Research Article

Magnetic Resonance in Chemistry

Received: 13 June 2008 Revised: 21 July 2008 Accepted: 28 July 2008 Published online in Wiley Interscience

(www.interscience.com) DOI 10.1002/mrc.2316

^{1}H and ^{17}O NMR relaxometric study in aqueous solution of Gd(III) complexes of EGTA-like derivatives bearing methylenephosphonic groups†

Lorenzo Tei,[a] Mauro Botta,[a]* Clara Lovazzano,[a] Alessandro Barge,[b] Luciano Milone[c] and Silvio Aime[c]*

The Gd(III) complexes of three new octadentate chelators, prepared by substitution of four, two, and one carboxylate groups of EGTA with phosphonate groups, have been investigated by ^{1}H and ^{17}O NMR relaxometric techniques in aqueous solutions. The analysis of the solvent proton relaxivity data as a function of pH, temperature, and magnetic field strength (nuclear magnetic relaxation dispersion (NMRD) profiles) in combination with the ^{17}O transverse relaxation rate data at variable temperature allowed assessing the hydration state of the complexes, the occurrence of pH-dependent oligomerization processes for the tetraphosphonate derivative, the presence of a well-defined second sphere of hydration that markedly contributes to the relaxivity, and the values of the structural and dynamic relaxation parameters. In addition, in the case of the monophosphonate derivative the presence of a coordinated water molecule has allowed evaluation of the kinetic parameters of the exchange process, highly relevant for the possible use of this Gd(III) complex as an MRI probe. The rate of exchange of the water molecule, $^{298}k_{ex} = 4.2 \times 10^{8}s^{-1}$, is one of the highest measured so far for a nonacoordinate Gd(III) chelate and optimal for developing contrast-enhancing probes of high efficacy at high magnetic fields.

Keywords: relaxivity; NMRD; ^{17}O NMR; Gd(III) complex; paramagnetic NMR; phosphonate

Introduction

For several decades, polyaminocarboxylate ligands have been extensively used for the coordination of metal ions in an extremely broad range of applications.[1–5] More recently, complexes of lanthanide(III) cations with this class of chelators have been the focus of great interest because of their increasing use in therapeutic and diagnostic clinical protocols.[6] This large attention has been stimulated by the possibility to exploit the different properties of the various members of the lanthanide series: optical (Eu, Tb, Yb), magnetic (Gd, Dy), and radionuclear (^{153}Sm, ^{166}Ho, ^{177}Lu). The coordination number, *CN*, of trivalent Ln cations typically being between 10 (lighter members) and 8 (heavier members), the ligand denticity is generally higher than 6 and most commonly 7/8. The higher denticity ensures a greater thermodynamic stability of the resulting metal chelates, a prerequisite of primary importance for biomedical studies.[6] In magnetic resonance imaging (MRI) applications as contrast-enhancing agents (CA), Gd(III) is the ion of choice because of the well-known favorable properties: seven unpaired electrons isotropically distributed, high magnetic moment, and long electronic relaxation times. Gd-based CA function by shortening the nuclear magnetic relaxation times of the water protons through a dipolar interaction mediated by the chemical exchange between the inner coordination sphere and the bulk water, as well as indirectly by the interaction involving the solvent molecules diffusing near the complex (outer coordination sphere).[7,8] The presence of at least one inner-space (IS) water molecule ($q = 1$) in fast exchange is essential to provide the complex with a good relaxation efficiency. The acyclic H_5DTPA (diethylenetriamine-*N*,*N*′,*N*″-pentaacetic acid) and macrocyclic H_4DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) chelators are both octadentate and form very stable mono aqua complexes with Gd(III) with tricapped trigonal prismatic and monocapped square antiprismatic coordination geometries, respectively.[7] Both $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(DOTA)(H_2O)]^{-}$ are approved CA currently used in the clinical practice and present similar chemical and pharmacokinetic properties and relaxation-enhancing ability.

The replacement of one or more acetic arms by methylenephosphonic groups has been achieved and investigated in several linear and cyclic ligands. This substitution may markedly influence several properties of the corresponding Ln(III) complexes: thermo-

* *Correspondence to: Mauro Botta, Dipartimento di Scienze dell'Ambiente e della Vita, Universit'a del Piemonte Orientale "A. Avogadro", Via Bellini 25/G, I-15100 Alessandria, Italy. E-mail: mauro.botta@mfn.unipmn.it*
Silvio Aime, Dipartimento di Chimica IFM, Universit'a degli Studi di Torino, Via P.Giuria 7, I-10125 Torino, Italy. E-mail: silvio.aime@unito.it

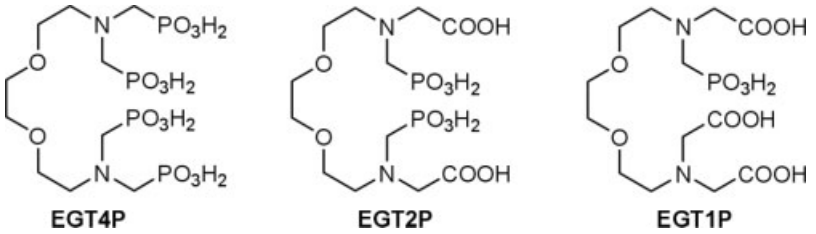
† *Dedicated to Prof. P. Pregosin on his 65th birthday.*

a *Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "A. Avogadro", Via Bellini 25/G, I-15100 Alessandria, Italy*

b *Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 7, I-10125 Torino, Italy*

c *Dipartimento di Chimica IFM, Università degli Studi di Torino, Via P. Giuria 7, I-10125 Torino, Italy*

Magnetic Resonance in Chemistry



Scheme 1. Chemical structure of the ligands.

dynamic stability, overall electric charge, solution structure, rate of coordinated water exchange (k_{ex}), and hydration number (q). The phosphonate derivatives of the Gd-based CA also show significant changes in the relaxometric properties: (i) the bulkier phosphonate groups may preclude the access of water to the coordination site of the metal ion, thus reducing the q value; (ii) for a similar reason the rate of exchange of the bound water molecule(s), k_{ex}, may be accelerated; and (iii) the ability of the phosphonate moieties to involve water molecules in H-bonding interactions often results in the formation of a well-defined second coordination sphere that enhances the relaxation efficiency of the Gd complex.

In the present work we have considered three novel phosphonate derivatives of H_4EGTA (ethyleneglycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid), an octadentate acyclic ligand that forms stable complexes with Ln(III) cations whose solution structures and relaxometric properties have been studied in detail (Scheme 1).[9] In particular, the most relevant feature of $[Gd(EGTA)(H_2O)]^-$ with respect to other related complexes is the presence of a fast rate of water exchange, a property of great relevance for the development of highly effective macromolecular MRI CA. The value of k_{ex}, determined by ^{17}O NMR techniques, is about an order of magnitude higher than for $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(DOTA)(H_2O)]^-$, and it is explained by the steric constraint of the oxoethylenic bridge on the coordinated water molecule that lowers the activation energy of the dissociative exchange process. We have synthesized and characterized with NMR relaxometric techniques the solution behavior of three derivatives of EGTA in which four,[10] two, and one carboxylates have been replaced by phosphonate groups.

Results and Discussion

Relaxometric properties

The ability of a Gd(III) chelate to enhance the water proton nuclear magnetic relaxation rates is evaluated in terms of the parameter relaxivity, r_{1p},[8] which refers to the increase of the longitudinal relaxation rate of the solvent observed in a 1 mM aqueous solution of the paramagnetic complex (Eqn (1)) at a given temperature and magnetic field strength (typically 298 K and 20 MHz, respectively):

$$r_{1p} = (R_1{}^{obs} - R_{1w})/(CA) \quad (1)$$

In Eqn (1), R_{1w} is the relaxation rate of a corresponding diamagnetic solution, which for pure water assumes the value of 0.38 s^{-1} at 20 MHz and 298 K. The r_{1p} values of monomeric Gd-based CA is given by the additive IS and outer-sphere (OS) contributions, which are roughly the same (2.5 mM^{-1} s^{-1}, 20 MHz, and 298 K) for a monoaquo complex. At high frequencies, the IS term increases proportionally with the increase of the molecular size of the complex (slower molecular tumbling, longer rotational correlation time, τ_R) and has a little dependence from the other parameters: the electronic relaxation times $T_{1,2e}$ (dependent on the trace of the square of the zero-field splitting, Δ^2, and on the correlation time describing the modulation of the zero-field splitting, τ_V), the residence lifetime of the bound water molecule τ_M ($\tau_M = 1/k_{ex}$), and the Gd–H_w distance r.

Characterization of GdEGT4P

The relaxivity of the tetraphosphonate Gd(III) complex has a value of 5.3 mM^{-1} s^{-1} at 20 MHz, 298 K, and pH = 7.5. This value is similar to that measured for GdEGTA (r_{1p} = 4.7) and several others q = 1 complexes of similar molecular size.[7,8] On the other hand, GdDOTP (H_8DOTP = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylenephosphonic acid), a complex that has no water molecules in its inner coordination sphere (q = 0), has a relaxivity (r_{1p} = 4.6) closely related to that of the carboxylate analogue GdDOTA (r_{1p} = 4.7). This was explained by the presence of a well-defined second sphere of hydration, favored by the presence of the phosphonic groups which are able to promote strong H-bonding interactions with the water molecules.[11–13] Clearly, the assessment of the hydration of the complex needs further investigation. Unlike GdEGTA, whose relaxivity is constant in the pH range 3–12, the relaxivity of GdEGT4P has a pronounced pH dependence. In the pH interval 12–8, r_{1p} assumes a constant value of 4.6 mM^{-1} s^{-1}, and then it increases monotonically on lowering the pH to reach a value of about 23 mM^{-1} s^{-1} at pH = 3 (Fig. 1). The constant value of relaxivity at basic pH, where the unbound oxygen atom of the phosphonic groups in the complex are fully deprotonated, suggests the presence in solution of a monomeric species characterized by q = 0. As for GdDOTP, the absence of a coordinated water molecule is compensated by the presence of a number of water molecules hydrogen-bonded to

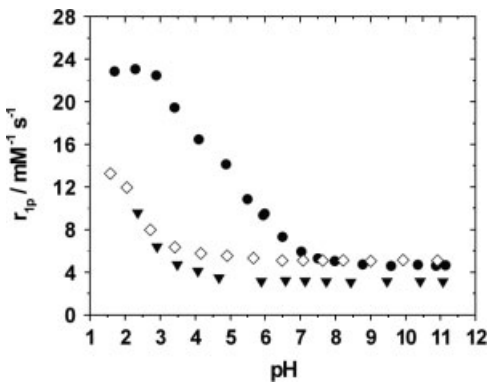


Figure 1. Plot of proton relaxivity r_{1p} at 20 MHz and 298 K *versus* pH for GdEGT4P (filled circles), GdEGT2P (open diamonds) and GdEGT1P (down triangles).

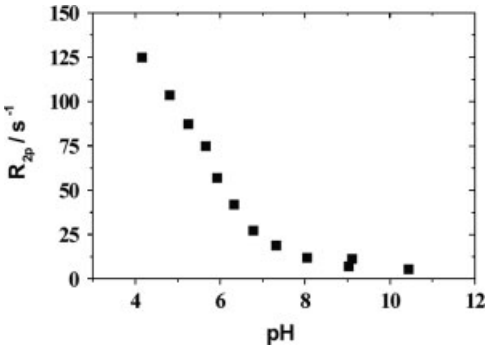


Figure 2. Plot of the paramagnetic contribution to the ^{17}O transverse relaxation rate R_{2p} as a function of pH at 2.1 T (12.2 MHz) and 298 K for a 8.3 mM aqueous solution of GdEGT4P.

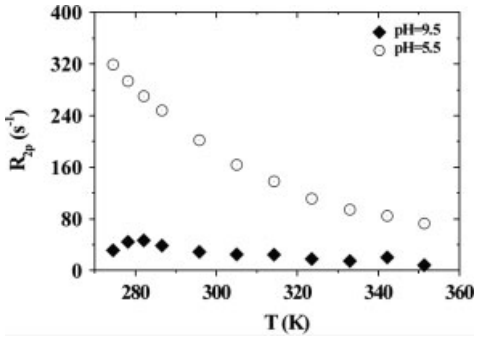


Figure 3. Temperature dependence of the paramagnetic contribution to the water ^{17}O NMR transverse relaxation rate R_{2p} for GdEGT4P. Experimental conditions: 18 mM, 2.1 T.

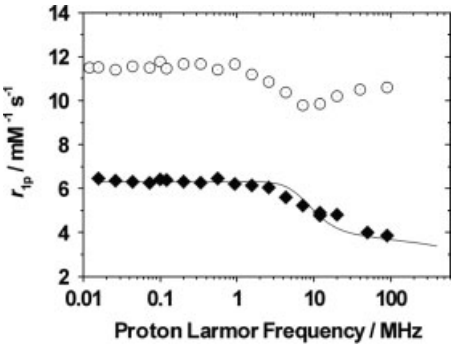


Figure 4. ^{1}H $1/T_1$ NMRD profiles of GdEGT4P at pH = 9.5 (filled diamonds) and 5.5 (open circles) at 298 K. The solid curve through the data points is calculated with the parameters reported in Table 1.

the phosphonic groups, and therefore at a distance sufficiently short from the gadolinium to contribute to the relaxivity. The increase of r_{1p} by lowering pH could be attributed to an increase of the hydration state of the complex resulting from the stepwise protonation of the phosphonic groups and to the formation of oligomers. Both the tendency to oligomerize and to increase the q value by passing from basic to neutral/acidic conditions have been previously observed and reported in the case of phosphonate complexes.[14–16] These two hypotheses can be verified by analysing ^{17}O NMR data and ^{1}H nuclear magnetic relaxation dispersion (NMRD) profiles.

The ^{17}O water transverse relaxation rates, R_2, are determined by the contact (through-bond) Gd–O_w interaction and provide direct access to information on the hydration state of the complex. This interaction is not influenced by the molecular reorientation of the complex, but it depends on the hydration number q, the electronic relaxation parameters, the rate of water exchange, and the scalar coupling constant $A/\hbar$.[17,18] The pH dependency of the ^{17}O R_2 values measured at 2.1 T and 298 K for a 31 mM solution of GdEGT4P is reported in Fig. 2. The behavior reproduces quite closely that observed for the ^{1}H R_1 data to indicate that the relaxivity changes involve the whole water molecule and do not simply depend on proton exchange processes. Strictly speaking, the ^{17}O R_2 pH dependency could arise either from hydration equilibria (q variations) and/or large variation of k_{ex}. In order to discriminate between these alternatives, it is useful to measure the temperature dependence of the ^{17}O R_2 data, a well-established procedure to obtain accurate estimates of q and k_{ex}. The data, recorded at both pH 5 and 9, are reported in Fig. 3. It is quite evident that at basic pH GdEGT4P does not possess any coordinated water molecule ($q = 0$). The Gd–O_W coupling involves only the dipolar interaction with the water molecules in the outer coordination shell, which is quite small owing to the low γ value of the ^{17}O nuclei. This result reproduces closely what is found in the case of GdDOTP. On the other hand, the profile at pH = 5.5 is typical of a system with $q > 0$ in the fast exchange regime.[18] Then, whereas the high steric encumbrance of the phosphonic groups does not allow access of a water molecule to the inner coordination sphere of the metal ion at basic pH (8/12), their protonation occurring at lower pH values favors an increase of the hydration state and the formation of aggregated species.

This latter hypothesis finds a clear support in the $1/T_1$ NMRD profiles measured at pH 9 and 5 (Fig. 4). The NMRD profile recorded at pH = 9 is characterized by the typical shape and amplitude of monomeric, low-molecular-weight complexes, featuring a single dispersion around 3–5 MHz.[8] We know from the ^{17}O NMR data that $q = 0$ and then we have to model the relaxation behavior in terms of the outer and second coordination sphere relaxation contributions only. By adopting standard values for the distance of closest approach of the water molecules (a = 4.0 Å) to the metal ion and for the relative diffusion coefficient ($D = 2.4 \times 10^{-5}$ cm^2 s^{-1}), and by assuming that each phosphonic group is H-bonded to a single water molecule, a satisfactory fit is obtained with the parameters listed in Table 1. It is worth underlining the fact that this analysis represents only a crude approximation that enables the estimation of the order of magnitude of the contribution of the solvent molecules of the second coordination shell of the complex. On the other hand, the NMRD profile measured at pH = 5 shows the characteristic features of a high-molecular-weight system: a minimum of the relaxivity at *ca* 10 MHz followed by a broad peak centred around 40 MHz.[8] This profile results from the occurrence of two conditions: $q > 0$ and slow molecular tumbling rate, as expected for oligomeric species. Clearly, the complexity of the species present in solution prevents us from any attempts to fit the data.

It has been shown that GdDOTP is able to interact with the positively charged domains of bovine serum albumin (BSA) and to form adducts endowed with high relaxivity ($r_{1p} \sim 22$ mM^{-1} s^{-1} at 20 MHz and 298 K), whose enhancement is attributed to exchangeable protons on the protein close to the interaction site of the complex and to a number of hydrogen-bonded water molecules in the second coordination sphere of the metal ion.[11] The behavior of GdEGT4P is expected to be rather similar owing

Table 1. Selected best-fit parameters obtained from the analysis of the $1/T_1$ NMRD profile (298 K, pH = 7.2) and ^{17}O NMR data (9.4 T) of the Gd complexes

Parameter	GdEGT4P	GdEGT2P	GdEGT1P	Gd–EGTA[a]
Δ^2 ($s^{-2} \cdot 10^{19}$)	6.2 ± 0.4	4.2 ± 0.2	4.3 ± 0.1	3.4 ± 0.2
$^{298}\tau_V$ (ps)	23 ± 0.3	22 ± 1	23 ± 1	24 ± 1
E_V (kJ mol^{-1})	–	–	1.5 ± 0.2	1.00
$^{298}\tau_M$ (ns)	–	–	2.4 ± 0.03	32.00
$\Delta H^{\#}_M$ (kJ mol^{-1})	–	–	28.9 ± 1.4	42.7 ± 3.1
$\Delta S^{\#}_M$ (J mol^{-1} K^{-1})	–	–	+17.2 ± 3	+42 ± 3
$^{298}\tau_R$ (ps)	–	–	70 ± 2	58 ± 6
r (Å)[b]	–	–	3.00	3.00
q[b]	0	0	1.00	1.00
a (Å)[b]	4.00	4.00	4.00	3.80
D (cm^2 s^{-1} × 10^{-5})[b]	2.24	2.24	2.24	2.0 ± 2
q' [c]	4.00	2.00	1.00	–
r' (Å)[d]	3.82	3.70	3.80	–

[a] From Ref. [9].
[b] Fixed during the least-squares procedure.
[c] Number of second sphere water molecules.
[d] Mean Gd–H (water) distance of the second sphere waters.

to the identical overall electric charge and number of phosphonic donor groups. We evaluated the binding interaction by titrating a dilute solution of the complex at pH 7 with the protein and measuring the changes of the longitudinal relaxation rate. The resulting binding isotherm was then analysed according to the proton relaxation enhancement (PRE)[19] equations to obtain the values of the affinity constant (K_A = 1440 M^{-1}) and of the relaxivity of the macromolecular adduct (r_{1p} = 19.5 mM^{-1} s^{-1}). As expected, these values reproduce quite closely the non-specific binding properties found for GdDOTP.

Characterization of GdEGT2P

The relaxivity of the bis-(methylenephosphonate) derivative at 20 MHz and 298 K is 3.7 mM^{-1} s^{-1} and is independent of pH in the range 4.5–11. Then, unlike the previous case, GdEGT2P follows the behavior of the parent compound GdEGTA and appears to be present in aqueous solution as a monomeric complex over a wide range of pH values. The value of the relaxivity is intermediate between the corresponding values of a pure OS complex ($r_{1p} \approx$ 2.0–2.5 mM^{-1} s^{-1}) and of a q = 1 complex ($r_{1p} \approx$ 4.5–5.5 mM^{-1} s^{-1}) of similar molecular size. This represents a good indication of the absence of water molecules in the inner coordination sphere of the metal ion and of the occurrence of a sizeable contribution of the second hydration sphere relaxation mechanism. The octacoordinate ground state of gadolinium in this complex is confirmed by the VT ^{17}O NMR R_2 data, which show a profile quite similar to that of GdEGT4P at basic pH (Fig. 3). The ^{1}H $1/T_1$ NMRD profile, recorded at 298 K and pH = 7.2 (Fig. 5), has a shape rather similar to that of the monomeric form of GdEGT4P. A good fit is indeed obtained by assuming the presence of two water molecules hydrogen-bonded to the two phosphonic groups of the ligand and by adopting standard values for the parameters a and D in the evaluation of the OS relaxivity. The best-fit parameters are listed in Table 1, which outline the similarity between the monomeric forms of GdEGT4P and GdEGT2P. Clearly, even the replacement of only two carboxylates with two phosphonic moieties introduces enough

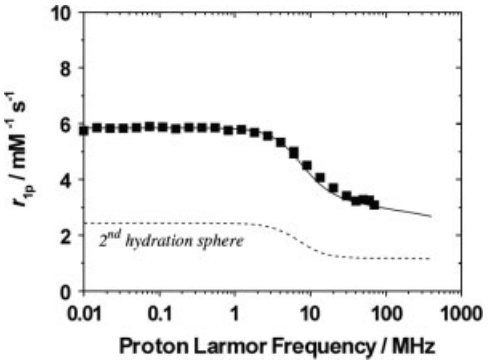


Figure 5. ^{1}H $1/T_1$ NMRD profiles of GdEGT2P at pH = 7.2 and 298 K. The solid curve through the data points and the second sphere contribution are calculated with the parameters reported in Table 1.

steric constraint in the first coordination shell of the Gd(III) ion to destabilize the nonacoordinate (q = 1) state in favor of an octacoordinate (q = 0) ground state in solution.

Characterization of GdEGT1P

The relaxivity of the gadolinium complex with the monophosphonate ligand EGT1P, r_{1p} = 5.4 mM^{-1} s^{-1}, is sensibly higher than that of GdEGT4P (+17% in the case of the monomeric form) and GdEGT2P (+46%) and even higher than that measured for GdEGTA. Therefore, we may safely assume that this complex is monohydrated and stable over a large range of pH values (Fig. 1). So, only the replacement of a single carboxylate with a phosphonic group maintains the hydration state of the parent GdEGTA complex. The steric compression of the oxoethylenic bridge on the adjacent bound water molecule, which is responsible of the fast rate of exchange, is reinforced by the introduction of the bulky phosphonic groups. When the substitution involves more than a single carboxylic group, the steric constraint is such as to invert the relative stabilities of the nona- and octacoordinate states, and only q = 0 complexes are found in solution. The high relaxivity value of GdEGT1P, larger than for other complexes of similar molecular weight, suggests also in this case a contribution from water molecule(s) present in the second coordination shell. The NMRD profile is reported in Fig. 6 and shows characteristic features of monohydrated Gd(III) complexes: the low frequency/high relaxivity plateau (r_{1p} > 9 mM^{-1} s^{-1}), the dispersion at ca 6–8 MHz, and then the high frequency/low relaxivity plateau ($r_{1p} \approx$ 5.0 mM^{-1} s^{-1}). Again, for the data-fitting it is necessary to make some reasonable estimate of the contribution of the second solvation sphere. In analogy with the previous cases, we associate a single water molecule to the phosphonic group of the ligand and then fit the data to a model that includes inner-, second-, and OS relaxation mechanisms. For the IS relaxivity we made the further assumption of q = 1, r = 3.0 Å (r is the Gd–Hw distance), and used for τ_M the value calculated from ^{17}O NMR data (see below). The different contributions to the relaxivity calculated with the parameters listed in Table 1 are shown in Fig. 6. At 20 MHz the inner-, second-, and OS relaxation mechanisms make a contribution to the global relaxivity of *ca* 50, 10, and 40%, respectively.

A rather crucial parameter that in general cannot be determined with accuracy by the analysis of the NMRD profiles is the mean residence lifetime of the coordinated water molecule, τ_M. As it is well known, the kinetic parameters of the water exchange can be

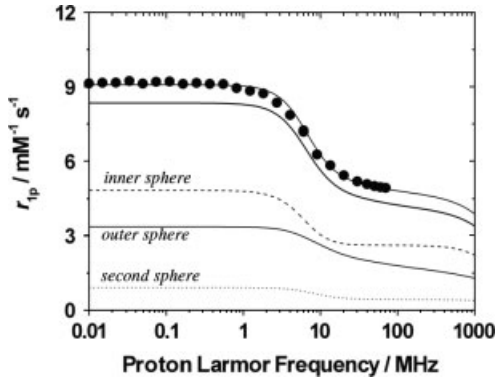


Figure 6. ^{1}H $1/T_1$ NMRD profiles of GdEGT1P at pH = 7.2 and 298 K. The solid curve through the data points and the different contributions to the relaxivity are calculated with the parameters reported in Table 1. The dashed curve is the calculated NMRD profile of GdEGTA, according to the data in Ref. [9].

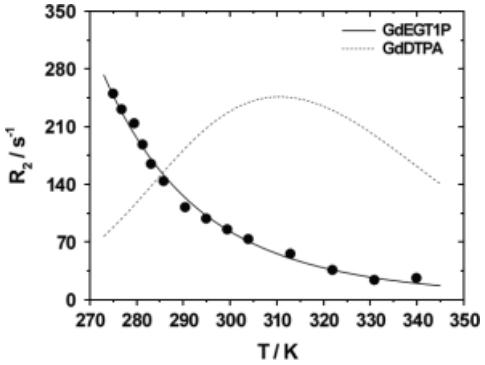
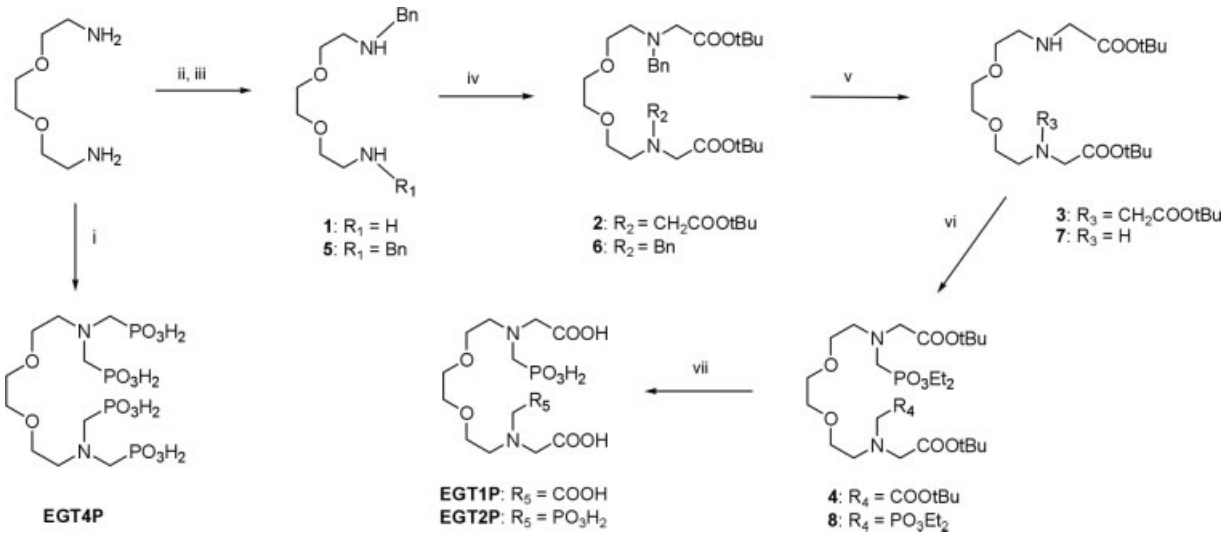


Figure 7. Temperature dependence of the paramagnetic contribution to the water ^{17}O NMR (9.4 T) transverse relaxation rate for a 25 mM aqueous solution of GdEGT1P. Solid curve fitted with the values of Table 1 for Δ^2, τ_V, E_V, and $A/\hbar = -3.80 \times 10^6$ rad s^{-1}. The dashed curve is the calculated profile of GdDTPA, according to the data in Ref. [17].

evaluated by measuring the temperature dependence of the ^{17}O transverse relaxation rate, R_2, of the water molecules in a solution of the paramagnetic complex.[17,18] This experiment was carried out at 9.4 T on a 25 mM solution of GdEGT1P at pH = 7.2. The results are reported in Fig. 7 and compared with the calculated curve of the corresponding complex $[GdDTPA(H_2O)]^{2-}$ for which a τ_M value of 300 ns (at 298 K) was calculated. The exponential increase of R_2 with decreasing temperature is indicative of a rate of water exchange much faster than for GdDTPA. For this latter, the maximum of the curve is at about 315 K, whereas for GdEGT1P it is well below 273 K. The fit of the experimental data to the Swift–Connick equations confirmed this qualitative analysis and provided the following values for the kinetic parameters: $^{298}\tau_M = 2.4$ ns ($k_{ex} = 4.1 \times 10^8$ s^{-1}), $\Delta H^{\#} = 28.9$ kJ mol^{-1}, $\Delta S^{\#} = +17.2$ J mol^{-1}. This is one of the fastest water exchange rates measured for a monohydrated Gd complex, about an order of magnitude faster than that of GdEGTA. The value of τ_M should not be too short so as to influence the overall correlation time and not too long to limit the relaxivity. Its optimal value decreases with increasing magnetic field strength and it can be estimated to be *ca* 30 ns at 20–40 MHz, and *ca* 2–4 ns at 80–100 MHz.[20] So, GdEGT1P is endowed with an optimal rate of water exchange for the attainment of enhanced relaxivity for its multimeric or macromolecular derivatives.

Synthesis

While the synthesis of EGT4P was accomplished following a reported procedure, that of EGT1P and EGT2P was carried out using similar synthetic procedures except for the first step (Scheme 2). In this first step, the reductive amination of 1,8-diaza-3,6-dioxaoctane with 2.5 or 1 M equivalents of benzaldehyde and $NaBH_4$ led to the formation of di- and monobenzyl amines, respectively. Whereas EGT2P was obtained starting from the dibenzyl derivative (*N*,*N*′-dibenzyl-1,8-diaza-3,6-dioxaoctane, **1**) in four steps, EGT1P was synthesized starting from the mono-benzyl derivative (*N*-benzyl-1,8-diaza-3,6-dioxaoctane, **5**) in the same number of steps. The alkylation with *tert*-butyl bromoacetate followed by hydrogenolysis of the *N*-benzyl groups with ammonium formate and Pd/C led to the formation of the derivatives **3** and **7** having two and one secondary amino groups, respectively. The phosphonic



Scheme 2. i: $ClCH_2PO_3H_2$, CH_2O, HCl; ii: PhCHO, CH_2Cl_2; iii: $NaBH_4$, CH_3CN; iv: $BrCH_2COOtBu$, K_2CO_3, NMP; v: $HCOONH_4$, Pd/C, MeOH; vi: HPO_3Et_2, CH_2O, toluene; vii: HBr.

groups were inserted by a Mannich reaction of **3** and **7** with diethyl phosphite and paraformaldehyde. Derivatives **4** and **8** were purified by column chromatography, and then the *tert*-butyl and ethylphosphonic esters were hydrolysed with a 33% solution of HBr in acetic acid. Ligands EGT2P and EGT1P were obtained as analytically pure white solids by precipitation with diethyl ether in overall 18 and 10% yield, respectively. All intermediates and products were characterized by ^{1}H and ^{13}C NMR spectroscopy and electrospray ionization mass spectra (ESI-MS).

Conclusions

The coordination polyhedron of GdEGTA is conveniently described as a tricapped trigonal prism in which the capping positions are occupied by the two nitrogen atoms and the IS water oxygen, and the trigonal faces are composed of the oxygen atoms of the ether and the carboxylate groups.[9] The substitution of the four carboxylate groups with four bulky and sterically demanding phophonate groups introduces significant differences in the properties of the corresponding Gd(III) complex. The coordinated water molecule is destabilized and, unlike GdEGTA, GdEGT4P has an octacoordinate ground state with $q = 0$. The steric interaction and electrostatic repulsion between phosphonate groups on the same trigonal face or on adjacent positions on opposite faces plays a predominant role in the formation of polymeric species. The tendency to release these electrostatic and steric interactions may induce de-coordination of one or more phosphonate groups and formation of oligomeric systems with concomitant access of water molecule(s) to the metal centre. This process is favored by the stepwise protonation of the $-PO_3^{2-}$ groups occuring at pH < 8.

We may suppose that in GdEGT2P the two methylenphosphonate groups are located in opposite positions on the two trigonal faces in order to minimize the repulsive interactions. In fact, no oligomerization processes were observed to indicate a higher stability of the system. On the other hand, the capping coordination site of the water molecule is still destabilized and the octacoordinate, $q = 0$, state is characterized by lower energy than the ninth coordinate, $q = 1$, state.

Finally, the presence of a single methylenphosphonate group allows the Gd(III) to maintain a ninth coordination site occupied by a water molecule while increasing its steric compression, which results in a extremely short residence lifetime. This finding, along with the presence of a sizeable contribution to relaxivity of second sphere water molecules, is relevant for the preparation of multimeric systems of high relaxivity optimized for use at high magnetic field strengths.[21]

Experimental

All chemicals were purchased from Sigma–Aldrich Co. and were used without purification, unless otherwise stated. NMR spectra were recorded on a JEOL ECP-400 (operating at 9.4 T). ESI mass spectra were recorded on a Waters Micromass ZQ.

Synthesis of 1,2-bis(*N*-benzyl-2-aminoethoxy)etane (1)

(1,2-bis(2-Aminoethoxy)etane) (3 ml, 20.2 mmol) in CH_2Cl_2 (5 ml) was added drop-wise (30 min) to a suspension of benzaldehyde (5.1 ml, 50.6 mmol) and Na_2SO_4 (7.2 g, 50.6 mmol) in CH_2Cl_2 (20 ml). After the addition, the reaction was stirred at room temperature overnight. The mixture was then filtered and dried under reduced pressure to obtain a yellow oil that was re-dissolved in acetonitrile (10 ml). $NaBH_4$ (1.5 g, 40.5 mmol) was added in portions at 0 °C and then the reaction was refluxed for 2 h. HCl concentrate was added slowly to the cooled solution until pH 1 is reached and then the reaction was refluxed for other 30 min. The product precipitated as a white solid, which was filtered, washed with cold diethyl ether (2 × 5 ml), and re-crystallized from H_2O. Yield: 5.5 g, 14 mmol (83%). ^{1}H NMR, CD_3OD, 400 MHz, 25 °C, δ 7.46 (m, 4H, H_{ar}), 7.45 (m, 6H, H_{ar}), 4.24 (s, 4H, CH_2Ph), 3.78 (t, $J = 5.2$ Hz, 4H, NCH_2CH_2O), 3.71 (s, 4H, CH_2O), 3.21 (t, $J = 5.2$ Hz, 4H, NCH_2CH_2O); ^{13}C NMR, CD_3OD, 100 MHz, 25 °C, δ 140.0, 128.4, 127.03 (C_{ar}), 70.6 (CH_2O), 70.4 (NCH_2CH_2O), 54.0 (CH_2Ph), 48.7 (NCH_2CH_2O). ESI-MS (*m/z*): 329.21 ($M + H^+$) (calc. 329.46).

1,2-bis(*N*-Benzyl-*N*′-*tert*-butoxycarbonylmethyl-2-aminoethoxy)etane (2)

1 (1.7 g, 4.2 mmol) and K_2CO_3 (4.1 g, 29.6 mmol) are suspended in 25 ml of anhydrous *N*-methylpyrrolidone (NMP) and stirred at room temperature for 30 min. Then, 2.5 M equivalent of tert-butyl bromoacetate (1.5 ml, 10.6 mmol) in 5 ml of NMP was added drop-wise in 45 min. The reaction was stirred at room temperature overnight and then 10 ml of H_2O was added. After extraction with diethyl ether (3 × 20 ml), the organic phase was dried over Na_2SO_4, filtered, and evaporated. The yellow oil obtained was purified by column chromatography (petrol ether/ethyl acetate 8.5/1.5; $R_f = 0.86$) to obtain a final white solid. Yield: 1.05 g, 1.9 mmol (45%). ^{1}H NMR, $CDCl_3$, 400 MHz, 25 °C, δ 7.32–7.28 (m, 10H, H_{ar}), 3.82 (s, 4H, CH_2Ph), 3.56 (t, $J = 6.1$ Hz, 4H, NCH_2CH_2O), 3.54 (s, 4H, CH_2O), 3.28 (s, 4H, NCH_2CO), 2.86 (t, $J = 6.1$ Hz, 4H, NCH_2CH_2O), 1.45 (s, 18H, $C(CH_3)$); ^{13}C NMR, $CDCl_3$, 100 MHz, 25 °C, δ 171.0 (COO-tBu), 139.4, 129.0, 128.3, 127.1 (C_{ar}), 80.8 ($C(CH_3)$), 70.4 (CH_2O), 70.2 (NCH_2CH_2O), 58.7 (CH_2Ph), 55.8 (NCH_2CO), 53.1 (NCH_2CH_2O), 28.3 ($C(CH_3)$). ESI-MS (*m/z*): 557.43 ($M + H^+$) (calc. 557.75).

1,2-bis(*N*-*tert*-Butoxycarbonylmethyl-2-aminoethoxy)etane (3)

2 (1.05 g, 1.9 mmol) was dissolved in MeOH (10 ml) and Pd/C (10%, 100 mg) was added. A solution of ammonium formate (0.5 g, 7.6 mmol) in MeOH (5 ml) was added to the stirring suspension at room temperature, and then the reaction was refluxed for 2 h. After cooling, the heterogeneous catalyst was filtered over celite and the solvent was evaporated. The oil obtained was re-dissolved in H_2O, basified with 1 M solution of NaOH, and extracted with CH_2Cl_2 (3 × 30 ml). The organic phase was then dried over Na_2SO_4, filtered, and evaporated to obtain a pale yellow oil. Yield: 0.57 g, 1.5 mmol (81%). ^{1}H NMR, $CDCl_3$, 400 MHz, 25 °C, δ 3.60 (s, 4H, CH_2O), 3.57 (t, $J = 5.2$ Hz, 4H, NCH_2CH_2O), 3.30 (s, 4H, NCH_2CO), 2.77 (t, $J = 5.2$ Hz, 4H, NCH_2CH_2O), 1.44 (t, 18H, $C(CH_3)$); ^{13}C NMR, $CDCl_3$, 100 MHz, 25 °C, δ 171.6 (*COO-t*Bu), 81.1 ($C(CH_3)$), 70.8 (CH_2O), 70.4 (NCH_2CH_2O), 51.8 (NCH_2CO), 48.8 (NCH_2CH_2O), 28.2 ($C(CH_3)$). ESI-MS (*m/z*): 377.31 ($M + H^+$) (calc. 377.50).

Ethylene glycol-bis-(2-aminoethylether)-*N*,*N*′-methyl-phosphonate-*N*,*N*′-*tert*-butylacetate (4)

Diethylphosphite (0.58 ml, 4.5 mmol) is added in 30 min to a solution of **3** (0.57 g, 1.5 mmol) in anhydrous toluene (12 ml) and under N_2. Then, a suspension of paraformaldehyde (0.2 g, 6 mmol) in 4 ml of toluene is added in 10 min and the solution

obtained is refluxed for 6 h. After cooling, the solvent is removed under reduced pressure and the oily product is purified by column chromatography (ethyl acetate/MeOH/NH_3 9.5/0.5/0.05, $R_f = 0.33$). Yield: 0.69 g, 1.04 mmol (68%). ^{1}H NMR, $CDCl_3$, 400 MHz, 25 °C, δ 4.11 (m, 8H, $POCH_2CH_3$), 3.56 (t, $J = 5.7$ Hz, 4H, NCH_2CH_2O), 3.54 (s, 4H, CH_2O) 3.51 (s, 4H, NCH_2CO), 3.19 (d, $J = 9.9$ Hz, 4H, NCH_2P), 2.96 (t, $J = 5.7$ Hz, 4H, NCH_2CH_2O), 1.42 (s, 18H, $C(CH_3)$), 1.31 (t, $J = 7.1$ Hz, 12H, $POCH_2CH_3$); ^{13}C NMR, $CDCl_3$, 100 MHz, 25 °C, δ 170.5 (*COO-t*Bu), 80.9 ($C(CH_3)$), 70.4 (CH_2O), 70.1 (NCH_2CH_2O), 62.0 ($POCH_2CH_3$), 56.8 (NCH_2CO), 55.0 (NCH_2CH_2O), 50.2 (d, $J = 162.1$ Hz, NCH_2P), 28.2 ($C(CH_3)$), 16.5 ($POCH_2CH_3$); ^{31}P NMR, CDCEGTP1, 25 °C, δ 24.3. ESI-MS (*m/z*): 677.46 ($M + H^+$) (calc. 677.73).

Ethylene glycol-bis-(2-aminoethylether)-*N,N′*-diphosphonic-*N,N′*-diacetic acid (EGT2P)

4 (0.68 g, 1 mmol) was dissolved in 5 ml of a 33% solution of HBr in acetic acid and stirred overnight at room temperature. The solvent was evaporated and the crude product was re-dissolved in acetonitrile; slow addition of excess diethyl ether led to precipitation of EGT2P as a white amorphous solid, and was isolated by centrifugation. This precipitation procedure was repeated twice obtaining analytically pure EGT2P. Yield: 0.400 g, 0.9 mmol (89%). ^{1}H NMR, D_2O, 400 MHz, 25 °C, δ 3.98 (s, 4H, CH_2O), 3.88 (t, $J = 5.2$ Hz, 4H, NCH_2CH_2O), 3.71 (s, 4H, NCH_2CO), 3.60 (t, $J = 5.2$ Hz, 4H, NCH_2CH_2O), 3.19 (d, $J = 11.3$ Hz, 4H, NCH_2P); ^{13}C NMR, D_2O, 100 MHz, 25 °C, δ 170.9 (*COOH*), 69.9 (CH_2O), 64.9 (NCH_2CH_2O), 58.1 (NCH_2CO), 55.5 (NCH_2CH_2O), 53.1 (d, $J = 124.6$ Hz, NCH_2P); ^{31}P NMR, D_2O, 25 °C, δ 6.75. ESI-MS (*m/z*): 403.13 ($M + H^+$) (calc. 403.29).

Synthesis of *N*-benzyl-1,8 diaza-3,6-dioxaoctane (5)

1,2-bis(2-Aminoetoxy)etane (10.0 g, 67.5 mmol) in CH_2Cl_2 (15 ml) was added dropwise (30 min) to a suspension of benzaldehyde (7.0 g, 67.5 mmol) and Na_2SO_4 (9.6 g, 67.5 mmol) in CH_2Cl_2 (20 ml). After the addition, the reaction was stirred at room temperature overnight. The mixture was then filtered and the solvent evaporated obtaining a yellow oil that was re-dissolved in MeOH (30 ml). $NaBH_4$ (2.5 g, 67.5 mmol) was added in small portions at 0 °C and then the reaction was refluxed for 2 h. HCl concentrate was added slowly to the cooled solution until pH 1 was reached and then the reaction was refluxed for another 30 min. The crude product precipitated as a pale yellow solid which was filtered, washed with cold diethyl ether (2 × 10 ml), dissolved in 10% solution of $NaHCO_3$, and extracted with CH_2Cl_2 (3 × 15 ml). The product was obtained as a white solid after purification by column chromatography ($CH_2Cl_2/CH_3OH/NH_3$ 9 : 1:0.1, $R_f = 0.12$). Yield 4.0 g, 16.8 mmol (56%). ^{1}H NMR, $CDCl_3$, 400 MHz, 25 °C, δ 7.31 (m, 5H, H_{ar}), 7.22 (m, 1H, NH), 3.79 (s, 4H, CH_2Ph), 3.59 (s, 6H, CH_2O), 3.48 (t, J = 5.2 Hz, 2H, CH_2O), 2.81 (m, 4H, NCH_2CH_2O); ^{13}C NMR, $CDCl_3$, 100 MHz, 25 °C, δ 140.0, 128.4, 127.0 (C_{ar}), 73.3, 70.6, 70.4 (CH_2O), 54.0 (CH_2Ph), 48.7 (Bz-$NHCH_2$), 41.7 (CH_2NH_2). ESI-MS (*m/z*): 239.12 ($M + H^+$) (calc. 239.34).

N-Benzyl-*N,N′,N′-tert*-butoxycarbonylmethyl-1,8-diaza-3,6-dioxaoctane (6)

5 (4.0 g, 16.8 mmol) and K_2CO_3 (11.6 g, 83.9 mmol) were suspended in 30 ml of anhydrous acetonitrile and stirred at room temperature for 30 min. Then, 3 M equivalents of *tert*-butyl bromoacetate (7.4 ml, 50.3 mmol) in 5 ml of acetonitrile were added drop-wise in 45 min. The reaction was stirred at room temperature overnight and then 10 ml of H_2O was added. After extraction with CH_2Cl_2 (3 × 30 ml), the organic phase was dried over Na_2SO_4, filtered, and evaporated. The crude obtained was purified by column chromatography ($CH_2Cl_2/MeOH/NH_3$ 9.5/0.5/0.05; $R_f = 0.4$) to obtain a final white solid. Yield: 3.66 g, 6.3 mmol (91%). ^{1}H NMR, $CDCl_3$, 400 MHz, 25 °C, δ 7.32–7.28 (m, 5H, H_{ar}), 3.85 (b s, 2H, CH_2Ph), 3.59 (t, J = 5.9 Hz, 4H, NCH_2CH_2O), 3.55 (s, 4H, CH_2O), 3.46 (s, 6H, NCH_2CO), 2.91 (t, J = 5.9 Hz, 4H, NCH_2CH_2O), 1.47 (s, 27H, $C(CH_3)$); ^{13}C NMR, $CDCl_3$, 100 MHz, 25 °C, δ 170.9 (COO-tBu), 139.4, 129.0, 128.4, 127.2 (C_{ar}), 80.9 ($C(CH_3)$), 70.5 (CH_2O), 70.3 (NCH_2CH_2O), 58.6 (CH_2Ph), 56.8 (NCH_2CO), 53.5, 53.1 (NCH_2CH_2O), 28.1 ($C(CH_3)$). ESI-MS (*m/z*): 571.56 ($M + H^+$) (calc. 571.78).

N,N,N′-tert-Butoxycarbonylmethyl-1,8-diaza-3,6-dioxaoctane (7)

6 (3.6 g, 6.2 mmol) was dissolved in MeOH (15 ml), and Pd/C (10%, 360 mg) was added. The mixture was introduced into a hydrogenation bottle, purged with nitrogen, and then stirred under hydrogen (1 atm.) for 4 h. The catalyst was removed by filtration on celite, the solvent was evaporated, and a pale yellow oil was obtained. Yield: 2.8 g, 5.7 mmol (92%). ^{1}H NMR, $CDCl_3$, 400 MHz, 25 °C, δ 3.58 (t, J = 5.8 Hz, 4H, NCH_2CH_2O), 3.54 (s, 4H, CH_2O), 3.42 (s, 6H, NCH_2CO), 2.89 (t, $J = 5.8$ Hz, 4H, NCH_2CH_2O), 1.46 (s, 27H, $C(CH_3)$); ^{13}C NMR, $CDCl_3$, 100 MHz, 25 °C, δ 171.0 (COO-tBu), 81.0 ($C(CH_3)$), 70.6 (CH_2O), 70.4 (NCH_2CH_2O), 56.7 (NCH_2CO), 53.6, 53.2 (NCH_2CH_2O), 28.1 ($C(CH_3)$). ESI-MS (*m/z*): 481.49 ($M + H^+$) (calc. 481.66).

Ethylene glycol-bis-(2-aminoethylether)-*N*,-methyl-phosphonate-*N,N′,N′-tert*-butylacetate (8)

Diethylphosphite (1.2 ml, 9.7 mmol) was added in 30 min to a solution of **7** (2.3 g, 4.8 mmol) in anhydrous toluene (20 ml) and under N_2. Then, a suspension of paraformaldehyde (0.3 g, 9.7 mmol) in 2 ml of toluene was added in 10 min and the solution obtained was refluxed for 4 h. After cooling, the solvent was removed under reduced pressure and the oily product was purified by column chromatography ($CH_2Cl_2/MeOH/NH_3$ 9/1/0.01; $R_f = 0.48$) Yield: 1.0 g, 1.56 mmol (42%). ^{1}H NMR, $CDCl_3$, 400 MHz, 25 °C, δ 4.14 (m, 4H, $POCH_2CH_3$), 3.77 (s, 4H, CH_2O), 3.61 (m, 4H, NCH_2CH_2O), 3.58 (s, 6H, NCH_2CO), 3.23 (d, $J = 9.9$ Hz, 2H, NCH_2P), 3.01 (m, 4H, NCH_2CH_2O), 1.46, 1.44 (s, 27H, $C(CH_3)$), 1.31 (t, $J = 7.0$ Hz, 6H, $POCH_2CH_3$); ^{13}C NMR, $CDCl_3$, 100 MHz, 25 °C, δ 170.5 (*COO-t*Bu), 81.4 ($C(CH_3)$), 70.3 (CH_2O), 70.1 (NCH_2CH_2O), 62.3 ($POCH_2CH_3$), 56.7, 55.3 (NCH_2CO), 55.2, 53.9 (NCH_2CH_2O), 50.1 (d, $J = 160.0$ Hz, NCH_2P), 28.2 ($C(CH_3)$), 16.6 ($POCH_2CH_3$); ^{31}P NMR, $CDCl_3$, 25 °C, δ 25.0. ESI-MS (*m/z*): 632.51 ($M + H^+$) (calc. 632.78).

Ethylene glycol-bis-(2-aminoethylether)-*N*-phosphonic-*N,N′,N′*-triacetic acid (EGT1P)

8 (0.65 g, 1 mmol) was dissolved in 3 ml of a 33% solution of HBr in acetic acid and stirred overnight at room temperature. The solvent was evaporated and the crude product was re-dissolved in acetonitrile; slow addition of excess diethyl ether led to the precipitation of EGT1P as a white amorphous solid, isolated by centrifugation. This precipitation procedure was repeated twice obtaining analytically pure EGT1P. Yield: 0.200 g, 0.5 mmol (50%). ^{1}H NMR, D_2O, 400 MHz, 25 °C, δ 4.32, 4.27 (s, 4H, NCH_2CO), 3.87 (s, 4H, CH_2O), 3.70–3.63 (m, 8H, NCH_2CH_2O), 3.53 (d, $J = 11.3$ Hz, 2H, NCH_2P); ^{13}C NMR, D_2O, 100 MHz, 25 °C, δ 168.4 (*COOH*),

69.9 (CH_2O), 65.1, 64.7 (NCH_2CH_2O), 56.1 (NCH_2CH_2O), 55.7, 55.4 (NCH_2CO), 51.4 (d, $J = 136.1$ Hz, NCH_2P);^{31}P NMR, D_2O, 25 °C, δ 7.82. ESI-MS (*m/z*): 367.16 ($M + H^+$) (calc. 367.32).

Synthesis of the Gd(III) complexes

The three complexes were synthesized as follows: the ligand (0.2 mmol) was dissolved in H_2O (5 ml) and the pH of the solution adjusted to 7.5 with NaOH 1N. To this solution, 3 ml of an aqueous solution of $GdCl_3$ (0.2 mmol) was added drop-wise by maintaining the pH at 7.5, again with NaOH 1N. At room temperature the complex formation was instantaneous. The pH of the solution was then increased to 8–9 by adding NaOH 1N in order to precipitate the possible excess of uncomplexed Gd(III) ions. The solution was then evaporated under reduced pressure and the residue dried at 70 °C overnight.

Proton relaxometric studies

The water proton $1/T_1$ longitudinal relaxation rates (20 MHz, 25 °C) were measured on a Stelar Spinmaster Spectrometer (Mede, Pv, Italy) on 0.5–2 mM aqueous solutions of the complexes. For the T_1 determinations the standard inversion–recovery method was used with typical 90°-pulse width of 3.5 μs, 16 experiments of 4 scans. The reproducibility of the T_1 data was estimated to be ±1%. The temperature was controlled with a Stelar VTC-91 airflow heater equipped with a copper–constantan thermocouple (uncertainty ±0.1 °C). In a typical titration experiment, several (five to eight) aqueous solutions at pH = 7.2 of the paramagnetic complex were prepared containing different concentrations of the anionic species (0–0.04 M) and the water proton relaxation rate of each solution was measured at 25 °C. The starting pH was adjusted by either HCl or KOH. Moreover, the pH of the solutions was controlled before and after the measurement. The proton $1/T_1$ NMRD profiles were measured on a fast field-cycling Stelar Spinmaster FFC relaxometer over a continuum of magnetic field strengths from 0.00024 to 0.5 T (corresponding to 0.01–20 MHz proton Larmor frequencies). The relaxometer operates under computer control with an absolute uncertainty in $1/T_1$ of ±1%. Additional data points at 20–70 MHz were obtained on a Stelar Relaxometer.

^{17}O NMR

Variable temperature ^{17}O NMR measurements were recorded on JEOL ECP-400 (9.4 T) and JEOL EX-90 (2.1 T) spectrometers equipped with a 5-mm probe and standard temperature control units. Aqueous solutions of the complexes (8–25 mM) containing 2.0% of the ^{17}O isotope (Cambridge Isotope) were used. The observed transverse relaxation rates were calculated from the signal width at half-height.

Acknowledgements

Financial support from PRIN 2005 (Grant no. 2005039914) is gratefully acknowledged. This work was carried out under the auspices of CIRCMSB and COST Action D38.

References

[1] J.-C. Bünzli, G. R. Choppin, *Lanthanide Probes in Life, Chemical and Earth Sciences*, Elsevier: Amsterdam, **1989**.
[2] J. Rocha, L. D. Carlos, *Curr. Opin. Solid State Mater. Sci.* **2003**, *7*, 199.
[3] D. Parker, *Chem. Soc. Rev.* **2004**, *33*, 156.
[4] J.-C. Bünzli, C. Piguet, *Chem. Soc. Rev.* **2005**, *34*, 1048.
[5] S. Pandya, J. Yu, D. Parker, *Dalton Trans.* **2006**, 2757.
[6] See the dedicated issue of *Chem. Soc. Rev.* **2006**, *35*, 499.
[7] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293.
[8] S. Aime, M. Botta, M. Fasano, E. Terreno, *Acc. Chem. Res.* **1999**, *32*, 941.
[9] S. Aime, A. Barge, A. Borel, M. Botta, S. Chemerisov, A. E. Merbach, U. Müller, D. Pubanz, *Inorg. Chem.* **1997**, *36*, 5104.
[10] L. A. Bromley, D. Cottier, R. J. Davey, B. Dobbs, S. Smith, *Langmuir* **1993**, *9*, 3594.
[11] M. Botta, *Eur. J. Inorg. Chem.* **2000**, 399.
[12] J. Ren, A. D. Sherry, *J. Magn. Reson.* **1996**, *B111*, 178.
[13] F. Avecilla, J. A. Peters, C. F. G. C. Geraldes, *Eur. J. Inorg. Chem.* **2003**, 4179.
[14] C. F. G. C. Geraldes, R. D. Brown 3rd, W. P. Cacheris, S. H. Koenig, A. D. Sherry, M. Spiller, *Magn. Reson. Med.* **1989;**, *9*, 94.
[15] C. F. G. C. Geraldes, A. D. Sherry, W. P. Cacheris, *Inorg. Chem.* **1989**, *28*, 3336.
[16] J. Gałezowska, R. Janicki, A. Mondry, R. Burgada, T. Bailly, M. Lecouvey, H. Kozłowski, *Dalton Trans.* **2006**, 4384.
[17] D. H. Powell, O. M. Ni Dhubhghaill, D. Pubanz, L. Helm, Y. S. Lebedev, W. Schlaepfer, A. E. Merbach, *J. Am. Chem. Soc.* **1996**, *118*, 9333.
[18] L. Helm, A. E. Merbach, *Chem. Rev.* **2005**, *105*, 1923.
[19] S. Aime, M. Botta, M. Fasano, E. Terreno, in *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, (Eds: A. E. Merbach, E. Tóth, John Wiley and Sons: Chichester, **2001**, pp 193.
[20] D. M. J. Doble, M. Botta, J. Wang, S. Aime, A. Barge, K. N. Raymond, *J. Am. Chem. Soc.* **2001**, *123*, 10758.
[21] J. B. Livramento, L. Helm, A. Sour, C. O'Neil, A. E. Merbach, E. Tóth, *Dalton Trans.* **2008**, 1195.

