Dalton Transactions

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ROYAL SOCIETY OF CHEMISTRY

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DOI: 10.1039/C4DT02971B

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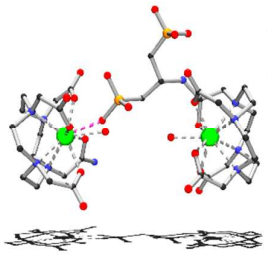
The self-assembling of two macrocyclic Gd-chelates results in a small-size dimeric system characterized by a remarkably high relaxivity, r_1.



YL^1-GdL^2 - r_1 = 12.4 mM^{-1} s^{-1}

YL^2-GdL^1 - r_1 = 12.3 mM^{-1} s^{-1}

GdL^1-GdL^2 - r_1 = 17.6 mM^{-1} s^{-1}

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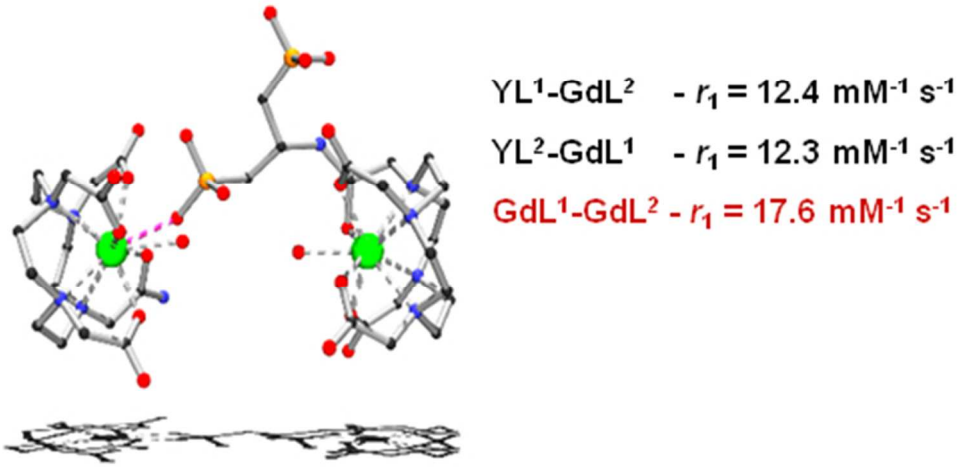

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Optimizing the high-field relaxivity by self-assembling of macrocyclic Gd(III) complexes

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Using recognition moieties that bind to the inner co-ordination sphere of a monomeric DO3A-type di-aqua complex, dimeric poly(aminocarboxylate) gadolinium(III) compounds can be formed with greatly enhanced relaxivities, arising from optimized contributions of inner- and second spheres of hydration.

Introduction

A set of important properties have contributed to the great success of magnetic resonance imaging (MRI) allowing the technique to establish itself as an outstanding diagnostic modality: absence of ionizing radiation during the acquisition of images; non-invasiveness and high patient acceptability; unlimited tissue penetration; impressive delineation of soft tissues resulting from a high degree of intrinsic contrast; excellent temporal and spatial resolution.[1] However, it is well known that the great impact of MRI in diagnostic imaging has been possible also thanks to the development of contrast agents (CA), which are used in more than one-third of the approximately 50 million examinations performed every year (43% in USA in 2013). Most of the currently available MRI CAs have been developed during the 1990's and are low-molecular weight, anionic and non-selective gadolinium(III) complexes that function by accelerating the rate of nuclear magnetic relaxation ($R_1 = 1/T_1$) of the protons of nearby water molecules in the tissues in which they are distributed.[2] A small concentration of these complexes (mM) is able to induce a significant increase of R_1 of water protons in tissues (approx. 90 M) thanks to the fast chemical exchange process of the water molecule between the inner coordination sphere of the Gd(III) and the bulk. The underlying mechanism is the electron-nuclear dipolar interaction between the electron magnetic moment of Gd(III) and the nuclear magnetic moment of the proton nuclei. The time fluctuation of this interaction arises from molecular rotation (τ_R), electron relaxation ($T_{1,2e}$) and chemical exchange (τ_M) and is responsible for the solvent relaxation enhancement. This latter property is expressed in terms of the parameter relaxivity (r_{1p}) which indicates the increase of solvent R_1 induced by a 1 mM concentration of the CA.[2,3]

The most important class of CA is represented by small chelates of Gd(III) with poly-aminocarboxylates ligands. These are intravenously injected (0.1–0.3 mmol kg^{-1} body weight) and are considered as extracellular fluid (ECF) agents, as they rapidly distribute between the intravascular and interstitial space and then are excreted primarily through the kidneys.[1] These commercially available, Gd-based complexes share a series of physical-chemical properties and molecular parameters: the presence of a single coordinated water molecule ($q = 1$); fast molecular reorientation (τ_R of ca. 60-90 ps); relatively long water residence lifetime ($\tau_M > 100$ ns) but not such as to affect the relaxivity at physiological temperature; overall negative or neutral electric charge, r_{1p} values of about 3-4 mM^{-1} s^{-1} (0.47 T, 37°C).[2,3] This low relaxivity is indicative of a limited contrast enhancement capability, much lower than that theoretically attainable. Over the past two decades, many studies have been directed to the in-depth understanding of the relationship between structure and dynamics in solution and the relaxivity of the Gd(III) complexes. This thorough understanding has contributed to the rational design and development of new probes, made more efficient through the optimization of several molecular parameters.[4] In particular, the relaxometric studies have clearly shown that the rotational dynamics represents the main limiting factor of r_{1p} for the low molecular weights Gd(III) chelates. Accordingly, there has been a particular focus on possible approaches to slow the rotation of the paramagnetic chelates, mainly through conjugation to large substrates. The reversible formation of adducts with human serum albumin (HSA) was one of the main routes to achieve a high relaxation enhancement because of the restricted tumbling rate ($1/\tau_R$) of the macromolecular adduct. More recently, better and more sophisticated systems have been developed in which Gd(III) chelates are linked to various macromolecular substrates or incorporated into nanoparticles.[5] Among these, we highlight the role of proteins, polymers, dendrimers, micelles, liposomes and viral capsids. These MRI nanoprobes allow the accumulation at the site of interest of a large

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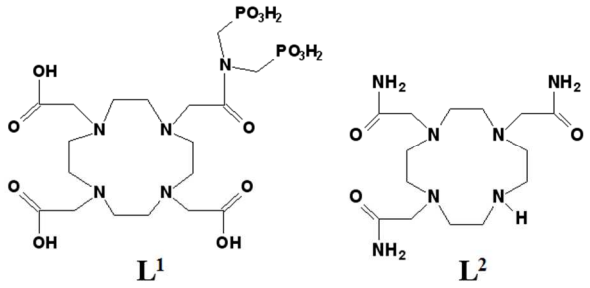
number of paramagnetic ions, thereby increasing the sensitivity of the technique. However, all these developments have allowed us to proceed towards the optimization of r_{1p} for magnetic field strengths around 0.5 - 1.0 T. Indeed, the appearance of a broad relaxivity peak with increasing τ_R values (> 200 ps) characterises such behaviour; the peak gradually becomes narrower and higher, while the maximum shifts to lower fields as molecular tumbling slows down.

Nowadays, the trend in MRI development is towards higher magnetic field strengths with the majority of MRI scanners used in clinics operating at 1.5 T. A large number of instruments operating at 3 T are already used in clinics and these comprise more than one-quarter of planned scanner purchases; 7 T instruments are widespread and increasingly utilized in clinical and pre-clinical studies. Higher fields, up to 12 T, are used in animal studies. The increasing availability of high-field systems requires a different strategy for relaxivity enhancement of metal-based probes. In fact, for the small Gd-chelates and their macromolecular derivatives the relaxivity decreases with increasing magnetic field strength as well as their effectiveness as an MRI CA. Two recent reviews have illustrated effectively and thoroughly these aspects.[6] We recall here only the most relevant conclusions: a valuable approach is based on the use of systems with q = 2, relatively fast exchange rate (τ_M ~ 10-100 ns) and rotational correlation times in the range of approximately 0.5-2 ns. Furthermore, a large contribution from water molecules in the second sphere of hydration should provide an additional major advantage at high fields. In accordance with this approach, some interesting strategies have been devised with the aim of enhancing r_{1p} at higher field strengths (≥ 1.5 T). Parker *et al*. developed monoaqua-GdDOTA derivatives placed at the centre of a hydrophilic dendritic structure. In this structural arrangement, the paramagnetic ion lies at the barycentre of the molecular complex so that the effects of rapid local rotation of the pendant chains are minimized and the motional coupling improved. In addition to a remarkable inner sphere contribution, the presence of a network of second sphere water molecules results in increased r_{1p} values at high field (ca. 22 and 18 mM^{-1} s^{-1} at 3 and 7 T, respectively).[7] Raymond has combined the advantages of a stable q = 2 complex, fast water exchange and nearly optimal rotational motion in a dendrimeric GdHOPO-derivative whose relaxivity is virtually constant in the range 1.5 - 3 T (r_{1p} = 17-19 mM^{-1} s^{-1}).[8] More recently, Tóth has described two interesting examples based on intermediate size multimeric systems formed by the assembly of three or six monomers (q = 2).[9,10]

Here, new results are presented on some first generation CAs, with the aim of demonstrating that a considerable relaxivity enhancement at high fields is possible based on the acquisition of new information on old systems. We report on an example of self-recognition between two macrocyclic Gd^{III} complexes in aqueous solution. This proof-of-concept study makes use of simple systems employed as a model for assessing the effect on the relaxometric properties of the individual species of the reversible formation of a dimeric adduct. In particular, we show that the self-assembling of two macrocyclic Gd-chelates results in a small-size dimeric system endowed with high relaxivity at high frequencies thanks to improved inner- and second-sphere relaxivity contributions. The binding interaction involves a mono-amide derivative of DOTA (**L**^{1}, q=1) bearing two pendant phosphate groups with a tris-amide derivative of DO3A (**L**^{2}, q=2) (Scheme 1).

Scheme 1. Chemical structures of the ligands.



Results and discussion

Gd**L**^{1} is a DOTA monoamide derivative bearing one inner sphere water molecule, in an axial position, and two pendant methylenphosphonic groups.[11] [Gd**L**^{2}]$^{3+}$ is a tris-amide DO3A derivative in which the metal ion features two coordinated water molecules that can be displaced by a number of oxy-anions. The aim of this work is to investigate the formation and the relaxometric properties of the dimer obtained through the displacement of a water molecule from the first coordination sphere of Gd**L**^{2} by a phosphonate group of Gd**L**^{1}. First to be illustrated will be the individual properties of the single complexes involved and afterwards their interaction will be discussed.

Characterisation of GdL1. In this complex, the central Gd^{III} ion is eight co-ordinated leaving space for one axial water molecule in the inner co-ordination sphere, which is in exchange with the bulk (Figure 1).

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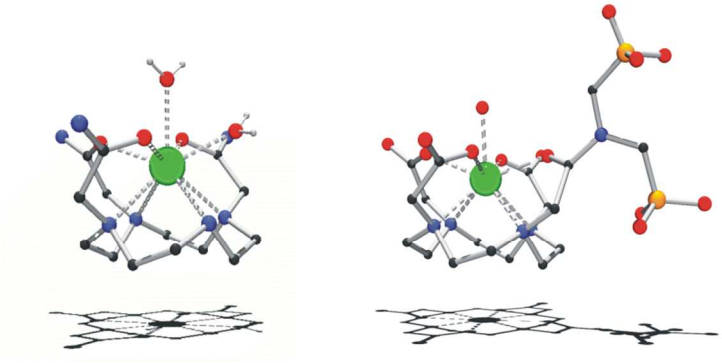
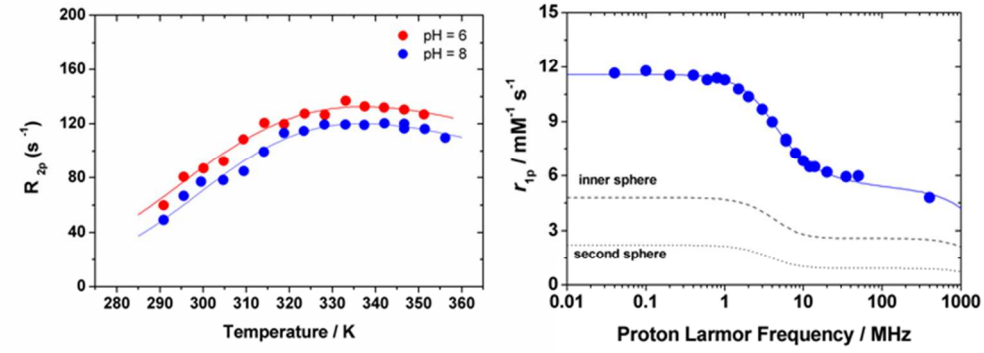
Figure 1. Ln(III) complexes of L^1 (right) and L^2 (left).



The relaxivity of this complex at 20 MHz and 25°C is 6.2 mM^{-1} s^{-1}, which is significantly higher than that of 4.7 mM^{-1} s^{-1} found for GdDOTA. The NMRD profile explains why: apart from the contribution of the slightly longer τ_R which increases the inner-sphere relaxivity, there is also a contribution from water molecules hydrogen bonded to the phosphonate groups which are close enough to the paramagnetic centre to be relaxed via the dipolar mechanism. Such an effect has been evaluated to be equivalent to the contribution of a single water molecule at a distance of 3.7 Å from the paramagnetic centre.[11] This estimated second sphere contribution is represented by the lower curve in the NMRD profile (Figure 2, right). The outer sphere contribution, not shown in the Figure, has been evaluated in terms of the well-established model of Freed (see ESI).[1-3] The axial water molecule has a rather long residence lifetime: about 1.9 μs at 25°C and pH 8. This has been assessed by measuring the ^{17}O transverse relaxation rate, R_{2p} ($=1/T_{2p}$), of a solution the complex as a function of temperature (Figure 2, left). The data represent new measurements and analysis that update the results previously reported.[11] Not only is this value almost an order of magnitude greater than that measured for GdDOTA but is considerably longer compared to the values found for other DOTA monoamide complexes.[2,3] Interestingly τ_M decreases by more than 30% at pH 6. A lowering of the pH does not cause a structural change or a modification of the coordination cage, as can be deduced from the invariance of the NMRD profile with the pH. However a partial protonation of the of the phosphonate groups does occur. We think that this behaviour can be explained in terms of a hydrogen bonding interaction between the inner-sphere water molecule and the phosphonate group(s), which makes the dissociation of the inner-sphere water energetically more difficult. The protonation step that occurs by lowering pH results in an increase in k_{ex} ($= 1/\tau_M$) by removing the interaction between the coordinated water and the phosphonic group(s).

Figure 2. Temperature dependence of the ^{17}O NMR (2.1 T) transverse relaxation rate of a 10 mM aqueous solution of GdL^1 (left) and NMRD profile at 25°C and pH=6.5 (right).



Characterisation of $[GdL^2]^{3+}$. This is a cationic complex, a triamide derivative of GdDO3A. These latter complexes have been shown to represent suitable systems for studying the binding interaction with anionic groups.[12] They show a well-defined structural geometry; they are sufficiently stable thermodynamically and kinetically in aqueous media; they possess two water molecules bound directly to the metal centre. Finally, the stepwise displacement of the water molecules by coordinating anionic groups is accompanied by a change in the relaxivity of the chelates.[13] These changes can be quantitatively analysed and provide useful information on the binding affinity and the structural features of the ternary complexes. Figure 1 shows the structure of the complex, outlining the square antiprismatic (SAP) geometry with one axial, and one equatorial bound water molecule. The two waters may well possess two different k_{ex} values and can each be displaced.[13]

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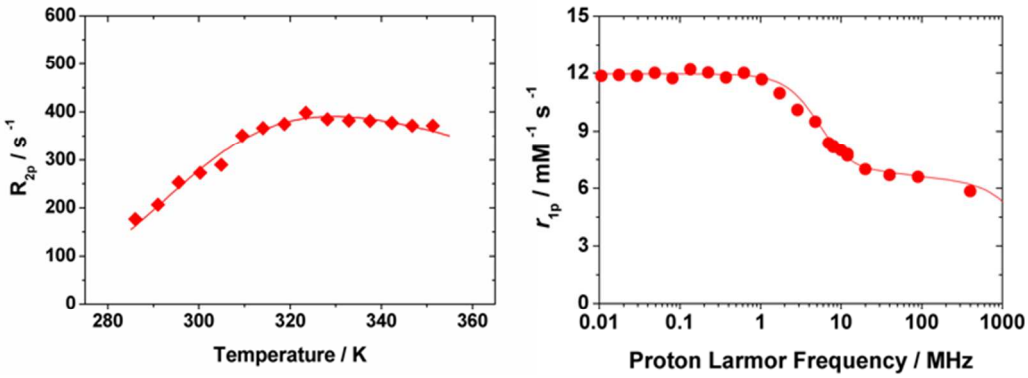
The r_{1p} value varies greatly with pH. There is a flat region at pH values below 6.8 where the relaxivity is about 7.0 mM^{-1} s^{-1}. This is quite typical of a complex with two bound water molecules (q = 2). With increasing pH, the relaxivity drops dramatically, to give a value of about 3 mM^{-1} s^{-1} (Figure S1, ESI). This value is more characteristic of q = 0 compounds where the relaxivity is derived only from the outer-sphere contribution. This behaviour is well understood: we are observing a change in the hydration number from q = 2 to 0 during this change in pH that is a consequence of the formation of a ternary complex with the carbonate present in the basic aqueous solution.[14] The rate of water exchange of the complex has been determined, at pH=6.5, by measuring the temperature dependence of the transverse relaxation rate ($R_2 = 1/T_2$) of the solvent using ^{17}O NMR at 2.1 T (Fig. 3, left). The k_{ex} value is rather long as expected of a cationic system and in full agreement with the values reported for related systems.[13]

Figure 3. Temperature dependence of the ^{17}O NMR (2.1 T) transverse relaxation rate of a 20 mM aqueous solution of Gd**L**^{2} (left) and NMRD profile at 25°C and pH=6.5 (right).



The NMRD profile (Figure 3, right) does not add a great deal of further information. By fixing the τ_M value obtained from the ^{17}O measurements, the data can be nicely fitted (Table 1). A reasonable τ_R value, of 80 ps, and typical values of the electronic relaxation parameters were calculated.

Table 1. Selected best-fit parameters obtained from the analysis of the ^1H NMRD profiles[a] and ^{17}O NMR data (2.1 T) for the Gd(III) complexes.

Parameter	Gd**L**^{1}	Gd**L**^{2}	Gd**L**^{1}-Y**L**^{2}	Gd**L**^{2}-Y**L**^{1}	Gd**L**^{1}-Gd**L**^{2}
r_1 (mM^{-1} s^{-1})[b]	5.7	6.7	11.9	14.8	17.2
Δ^2 (10^{19} s^{-2})	1.8±0.2	3.8±0.4	1.8±0.3	6.6±0.5	2.0±0.3
τ_V^{298} (ps)	22±3	18±2	36±3	23±3	50±4
τ_R^{298} (ps)	85±3	80±2	195±3	215±3	210[c]
τ_M^{298} (μs)	1.4±0.2	1.5±0.3	0.81±0.04	0.25±0.02	0.45[c]
$\Delta H^{\#}_M$ (kJ mol^{-1})	33.4±0.4	44.1±0.6	23.3±0.8	43.1±1.2	/
E_V (kJ mol^{-1})[c]	1	1	1	1	
$A_O/\hbar$ (10^6 rad s^{-1})	-3.8±0.1	-3.7±0.2	-3.3±±0.2	-3.6±0.4	/
q[c]	1	2	1	1	1
r (Å)	3.0[c]	3.0[c]	3.0[c]	3.0[c]	3.0[c]
q'	1[c]	/	2[c]	2[c]	4.0±0.8
r' (Å)	3.7±0.1	/	3.6±0.2	3.5±0.1	3.5±0.2

[a] The outer-sphere component of the relaxivity was estimated by using standard values for the distance of closest approach a (4.0 Å) and the relative diffusion coefficient of solute and solvent D (2.24 cm^2 s^{-1}); [b] per Gd, at 60 MHz and 298 K; [c] fixed during the fitting procedure.

It is well established that CO_3^{2-} is not the only species that can form ternary complexes with triamide DOTA derivatives. Similar ternary complexes can also be formed with a variety of oxyanions.[12,13] Some of these behave as bidentate ligands and displace both water molecules (lactate, citrate); others like acetate, fluoride and phosphate bind in a monodentate manner and so displace a single water molecule. In particular, in a previous work we have shown that in the case of the ternary phosphate complex, in spite

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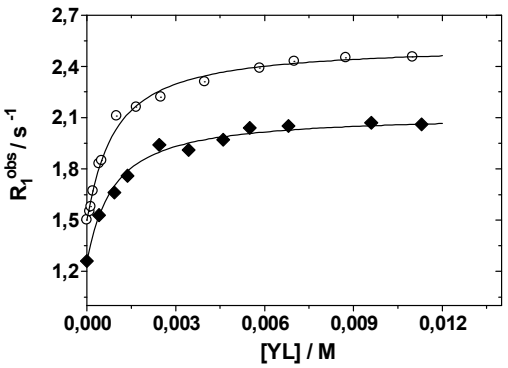
of the loss of a water molecule, the decrease in the relaxivity is almost negligible. This was attributed to the presence of a network of hydrogen-bonded water molecules promoted by the phosphate anion that gives a strong relaxation enhancement.[13]

Binding Interaction. The central aim of this project is to prepare and characterise, by NMR relaxometric techniques, a dimeric "self-recognition" compound made from the two macrocyclic DOTA-like gadolinium complexes joined by the interaction of a phosphate pendent arm of one Gd**L**^{1} with the inner co-ordination sphere of the other Gd**L**^{2}. The relaxometric characterisation will separate the contributions of the two constituent parts and characterise the effect that the binding has had on each gadolinium centre at a time. This will be done by making one part of the dimeric self-recognition complex diamagnetic, by using Y(III) instead of Gd(III), characterising by ^{1}H and ^{17}O relaxation rate measurements, and then swapping the diamagnetic and paramagnetic moieties and repeating the characterisation. In this way, it is possible to study the effect of the binding interaction on each monomer separately from the other as only the paramagnetic half will be "seen" by the instrumentation. This, coupled with the characterisation of the starting reagents, will provide a complete picture of the dimer formation.
In order to assess the value of the binding constant between the two species the Proton Relaxation Enhancement Method (PRE) has been used.[15] This technique uses the change in the relaxation rates that occurs following the addition of a reagent that binds to a complex to extract the affinity constant (K_A) and the relaxivity of the bound complex (r_{1p}^{b}). To this end, the titration of a solution of 0.17 mM of [Gd**L**^{2}]$^{3+}$ with varying concentrations of Y**L**^{1} was performed at 20 MHz and a pH of 6.5 (Figure 4). An increase in the concentration of the Y(III) complex causes an increase of R_1 of the solution signalling the interaction of the complexes with the formation of the dimer and consequent decrease in the molecular tumbling (longer τ_R). The calculated affinity constant is not particularly high, but adequate for the purposes of this study (Table 2). The K_A value of 1220 (± 48) M^{-1} implies that the Gd(III) complex will be fully bound in the presence of a ca. 6-fold excess of the diamagnetic complex. The value of r_{1p}^{b}, 12.4 mM^{-1} s^{-1}, corresponds to a significant relaxivity enhancement despite the fact that the dimer formation involves a reduction of the hydration state of the complex (from q = 2 to q = 1). This is due in part to the fact that the adduct has a much larger molecular size and the increase in τ_R causes a strong enhancement of the inner-sphere relaxivity. In the case of the binding to Na_3PO_4, the change in r_{1p} is barely observed because the loss of a water molecule is fully compensated for by an increase in the second-sphere relaxivity.[13,16]

Figure 4. Plot of the water proton longitudinal relaxation rate of a solution of the complexes Gd**L**^{1} (diamonds; 0.14 mM) and Gd**L**^{2} (open circles; 0.17 mM) as a function of concentration of Y**L**^{2} and Y**L**^{1}, respectively, at 20 MHz, 25°C and pH = 6.5.



A very similar situation is observed in the case the titration of Gd**L**^{1} with Y**L**^{2}. The calculated K_A value is identical, within operational errors, and the effect on the r_{1p} of the paramagnetic adduct is also quite similar (12.3 mM^{-1} s^{-1}; Table 2).

Table 2. Affinity constants for the formation of the self-recognition compounds and relaxivity values (20 MHz, 25°C).

Dimer Gd-Y	Affinity constant (K_A, M^{-1})	$^{20}r_{1p}^{b}$ (mM^{-1} s^{-1})
Y**L**^{1}-Gd**L**^{2}	1220±48	12.4±0.2
Y**L**^{2}-Gd**L**^{1}	1228±45	12.3±0.3
Gd**L**^{2}-Gd**L**^{1}	1246±64	17.6±0.6

^{17}O NMR. The ^{17}O-R_{2p}-variable temperature profiles of the two adducts were then measured under experimental conditions that ensure the complete binding of the paramagnetic complex, (1:6). In each case, the curves show that the peaks are shifted towards lower temperature with respect to their monomer forms. This indicates a faster rate of exchange of the water bound to the

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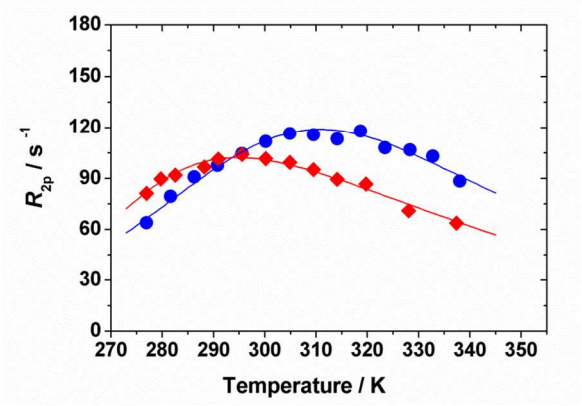
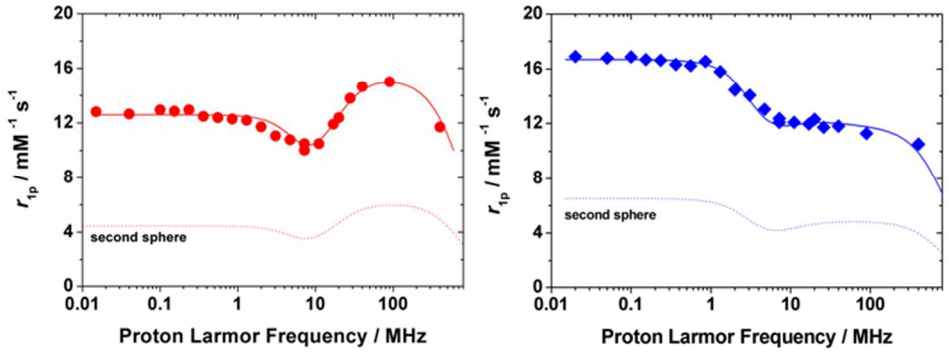
paramagnetic centre.[17] The decrease of τ_M for Y**L**^{1}-Gd**L**^{2}, from 1.6 to 0.3 μs at 25°C, is particularly evident and might be attributed to two reasons: 1) the decrease in the positive charge upon the binding to an alkylphosphonate group; 2) steric compression of the phosphonate group at the water binding site that destabilises the nine-coordinate ground state in the dissociative exchange mechanism.[18] In the case of Y**L**^{2}-Gd**L**^{1}, it is probable that only steric effects are operative and so the change in τ_M is less pronounced (from 1.4 to 0.8 μs).

Figure 5. Plot of the temperature dependence of the transverse ^{17}O relaxation rate of Y**L**^{1}-Gd**L**^{2} (7 mM; red diamonds) and Y**L**^{2}-Gd**L**^{1} (9 mM; blue circles) at 2.1 T.



NMRD profiles. The NMRD profile of the Y**L**^{1}-Gd**L**^{2} adduct shows a marked increase of relaxivity over the monomer, particularly pronounced in the high field region. This is a clear consequence of a longer τ_R value, as evidenced by the presence of the broad hump centred around 100 MHz, which is quite close to the operating frequency of the new generation of MRI scanners. However, even assuming that the adduct is a compact and rigid system and that the increase of molecular mass is translated entirely in a corresponding increase of τ_R (from 85 to approx. 200 ps), it is necessary to take into account an additional contribution to relaxivity in order to obtain a good fit. This consists in a sizeable second-sphere contribution, which corresponds to that associated with the presence of two water molecules at a distance of 3.5 Å from the paramagnetic centre. Of course, it is more likely that this contribution derives itself from several second sphere water molecules with a variety of *r*' and τ_R' values.
We observed very similar results also in the case of Y**L**^{2}-Gd**L**^{1}. The shape of the profile is different as the value of the electronic relaxation time of the DOTA-like Gd(III) complex is longer and so the "peak" at high fields is less pronounced. In addition, the water exchange rate at the Gd**L**^{1} centre is rather low and limits the relaxation enhancement. Even in this case to obtain a reasonable fit we had to assume the presence of a large contribution of second sphere water molecules.[19]

Figure 6. ^{1}H NMRD profiles of Y**L**^{1}-Gd**L**^{2} and Y**L**^{2}-Gd**L**^{1} at 25°C and pH=6.5



In a final experiment, we took into consideration the case of the interaction between the two paramagnetic Gd(III) complexes. Despite the fact that the strength of the interaction has already been evaluated in the case of the mixed Y(III) and Gd(III) chelates, we repeated the relaxometric titration adding increasing amounts of Gd**L**^{2} to a dilute solution of Gd**L**^{1} (Figure 7, left), at 25 °C and 20 MHz. In this case, the value of R_1 continues to increase following the addition of Gd**L**^{2} and therefore it is not possible to observe an asymptotic value. However, it appears evident that the values of R_1 measured are far greater than expected in the

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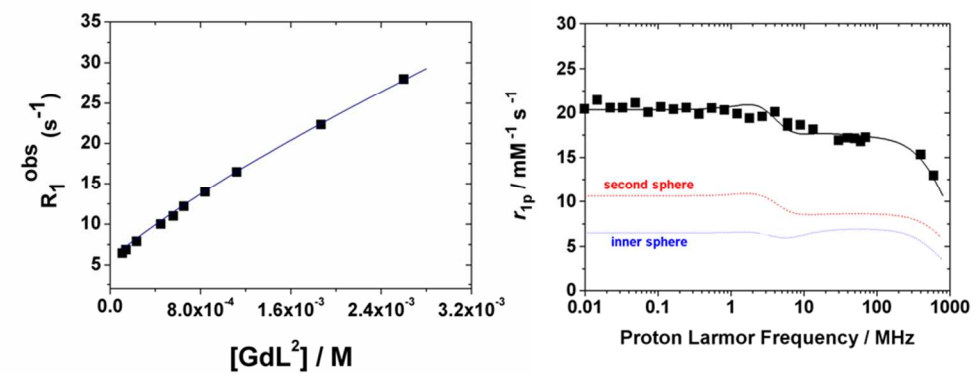
DOI: 10.1039/C4DT02971B PREPRINT

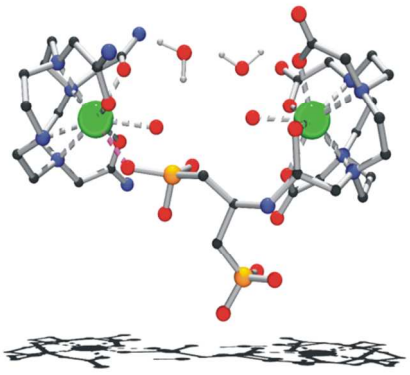
absence of interaction. The fit of the data provides a value of K_A completely analogous to those found for the Y-Gd dimers (Table 2) and a value of r_{1p}[b] as high as 17.6 mM^{-1} s^{-1} (see ESI). Whereas the relaxivity of the mixed dimers seem very similar, in the case of the Gd-Gd adduct we observe a further ca. 40% increase of r_{1p} at 20 MHz.

Figure 7. Titration experiment (left) of Gd**L**[1] (0.7 mM) with Gd**L**[2] at 20 MHz and 25°C. Right: ^{1}H NMRD profile of the adduct Gd**L**[1]-Gd**L**[2] at 25°C and pH=6.5.



Finally, the NMRD profile of the fully paramagnetic, Gd**L**[1]-Gd**L**[2], complex is presented (Figure 7, right). For ensuring complete complexation, a molar excess of 6:1 of one of the components was needed. This profile is then affected by errors as it is calculated from the observed relaxation rates, the affinity constant and the known relaxivity values of the individual complex, in excess, at all measured proton Larmor frequencies. The relaxivity varies only very little from 0.5 to 5 T as it decreases only at very high frequencies (> 300 MHz). At the clinically relevant fields, the r_{1p} assumes a value of ca. 18.0 mM^{-1} s^{-1}, which is sensibly higher than that found for the mixed Gd-Y dimers (~ 20-40% at 2.1 T). The interpretation of this result is quite difficult. A possible explanation could be the preferred geometry of the dimeric complex, in which the two hydrophobic regions are pointing in opposite directions from each other. The two hydrophilic regions face each other, allowing the presence of a hydrogen bonded network of water molecules that contribute greatly to the relaxivity (Scheme 2). However, while this is a plausible hypothesis to account for the high relaxivity of the Gd-Y dimers, it is more difficult to interpret the increase of r_{1p} for the Gd-Gd compound. This could be associated with small structural changes that might occur on replacing Y with Gd. For example, a different relative population of the isomers SAP (square antiprismatic) and TSAP (twisted square antiprismatic) which induces a variation of the rate of water exchange. In addition, minor conformational changes could affect the Gd-Gd distance or the number of water molecules in the second hydration sphere.

Scheme 2. Schematic representation of the dimeric adduct showing the second sphere water molecules in the hydrophilic region in between the two Gd(III) ions.



Therefore, a sound analysis of the NMRD profile is not possible based solely on the data available. Tentatively, we carried out a least-square fit by fixing some parameters: q, r, τ_R (210 ps; it cannot differ from the value of the mixed Gd-Y dimers), τ_M (arbitrarily set to 0.45 μs), a, D, τ_R'. Instead, we considered as adjustable parameters Δ^2, τ_V, r' and q'. Clearly, this analysis is quite coarse and rather qualitative because the two paramagnetic centres are characterized by different electronic relaxation times and rate of exchange of the bound water molecule. This analysis, however, allows a gross estimation of the order of magnitude of the

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DOI: 10.1039/C4DT02971B

various contributions. Despite the significant limitations of the approach, we obtained a good fit of the data based on the following parameters: $\Delta^2 = 2.0\text{x}10^{19}$ s^{-2}, $\tau_V = 50$ ps, $r' = 3.5$ Å and $q' = 4$ (Table 1). At 3 T, the relaxivity enhancement, as compared to Gd**L**^{2}-Y**L**^{1}, is of +42% for the second sphere term while the contribution of the inner sphere relaxation mechanism is essentially unaltered. Both these two contributions show a small field-dependence in the range of about 0.5 – 4 T (Fig. 7).

Conclusions

Heterodimeric compounds of limited size and molecular weight have been shown to possess values of relaxivity (Gd) two to three times higher than those of related commercial complexes. High relaxivity values are observed over a wide range of proton Larmor frequencies and conveniently cover the region of interest for high-field MRI. The mixed Gd-Y dimers demonstrate that the coordination of a pendant donor group of a complex on another, coordinatively unsaturated, complex gives rise to a compact and rigid system characterized by optimal values of the rotational correlation time (~ 0.2 ns). It is also clear that there exists an additional contribution from water molecules in the second coordination sphere. This contribution is very significant and represents about 30-40% of the global relaxation enhancement. It can be hypothesized that this is achieved thanks to the face-to-face dimer conformation that promotes the formation of a network of H-bonded water molecules around the more hydrophilic components of the two chelating units (molecular planes containing carboxylic, amidic and phosphonic groups; Scheme 2). In the case of the Gd-Gd system, the water molecules are affected by the presence of two paramagnetic centres and this might contribute to a further increase in relaxivity. The relaxivity values observed are comparable to those of multimeric or macromolecular systems, which have a significantly larger size.
Clearly, for a possible practical application, we need a higher level of selectivity and so we will need to increase the strength of the interaction or develop systems in which the two chelating units are connected by an appropriate linker.

Experimental

The ligand **L**^{1}, prepared following a procedure reported, was provided by Prof. G. B. Giovenzana (Università del Piemonte Orientale).[11] The ligand **L**^{2} was synthesized according to the procedure detailed in ref. 21. Lanthanide(III) trifluoromethanesulfonate, $Ln(CF_3SO_3)_3$, and chloride, $LnCl_3 \cdot 6H_2O$, (Ln = Y, Gd), were purchased from Aldrich.
The Y**L**^{1} and Gd**L**^{1} complexes were prepared by adding stoichiometric amounts of the corresponding $LnCl_3$ to the aqueous solution of the ligand, at neutral pH and 40 °C. The resulting solution was stirred for about 24 h to ensure complete complexation. The Y**L**^{2} and Gd**L**^{2} complexes were prepared by mixing the ligand and the corresponding $Ln(CF_3SO_3)_3$ in methanol, according to the published procedure.[21]
The formation of the Y(III) complexes was verified by recording the ^{1}H NMR spectra on a JEOL ECP-400 spectrometer. The absence of free gadolinium in the solutions of Gd**L**^{1} and Gd**L**^{2} was checked using the xylenol orange test.[22]

^{1}H and ^{17}O NMR measurements.

The water proton longitudinal relaxation rates were measured on about 0.5 - 2.5 mM solutions of the Gd(III) complex in non-deuterated water. The $1/T_1$ NMRD profiles were measured on a fast field-cycling Stelar SmartTracer relaxometer (Stelar s.r.l., Pavia, Italy) over a continuum of magnetic field strengths from 0.00024 to 0.25 T (corresponding to 0.01-10 MHz proton Larmor frequencies). The relaxometer operates under computer control with an absolute uncertainty in $1/T_1$ of ± 1%. The temperature was controlled with a Stelar VTC-91 airflow heater equipped with a calibrated copper–constantan thermocouple (uncertainty of ±0.1 K). Additional data points in the range 15-70 MHz were obtained on a Stelar Relaxometer equipped with a Bruker WP80 NMR electromagnet adapted to variable-field measurements (15-80 MHz proton Larmor frequency). High fields data were obtained on high-resolution JEOL ECP-400 or Bruker Avance III 500 spectrometers. The exact concentration of Gd^{III} was determined by measurement of bulk magnetic susceptibility shifts of a *t*BuOH signal.[23]
Variable-temperature ^{17}O NMR measurements were recorded on a JEOL EX-90 spectrometer (2.1 T) equipped with a 5 mm probe and standard temperature control unit. Aqueous solutions of the complex (7-20 mM) containing 4.0% of the ^{17}O isotope (Cambridge Isotope) were used. The observed transverse relaxation rates were calculated from the signal width at half-height. The bulk magnetic susceptibility contribution was subtracted from the ^{17}O NMR shift data using the ^{1}H NMR shifts of the *t*BuOH signal as internal reference.

Acknowledgements

This was supported by MIUR (PRIN 2012). MB thanks financial support of the "Compagnia di San Paolo" (CSP-2012 NANOPROGLY Project).

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DOI: 10.1039/C4DT02971B

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