_{em} = 594$ nm).[12] As this corresponds closely to one peak of enhanced relaxivity, this is at least consistent with a relationship between partial protonation of the pyridyl substituents in this complex and the relaxivity enhancement observed near pH 3. The temperature dependence of the bulk water proton relaxation rate of Gd(**3**)$^{3+}$ at three pH values, 3.3, 5.3 and 8.5, is shown in Figure 3. Although the temperature profile at pH 3.3 and 8.5 are marginally steeper than at pH 5.3, the shape of each profile is quite similar. This is consistent with similar water molecule and prototropic exchanges for Gd(**3**)$^{3+}$ at all three pH values.

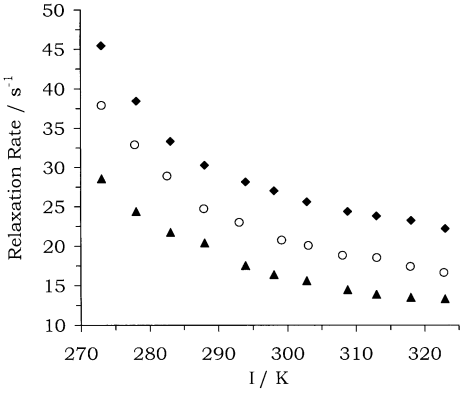


Figure 3. Longitudinal water proton relaxation rates of 1 mM solutions of Gd(**3**)$^{3+}$ at pH 3.3 (○), pH 5.3 (▲) and pH 8.5 (◆) measured as a function of temperature at 20 MHz.

To help identify the source of the relaxivity peaks near pH 3 and 8.5, we rationalized that slowing rotation may serve to amplify any small differences in prototropic exchange that may occur in Gd(**3**)$^{3+}$. Given that serum albumin has been shown to bind other Gd^{3+} complexes with aryl substituents,[18–20] it was reasoned that the aryl groups of Gd(**3**)$^{3+}$ may serve as a suitable means for binding to albumin. Figure 4

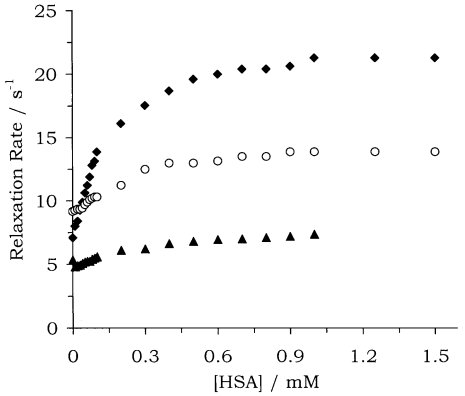


Figure 4. Titrations of Gd(**3**)$^{3+}$ at pH 3.3, 1.5 mM (○), pH 5.5, 1.2 mM (▲) and pH 8.5, 1.2 mM (◆) with human serum albumin (HSA) plotted against longitudinal water proton relaxation rate, measured at 20 MHz and 25 °C.

shows that the water relaxation rate of Gd(**3**)$^{3+}$ increases substantially upon addition of human serum albumin (HSA) and that the increase in relaxivity is greatest at pH 8.5, intermediate at pH 3.3, and smallest at pH 5.5.

In the presence of only ≈0.6 equivalents of HSA, a relaxivity enhancement of 186% was observed for Gd(**3**)$^{3+}$ on passing from pH 5.5 to 8.5, with a maximum relaxivity value of 21 mM^{-1}s^{-1}. This shows that as the rotational correlation time (τ_R) of the complex increases, any small differences in prototropic exchange are amplified. The fact that addition of more HSA did not enhance the effect substantially suggests the involvement of multiple protein binding sites. The effect of changing pH is likely to affect the binding between complex and protein and each binding site is expected to have a different pH dependent binding interaction. This renders meaningful fitting of the binding data difficult. Nonetheless these results are consistent with a more rapid prototropic exchange at pH 3.3 than at pH 5.5. The data also suggest that there is even more rapid exchange at pH 8.5, contradicting the results of the variable temperature relaxation measurements. However, it is difficult to conclude with certainty that prototropic exchange is responsible for the relaxivity enhancement at pH 8.5 because luminescence studies on Eu(**3**)$^{3+}$ indicate that the phenolic protons have pK_a values in excess of 11. Consequently there is no reason to expect a change in the ability of the substituents to catalyze prototropic exchange on passing from pH 6 to 8.5.

Gel filtration: Solomon–Bloembergen–Morgan theory indicates that there are only two parameters that could be altered with pH to effect changes in relaxivity, namely τ_M and τ_R. If there is no significant increase in prototropic exchange rate between pH 5.5 and 8.5, one could reason that there is a change in the rotational correlation time that is modulated by pH. The only feasible explanation for such a change in τ_R would be an aggregation that occurs on passing from pH 5.5 to 8.5. In order to investigate this possibility the extent of aggregation was investigated by gel filtration. 10 mM solutions of Gd(**3**)$^{3+}$ were eluted through G15-120, G25-150 and G50-40 Sephadex columns with molecular weight cut-offs of <1500, 1000–5000 and 1500–30000, respectively. The void volumes were determined by elution of BSA (67.5 kDa) while vitamin B12 was used as a molecular weight standard ($F_W = 1355$). Elution was monitored in-line by UV absorbance at 254 nm. The elution traces for Eu(**3**)$^{3+}$ at pH 5.5 and 8.5 are shown in Figure 5.

There are two features of these traces worthy of note. Firstly, the apparent hydrodynamic size of the complex on each column is ≈1500 while the molecular weight of the complex is only 980 Daltons. Secondly, the apparent size of the complex at pH 8.5 is somewhat smaller than at pH 5.5. The apparent size of the complex at pH 5.5 is too small to support dimerization or aggregation, rather it suggests that the molecular volume of the complex is larger than predicted based upon a spherical model. The diminution in the size of the complex as the pH increases may then be rationalized in terms of alterations in morphology resulting in a more compact structure at higher pH. This hypothesis is supported by the luminescence results discussed below. It must be

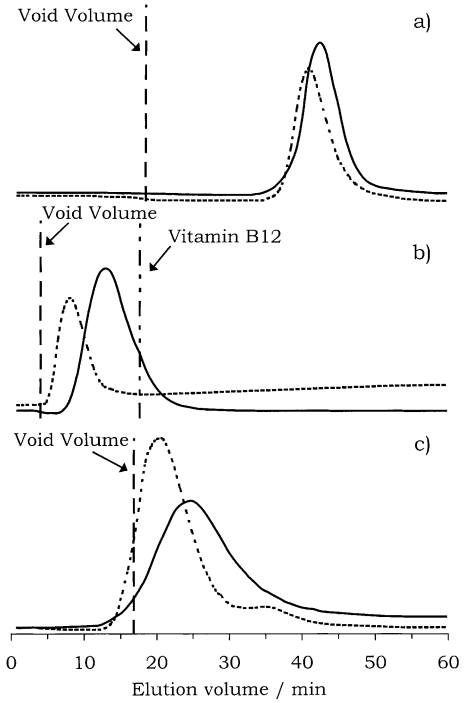


Figure 5. Gel filtration elution traces of Gd(**3**)$^{3+}$ over a) Sephadex G50-40 (1.5–30 kDa), b) Sephadex G25-150 (1–5 kDa) and c) Sephadex G15-120 (<1500 Da) at pH 5.5 (----) and pH 8.5 (——).

concluded, however, that these complexes are monomeric across the pH range in question and aggregation may be ruled out as the source of the relaxivity enhancement.

Electrochemistry and luminescence studies: The luminescence spectrum of Eu(**3**)$^{3+}$ used to estimate the pK_a values of the aryl substituents indicated that this complex has some unanticipated features.[12] Above pH ≈5, the luminescence emission intensity decreased with increasing pH regardless of the excitation wavelength employed and nearly disappeared by pH ≈8. These changes paralleled the pH range over which the enhancement in relaxivity was observed (Figure 6).

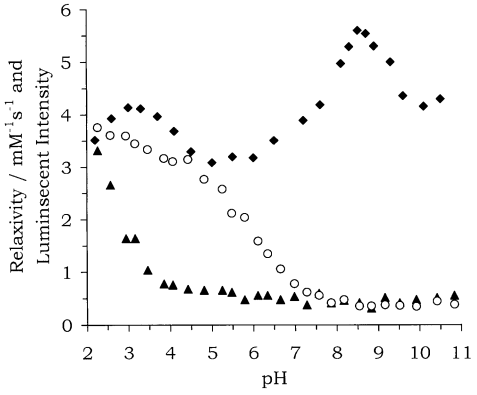


Figure 6. Relaxivity (r_1) pH profile of Gd(**3**)$^{3+}$ (◆) shown alongside the pH profile of the luminescent intensity of the excitation bands of Eu(**3**)$^{3+}$, monitored at 594 nm and exciting the protonated chromophore (▲) and the europium ion directly (○).

The oxidation potential of phenol is known to decrease with increasing pH until the phenol is fully deprotonated at which point the oxidation potential abruptly stabilizes.[21] As this was considered the source of the luminescence changes described above, cyclic voltammetry was used to measure the oxidation potential of both **3** and Gd(**3**)$^{3+}$ as a function of pH (Figure 7).

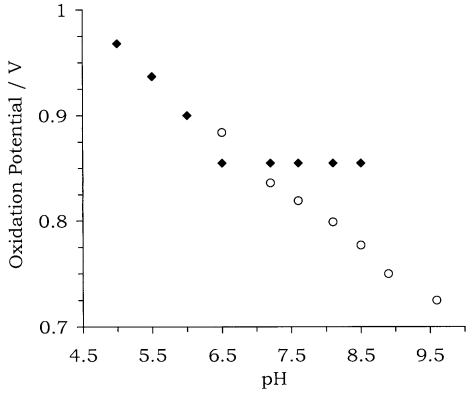
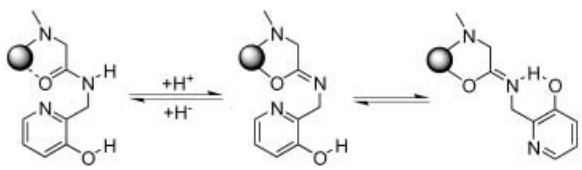


Figure 7. Plot of oxidation potential versus pH for Gd(**3**)$^{3+}$ (◆) and the free ligand **3** (○).

The oxidation potential of **3** exhibits the same behaviour as other phenols, decreasing linearly with increasing pH. In contrast the oxidation potential of Gd(**3**)$^{3+}$ decreases in a linear fashion below pH 6.5 and thereafter remains constant at 855 mV. This behavior is indicative of a phenol incapable of further deprotonation above this pH value. Since the free ligand continues to be affected by further increases in pH, it is clear that this is not the result of the phenols in this system having unusually low pK_a values but rather is a feature related to the presence of the Gd^{3+} ion.

The pK_a values of amide NH protons are drastically reduced upon coordination of the amide oxygen to a metal ion. The coordinated amide protons in Eu(**3**)$^{3+}$ may be observed under acidic conditions by ^{1}H NMR spectroscopy when the spectrum is recorded in H_2O (δ_{NH} ≈0 ppm). The deprotonation of coordinated amides may then be observed by altering the pH of the solution ^{1}H NMR spectroscopy. The deprotonation of the coordinated amide is observed to occur over this same pH range (see Supporting Information).[12] The spatial arrangement of the ligand is such that the phenols may form a six-membered acid–base pair with the deprotonated amide (Scheme 1). Such an acid–base pair would cause the



Scheme 1. Deprotonation of the coordinating amides results in formation of an intramolecular acid–base pair with the phenolic protons.

observed flattening out of the oxidation potential of Gd(**3**)$^{3+}$. Furthermore, the first pK_a of the amide protons of Gd(**3**)$^{3+}$ estimated by cyclic voltammetry (6.5) correlates well with the value determined by potentiometric titration. Between pH 4

and 10, four equivalents of base were consumed, pK_a values of 6.6, 7.1, 8.5 and 9.6 were obtained consistent with the amides becoming deprotonated over the same pH range as the enhancement in relaxivity. Significantly, the last pK_a value occurs beyond the pH range of this relaxivity enhancement. It is noteworthy that these pK_a values are somewhat lower than those reported for a related compound.[15] These differences are thought to originate from the differing natures of the amide substituents in these complexes.

This acid–base interaction develops over much the same pH range as the enhancement in relaxivity of Gd(**3**)$^{3+}$ and is supported by the observation that the apparent molecular volume of the complex decreases with increasing pH over the pH range 5.5–8.5. Formation of such an acid–base pair would be expected to result in a more ordered, compact structure in which the motion of the aryl substituents is restricted, the hydrodynamic volume of the complex would therefore be smaller. Similar behavior might be expected for Gd(**2**)$^{3+}$ yet this complex does not exhibit the same increase in relaxivity. It appears, therefore, that the pyridyl moiety is crucial to the mechanism by which the relaxivity is enhanced.

Effect of oxidant on relaxivity: Hydroxypyridines may be oxidized to two possible products, either oxidation of the phenol to a quinoid type structure or oxidation of the pyridyl to an *N*-oxide radical. By examining the relaxivity of Gd(**3**)$^{3+}$ after treatment with a variety of oxidants, the importance of each aspect of the hydroxypyridyl moiety could be assessed. In addition, this experiment would test the viability of Gd(**3**)$^{3+}$ as an oxidant sensitive contrast agent. Accordingly, samples of Gd(**3**)$^{3+}$ were treated with three chemical oxidants expected to form *N*-oxides with the pyridyl moiety, H_2O_2, $NaBO_3$ and $NaIO_4$. In each case a marked decrease in relaxivity of the complex was observed at pH 8.5 (Table 1). These changes were dependent upon the oxidant employed, presumably reflecting their differing oxidation efficiencies at this pH. The formation of the *N*-oxide was reversible in each case. Addition of an excess of a second amine was found to restore the relaxivity to its original value.

Table 1. The relaxivity of Gd(**3**)$^{3+}$ in the presence of a variety of oxidants at pH 3 and 8.5.

Oxidant	Relaxivity/ mM^{-1}s^{-1}	
	pH 3.3	pH 8.5
none	4.1	5.6
$NaBO_3$	3.3	4.4
H_2O_2	–	3.1
$NaIO_4$	3.6	4.5
electrochemical	–	4.5

Formation of *N*-oxides also affected the relaxivity at pH 3.3 supporting the hypothesis that partially protonated pyridyl moieties initiate a small increase in prototropic exchange. When Gd(**3**)$^{3+}$ was exposed to electrochemical oxidation (6 mM in 50% TRIS buffer, 50% acetonitrile, 25 mM Bu_4NClO_4, oxidation potential = 1.05 V for 2 h), a similar decrease in relaxivity (≈20%) was observed at pH 8.5. This decrease was expected on the basis that the phenol is instrumental in enhancing the relaxivity of the complex at this pH. Since the oxidation potential of Gd(**3**)$^{3+}$ is much higher (>1.0 V) at acidic pH values, attempts to oxidize and measure relaxivity at this pH were not attempted. These observations are consistent with the idea that both the hydroxyl and pyridyl groups are essential to the enhancement of relaxivity between pH 6 and 9. However, these groups apparently do not catalyze exchange of protons with bulk water but rather they alter the relaxivity of Gd(**3**)$^{3+}$ via a structural rearrangement.

Discussion and Conclusions

The tetraamide DOTA complexes Gd(**1**)$^{3+}$, Gd(**2**)$^{3+}$ and Gd(**3**)$^{3+}$ exist primarily as square antiprismatic coordination complexes in solution and exhibit slow water exchange. Neither Gd(**1**)$^{3+}$ nor Gd(**2**)$^{3+}$ possess the requisite features to catalyze the prototropic exchange of this slowly exchanging water molecule so the relaxivity profiles of these two complexes are similar to those obtained for simple tetraamides for example DOTAM. Gd(**3**)$^{3+}$ does, however, show two regions of enhanced water relaxivity, suggesting that it may be necessary to have a minimum of two hydrogen bonding groups on an amide side-chain group to catalyze prototropic exchange. A small enhancement of 25% is observed in a pH region that corresponds closely to the pK_a value of the pyridyl group (2.9). Although no substantial increase in the rate of exchange was observed in variable temperature relaxation studies, binding studies with HSA suggest that in fact a discernable increase in the overall exchange processes had occurred. This suggests that when the pH of a solution of Gd(**3**)$^{3+}$ is lowered to near 3, the partially protonated pyridyl moieties work in concert with the phenolic groups to catalyze prototropic exchange.

In contrast, when the pH of this solution is raised above 7, the amide protons begin to dissociate and this has two effects that work in concert to increase the relaxivity of the complex. First, the complex gradually looses its positive charge and eventually becomes monoanionic at high pH (Gd(**3**)$^{3+}$ → Gd(**3**)$^{-}$). Tricationic complexes of this sort are associated with three anions, in this case chloride ions (Figure 8). The pioneering work of Parker and co-workers has demonstrated that the counter-ions in this type of tetraamide complexes are closely associated with the complex and occupy space around the amide substituents above the coordinated water molecule.[15, 22, 23] As the charge on the complex is neutralized these ions are able to move away from the complex and, in so doing, increase access of bulk solvent to the complex. The second result of the increasing deprotonation of the coordinating amides is that they begin to form acid–base pairs with the phenolic groups. In the pH region close to the pK_a of the coordinating amides in Gd(**3**)$^{3+}$ the rate of deprotonation and reprotonation will be extremely rapid. Thus, a rapid three-pool equilibrium between the amide, imide and acid–base paired imide forms of each pendant arms is to be expected. Around pH 8.5 the time-averaged position of the aryl groups is further away from the coordinated water molecule than at

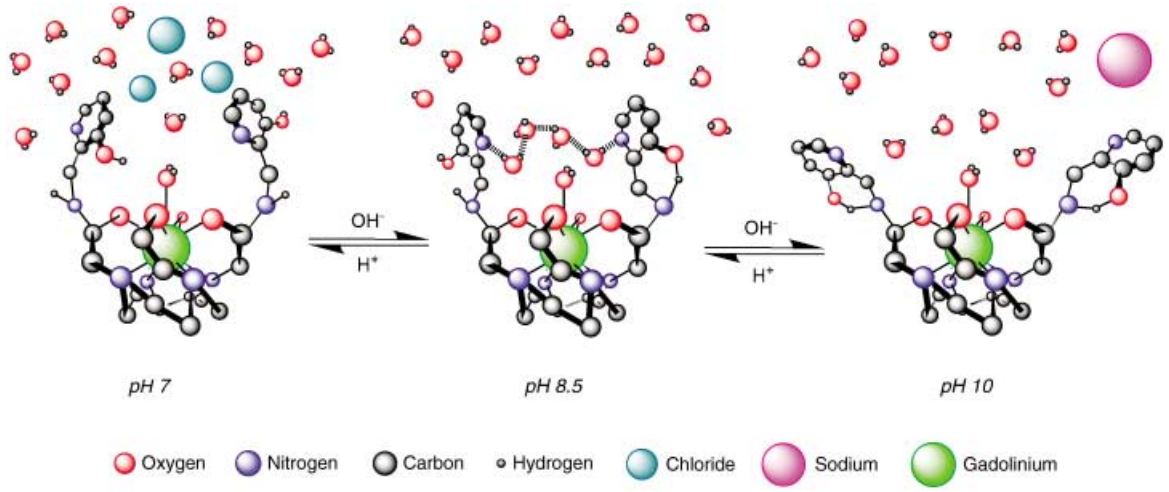


Figure 8. Schematic representation of the conformation of Gd(**3**)$^{3+}$ (two of the amide substituents have been omitted for clarity). At low and intermediate pH the aryl substituents point upwards excluding water from a second hydration sphere. As the pH of the solution is increased the coordinating amides become deprotonated. This sets up an equilibrium in which the phenolic protons form acid–base pairs with the resulting imides. At and around pH 8.5 the time-averaged position of the pyridyl group opens up the top of the complex allowing access of water to form a second hydration sphere. The time-averaged position of the four pyridyl units is such that they tend to organize a second hydration sphere of water molecules across the top of the complex. As the pH is raised further and the equilibrium tends to the deprotonated form of the amides, the time-averaged position of the aryl groups lies away from the top of the complex. This allows access of a second hydration sphere to the top side of the complex but without organization the residence lifetime of a water molecule in this second hydration sphere is short.

lower pH. This allows access of water to form a second hydration sphere above the coordinated water molecule.

The contributions of second hydration spheres to the relaxivity of a complex have been thoroughly described.[24] However, the same effect must also occur with Gd(**2**)$^{3+}$ and yet no relaxivity enhancement is observed in this case. But Gd(**2**)$^{3+}$ lacks the additional pyridine donors of Gd(**3**)$^{3+}$. It is anticipated that the pyridine nitrogens of Gd(**3**)$^{3+}$ are caused to point across the top of the coordinated water molecule by the formation of the acid–base pairs. The second hydration sphere of water molecules is able to hydrogen bond to these nitrogen donors. This causes a degree of organization within this second hydration sphere that prolongs the length of time these water molecules reside in close to proximity to the gadolinium ion. Only with this prolonged residence in the second hydration sphere are water molecules able to experience paramagnetic relaxation by the gadolinium ion. Hence, no relaxivity enhancement is observed for Gd(**2**)$^{3+}$ in which no organizational effects are possible. This organizational effect of the pyridyl groups is also lost as the pH is further increased above 8.5. At pH 10 and above the amides become completely deprotonation and the equilibrium is shifted to favour the acid–base pairing of the imide and phenols. The effect of this is to move the time-averaged position of the aryl groups yet further from the coordinated water molecule. While this allows unfettered access of water molecules to the second hydration sphere there are now no points of anchorage around which it may be organized. Consequently, the residence lifetime of a water molecule of the second hydration sphere at higher pH is short, so they are not efficiently relaxed by gadolinium and the relaxivity falls as a result. The subsequent increase in relaxivity above pH 10 is the result of external base catalyzed prototopic exchange of the coordinated water molecule.

Since no increase in the rate of water molecule exchange is observed resulting from the presence of this second hydration sphere, it is exchange of water molecules in the second hydration sphere with those of the bulk solvent that appears to account for the relaxivity enhancement. Given the comparatively short expected residence lifetime of a second sphere water molecule and the distance of closest approach to the gadolinium center, the relaxation effects experienced by these water molecules should be comparatively inefficient. Hence only a modest increase in relaxivity is observed when the residence lifetime of second sphere water molecules is long. When the residence lifetime in the second sphere is too short, in Gd(**3**)$^{3+}$ at high pH and in Gd(**2**)$^{3+}$, no relaxation occurs and therefore no enhancement is observed. In conclusion, the larger relaxivity enhancement (84%) can be attributed to the pH responsive formation of an organized second hydration sphere in Gd(**3**)$^{3+}$. This provides new insights toward the design of pH responsive contrast media. The significant relaxivity enhancement observed for Gd(**3**)$^{3+}$ in the presence of HSA on passing from pH 5.5 to 8.5 (186%) could render this complex useful as a pH or oxidant sensitive blood pool agent.

Experimental Section

^{1}H and ^{17}O NMR spectra were recorded on a Varian Inova 500 spectrometer operating at 499.99 and 67.72 MHz, respectively. Relaxation measurements were made using an MRS-6 NMR Analyzer from the Institut "Jožef Stefan", Ljubljana, Slovenjia operating at 20 MHz. Samples were run either in buffered solution using phosphate/citrate, tris or carbonate buffer or after adjusting the pH with hydrochloric acid and potassium hydroxide solution.

Due to the insolubility of all Ln(**2**)$^{3+}$ complexes across the pH range measurements performed on this complex were made using solutions in

50% aqueous methanol. ^{17}O NMR data for $Gd(\mathbf{2})^{3+}$ were measured in 50% aqueous acetonitrile.

Luminescence spectra were recorded on Perkin Elmer LS55 fluorimeter. The pH of luminescence samples were measured using a Fisher Accumet 925 pH meter equipped with an Orion 8103 Ross combination pH electrode. The pH of luminescence samples was altered by addition of solid lithium hydroxide monohydrate or *p*-toluenesulfonic acid. Cyclic voltammograms were recorded on a BAS CV-50W voltammetric analyzer using a glassy carbon working electrode, platinum wire auxiliary electrode and Ag/AgCl reference electrode. Samples were prepared at 0.6 mM concentrations using a 50 mM solution of tetrabutylammonium perchlorate in acetonitrile and a buffer solution of either phosphate-citrate or tris buffer.

Titrations of $Gd(\mathbf{3})^{3+}$ with HSA were performed by addition of a solution 5 mM in HSA and 1 mM in $Gd(\mathbf{3})^{3+}$ to a 1 mM buffered solution of $Gd(\mathbf{3})^{3+}$. Samples were mixed and then allowed to equilibrate for 5 min after addition of HSA before the measurement was taken. The effect of chemical oxidants upon relaxivity was measured by addition of 5 equiv oxidant to a buffered solution of the complex. Relaxivity measurements were made at 0.5, 1, 2 and 24 h after addition of the oxidant. Gel filtration experiments were performed using a 25 cm column of the appropriate sephadex resin. The elution was monitored in-line using an ISCO type 6 optical unit monitoring at 254 nm and an ISCO UA-5 chart recorder.

The following procedure for the preparation of $Gd(\mathbf{3})^{3+}$ is typical of that used for preparing complexes of these ligands.

Gadolinium oxide (20 mg, 54 μmol) was added to conc. HCl (2 mL) and heated until dissolved. The solvents were removed under reduced pressure to afford the corresponding chloride as a colorless solid. The chloride salt was dissolved in water (3 mL) and the solution added to a solution of the ligand **3** (100 mg, 121 μmol) in water (3 mL). After heating for 18 h at 60 °C, the solvents were removed under reduced pressure to afford the complex $Gd(\mathbf{3})^{3+}$ as a colorless solid.

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