New Bifunctional Contrast Agents: Bis-Amide Derivatives of C-Substituted Gd-DTPA

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In order to expand upon and confirm previous observations on the positive and adverse roles played by the functionalization and substitution of the parent MRI contrast agent Gd-DTPA, two new bi-functionalized contrast agents namely Gd-(S)-EOB-DTPA-BMA and Gd-(S)-C₄Bz-DTPA-BMA have been synthesized, purified, and characterized by multinuclear NMR studies. Water ^1H nuclear magnetic relaxation dispersion profiles were recorded at several temperatures. τ_R was determined by ^2H NMR on the diamagnetic lanthanum complex. τ_M, the water residence time, was obtained by ^{17}O NMR measurements and the stability versus transmetallation by zinc was evaluated by proton relaxometry. The residence time of water molecules in the first coordination sphere of the Gd complexes has confirmed the antagonistic effects of amide functions and C-4 substitution on the reduction of the water residence time, a critical parameter of relaxivity. This C-4 position is thus particularly appropriate for the substitution required by the design of new contrast agents covalently bound to macromolecules or carrying specific vectors.

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Introduction

The efficacy of the gadolinium complexes currently used as contrast agents in Magnetic Resonance Imaging results partly from the presence of water molecules in the first coordination sphere of the ion and from the exchange of these coordinated molecules with the bulk. An exchange rate which is too slow or too fast limits the efficacy of the contrast agent. In a previous report,[1] we have shown that Gd-DTPA derivatives carrying benzyl groups are characterized by water exchange rates which differ according to the position of the group: the complex C-substituted on an ethylenic carbon i.e. [Gd-(S)-C₄Bz-DTPA] has a faster water exchange rate and an higher relaxivity, the bis(benzylamide) (Gd-DTPA-BBzA) has a slightly higher proton relaxivity at 37 °C than the parent compound but the longer residence time of its coordinated water molecules (τ_M) clearly limits the relaxivity at low temperatures and, finally, the bis-ester derivative (Gd-DTPA-BBzE) behaves like Gd-DTPA. Another C-substituted derivative, Gd-(S)-EOB-DTPA, was also shown to be characterized by an increased water exchange rate compared with the parent compound.[2] On the contrary, amide derivatives of Gd-DTPA or Gd-DOTA are known to be characterized by slower water exchange rates than those of their parent structures.[3−5]

In this work, the influence of the C-substitution on the behavior of bis-amide derivatives was thus studied on two compounds, both substituted on the ethylenic C-4 carbon. Gd-(S)-EOB-DTPA-BMA (1) and Gd-(S)-C₄Bz-DTPA-BMA (2) (Figure 1) were synthesized and their gadolinium complexes were characterized by: i) proton relaxation rate measurements between 0.5 mT and 7.05 T (NMRD profiles), ii) estimation of their rotational correlation times through the analysis of the deuterium relaxation rates of their specifically labeled La-analogues, and iii) determination of the water residence times of their coordinated water molecules through the analysis of the temperature dependence of the ^{17}O transverse relaxation rates of water. Their stability versus transmetallation by Zn^{2+}, an important endogenous ion, was also studied. Finally, all these parameters, compared with those of parent and analogous compounds, are discussed in the context of the structure−relaxivity relationship.
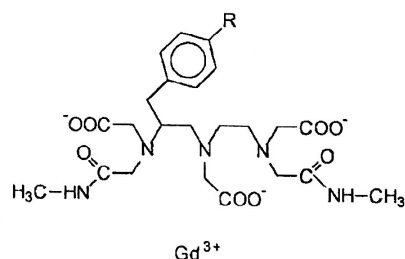
Gd-(S)EOB-DTPA-BMA 1 : R = -OC₂H₅

Gd-(S)C₄Bz-DTPA-BMA 2: R = -H

Figure 1. Chemical structures of Gd-(S)-EOB-DTPA-BMA (1) and Gd-(S)-C₄Bz-DTPA-BMA (2)

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Results and Discussion

The bis-amide complexes **1** and **2** (Figure 1) were synthesized by the classical method starting from the reaction of the pentacarboxylated ligands with acetic anhydride in the presence of pyridine to produce *C*-functionalized DTPA bis-anhydrides. The subsequent amination was carried out with an excess of methylamine.

NMRD profiles recorded at 310 K show that, in general, the relaxivities (r_1) of all the *C*-substituted derivatives are higher than those of Gd-DTPA and Gd-DTPA-BMA (Figure 2). At low magnetic fields, the relaxivities of the bis-amide derivatives of the *C*-substituted complexes are slightly lower than those of their carboxylate analogues. At higher fields, however, the relaxivities of the bis-amides **1** and **2** are similar to those of their parent compounds Gd-(*S*)-EOB-DTPA (**5**) or Gd-(*S*)-C$_4$Bz-DTPA (**6**) but still markedly higher than those of the unsubstituted structures Gd-DTPA (**3**) or Gd-DTPA-BMA (**4**). At 0.47 T (20 MHz) and 310 K, the relaxivity values of Gd-(*S*)-EOB-DTPA (**5**), Gd-(*S*)-EOB-DTPA-BMA (**1**), Gd-(*S*)-C$_4$Bz-DTPA (**6**), Gd-(*S*)-C$_4$Bz-DTPA-BMA (**2**), Gd-DTPA (**3**), and Gd-DTPA-BMA (**4**) are 5.5, 5.0, 4.8, 4.7, 3.9, and 3.8 s^{-1}·mM^{-1} respectively.
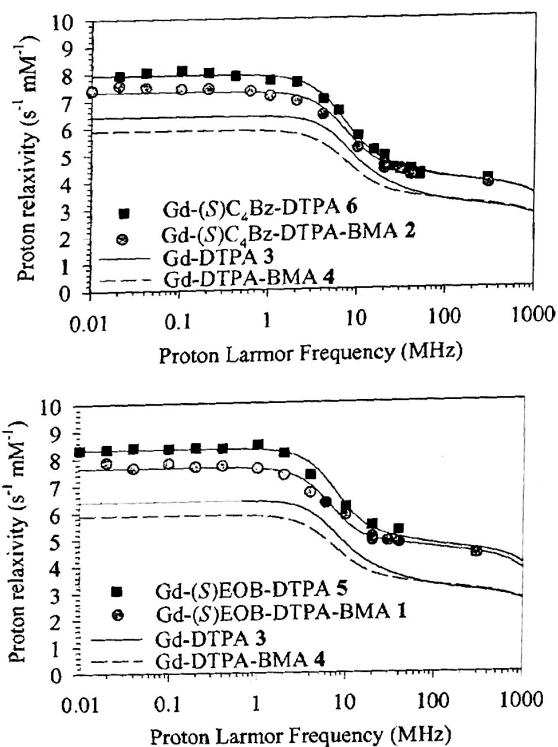

Figure 2. ^{1}H NMRD relaxivity profiles of Gd-(*S*)-C$_4$Bz-DTPA-BMA (**2**) and Gd-(*S*)-C$_4$Bz-DTPA (**6**) in water at 310 K ([Gd complex] = 1 mM) (top graph) and of Gd-(*S*)-EOB-DTPA-BMA (**1**) and Gd-(*S*)-EOB-DTPA (**5**) in water at 310 K ([Gd complex] = 1 mM) (bottom graph). The fittings of the NMRD profiles of Gd-DTPA (**3**) and Gd-DTPA-BMA (**4**) are shown for comparison. The lines correspond to the theoretical fittings of the data points.

The longitudinal relaxivity, r_1, arises from both short (inner sphere) and long (outer sphere) distance magnetic interactions. The inner sphere contribution can be described by the Solomon−Bloembergen equations,[6−7] while the outer sphere contribution can be accounted for by the Freed model[8] (see ref.[2] for the description of the models). A rather large number of structural parameters is included in those models: q the number of water molecules in the first coordination sphere, τ_M the residence time describing the exchange between coordinated and bulk water, r the distance between coordinated water protons and the unpaired electron spin, d the distance of closest approach, D the relative diffusion constant, τ_R the rotational correlation time of the hydrated complex and $\tau_{s1,2}$ the longitudinal and transverse relaxation times of the electron. These latter parameters are field dependent with τ_{SO} being the value of $\tau_{s1,2}$ at zero field and τ_v the correlation time modulating the interaction responsible of the electronic relaxation.

Therefore, the analysis of the proton NMRD curves by a multi-parametric fitting procedure takes advantage of the knowledge of some of the parameters.[2,9] In previous studies,[2,4,9−12] it has been shown that q, τ_R, and τ_M could be evaluated by specific NMR techniques and reduce the ambiguity of the analysis of the proton NMRD curves. By analogy with similar complexes [Gd-(*S*)-EOB-DTPA (**5**), Gd-DTPA-BMA (**4**) or Gd-DTPA (**3**)], q was assumed to be equal to one and d and D^{310} were set to 0.36 nm and 3.3 × 10^{-9} m^2·s^{-1} respectively.

The determination of τ_R was performed at five temperatures (from 278 K to 318 K) on the diamagnetic lanthanum complexes deuterated at the α positions of the carbonyl groups[13] through the analysis of the deuterium longitudinal rates.[2] At 310 K, the values of τ_R are fairly close {τ_R [La-(*S*)-[D$_8$]EOB-DTPA-BMA] = 63 ± 7 ps; τ_R [La-(*S*)-[D$_{10}$]EOB-DTPA] = 66 ± 7 ps; τ_R [La-(*S*)-[D$_8$]C$_4$Bz-DTPA-BMA] = 60 ± 6 ps; τ_R [La-(*S*)-[D$_{10}$]C$_4$Bz-DTPA] = 65 ± 7 ps; τ_R [La-[D$_8$]DTPA-BMA] = 67 ± 7 ps; and τ_R [La-[D$_{10}$]DTPA] = 60 ± 6 ps}. The activation energies for the rotations calculated from the evolutions of τ_R with temperature are comparable for all the complexes (E_R = 20 −24 kJ/mol).

Among the two relaxation mechanisms, i.e. the outer sphere and the inner sphere contributions, only the latter is influenced by the water residence time and can thus be quenched at low temperatures while the former always increases when the temperature is lowered. Therefore, as long as the water residence time is not limiting the propagation of the relaxation ($\tau_M < T_{1M}$ where T_{1M} is the relaxation time of the protons of the coordinated water molecule), the global relaxivity increases when temperature decreases. If this condition is not fulfilled, the global relaxivity may reach a plateau or even decrease at low temperatures. The possible influence of τ_M on the proton relaxivity was evaluated at 0.47 T over a range of temperatures between 278 K and 318 K (Figure 3). Like Gd-DTPA itself, its *C*-functionalized derivatives exhibit a continuous increase in r_1 when the temperature is lowered, revealing that τ_M does not limit the relaxation rate. On the contrary, the bis-amide

complexes Gd-DTPA-BMA **4**, Gd-(S)-C₄Bz-DTPA-BMA **2**, and Gd-(S)-EOB-DTPA-BMA **1** show a clear limitation of their relaxivity by the water exchange below 310 K.
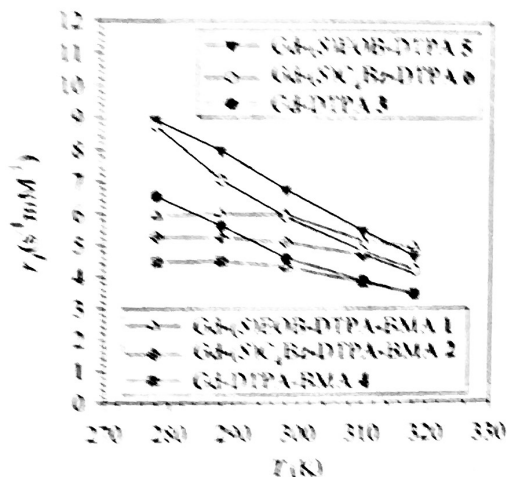

Figure 3. Temperature dependence of the proton relaxivities of the various Gd complexes at 0.47 T.

A quantitative estimation of the residence times of water molecules in the first coordination spheres of the gadolinium complexes (τ_M) was obtained from the analysis of the temperature dependence of the ^{17}O transverse relaxation rates of solutions containing 13–50 mm of the gadolinium complexes. Various parameters are involved in the theoretical fitting of the ^{17}O data: A/ℏ, the hyperfine coupling constant between the oxygen nucleus of bound water molecules and the Gd³⁺ ion; τ_V, the correlation time modulating the electronic relaxation of Gd³⁺; E_V, the activation energy related to τ_V; Δ^2, the mean-square zero field splitting energy (Δ^2 is related to τ_{SO} by $\tau_{SO} = [12 \Delta^2 \tau_V]^{-1}$); and $\Delta H^{\neq}$ and $\Delta S^{\neq}$, the enthalpy and entropy of the water exchange process. The theoretical fitting of the transverse relaxation rates obtained over a temperature range from 280 K to 350 K gives values of τ_M^{310} for Gd-(S)-EOB-DTPA-BMA (**1**) and

Gd-(S)-C₄Bz-DTPA-BMA (**2**) of 472 ± 38 ns and 429 ± 23 ns respectively. These values are between those reported for pentacarboxylate complexes [τ_M^{310} [Gd-DTPA (**3**)] = 143 ± 25 ns; τ_M^{310} [Gd-(S)-EOB-DTPA (**5**)] = 82 ± 21 ns; τ_M^{310} [Gd-(S)-C₄Bz-DTPA (**6**)] = 86 ± 8 ns] and the bis-amide parent structure (τ_M^{310} [Gd-DTPA-BMA (**4**)] = 967 ± 36 ns) (Table 1). These results are in good agreement with the temperature dependence of r_1 at 0.47 T and they confirm that the presence of amide functions on the structure of the DTPA ligand considerably decreases the exchange rate and that a conformational or steric effect, induced by substitution on the C₄ of the ligand, counteracts this unfavorable effect.

The fittings of the proton NMRD profiles were performed with the distance r fixed to the usual value of 0.31 nm for Gd-DTPA (**3**) and Gd-DTPA-BMA (**4**). For the C-functionalized complexes, such a distance results in τ_R values significantly larger than those obtained by deuterium relaxometry (τ_R^{310}= 112 ps, 91 ps, 109 ps and 84 ps for complexes **1**, **2**, **5**, and **6** respectively). Shorter effective distances (0.291 nm and 0.3 nm) were therefore used to fit the data (Table 2). For complexes **1** and **5**, τ_R values obtained for the distance r equal to 0.291 nm are in better agreement with the values obtained by deuterium relaxometry, whereas for complexes **2** and **6**, both distances (0.291 nm and 0.3 nm) can account for the experimental results. The fitted values of τ_{SO} and τ_V (Table 2) are in the same range for all the complexes and agree with those obtained by the fitting of the ^{17}O data (Table 1). At low magnetic fields, the lower relaxivity of the bis-amide C-substituted complexes **1** and **2**, compared with their parent complexes **5** and **6**, can be related to their longer τ_M values which result in a more marked limitation of the relaxivity at low rather than at high fields.

The NMRD profiles of Gd-(S)-EOB-DTPA-BMA (**1**) and Gd-(S)-C₄Bz-DTPA-BMA (**2**) at 298 K and 310 K are shown in Figure 4. The data recorded at 298 K are only slightly greater than those obtained at 310 K, confirming that τ_M is indeed limiting the relaxivity. The parameters obtained from the theoretical fitting are shown in Table 2.

Table 1. Parameters obtained from the fitting of the reduced transverse relaxation rate of ^{17}O

	Gd-(S)-EOB-DTPA-BMA (**1**)	Gd-(S)-C₄Bz-DTPA-BMA (**2**)	Gd-DTPA (**3**)	Gd-DTPA-BMA (**4**)	Gd-(S)-EOB-DTPA (**5**)	Gd-(S)-C₄Bz-DTPA (**6**)
Δ^2 (10^{20} s⁻²)	0.98 ± 0.10	0.92 ± 0.03	1.08 ± 0.03	0.85 ± 0.03	1.05 ± 0.12	1.17 ± 0.03
τ_V^{310} (ps)	16 ± 2.0	16 ± 0.4	23 ± 0.3	16 ± 0.6	17 ± 2	10 ± 0.3
|A/ℏ| (10^6 rad s⁻¹)	2.99 ± 0.23	2.80 ± 0.20	3.4 ± 0.1	3.16 ± 0.04	4.1 ± 0.5	3.19 ± 0.06
$\Delta H^{\neq}$ (kJ/mol)	43.9 ± 0.14	37.1 ± 0.07	51.5 ± 0.3	48 ± 0.05	53.5 ± 0.3	50.1 ± 0.13
$\Delta S^{\neq}$ (J/mol K)	17.6 ± 0.23	−3.54 ± 0.23	52.1 ± 0.6	24.9 ± 0.2	63 ± 1.3	51.8 ± 0.4
E_V (kJ/mol)	0.95 ± 1.44	0.90 ± 12.4	4.5 ± 4.2	15.0 ± 10.9	7.1 ± 5.9	8.7 ± 3.9
τ_M^{310} (ns)	472 ± 38	429 ± 23	143 ± 25	967 ± 36	82 ± 21	86 ± 8
τ_{SO} (ps)[a]	54 ± 27	58 ± 4	67 ± 11	56 ± 4	58 ± 14	69 ± 4

[a] Calculated from the values of Δ^2 and τ_V^{310}.

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Table 2. Values of τ_R, τ_{SO}, τ_V, and r obtained by fitting of the proton NMRD data at 310 K and 298 K

T = 310 K	τ_R (ps)	τ_M (ns)[a]	τ_{so} (ps)	τ_v (ps)	r (nm)
Gd-(S)-EOB-DTPA-BMA (1)	71 (63)[b]	472	72	16	0.291
	92	472	79	21	0.3
Gd-(S)-C$_4$Bz-DTPA-BMA (2)	61 (60)[b]	429	76	11	0.291
	70	429	86	16	0.3
Gd-DTPA (3)	57 (60)[b]	143	83	23	0.31
Gd-DTPA-BMA (4)	64 (67)[b]	967	74	12	0.31
Gd-(S)-EOB-DTPA (5)	71 (66)[b]	82	76	23	0.291
	86	82	88	28	0.3
Gd-(S)-C$_4$Bz-DTPA (6)	61 (65)[b]	86	77	14	0.291
	72	86	91	17	0.3

T = 298 K	τ_R (ps)	τ_M (ns)[a]	τ_{so} (ps)	τ_v (ps)	r (nm)
Gd-(S)-EOB-DTPA-BMA (1)	84 (86)[b]	976	69	17	0.291
	97	976	78	24	0.3
Gd-(S)-C$_4$Bz-DTPA-BMA (2)	73 (83)[b]	798	72	13	0.291
	82	798	88	15	0.3

[a] τ_M values were obtained from ^{17}O NMR. [b] The values in parentheses are those obtained by ^{2}H NMR spectroscopy on the lanthanum analogue.
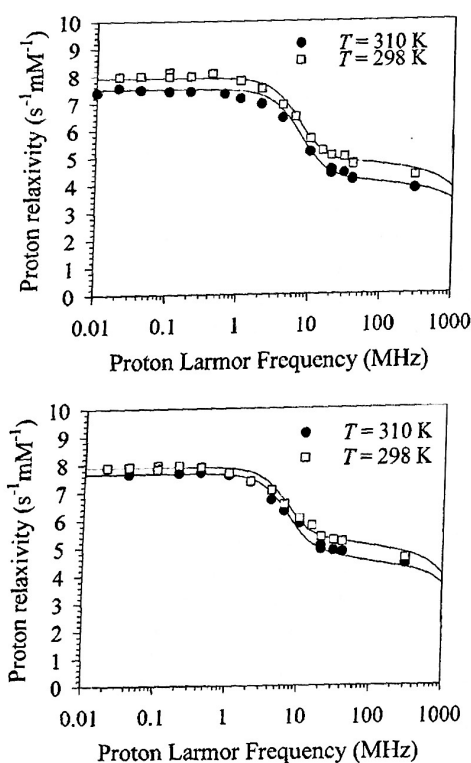

Figure 4. ^{1}H NMRD relaxivity profiles of Gd-(S)-C$_4$Bz-DTPA-BMA (2) (top graph) and Gd-(S)-EOB-DTPA-BMA (1) (bottom graph) in water at 298 K and 310 K ([Gd complex] = 1 mM). The lines correspond to the theoretical fittings of the data points

Finally, the transmetallation of the gadolinium complex by Zn^{2+} has been assessed. In a phosphate buffer (pH = 7), a precipitation of gadolinium ions expelled from the complex by Zn^{2+} occurs resulting in a decrease of the pro-

ton paramagnetic relaxation rate of the solution. The time evolution of the proton relaxation rate was monitored at 0.47 T and 310 K. Previous results showed that, on the one hand, the C-functionalized carboxylate complexes are kinetically and thermodynamically more stable than the parent compound Gd-DTPA and, on the other hand, that bis-amide derivatives are less stable.[14] For the bis-amide C-substituted compounds, the C-substitution and the bis-amide functions have opposite effects. The "kinetic index", which is the time required to reach 80 percent of the initial R_1^P value, and the "thermodynamic index", which is the R_1^P (t)/ R_1^P (0) value measured after 3 days, indeed show a faster and more extensive transmetallation of Gd-(S)-EOB-
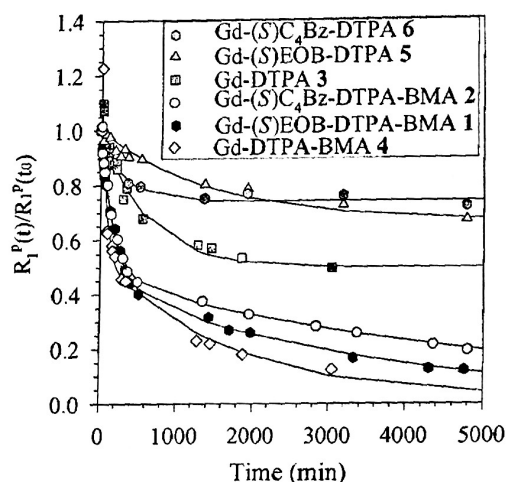

Figure 5. Evolution of the R_1^P (t)/ R_1^P (t = 0) ratio as a function of time for Gd-(S)-C$_4$Bz-DTPA (6), Gd-(S)-EOB-DTPA (5), Gd-DTPA (3), Gd-(S)-C$_4$Bz-DTPA-BMA (2), Gd-(S)-EOB-DTPA-BMA(1), and Gd-DTPA-BMA (4) (concentrations of Gd complexes and ZnCl$_2$ are 2.5 mM in a phosphate buffer of pH = 7, T = 310 K, B_0 = 0.47 T)

Table 3. Values of the kinetic and thermodynamic indexes obtained during the transmetallation process

	Kinetic index (min)[a]	Thermodynamic index (%)[b]
Gd-(S)-EOB-DTPA-BMA (1)	70	13
Gd-(S)-C₄Bz-DTPA-BMA (2)	80	22
Gd-DTPA (3)[c]	260–280	49
Gd-DTPA-BMA (4) [c]	50–60	9
Gd-(S)-EOB-DTPA (5) [c]	1500	69
Gd-(S)-C₄Bz-DTPA (6) [c]	300	73

[a] The kinetic index is defined here as the time required to attain 80 % of the initial value of R_1^P. [b] The thermodynamic index is defined here as the ratio between R_1^P at 4320 min and the initial R_1^P. [c] From ref.[14].

DTPA-BMA (1) and Gd-(S)-C₄Bz-DTPA-BMA (2) than that observed for the parent compound Gd-DTPA (3). Only a slight stabilization was observed with respect to the unsubstituted bis-amide derivative (Figure 5 and Table 3).

Conclusion

Two new bi-functionalized compounds, Gd-(S)-EOB-DTPA-BMA (1) and Gd-(S)-C₄Bz-DTPA-BMA (2), have been successfully synthesized. The τ_M values obtained by ^{17}O NMR spectroscopy confirm the lengthening and adverse effect of the amide functions and demonstrate the beneficial effect of a C-4 substitution on the reduction of the water residence time. As assessed by the Zn^{2+} transmetallation process, the two new bis-amides are less stable than Gd-DTPA and slightly more stable than Gd-DTPA-BMA (4). In conclusion, although more difficult to synthesize, C-4-substituted DTPA complexes are better suited for the covalent binding to macromolecules or to specific vectors in the context of the design of new specific contrast agents for molecular imaging.

Experimental Section

General

Chemicals: Gd-DTPA (3), Gd-(S)-EOB-DTPA (5), and the ligand EOB-DTPA were provided by Schering AG (Berlin, Germany). The ligand (S)-C₄Bz-DTPA and the corresponding gadolinium complex were obtained as described earlier.[1] The organic reactants and intermediates were purchased from Aldrich (Bornem, Belgium). NMR Spectroscopy: ¹H and ¹³C NMR spectra were recorded with a Bruker AMX-300 instrument (Bruker, Karlsruhe, Germany). Chemical shifts (in ppm) are referred to the solvent peak. For ¹³C NMR, the methyl signal of *tert*-butanol was used as reference (δ = 31.2 ppm). Mass Spectrometry: Mass spectra (LSIMS) were obtained with a VG AUTOSPEC mass spectrometer (VG Analytical, Manchester, UK). Samples were dissolved in water and deposited in a glycerol matrix. Chromatography: High-performance liquid chromatography (HPLC) was performed with a Waters 600 multisolvent delivery system equipped with a Rheodyne injection valve (20μL loop) and controlled by the Millenium software (Waters, Milford, USA). A Novapak C18 column (4.56 mm × 150 mm) was used. Elution was performed with a linear gradient of pure 0.05 M triethylammonium acetate (pH = 6) to 100 % methanol at a flow rate of 1 mL/min for 20 min. A fluorescence detector was used to monitor the elution of the gadolinium complex. Relaxometry: Proton Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were recorded with a Field Cycling Relaxometer working between 0.02 and 50 MHz. Additional points at 20 and 300 MHz respectively were obtained on a Bruker Minispec PC-20 thermostatically controlled by a tetrachloroethylene flow and with a Bruker AMX 300 spectrometer. Fitting of the ¹H NMRD curves was performed with previously described software using minimization routines (Minuit, CERN Library).[15,16] The deuterium and ¹⁷O measurements were carried out with 2 mL samples contained in 10-mm o.d. tubes with a Bruker AMX-300 spectrometer equipped with a broadband probe. The field homogeneity was optimized on the proton free induction decay using the decoupling coil. The temperature was regulated by air or nitrogen flow (Bruker BVT-2000 unit). Deuterium longitudinal relaxation rates were measured using an inversion recovery Fourier transform sequence on solutions containing the labeled diamagnetic lanthanum complex dissolved in deuterium depleted water (Aldrich, Bornem, Belgium). The experimental data were fitted with a three-parameter minimization routine. Diamagnetic transverse relaxation times of ¹⁷O water (pH ≈ 6.5) were measured at natural abundance using a Carr−Purcell−Meiboom−Gill sequence and a two-parameter fit of the data. ¹⁷O transverse relaxation times of water (natural abundance) in solutions containing paramagnetic complexes were calculated directly from the linewidth. All ¹⁷O NMR spectra were proton decoupled. The theoretical fitting of the data was performed as described previously.[1,2,9]

Synthesis of Ligands

3,6,9-Tris(carboxymethyl)-4-ethoxybenzyl-1,11-bis(methylamino)-3,6,9-triazaundeca-1,11-dione [(S)-EOB-DTPA-BMA (1)]: A mixture of (S)-EOB-DTPA (0.4 g), acetic anhydride (0.6 mL), and pyridine (0.5 mL) was heated at 70 °C for 24 h. The suspension was cooled, a 2 M methylamine (methanol solution) was added (3 mL) and the mixture was stirred for around 24 h. After addition of methanol (50 mL), the mixture was filtered. The filtrate was evaporated to dryness under reduced pressure. Water (5 mL) was then added and the pH was adjusted to 2 with 2 N HCl. The solution was passed through a DOWEX AG 50 W-X8 (H⁺ form) cation-exchange resin which was first eluted with water and the product was recovered from the resin by elution with 2 N aqueous ammonia. The residue obtained after evaporation of the solution was dissolved in acetic acid and precipitated with ethyl acetate. The crude product was recovered by filtration. Yield: 0.16 g (38 %). ¹H NMR (D₂O): δ = 6.9–6.7 (4 H, AA′BB′, Ph), 3.8 (q, 2 H, CH₂), 3.6–2.8 (m, 19 H, 8 × CH₂, CH), 2.5 (s, 3 H, CH₃), 2.45 (s, 3 H, CH₃), 1.1 (t, 3 H, CH₃) ppm. ¹³C NMR (D₂O): δ = 178.1, 171.8, 171.2, 170.6, 170.4, 166.1, 114.2, 128.9, 130.4, 65.1, 62.5, 61.1, 58.9,

57.3, 57.2, 56.5, 55.1, 54.7, 52.3, 51.8, 26.4, 26.3, 14.3 ppm. LSIMS: [M + H]⁺: 554⁺. HPLC: 13.3 min.

4-Benzyl-3,6,9-tris(carboxymethyl)-1,11-bis(methylamino)-3,6,9-triazaundeca-1,11-dione [(S)-C₄Bz-DTPA-BMA (2)]: This compound was obtained from (S)-C₄Bz-DTPA[1] as described for (S)-EOB-DTPA-BMA above. Yield: 0.142 g (32 %). ¹H NMR (D₂O): δ = 7.3–7.0 (m, 5 H, Ph), 3.45–2.6 (m, 19 H, 9 × CH₂, CH), 2.5 (s, 3 H, CH₃), 2.45 (s, 3 H, CH₃) ppm. ¹³C NMR (D₂O): δ = 177.9, 171.9, 171.3, 170.6, 170.3, 138.8, 128.6, 128.3, 125.8, 62.4, 61.0, 58.8, 57.2, 57.1, 56.6, 55.2, 54.6, 52.4, 51.7, 26.4, 26.3 ppm. LSIMS: [M + H]⁺: 536⁺. HPLC: 12.5 min.

Complexation: The La(III) or Gd(III) complexes were prepared by mixing aqueous solutions of equimolar amounts of hydrated LaCl₃ or GdCl₃ and the ligand. The pH was adjusted to between 6.5 and 7. The absence of free La or Gd ions was checked with xylenol orange indicator. The complexes were passed through a Sep-Pak column (Waters, Accell Plus QMA Cartridges) and the eluted solutions were freeze-dried. The purities of the chelates were verified by HPLC and their masses confirmed by LSIMS.

Deuteration: Deuteration of the ligands [(S)-EOB-DTPA-BMA and (S)-C₄Bz-DTPA-BMA] or of the lanthanum complexes at the α position of the carboxylic groups was performed by the procedure described by Wheeler and Legg.[13] The ligand or complex (4 mmol) was dissolved in D₂O (40 mL), the pD was adjusted to 10.6 by addition of K₂CO₃, and the mixture was heated to reflux for 24 h with stirring. The pD was then adjusted to 2 (ligand solution) or 6 (lanthanum complex solution) with concentrated hydrochloric acid, the solution was evaporated to a final volume of 10 mL and the solid KCl was removed by filtration. Acetone was added to induce precipitation of the deuterated compounds, which were recovered by filtration, dissolved in H₂O and isolated after lyophilization. The deuteration (> 95 %) was confirmed by ¹H NMR spectroscopy.

(S)-[D₄]EOB-DTPA-BMA: NMR (D₂O, pH = 10): δ = 6.9–6.7 (4 H, AA′BB′, Ph), 3.8 (q, 2 H, CH₂), 3.6–2.8 (m, 11 H, 5 × CH₂, CH), 2.5 (s, 3 H, CH₃), 2.45 (s, 3 H, CH₃), 1.1 (t, 3 H, CH₃) ppm.

(S)-[D₄]C₄Bz-DTPA-BMA: NMR (D₂O, pH = 10): δ = 7.4–7.1 (m, 5 H, Ph), 3.45–3.0 (m, 5 H, 2 × CH₂, CH), 2.9–2.6 (m, 6 H, 3 × CH₂), 2.5 (s, 3 H, CH₃), 2.45(s, 3 H, CH₃) ppm.

Kinetics of the Transmetallation: The technique is based on the evolution of the paramagnetic proton longitudinal relaxation rate (R_1) of a phosphate buffer solution (pH, 7, [H₂PO₄⁻] + [HPO₄²⁻] + [PO₄³⁻] = 67 mM) containing 2.5 mM of gadolinium complex

and 2.5 mM of ZnCl₂.[14] The measurements were performed with a Bruker Minispec PC 120 (20 MHz) spin analyzer at 310 K.

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