DOI: 10.1002/chem.201001548

# Critical In Vitro Evaluation of Responsive MRI Contrast Agents for Calcium and Zinc**

Anurag Mishra,*[a, b] Nikos K. Logothetis,[b] and David Parker*[a]

**Abstract:** The synthesis of two gadolinium(III) complexes that exhibit an increase in proton relaxivity in the presence of added $Ca^{2+}$ or $Zn^{2+}$ ions is reported. The complexes increase their hydration state from zero to one following metal-ion binding, confirmed by spectral measurements on the corresponding $Eu^{III}$ complexes. At a field of 1.4 T and 310 K, modulation of relaxivity of the order of 30–40% was observed in mouse serum in each case. The dissociation constants for $Ca^{2+}$ and $Zn^{2+}$ binding were sensitive to the presence of added bicarbonate, and were 450 μM ($Ca^{2+}$) and 200 μM ($Zn^{2+}$) in serum. Such systems may, therefore, be considered for use as magnetic resonance imaging (MRI) contrast agents to track the restoration of changes in metal-ion concentration in the cerebrospinal fluid of the brain, following neural stimulation.

**Keywords:** calcium · gadolinium · imaging agents · lanthanides · zinc

## Introduction

Calcium plays a pre-eminent role as a signalling ion in biology, with an important function as a second messenger in the brain. The concentration of calcium within cells falls within the range of 20–100 nM, whereas in extracellular fluids the concentration is of the order of 1.5 mM. Levels of extracellular calcium decrease by between 15 and 30% during normal brain function, whereas reductions of as much as 90% in the cerebrospinal fluid can occur during major trauma, epilepsy, or anoxia. This variation in extracellular $[Ca^{2+}]_0$ in the brain is strongly linked to the action of the parathyroid hormone, and is a significant determinant of neural function in healthy and diseased states.[1,2] Observation of changes in ionisable $[Ca^{2+}]_0$ are typically undertaken by using specific ion-selective microelectrodes or with the aid of fluorescent calcium-selective probes. Each of these methods suffers from drawbacks of invasiveness and limited depth penetration, stimulating a search for alternative methods to track changes with appropriate spatial and temporal resolution. Amongst these, the development of responsive magnetic resonance imaging (MRI) contrast agents shows considerable promise,[3,4] and systems responding selectively to changes in $[Ca^{2+}]$,[3,4] copper,[5] and $[Zn^{2+}]$[6,7] have been devised.

A key aspect of probe design is to ensure that the dissociation constant of the responsive contrast agent matches the resting concentration of the target species in the local biological medium. This issue inhibits the pragmatic development of calcium-sensitive MRI probes for intracellular use.[3b] A similar inherent problem exists with copper-sensitive probes, as the concentration of "kinetically labile" $Cu^{2+}$ in vivo is likely to be extremely low (i.e., sub-nanomolar).[5] It has been postulated that concentrations of zinc in cerebrospinal fluid may rise from 10 nM to values as high as 250 μM, following stress-induced release of zinc ions from neuronal synaptic vesicles, which suggests a possible application for zinc-activated MRI probes.[6]

In this work, we have set out to create and evaluate MRI contrast agents for $Ca^{2+}$ and $Zn^{2+}$ that exhibit an increase in relaxivity on metal-ion binding to the probe. Thus, as the local concentration of, for example, $Ca^{2+}$ increases with time upon returning to the normal higher equilibrium value, the relaxivity of the probe must also increase to signal this change. Obviously, the timescale for image acquisition must be fast with respect to the time taken for the $[Ca^{2+}]$ to return to its equilibrium value. A convenient way of engi-

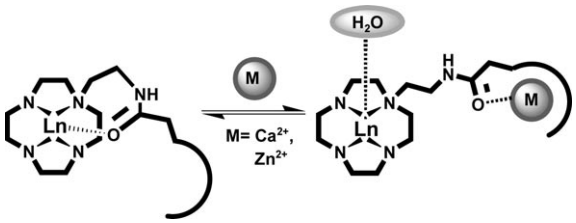
[a] Dr. A. Mishra, Prof. D. Parker
Department of Chemistry, Durham University
South Road, Durham, DH1 3LE (UK)
Fax: (+44) 1913342051
E-mail: anurag.mishra@dur.ac.uk
david.parker@dur.ac.uk

[b] Dr. A. Mishra, Prof. N. K. Logothetis
Max Planck Institute for Biological Cybernetics
Department of Physiology of Cognitive Processes
Spemannstrasse 38, Tubingen, 72076 (Germany)

[**] MRI = magnetic resonance imaging.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201001548.

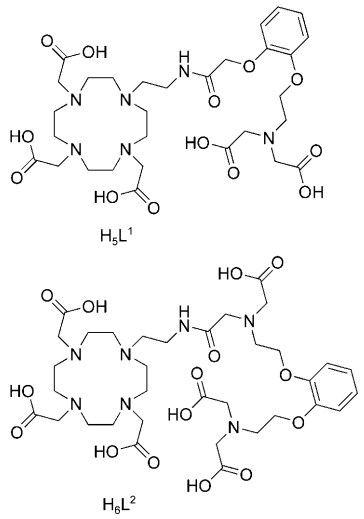
  




Scheme 1. Schematic representation of metal-ion responsive CAs.

neering this response is to develop a system in which the contrast agent increases its hydration state from zero to one following reversible metal-ion binding, (Scheme 1). In this hypothetical case, the amide carbonyl oxygen binds to the $Gd^{3+}$ ion in the unactivated state to give a 7-ring chelate. However, it preferentially coordinates to the added metal ion ($Ca^{2+}$ or $Zn^{2+}$), liberating space for a water molecule to occupy the vacated site at Gd, enhancing relaxivity proportionately. The enhancement of relaxivity associated with local hydration changes has been reported previously in the context of pH-responsive systems[7] and metal-ion activated systems.[4,6]

The metal-ion binding site in the responsive agent needs to be designed with the coordination number, donor preference, and desired overall affinity borne in mind. In this work, we utilised six and eight-coordinate pyro-EGTA-based ion-binding moieties for $Zn^{2+}$ and $Ca^{2+}$, which are variants of structurally related analogues,[8] respectively. Thus, we have set out to examine the behaviour of gadolinium(III) complexes of $L^1$ for $Zn^{2+}$ and $L^2$ for $Ca^{2+}$ (Scheme 2). In addition, we have examined the emission spectral properties of the $Eu^{III}$ analogues. Complexes of europium(III) exhibit an emission spectral profile that is sensitive to the europium coordination environment.[7,9,10] In this context, the spectral behaviour of structurally related DO3A-monoamide europium(III) complexes, with similar $CH_2CH_2NHCOR$ substituents, provides a useful reference point for comparative emission spectral analyses. Examples




Scheme 2. Structures of ligands.

of complexes with and without a bound water were identified in this work, and the hydration change was signalled clearly by a reduction in the emission intensity ratios of the $\Delta J=2/\Delta J=1$ bands, from 2:1 ($q=1$) to 3:1 ($q=0$).[11] Europium emission spectra are particularly sensitive to changes in the coordination environment of the metal ion, especially if the axial donor is varied.[9]

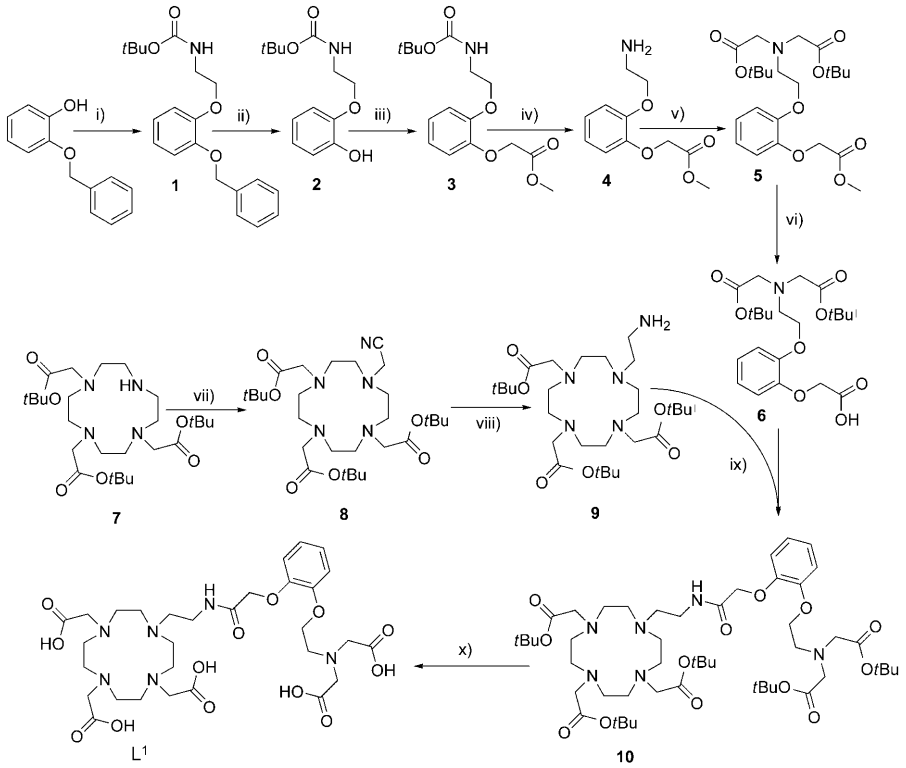
## Results and Discussion

**Ligand and complex synthesis**: Ligand $L^1$ was synthesized in 10 steps according to Scheme 3. Stepwise alkylation of 2-(benzyloxy)phenol with *tert*-butyl 2-bromoethylcarbamate in acetonitrile afforded compound **1**, which was debenzylated by hydrogenation, using Pd/C as the catalyst, to give **2** in 80% yield. This phenol was alkylated with methylbromoacetonitrile in MeCN to give compound **3**, from which the amine **4** was obtained by acid cleavage of the Boc groups. *N,N*-Dialkylation of **4** with *tert*-butylbromoacetate in anhydrous DMF proceeded in 68% yield and the mono-acid **6** was obtained by selective deprotection of the methyl group with LiOH. Alkylation of tris-*tert*-butyl-DO3A **7** with bromoacetonitrile gave the nitrile **8**, from which the corresponding amine **9** was obtained by hydrogenation over Raney nickel (7 M $NH_3$/MeOH, 20 °C).[12,13] The penta-ester **10** was synthesized by coupling the amine **9** and acid **6** by using *N′*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC), 1-hydroxybenzotriazole (HOBt) and *N*-methylmorpholine (NMM) in DMF, from which $L^1$ was obtained by hydrolysis with neat trifluoroacetic acid (TFA; 51%).

The related ligand $L^2$ was synthesized in 9 steps as shown in Scheme 4. Alkylation of the phenol **2** with benzyl-2-bromoethylcarbamate gave the carbamate **11**, which was further treated with mild acid, cleaving the Boc group to afford the amine **12**. Reductive amination (benzene, 4 Å molecular sieves) of the amine **12** with benzaldehyde yielded the secondary amine **13**, from which the methyl ester **14** was obtained by alkylation with methylbromoacetate. The benzylcarbamate was hydrogenated over Pd/C to yield the diamine **15**. *N,N,N′*-Trialkylation with *tert*-butylbromoacetate (DMF, $K_2CO_3$) proceeded in 74% yield. Following basic hydrolysis of **16**, the resultant acid **17** was coupled with the macrocyclic primary amine **9** to give the hexa-ester **18**. Finally $L^2$ was obtained by acid hydrolysis using TFA. Ligands, $L^1$ and $L^2$ were purified by reverse-phase (RP)-HPLC and loaded with $Ln^{3+}$ ($Gd^{3+}$ or $Eu^{3+}$) using $LnCl_3{\cdot}6H_2O$ in water at pH 6.5. The final concentration of $Ln^{3+}$ was determined by inductively coupled plasma optical emission spectrophotometry (ICP-OES).

**Modulation of proton longitudinal relaxivity with calcium and zinc**: The proton longitudinal relaxivity ($r_{1p}$) of [Gd·$L^1$] and [Gd·$L^2$] were measured to be 3.80 and 3.29 $mM^{-1}s^{-1}$ in buffered aqueous solution (310 K, 1.4 T, pH 7.4, 0.1 M 3-morpholinopropanesulfonic acid (MOPS)). These values slightly change (up to 5%) over the pH range 3.5 to 8, and mea-



Scheme 3. Reagents and conditions: i) *tert*-butyl 2-bromoethylcarbamate, $K_2CO_3$, anhydrous MeCN, 72%; ii) Pd-C (10%), $H_2$, MeOH, 40 psi, 80%; iii) methylbromoacetate, $K_2CO_3$, anhydrous MeCN, 88%; iv) 10% TFA in $CH_2Cl_2$, 92%; v) *tert*-butylbromoacetate, $K_2CO_3$, anhydrous DMF, 68%; vi) LiOH, THF/MeOH/$H_2O$ (3:2:2); vii) bromoacetonitrile, $K_2CO_3$, anhydrous MeCN, 78%; viii) 7 M $NH_3$·MeOH, Raney nickel, $H_2$, MeOH, 72%; ix) NMM, EDC, HOBt, anhydrous DMF, 28%; x) neat TFA, 51%.

sured relaxation rates varied linearly with complex concentration over the range 0.5 to 5 mM. Taken together, this suggests that neither inter- nor intramolecular ligation by a remote ligand carboxylate was occurring. These relaxivity values were also measured in solutions containing various added cations ($K^+$, $Na^+$, $Mg^{2+}$, $Ca^{2+}$, $Zn^{2+}$ and $Cu^{2+}$) at concentrations similar to, or greater than, those encountered in extracellular biological fluids (Figure 1). To a first approximation, it is evident that [Gd·$L^1$] is sensitive to added zinc (73% enhancement) and [Gd·$L^2$] selectively responds to calcium (67% enhancement). Neither exhibit a significant change (<25%) in the presence of added $Na^+$, $K^+$, $Mg^{2+}$, $Cu^{2+}$ and $Zn^{2+}$or $Ca^{2+}$. The relatively low change in relaxivity observed in the presence of copper (II) may be tentatively linked to its preference to bind to the more remote amino-diacetate moiety in [Gd·$L^1$] and [Gd·$L^2$]. Since copper(II) prefers 4-coordinate square-planar geometry, it is unable to bind cooperatively to the amide carbonyl group that $Zn^{2+}$or $Ca^{2+}$ will ligate. The relaxivity values measured for [Gd·$L^1$] and [Gd·$L^2$] (310 K, 1.4 T) in the absence of added metal ions were somewhat higher than might be expected for a $q=0$ complex, unless a significant second sphere contribution to the overall relaxivity is present. The importance of second-sphere contributions to relaxivity has been emphasised[14] previously. On the other hand, the limiting relaxivity values measured in the presence of added zinc and calcium (6.3 and 5.8 $mM^{-1}s^{-1}$, respectively) are reasonable values for mono-aqua Gd complexes with proximate hydrophilic moieties that are likely to create a strong second sphere of hydration. The origin of the observed modulation was considered first to involve a hydration change at the Gd centre. Accordingly, the hydration state ($q$) of the analogous pair of Eu complexes was measured. This process involved determining the radiative lifetime of the Eu $^5D_0$ excited state in $H_2O$ and $D_2O$, in the presence and absence of added $Zn^{2+}$ or $Ca^{2+}$, Table 1.

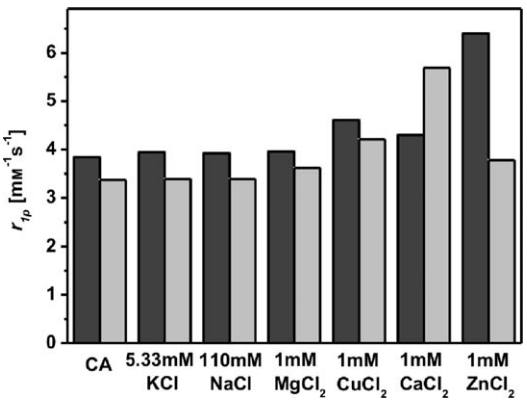


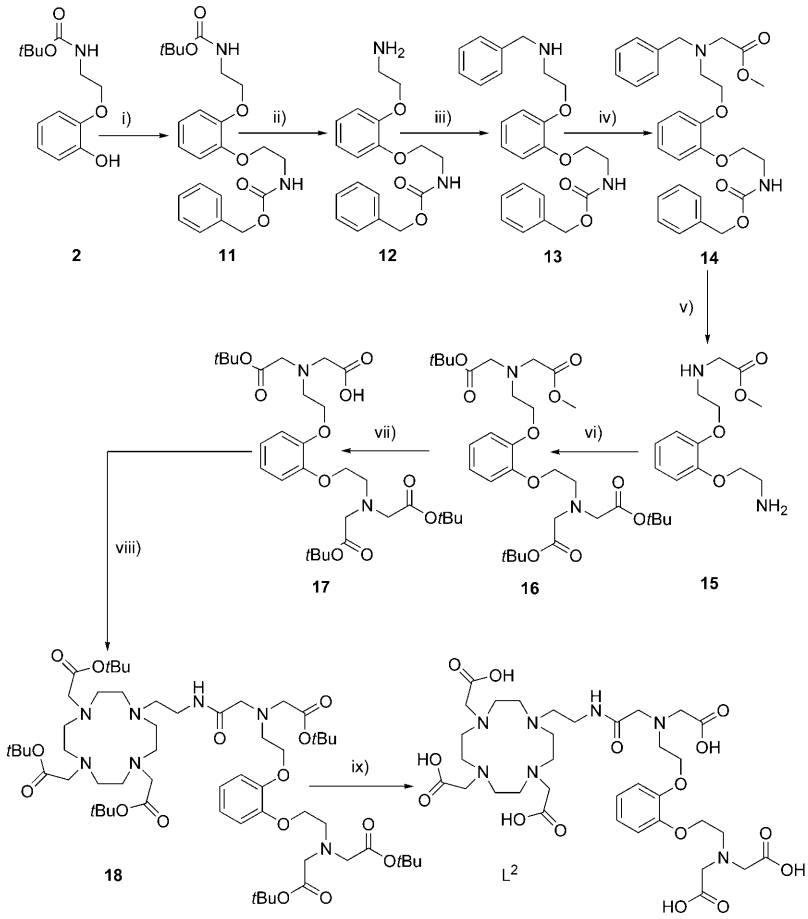

Figure 1. Relaxivity values for aqueous solutions containing [Gd·$L^1$] (dark grey) and [Gd·$L^2$] (light grey) in the presence of the stated salts (310 K, 1.4 T, pH 7.4, 0.1 M MOPS, 0.5 mM complex).

Table 1. Rate constants ($k$) defining the decay of the Eu $^5D_0$ excited state (298 K, $H_2O$ or $D_2O$, 0.1 M MOPS, pH 7.4, 3 mM complex).

| Complex | $k_{H_2O}$ [$ms^{-1}$] | $k_{D_2O}$ [$ms^{-1}$] | $q$[a,b,c] |
|---|---|---|---|
| [Eu·$L^2$] | 1.49 | 0.87 | 0.35 |
| [Eu·$L^2$]+$Ca^{2+}$(0.5 mM) | 1.57 | 0.77 | 0.57 |
| [Eu·$L^2$]+$Ca^{2+}$ (2 mM) | 1.68 | 0.70 | 0.88 |
| [Eu·$L^2$]+$Ca^{2+}$ (3 mM) | 1.83 | 0.69 | 0.97 |
| [Eu·$L^1$] | 1.46 | 0.80 | 0.40 |
| [Eu·$L^1$]+$Zn^{2+}$ (0.5 mM) | 1.53 | 0.77 | 0.52 |
| [Eu·$L^1$]+$Zn^{2+}$ (1 mM) | 1.72 | 0.87 | 0.63 |
| [Eu·$L^1$]+$Zn^{2+}$ (3 mM) | 2.06 | 0.93 | 1.00 |

[a] $q$ values possess an error of 20%; [b] no changes in $q$ (>10%) were measured in the presence of added $MgCl_2$ and $CaCl_2$ or $ZnCl_2$ (1 equiv); [c] $q$ values in the absence of added metal ions were the same at pH 7.4 and 3.5 and the Eu spectral form was the same in each case also.




Scheme 4. Reagents and conditions: i) benzyl-2-bromoethylcarbamate, $K_2CO_3$, anhydrous MeCN, 66%; ii) 10% TFA in $CH_2Cl_2$, 72%; iii) benzaldehyde, benzene; MeOH, $NaBH_4$, 58%; iv) methylbromoacetate, $K_2CO_3$, anhydrous MeCN, 82%; v) Pd/C (10%), $H_2$, MeOH, 50 psi, 80%; vi) *tert*-butylbromoacetate, $K_2CO_3$, anhydrous DMF, 74%; vii) LiOH, THF/MeOH/$H_2O$ (3:2:2); viii) compound **9**, NMM, EDC, HOBt, anhydrous DMF, 33%; ix) neat TFA, 55%.

The empirical relationship that accounts for vibrational deactivation of the metal excited state by directly coordinated, closely diffusing water molecules and proximate NH oscillators was used.[15] Hence, $q$ values were estimated. This data confirmed that each complex underwent a change from a low $q$ value ($q \leq 0.4$) to $q = 1$, in the presence of more than one equivalent of $Ca^{2+}$ [Gd·$L^2$], or $Zn^{2+}$ [Gd·$L^1$]. This hydration switch accounts for the observed relaxivity increase that is expected for Gd complexes of this molecular volume. The overall relaxivity will also contain a significant second-sphere contribution that is likely to vary from the free to the metal-bound system. It is also possible that the free Gd complexes exist in solution as a mixture of $q = 0$ and $q = 1$ complexes in differing ratio and hence give rise to the non-integral hydration state (Eu) and the slightly elevated relaxivity values.

**Calcium activation of [Gd·$L^2$] and modulation of [Eu·$L^2$]**: Incremental addition of $CaCl_2$ to [Gd·$L^2$] resulted in increases in the proton relaxivity, up to a 1:1 stoichiometry, (Figure 2). The observed variation in $r_{1p}$ with [$Ca^{2+}$] was fitted to a 1:1 binding isotherm, notwithstanding the evidence for "$ML_2$" formation (i.e., two lanthanide complexes per $Ca^{2+}$ ion) at low added $Ca^{2+}$ values. This gave an apparent association constant, $\log K = 3.59$ ($\pm 0.03$) (310 K, pH 7.4, 0.1 M MOPS). Subsequent addition of ethylenediaminetetraacetic acid (EDTA) to this solution reversed the observed increases and, in the limit, restored the initial relaxivity value (Figure 2). Such behaviour is consistent with preferential binding of $Ca^{2+}$ to EDTA, and accords with the reversibility of $Ca^{2+}$ binding to [Gd·$L^2$]. Changes to the lanthanide(III) ionic environment with added calcium could also be monitored by observing the changes to the form of the [Eu·$L^2$] emission spectrum, (Figure 3). In the presence of added $CaCl_2$, the ratio of the $\Delta J = 2/\Delta J = 1$ band intensity (centred at 615/592($\pm 10$) nm) decreased from 3:1 to 2:1 and was reversed upon addition of EDTA. A strikingly similar spectral change had been noted earlier with structurally analogous europium complexes and was attributed to the change in hydration,[11] from zero to one bound water molecule.

Human serum albumin (HSA) is the major endogenous protein in most extracellular fluids and its effect on the measured relaxivity of [Gd·$L^2$] was assessed in the absence and presence of 1 equivalent of $CaCl_2$. In the absence of $CaCl_2$, addition of HSA led to a gentle increase in $r_{1p}$ of up to 33% (4.36–5.80 $mM^{-1}s^{-1}$, at 0.6 mM added protein), compared with a rise of 14% (6.92–7.94 $mM^{-1}s^{-1}$) in the presence of an equivalent of $CaCl_2$. No significant spectral changes were observed in parallel series of experiments with [Eu·$L^2$], consistent with no variation in the Eu coordination environment associated with the presence of added protein.

Anions in extracellular fluids can bind reversibly to lanthanide ion centres that are coordinatively unsaturated.[16] The most abundant anion is bicarbonate and it is commonly found at concentrations of the order of 25 mM, compared with 2.3 mM for lactate and 1 mM for various phosphoanions.[16,17] It has been shown that carbonate can chelate to a $Ln^{III}$ centre, displacing any bound water in $q = 1$ or $q = 2$ complexes with heptadentate macrocyclic ligands.[17,18] This is the case for the $Ca^{2+}$ ternary adduct of [Gd·$L^2$],

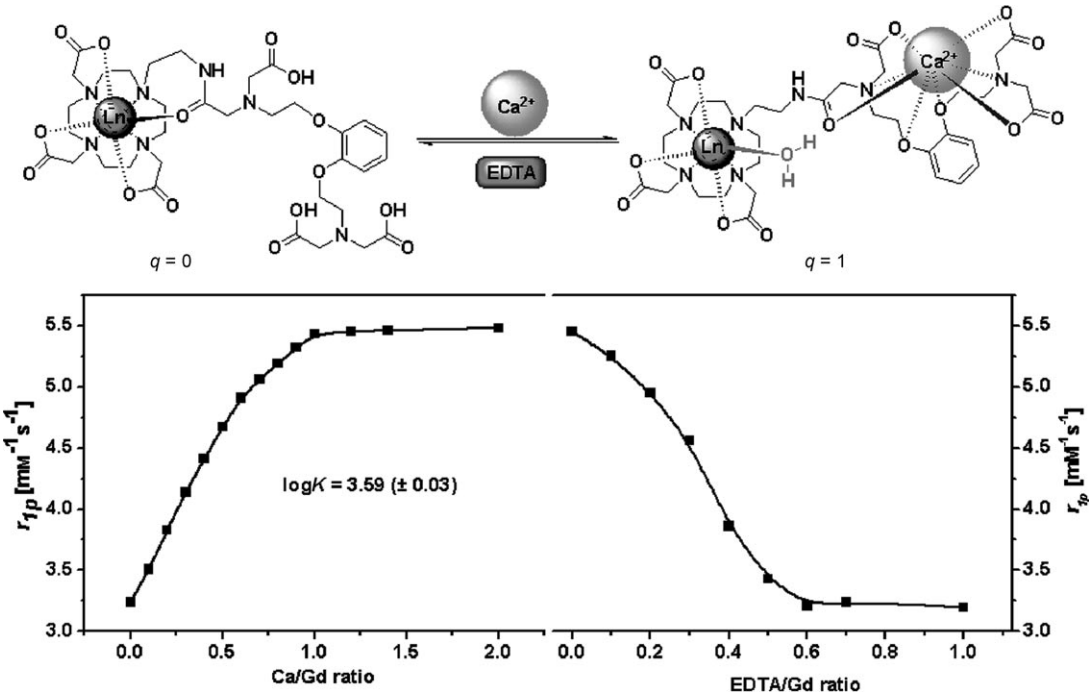



Figure 2. Variation of relaxivity with added $Ca^{2+}$ and subsequent EDTA addition for an aqueous solution containing [Gd·$L^2$] (pH 7.4, 0.1 M MOPS, 310 K, 1.4 T, 1 mM complex).

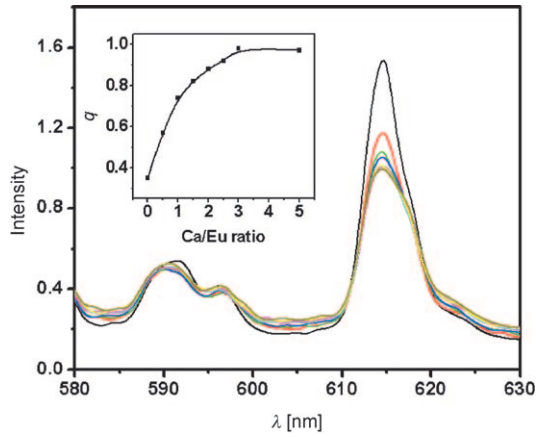



Figure 3. Changes in the europium emission spectrum of [Eu·$L^2$] with added $CaCl_2$, also showing (inset) the concomitant changes in the hydration state, $q$ (3 mM complex, 0.1 M MOPS, pH 7.4, $\lambda_{exc}$ 397 nm).

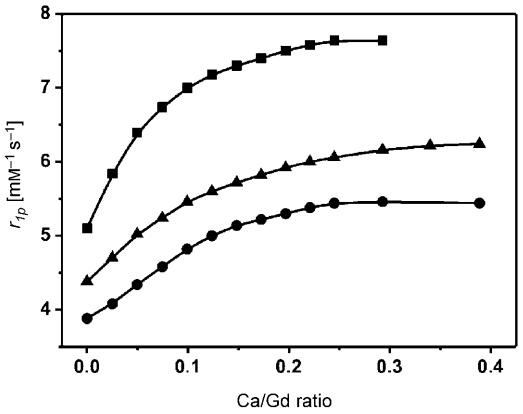



Figure 4. Variation of relaxivity in a solution containing [Gd·$L^2$] in the presence of 0.5 mM human serum albumin (square), 25 mM $NaHCO_3$ (circle) and 0.5 mM HSA plus 25 mM $NaHCO_3$ (triangle) (pH 7.4, 0.1 M MOPS, 310 K, 1.4 T, 0.5 mM complex).

(Figure 4). Incremental addition of $NaHCO_3$ to [Gd·$L^2$] was undertaken at pH 7.4 in buffered solution in the absence and presence of one equivalent of $CaCl_2$. In the absence of added $Ca^{2+}$, no significant variation in proton relaxivity was observed for aqueous solutions containing 0.5 mM [Gd·$L^2$] adding up to 50 mM bicarbonate. In contrast, a 25% (6.72–5.34 $mM^{-1}s^{-1}$) reduction in $r_{1p}$ of $Ca^{2+}$ bound [Gd·$L^2$] was measured over the same range of added bicarbonate. Parallel series of experiments were undertaken with [Eu·$L^2$] (3 mM complex, $\lambda_{exc}$ = 397 nm) observing the emission spectral profile. No change in spectral form was observed in the absence of added $Ca^{2+}$, but in its presence the $\Delta J = 2/\Delta J = 1$ intensity ratio increased to 4:1, accompanied by a 30% reduction in the intensity of the $\Delta J = 0$ transition at 579 nm. These spectral changes are typical of the presence in solution of a carbonate-chelated species, and have been observed on numerous occasions for carbonate adducts with $Eu^{III}$ complexes of *N*-alkyl DO3A complexes.[18]

The variation of the proton relaxivity with added $CaCl_2$ was monitored for three sets of aqueous solutions containing 0.5 mM HSA, 25 mM $NaHCO_3$ and 0.5 mM HSA plus 25 mM $NaHCO_3$ (Figure 4). In the first two cases, added $Ca^{2+}$ caused a 47% (5.10–7.51 $mM^{-1}s^{-1}$) and 43% (3.86–5.50 $mM^{-1}s^{-1}$) change in $r_{1p}$, respectively, over the range of

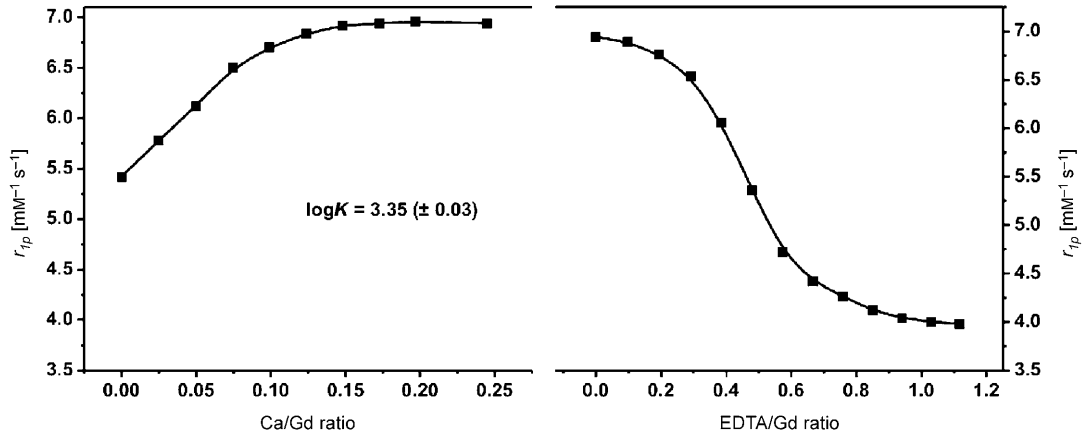



Figure 5. Proton relaxivity changes observed in mouse serum in the presence of [Gd·L$^2$] as a function of added $CaCl_2$ and subsequent (right) addition of EDTA (pH 7.4, 0.1 M MOPS, 310 K, 1.4 T, 0.5 mM complex).

zero to one added equivalent. In the latter case, this diminished only slightly to 40% (4.38–6.16 mM$^{-1}$s$^{-1}$). Furthermore, the apparent association constant was estimated to be $\log K = 3.35(\pm 0.03)$, that is, $K_d = 450$ μM, in the background of 25 mM $NaHCO_3$ and in the protein bicarbonate medium (pH 7.4, 0.1 M MOPS, 310 K).

Finally, relaxivity changes as a function of added $Ca^{2+}$ were assessed in mouse serum. Studies had not been reported earlier in this medium by workers examining calcium activated contrast agents.[3–6,13] In this case, there is an initial concentration of $CaCl_2$ in the serum. This was measured to be 0.64(±0.05) mM using a biocompatible $Ca^{2+}$ selective electrode (ISE). This method quantifies only ionised $Ca^{2+}$ in mouse serum. A titrimetric analysis study was undertaken measuring $r_{1p}$ in mouse serum containing [Gd·L$^2$], as a function of added $CaCl_2$ followed by incremental addition of EDTA (Figure 5). The observed relaxivity changes were fitted by iterative least-squares analysis to give an affinity constant for $Ca^{2+}$ binding, $\log K = 3.40(\pm 0.04)$. This value is consistent with the idea that it is competitive binding of bicarbonate to the Ca-bound adduct that most significantly perturbs the equilibrium between [Gd·L$^2$] and [Gd·L$^2$-($H_2O$)Ca]. It may also be noted that addition of excess EDTA to the mouse serum mops up both the initial and the added $Ca^{2+}$, so that $r_{1p}$ goes to a lower final value than the initial one.

**Zinc modulation of [Gd·L$^1$] and [Eu·L$^1$]**: Parallel series of experiments were undertaken with [Gd·L$^1$], exploring the variation of measured relaxivity in buffer solution and in mouse serum (Figure 6). The percentage change in relaxivity over the range zero to one added equivalent of $ZnCl_2$ was 73% (3.73–6.40 mM$^{-1}$s$^{-1}$) in the former case and 40% (4.00-5.61 mM$^{-1}$s$^{-1}$) in the latter. Addition of EDTA reversed the relaxivity increases and the $r_{1p}$ value returned to the original value in the limit. This behaviour is consistent with the reversibility of the binding of $Zn^{2+}$ to the putative hexadentate binding moiety in L$^1$. Analysis of the zinc binding isotherms indicated a $\log K$ value of 3.90(±0.02) in aqueous buffer (pH 7.4, 310 K, 0.1 M MOPS) and of 3.50(±0.02) in mouse serum.

Incremental addition of $ZnCl_2$ to [Eu·L$^1$] gave rise to changes in the spectral form and the europium emission lifetime, that were also reversed by adding EDTA (Figure 7). The form of the three allowed transitions in the magnetic-dipole-allowed $\Delta J = 1$ manifold (centred around 590 nm) changed upon addition of $ZnCl_2$. More striking was the variation in the intensity of the $\Delta J = 2/\Delta J = 1$ manifolds, that reduced from 3:1 to 2:1 following addition of one equivalent of $ZnCl_2$. Such behaviour closely resembles that observed with [Gd·L$^2$] in the presence of $Ca^{2+}$, and is consistent with a reversible change in the europium coordination environment (Figure 6) associated with the change in metal hydration state.

## Conclusion

The experiments reported herein, serve to establish the modulation of relaxivity induced by the selective, reversible binding of $CaCl_2$ to [Gd·L$^2$] and $ZnCl_2$ to [Gd·L$^1$]. In each case, the variation can be attributed to a change in the lanthanide(III) hydration state, enhancing relaxivity in the metal-ion-bound form. A judicious estimate can be made to define the theoretical relaxivity changes that may occur when $[Ca^{2+}]_0$ or local $[Zn^{2+}]$ vary. In the case of a $Zn^{2+}$ burst in the cerebrospinal fluid of the brain, the transient concentration may increase from near 0 to 250 μM locally. Such a change would be signalled by a 12% increase in relaxivity that would diminish with time as the zinc concentration returned to equilibrium. Modulation of ionisable $[Ca^{2+}]_0$ in cerebrospinal fluid, based on microelectrode studies, is hypothesised to involve a rapid reduction in $[Ca^{2+}]_0$ of 30% under maximal stimulation, and up to 90% for a traumatic event. The variation of $r_{1p}$ with time might allow tracking of the restoration of the $[Ca^{2+}]_0$ to its equilibrium value by

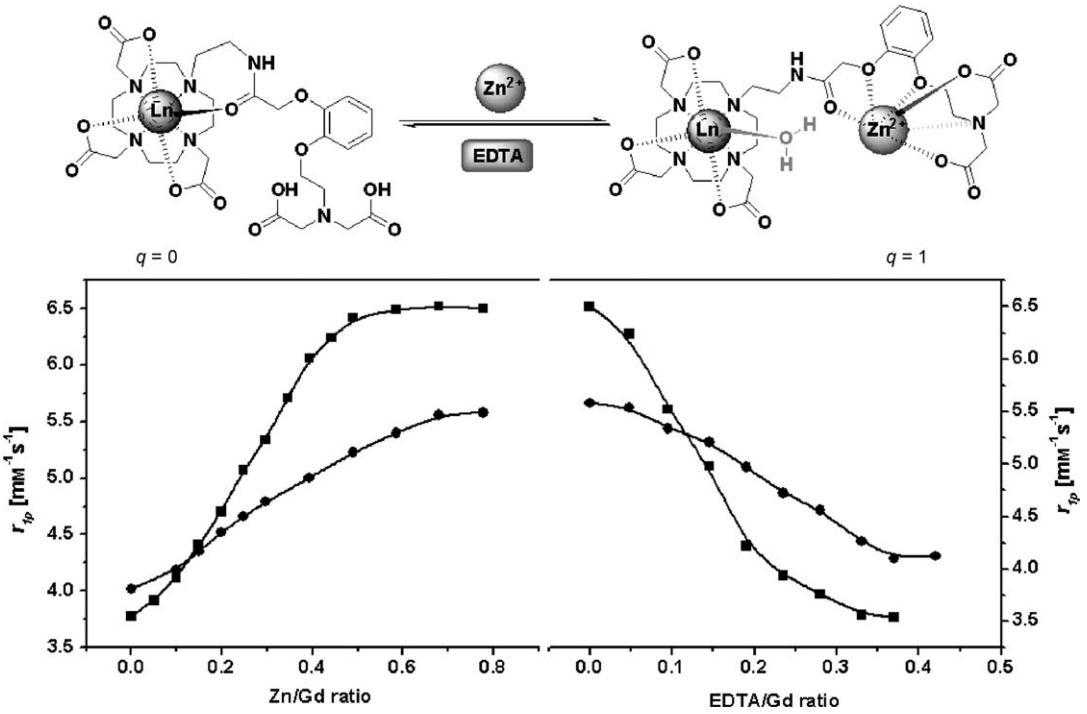



Figure 6. Proton relaxivity changes in aqueous buffer (square) and mouse serum (circle) in the presence of [Gd·L[1]], as a function of added $ZnCl_2$ with subsequent (right) addition of EDTA (0.1 M MOPS, pH 7.4, 310 K, 1.4 T, 0.5 mM complex).

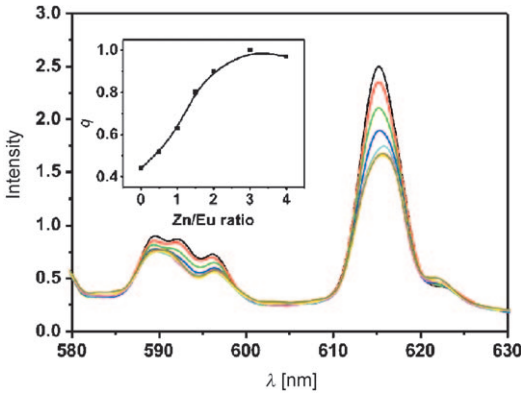



Figure 7. Europium emission spectral changes for [Eu·L[1]] in the presence of added $ZnCl_2$, also showing (inset) the accompanying variation in Eu hydration state, $q$ (3 mM complex, $\lambda_{exc}$ 397 nm, pH 7.4, 0.1 M MOPS).

MRI of the brain. The two cited cases would be characterised by 4 and 22% modulations of $r_{1p}$ (310 K, 1.4 T). These calculations are only valid if the timescale for MRI data acquisition is faster than the time taken for the metal-ion concentration to return to equilibrium. Modern imaging techniques allow the acquisition of a single 2 mm thick volume element within less than 50 msec (typically you take more than 20 slices per second), and it is believed that the relaxation time characterising re-establishment of ionic equilibrium is of the order of several seconds.[1,2] Furthermore, over each period the local concentration of the contrast agent in the volume element examined must be assumed not to change, which is a reasonable assumption for this short timescale. Finally, it is appropriate to note that these relaxivity changes have been measured at 1.4 T and 310 K. At higher fields, the modulation of $r_{1p}$ will differ, although this change is not expected to be very significant at 3 or 7 T. Measurements of relaxivity made at 7 T for [Gd·L[2]] gave values that were only 10% lower than those measured at 1.4 T. Indeed, the NMRD profiles for such low MW contrast agents that show little protein association exhibit a fairly flat-field dependence of relaxivity after this field range.[19]

## Experimental Section

**General**: The chemicals were purchased from commercial suppliers (Acros, Aldrich, Fluka, Merck, Strem and VWR) and were used without further purification unless otherwise stated. Solvents were dried using an appropriate drying agent when required ($CH_3CN$ over $CaH_2$, $CH_3OH$ over $Mg(OMe)_2$ and THF over Na/benzophenone). Unless otherwise mentioned, all the reactions were carried out under a nitrogen atmosphere and the reaction flasks were pre-dried with a heat gun under vacuum. All the chemicals, which were air or water sensitive, were stored under an inert atmosphere. Ultra pure deionised water (18 $M\Omega\,cm^{-1}$) was used throughout. All glassware was washed with a mixed acid solution and thoroughly rinsed with deionised, distilled water.

**Chromatography**: Flash column chromatography was performed by using flash silica gel 60 (70–230 mesh) from Merck. Thin layer chromatography (TLC) was performed on aluminium-backed silica gel plates with 0.2 mm thick silica gel 60 $F_{254}$ (E. Merck) using different mobile phases. The compounds were visualized by UV irradiation (254 nm) or iodine staining.

HPLC was performed at room temperature on a Varian PrepStar Instrument, equipped with PrepStar SD-1 pump heads. UV absorbance was measured using a ProStar 335 photodiode array detector at 254 nm. The detector was equipped with a dual-path length flow cell which enables

measurement of absorption of analytical and semi-preparative samples without changing the flow cell. Reversed-phase (RP) analytical HPLC was performed in a stainless steel Chromsep (length 250 mm, internal diameter 4.6 mm, outside diameter 3/8 inch and particle size 8 μm) $C_{18}$ column and semi-preparative RP-HPLC was performed on a stainless steel Chromsep (length 250 mm, internal diameter 21.2 mm and particle size 5 μm) $C_{18}$ column (Varian, Advanced Chromatographic Solutions). The compounds were purified using the following method: the gradient was used with the mobile phase starting from 90% solvent A (0.1% TFA in water) and 10% of solvent B (0.1% TFA in MeCN) to 70% B in 10 min, 100% B in 14 min, 100% B isocratic till 16 min and decreased to 10% B in 20 min. The flow rate used for analytical HPLC was 1 mL min$^{-1}$ and for preparative HPLC was 15 mL min$^{-1}$. All the solvents for HPLC were filtered through a nylon-66 Millipore filter (0.45 μm) prior to use.

**Spectroscopy**: $^{1}$H and $^{13}$C NMR spectra were recorded on a Bruker 300 MHz spectrometer ($^{1}$H; internal reference $CDCl_3$ at 7.27 ppm or $D_2O$ at 4.75 ppm); 75 MHz spectrometer ($^{13}$C; internal reference $CDCl_3$ at 77.0 ppm). All experiments were performed at 23 °C.

Electrospray mass spectra (ESI-MS) were recorded on SL 1100 system (Agilent, Germany) with ion-trap detection in positive and negative ion mode. HRMS were measured on a Thermo Finnigan LQT.

Emission spectra were measured on an ISA Joblin-Yvon Spex Fluorolog-3 luminescence spectrometer (using DataMax v2.20 software), whereas lifetimes were measured on a Perkin–Elmer LS55 luminescence spectrometer (using FL Winlab software). All samples were contained in quartz cuvettes with a path length of 1 cm and polished base. Measurements were obtained relative to a reference of pure solvent contained in a matched cell. Luminescent titrations were carried out by normalising the emission spectra with the absorption spectra in each point, to account for the decrease in the sample concentration caused by addition of cation/anion stock solution, where appropriate. All measurements were carried in 0.1 M MOPS buffer at pH 7.4. To avoid the undesirable evolution of carbon dioxide in buffer, argon was bubbled through the solution for 30 min before proceeding to titrations.

**Lifetime measurements**: Lifetimes of europium complexes were measured by direct excitation of $Eu^{III}$ ion using a short pulse of light (397 nm) followed by monitoring the integrated intensity of light (for europium 615 nm) emitted during a fixed gate time ($t_g$) after a delay time ($t_d$). At least 20 delay times were used covering 3 or more lifetimes. A gate time of 0.1 ms was used, and the excitation and emission slits were set to 10 and 2.5 nm band-pass respectively. The obtained exponential decay curves were fitted to the equation below, using Origin 6.0 software (Data Analysis & Technical Graphics):

$$I = I_0 + A_1 e^{(-kt)} \quad (1)$$

in which: $I$ = intensity at time $t$ after the flash, $I_0$ = intensity after the decay has finished, $A_1$ = pre-exponential factor and $k$ = rate constant for decay of the excited state. The excited state lifetime ($\tau$) is the inverse of the radiative rate constant ($k$).

To examine the influence of some biologically common cations/anions on Eu complexes, luminescent titrations were carried out by using different concentrations of $Ca^{2+}$, $Zn^{2+}$ and $NaHCO_3$ in 0.1 M MOPS buffer at pH 7.4. All of these measurements were carried out by adding the selected cations/anions as liquid concentrated stock solutions and the addition at each point was approx. 0.05–0.5% in volume of the original solution observed, to avoid significant increase in sample volume. HSA was added as a solid. Each Eu emission spectrum was corrected for dilution.

**Inner-sphere hydration number ($q'$) determination**: Inner-sphere hydration numbers ($q'$) were determined after excitation at the $^{5}L_6 \leftarrow {}^{7}F_0$ band (397 nm) and emission at 615 nm. 3 mM solutions of the Eu-complexes were prepared in 0.1 M MOPS buffer at pH 7.4 in $H_2O$ and in $D_2O$. Hydration numbers ($q'$) were calculated according to Equation (2):

$$q'\mathrm{Eu} = 1.2[(k_{H_2O} - k_{D_2O} - 0.25) - 0.075n] \quad (2)$$

In the case of coordinated amide NH oscillators present close to the lanthanide ion, another correction is made to obtain $q'$ [Eq. (2)], in which $n$ refers to the number of coordinated amide NH oscillators.

**Relaxivity measurements**: Relaxivity measurements were carried out at 37 °C, 60 MHz (1.4 T) on a Bruker Minispec mq60 instrument and at 23 °C, 300 MHz on a vertical 7 T/60 cm MRI Biospec system (Bruker Biospin). The mean value of three separate measurements was recorded and averaged. The relaxivities of the compounds were calculated as the slope of the function shown in Equation (3):

$$1/T_{1,obs} = 1/T_{1,d} + r_1 \times [\mathrm{GdL}_n] \quad (3)$$

in which $T_{1,obs}$ is the measured $T_1$, $T_{1,d}$ is the diamagnetic contribution of the solvent (calculated to be 4000 ms) and $[\mathrm{GdL}_n]$ is the concentration in mM of the appropriate $Gd^{III}$ complex ($n$ = 1 and 2). The errors for all relaxivity values were less than (0.6) mM$^{-1}$ s$^{-1}$.

Inductively coupled plasma optical emission spectrometry (ICP-OES) for [Gd and Eu] analyses was performed using a Jobin–Yvon Ultima 2 spectrometer.

The apparent binding constant of the selected cation was calculated using Equation (4)[20]:

$$[\mathrm{X}] = \frac{\frac{(R-R_0)}{(R_1-R_0)}}{K} + [\mathrm{GdL}] \times \frac{(R-R_0)}{(R_1-R_0)} - [\mathrm{GdL}] \times \left(\frac{(R-R_0)}{(R_1-R_0)}\right)^2}{1-\frac{(R-R_0)}{(R_1-R_0)}} \quad (4)$$

$$K = \frac{[\mathrm{GdX}]}{[\mathrm{X_f}][\mathrm{Gd_f}]} \quad (5)$$

in which [X] is the total concentration of cation in the solution; [GdL] the total concentration of the complex; $K$ the binding constant; $R$ the relaxation rate of a given concentration of X; $R_0$ the initial relaxation rate; $R_1$ the final relaxation rate; [GdX]: the concentration of the cation-coordinated complex; $[X_f]$ the concentration of free cation in the mixture; $[Gd_f]$ the concentration of the free complex.

**Synthesis of compound 10**: A solution of 2-(2-(2-(bis(2-*tert*-butoxy-2-oxoethyl)amino)ethoxy)phenoxy)acetic acid (0.6 g, 1.37 mmol), compound **9** (0.69 g, 1.23 mmol), NMM (0.38 mL, 2.73 mmol) and HOBt (0.2 g, 1.5 mmol) in anhydrous DMF (5 mL) was stirred at 0–5 °C for 15 min and then *N*′-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide (EDC) (0.29 g, 1.5 mmol) was added. The reaction mixture was stirred for 12 h at room temperature. The completion of the reaction was verified by ESI-MS then the solution was poured into water (40 mL) and extracted with EtOAc (3×50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, 10% MeOH in $CH_2Cl_2$, $R_f$ = 0.15) to give 10 as a dark yellow gum (0.34 g, 28%). $^{1}$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.46 (s, 18H; $CH_3$), 1.47 (s, 27H; $CH_3$), 2.05–3.00 (br m, 16H; $NCH_2$, $CH_2$ of ring), 3.03–3.40 (br m, 8H; $NCH_2CO$ and $CH_2$ of ring), 3.44–3.73 (br m, 6H, $NCH_2CO$), 3.81 (s, 2H; $CH_2NH$), 4.15–4.21 (m, 2H; $CH_2O$), 4.69 (s, 2H; $OCH_2CONH$), 6.83–7.01 (m, 4H; ArH), 7.51 ppm (br s, 1H; NH); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 27.8, 27.9, 28.1, 37.9, 52.0, 53.3, 53.5, 55.6, 55.7, 55.8, 56.9, 58.0, 58.2, 67.0, 68.3, 80.9, 81.1, 81.8, 114.1, 115.9, 121.0, 122.8, 147.7, 149.2, 169.3, 170.7, 172.7, 172.9 ppm; HRMS (ESI$^+$): $m/z$: calcd for $C_{50}H_{87}N_6O_{13}$: 979.6331 $[M+H]^+$; found 979.6307.

**Synthesis of $L^1$**: [4,7-Bis-butoxycarbonylmethyl-10-(2-(2-(2-(2-(bis(2-*tert*-butoxy-2-oxoethyl)amino)ethoxy)phenoxy)acetamido)ethyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (0.3 g, 0.3 mmol) was dissolved in neat TFA (10 mL) and stirred overnight. The completion of the reaction was verified by ESI-MS and the solvent was evaporated under reduced pressure. The residue was then purified by preparative HPLC ($t_R$ = 4.8 min). After lyophilization, ligand $L^1$ was obtained as a light yellow sticky solid (0.011 g, 51%). $^{1}$H NMR (300 MHz, $D_2O$): $\delta$ = 2.95–3.29 (br m, 12H; $NCH_2$ and $CH_2$ of ring), 3.30–3.8 (br m, 12H; $NCH_2CO$ and $CH_2$ of ring), 3.81–4.10 (m, 4H; $CH_2N$ and $CH_2NH$), 4.24–4.33 (m, 2H; $NCH_2CO$), 4.37 (s, 2H; $NCH_2CO$), 4.40–4.51 (m, 2H; $CH_2O$), 4.80 (s, 2H; $OCH_2CONH$), 6.94–7.11 ppm (m, 4H; ArH); $^{13}$C NMR (75 MHz, $D_2O$): $\delta$ = 34.6, 49.0, 49.7, 52.1, 53.6, 54.3, 55.9, 56.4,

56.6, 63.8, 65.4, 113.8, 114.4, 118.2, 122.7, 146.5, 146.9, 166.0, 168.5, 171.6, 173.0 ppm; MS (ESI$^+$): $m/z$: calcd for $C_{30}H_{47}N_6O_{13}$: 699.3 $[M+H]^+$; found 699.4.

**Synthesis of compound 18**: A solution of 2-((2-(2-(2-(bis(2-*tert*-butoxy-2-oxoethyl)amino)ethoxy)phenoxy)ethyl)(2-*tert*-butoxy-2-oxoethyl)amino)-acetic acid (0.29 g, 0.48 mmol), compound **9** (0.24 g, 0.43 mmol), NMM (0.1 g, 0.96 mmol) and HOBt (0.07 g, 0.53 mmol) in anhydrous DMF (5 mL) was stirred at 0–5 °C for 15 min and then EDCI (0.1 g, 0.53 mmol) was added. The reaction mixture was stirred overnight at room temperature. After ensuring completion of the reaction, the reaction mixture was poured into water (40 mL) and extracted with EtOAc (3×50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$ and filtered. The filtrate was evaporated under reduced pressure and the residue purified by column chromatography (silica gel, 10% MeOH in $CH_2Cl_2$, $R_f$= 0.15) to give **18** as a pale yellow gum (0.16 g, 33%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=1.36 (s, 9H; $CH_3$), 1.37 (s, 18H; $CH_3$), 1.40 (s, 18H; $CH_3$), 1.41 (s, 9H; $CH_3$), 2.49–2.74 (m, 16H; $NCH_2$ and $CH_2$ of ring), 2.95–3.24 (m, 18H; $CH_2$ of ring, $NCH_2CO$ and $CH_2NH$), 3.40–3.60 (m, 4H; $NCH_2CO$), 3.83–4.14 (m, 4H; $CH_2O$), 6.68–6.89 ppm (m, 4H; ArH); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$=27.4, 27.6, 27.7; 53.1, 54.2, 54.5, 55.3, 55.4, 56.1, 56.4, 56.7, 57.1, 58.7, 59.2, 61.8, 67.0, 67.6, 80.6, 82.0, 82.3, 82.4, 114.4, 120.9, 148.5, 148.7, 170.2, 171.2, 172.0, 172.2, 173 ppm; HRMS (ESI$^+$): $m/z$: calcd for $C_{58}H_{102}N_7O_{15}$: 1136.7428 $[M+H]^+$; found 1136.7420.

**Synthesis of L$^2$**: [4,7-Bis-butoxycarbonylmethyl-10-(2-(2-((2-(2-(2-(bis(2-*tert*-butoxy-2-oxoethyl)amino)ethoxy)phenoxy)ethyl)(2-*tert*-butoxy-2-oxoethyl)amino)acetamido)ethyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (0.90 g, 1.3 mmol) was dissolved in neat TFA (10 mL) and stirred at room temperature for 18 h. The completion of the reaction was confirmed by ESI-MS. The solvent was evaporated under reduced pressure and the residue was purified by preparative HPLC ($t_R$=4.2 min). After lyophilization, ligand L$^2$ was obtained as a light yellow sticky solid (0.062 g, 55%).$^1$H NMR (300 MHz, $D_2O$): $\delta$=2.90–3.18 (m, 14H; $NCH_2$ and $CH_2$ of ring), 3.19–3.45 (m, 8H; $NCH_2CO$), 3.46–3.75 (m, 5H; $CH_2NH$, $CH_2N$, $NCH_2CO$), 3.76–3.97 (m, 5H; $CH_2N$, $NCH_2CO$), 4.27 (br s, 4H; $NCH_2CO$), 4.30 (s, 2H, $NCH_2CONH$), 4.30–4.45 (br m, 4H; $CH_2O$), 6.91–7.04 (br m, 4H; ArH); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$=42.0, 51.9, 52.5, 54.9, 55.8, 56.5, 57.1, 58.2, 58.3, 58.7, 58.9, 59.3, 59.6, 65.5, 65.8, 121.1, 125.2, 149.4, 149.5, 168.6, 171.2, 171.3, 171.5; MS (ESI$^+$): $m/z$: calcd for $C_{34}H_{54}N_7O_{15}$: 800.3 $[M+H]^+$; found 800.4.

**General preparation of lanthanide complexes of L$^1$ and L$^2$**: Lanthanide complexes of L$^1$ and L$^2$ were prepared from the corresponding solutions of the ligands (1 equiv) and solutions of $GdCl_3{\cdot}6H_2O$/$EuCl_3{\cdot}6H_2O$ (1.0 equiv). The reaction mixture was stirred at 60 °C for 20 h. The pH was periodically checked and adjusted to 6.5 with solutions of NaOH (1 M) and HCl (1 N) as needed. After completion, the reaction mixture was cooled and passed through chelex-100 to trap free $Ln^{3+}$ ions, and the Ln-loaded complexes were eluted. The fractions were dialyzed (500 MW cutoff; Spectra/Pro biotech cellulose ester dialysis membrane, Spectrum Laboratories) and lyophilized to obtain off-white solids. The absence of free $Ln^{3+}$ was checked with xylenol orange indicator. These complexes were characterized by ESI-MS in positive and negative mode and the appropriate isotope pattern distribution for $Gd^{3+}$ and $Eu^{3+}$ were recorded.

*[Gd.L$^1$]*: MS (ESI$^-$): $m/z$: calcd for $C_{30}H_{42}{}^{155}GdN_6O_{13}$: 852.2 $[M-H]^-$; found 852.1, $r_{1p}$=3.78 $mM^{-1}s^{-1}$ at 60 MHz and 3.35 $mM^{-1}s^{-1}$ at 300 MHz; $r_{2p}$=6.79 $mM^{-1}s^{-1}$ at 300 MHz.

*[Eu.L$^1$]*: MS (ESI$^-$): $m/z$: calcd for $C_{30}H_{42}{}^{153}EuN_6O_{13}$: 845.2 $[M-H]^-$; found 852.2, $\tau_{(H_2O)}$ 0.68 ms; $\tau_{(D_2O)}$ 1.25 ms and $q$=0.4.

*[Gd.L$^2$]*: MS (ESI$^+$): $m/z$: calcd $C_{30}H_{51}{}^{155}GdN_7O_{15}$: 955.2 $[M+H]^+$; found 955.2, $r_{1p}$=3.27 $mM^{-1}s^{-1}$ at 60 MHz and 2.85 $mM^{-1}s^{-1}$ at 300 MHz; $r_{2p}$= 4.56 $mM^{-1}s^{-1}$ at 300 MHz.

*[Eu.L$^2$]*: MS (ESI$^-$): $m/z$: calcd for $C_{34}H_{48}{}^{151}EuN_7NaO_{15}$: 968.2 $[M-H]^-$; found 968.1, $\tau_{(H_2O)}$ 0.69 ms; $\tau_{(D_2O)}$ 1.15 ms and $q$=0.35.

## Acknowledgements

We thank ESF COST Action D38, the Max Planck Society and the Hertie Foundation for support and the EC for a Marie Curie Intra-European Fellowship (PIEF-GA-2009-237253).

[1] G. Breitwieser, *Int. J. Biochem. Cell Biol.* **2008**, *40*, 1467; S. Hrabtova, D. Masri, L. Tao, F. Xiao, C. Nicholson, *J. Physiol.* **2009**, *587*, 4029.
[2] S. Zanotti, C. Andrew, *J. Neurochem.* **1997**, *69*, 594.
[3] a) J. L. Major, T. J. Meade, *Acc. Chem. Res.* **2009**, *42*, 893; b) W-H. Li, S. E. Fraser, T. J. Meade, *J. Am. Chem. Soc.* **1999**, *121*, 1413; c) E. L. Que, C. J. Chang, *Chem. Soc. Rev.* **2010**, *39*, 51.
[4] a) K. Dhingra, M. E. Maier, M. Beyerlin, G. Angelovski, N. K. Logothetis, *Chem. Commun.* **2008**, 3444; b) K. Dhingra, P. Fousková, G. Angelovski, M. E. Maier, N. K. Logothetis and E. Toth, *J. Biol. Inorg. Chem.* **2007**, *13*, 35; c) C. S. Bonnet, E. Toth, *Future Med. Chem.* **2010**, *2*, 367384.
[5] M. Andrews, A. J. Amoroso, C. P. Harding, S. J. A. Pope, *Dalton Trans.* **2010**, *39*, 3407; E. L. Que, E. Giandio, S. L. Baker, S. Aime, C. J. Chang, *Dalton Trans.* **2010**, *39*, 469; Y. You, E. Tomat, K. Hwang, T. Atanasijevic, W. Nam, A. P. Jasanoff, S. J. Lippard, *Chem. Commun.* **2010**, *46*, 4139.
[6] J. L. Major, R. M. Boiteau, T. J. Meade, *Inorg. Chem.* **2008**, *47*, 10788; J. L. Major, G. Porigi, C. Luchinat, T. J. Meade, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 13881.
[7] M. P. Lowe, D. Parker, *Chem. Commun.* **2000**, 707; M. P. Lowe, D. Parker, O. Reany, S. Aime, M. Botta, G. Castellano, E. Gianolio, *J. Am. Chem. Soc.* **2001**, *123*, 7601.
[8] a) O. Reany, T. Gunnlaugsson, D. Parker, *J. Chem. Soc. Perkin Trans. 2* **2000**, 1819; b) G. Angelovski, P. Fouskova, I. Mamedov, S. Canals, E. Toth, N. K. Logothetis, *Biochem. Chem.* **2008**, *9*, 1729; c) G. C. R. Ellis-Davies, R. J. Barsotti, *Cell Calcium* **2006**, *39*, 75; d) L. Tei, Z. Baranyai, M. Botta, L. Piscopo, S. Aime, G. B. Giovenzana, *Org. Biomol. Chem.* **2008**, *6*, 2361.
[9] R. S. Dickins, J. I. Bruce, D. Parker, D. J. Tozer, *Dalton Trans.* **2003**, 1264; D. Parker, *Chem. Soc. Rev.* **2004**, *33*, 156.
[10] R. Pal, D. Parker, *Org. Biomol. Chem.* **2008**, *6*, 1020; R. Pal, D. Parker, L. C. Costello, *Org. Biomol. Chem.* **2009**, *7*, 1525.
[11] A. Congreve, D. Parker, E. Gianolio, M. Botta, *Dalton Trans.* **2004**, 1441.
[12] S. Aime, G. Cravotto, S. G. G. Crich, G. B. Ferrari, G. Palmisano, M. Sisti, *Tetrahedron Lett.* **2002**, *43*, 783.
[13] A. Mishra, P. Fouskova, G. Angelovski, E. Balogh, A. K. Mishra, N. K. Logothetis, E. Toth, *Inorg. Chem.* **2008**, *47*, 1370.
[14] a) M. Botta, *Eur. J. Inorg. Chem.* **2000**, 399; b) S. Aime, M. Botta, D. Parker and J. A. G. Williams, *J. Chem. Soc. Dalton Trans.* **1996**, 17.
[15] A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams, M. Woods, *J. Chem. Soc. Perkin Trans. 2* **1999**, 493.
[16] R. S. Dickins, J. A. K. Howard, D. Parker, A. S. Batsanov, M. Botta, J. I. Bruce, C. S. Love, R. D. Peacock, H. Puschmann, *J. Am. Chem. Soc.* **2002**, *124*, 12697.
[17] J. I. Bruce, R. S. Dickins, T. Gunnlaugsson, S. Lopinski, M. P. Lowe, D. Parker, R. D. Peacock, J. J. B. Perry, S. Aime, M. Botta, *J. Am. Chem. Soc.* **2000**, *122*, 9674.
[18] Y. Brettonière, D. Parker, R. Slater, M. J. Cann, *Chem. Commun.* **2002**, 1930; Y. Brettonière, M. J. Cann, D. Parker, R. Slater, *Org. Biomol. Chem.* **2004**, *2*, 1624.
[19] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293.
[20] R. Pal, D. Parker, *Chem. Commun.* **2007**, 474.

Received: June 2, 2010
Published online: January 5, 2011