Synthesis, Variable Temperature and Pressure ^{17}O NMR Study of Bis(alkylamide) Derivatives of $[(Gd\text{-}DTPA)(H_2O)]^{2-}$ – An Assessment of the Substitution Effect on Water Exchange Kinetics

François Botteman,[a] Gaëlle M. Nicolle,[b] Luce Vander Elst,[a] Sophie Laurent,[a] André E. Merbach,[b] and Robert N. Muller*[a]

Keywords: Water exchange / MRI contrast agents / DTPA-bis(amide) derivatives / Substituent effects / ^{17}O

In this work, primary, secondary and tertiary bis(amide) derivatives of DTPA have been synthesized and the residence time of the water molecule coordinated to their gadolinium complexes has been measured by variable temperature ^{17}O NMR spectroscopy. The influence of substituent characteristics (molecular mass, hydrophobicity, volume) has been studied. For the primary and secondary bis(amide) complexes, increasing the length of the lateral chains decreases the water residence time. It varies from 1171±38 ns for [Gd(DTPA-BA)(H_2O)] to 673±32 ns for [Gd(DTPA-BHeptA)(H_2O)]. The activation volumes of 5 bis(amide) complexes {[Gd(DTPA-BA)(H_2O)], [Gd(DTPA-BEA)(H_2O)], [Gd(DTPA-BnBA)(H_2O)], [Gd(DTPA-BAA)(H_2O)], and [Gd(DTPA-BHA)(H_2O)]} have been measured by a variable pressure ^{17}O NMR spectroscopic study. No change in the activation volume is observed when the substituent size is increased. The inner sphere is therefore not disturbed by the substitution on the amide groups. The slight decrease in water residence time can be attributed to the hydrophobicity increase of the complexes which, in turn, leads to a hydrophobic environment favorable to water molecule exchange. The water residence time of tertiary bis(amide) complexes is similar to the fastest secondary bis(amide) complex. For the tertiary bis(amide) complexes, a length increase by the substituent does not change the residence time. According to these results, we suspect that a disruption of the hydration shell of the complexes by amide substituents occurs that can influence coordinated water exchange by a factor of two, at most.

Introduction

Most of the contrast agents used in Magnetic Resonance Imaging (MRI) are paramagnetic complexes of gadolinium. Their efficiency, expressed by their relaxivity, r_1 (water proton relaxation rate enhancement induced by 1 millimole per liter of paramagnetic complex), depends on water exchange kinetics, electronic relaxation, and molecular tumbling, as well as on the number of water molecules in the first coordination sphere of the gadolinium cation (q). The influence of these parameters has been extensively described and the ways in which to modify some of them, for instance the rotational correlation time of the complex (τ^r) and the number q, are well known.[1] By comparison, the strategies towards changing the electronic relaxation times (τ_{Si}, $i = 1, 2$) and the residence time of the water-coordinated molecule (τ_M) are poorly understood.

[a] NMR Laboratory, Department of Organic Chemistry, University of Mons-Hainaut,
7000 Mons, Belgium
Fax: (internat.) + 32-65/373520
E-mail: robert.muller@umh.ac.be
[b] Institute of Molecular and Biological Chemistry, Swiss Federal Institute of Technology, EPFL-BCH,
1015 Lausanne, Switzerland

τ_M is not a limiting factor for the relaxivity of commercially available T_1 low molecular weight agents, like Magnevist® (Schering, Berlin, Germany), Omniscan® (Amersham Health, Amersham, United Kingdom), Dotarem® (Guerbet, Aulnay, France) or Prohance® (Bracco, Milan, Italy), at physiological temperatures and magnetic fields normally used in MRI, but this parameter may play an important role when covalent or noncovalent coupling to a macromolecule is involved.[2,3] Indeed, in order to improve the relaxivity, high molecular weight contrast agents have been developed. To reach optimal relaxivity, the residence time must, however, fulfil the condition: $T_{1M} > \tau_M > \tau_{Si}$, τ_R, where T_{1M} is the longitudinal relaxation time of nuclei in the first coordination sphere. The adjustment of τ_M is therefore of paramount importance.

Up to now, it has been reported that the water exchange rate of monoaquagadolinium chelates decreases by several orders of magnitude as compared to the aqueous ion.[4] Micksei et al. showed that the water exchange rate of $[Gd(DTPA)(H_2O)]^{2-}$ is about 200 times slower than that for aqueous gadolinium $[Gd(H_2O)_8]^{3+}$.[5] The presence of amide groups further decreases the exchange rate of Ln[III]-DTPA derivatives.[6–9] The determination of water exchange rates for a set of lanthanide complexes of DTPA-

BMA (from Nd^{3+} to Ho^{3+}) has shown that the exchange rate increases when the ionic radius of the lanthanide decreases.[10] These two latter observations show the importance of steric hindrance in the coordination cage on the water exchange rate. Furthermore, the charge has also been invoked as a factor affecting residence times which are particularly long, as for $[Gd(DTMA)(H_2O)]^{3+}$ (τ_M= 17 μs at 278 K),[11] or short, as for $[Gd(PCTP\text{-}[13])(H_2O)]^{3-}$ (τ_M = 8 ns at 278 K).[12] Further substitution of the DTPA backbone on the ethylene bridge decreases the residence time by about 30% as compared to $[Gd(DTPA)(H_2O)]^{2-}$ at 310 K as is the case for the Gd^{III} chelates of EOB-DTPA and MS-325.[13,14]

From the literature, no simple relationship can be clearly established between the structure of Gd complexes and water exchange kinetics. To unravel some relationship, we have studied a series of equally charged but diversely amide-substituted chelates of Gd-DTPA bis(amide) derivatives (Table 1). Proton and oxygen-17 relaxometry at variable temperatures and pressures have been carried out to analyze the influence of the lengthening of the amide substituent on the water residence time.

Results and Discussion

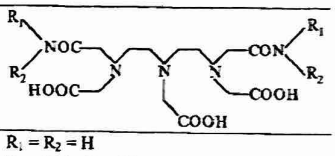
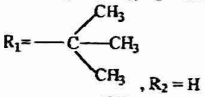
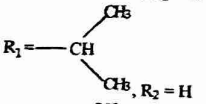
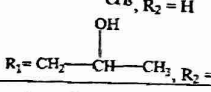
Primary and Secondary Bis(amide) DTPA Gd^{III}-Chelates

The proton relaxivity of primary and secondary chelates measured at 20 MHz and 310 K is given in Table 2. The data for the variable temperature study is presented in Figure 1. The proton longitudinal relaxivity reflects the molecular mass increase of the complexes as expected by the Solomon–Bloembergen theory.[15,16] The relaxivity is the sum of inner- and outer-sphere contributions. The outer-sphere relaxation is not influenced by the residence time of water and increases when the temperature decreases due to the reduction of the water molecule translational diffusion. On the contrary, the inner-sphere contribution depends on the water residence time as expressed in Equations (1) and (2).

When the water residence time is short as compared to T_{1M} ($\tau_M << T_{1M}$), the inner-sphere contribution increases and the total relaxivity is enhanced when the temperature decreases, as for $[Gd(DTPA)(H_2O)]^{2-}$.[17] If the water exchange is slow, the inner-sphere contribution decreases when the temperature is reduced, as is the case for [Gd(DTPA-BMA)(H_2O)]. As a result, the total relaxivity may remain approximately constant or decrease at low temperature. The proton relaxivity of all the bis(amide) complexes studied in this work is clearly limited at low temperatures and reflects a long water residence time (Figure 1).

For the determination of the residence time from ^{17}O NMR spectroscopic measurements, the experimental ^{17}O NMR spectroscopic data were fitted assuming q = 1 (the temperature dependence of the logarithm of the ^{17}O reduced transverse relaxation rates is shown in Figure 2 for four of the studied complexes). This assumption was supported by the values of the hyperfine-coupling constant ($A/\hbar$) and relaxivities that are similar to those measured for DTPA complexes with one coordinated water molecule. Table 2 shows the parameters obtained by the fitting of the experimental points. Since the ^{17}O transverse relaxation rates have been measured at one magnetic field (7.05 T in this study), the electronic parameters obtained are relatively

Table 1. Structures of the ligands

	Bis(amide) derivative	Ligand abbreviation	R_1
			
1	*Bis(amide)*	DTPA-BA	$R_1 = R_2 = H$
2	*Bis(methylamide)*	DTPA-BMA	$R_1 = CH_3$, $R_2 = H$
3	*Bis(ethylamide)*	DTPA-BEA	$R_1 = CH_2CH_3$, $R_2 = H$
4	*Bis(n-propylamide)*	DTPA-BnPA	$R_1 = CH_2CH_2CH_3$, $R_2 = H$
5	*Bis(n-butylamide)*	DTPA-BnBA	$R_1 = CH_2CH_2CH_2CH_3$, $R_2 = H$
6	*Bis(amylamide)*[a]	DTPA-BAA	$R_1 = CH_2CH_2CH_2CH_2CH_3$, $R_2 = H$
7	*Bis(hexylamide)*	DTPA-BHA	$R_1 = CH_2CH_2CH_2CH_2CH_2CH_3$, $R_2 = H$
8	*Bis(heptylamide)*	DTPA-BHeptA	$R_1 = CH_2CH_2CH_2CH_2CH_2CH_2CH_3$, $R_2 = H$
9	*Bis[bis(methylamide)]*	DTPA-BBMA	$R_1 = CH_3$, $R_2 = CH_3$
10	*Bis[bis(ethylamide)]*	DTPA-BBEA	$R_1 = CH_2CH_3$, $R_2 = CH_2CH_3$
11	*Bis[bis(n-propylamide)]*	DTPA-BBnPA	$R_1 = CH_2CH_2CH_3$, $R_2 = CH_2CH_2CH_3$
12	*Bis(t-butylamide)*	DTPA-BtBA	 , $R_2 = H$
13	*Bis(i-propylamide)*	DTPA-BiPA	 , $R_2 = H$
14	*(R,S)*-Bis(2-*hydroxypropylamide*)	DTPA-(R,S)BHPA	 , $R_2 = H$

[a] The abbreviation BAA [*Bis(amylamide)*] for the pentyl derivative has been selected to avoid confusion with the propyl derivative (BnPA)

Table 2. Parameters obtained from the fitting of experimental ^{17}O NMR spectroscopic data at variable temperature and proton longitudinal relaxivity of the studied bis(amide) complexes

	Complexes	r_1 [s^{-1} mM^{-1}] (310 K at 20 MHz)	τ_M^{310} [ns]	$\Delta H^{\neq}$ [kJ mol^{-1}]	$\Delta S^{\neq}$ [Jmol^{-1} K^{-1}]	$A/\hbar$ [10^6 rad s^{-1}]	B [10^{20} s^{-2}]	τ_v [ps] (298 K)
	$[Gd(DTPA)(H_2O)]^{2-}$ [a]	3.8	143±25	51.5±0.3	52.1±0.6	−3.4±0.1	2.60±0.06	12.3±0.3
1	$[Gd(DTPA\text{-}BA)(H_2O)]$	3.6	1171±38	43.3±0.1	8.2±0.1	−2.8±1.5	2.45±0.07	36.5±1.2
2	$[Gd(DTPA\text{-}BMA)(H_2O)]$[b]	3.8	967±36	48.0±0.1	24.9±0.2	−3.2±0.1	2.04±0.06	21.2±0.6
3	$[Gd(DTPA\text{-}BEA)(H_2O)]$	3.7	927±31	46.8±0.1	21.2±0.1	−2.8±1.3	2.73±0.10	39.2±1.4
4	$[Gd(DTPA\text{-}BnPA)(H_2O)]$	4.0	845±35	47.2±0.1	23.4±0.2	−2.8±0.1	2.09±0.18	24.8±2.1
5	$[Gd(DTPA\text{-}BnBA)(H_2O)]$	4.4	713±31	48.5±0.1	29.2±0.2	−3.4±0.2	2.03±0.21	17.1±1.9
6	$[Gd(DTPA\text{-}BAA)(H_2O)]$	4.3	575±29	48.4±0.1	20.4±0.3	−3.6±0.1	1.96±0.17	14.8±1.3
7	$[Gd(DTPA\text{-}BHA)(H_2O)]$	4.5	681±23	45.7±0.1	20.5±0.2	−2.8±1.6	1.37±0.05	25.2±0.8
8	$[Gd(DTPA\text{-}BHeptA)(H_2O)]$	4.5	673±32	45.7±0.1	20.5±0.2	−3.0±0.3	2.27±0.14	17.9±1.1
9	$[Gd(DTPA\text{-}BBMA)(H_2O)]$	3.8	624±44	48.9±0.1	31.5±0.4	−3.5±0.1	3.46±0.31	25.9±2.4
10	$[Gd(DTPA\text{-}BBEA)(H_2O)]$	3.9	654±78	48.3±0.1	29.2±0.6	−3.3±0.4	2.00±0.16	18.5±1.5
11	$[Gd(DTPA\text{-}BBPA)(H_2O)]$	4.5	672±38	42.3±0.1	9.4±0.2	−3.0±0.3	1.67±0.28	22.2±3.8
12	$[Gd(DTPA\text{-}BiPA)(H_2O)]$	4.1	654±29	52.6±0.1	42.9±0.1	−4.5±1.4	3.34±0.31	15.2±1.3
13	$[Gd(DTPA\text{-}BtBA)(H_2O)]$	4.2	659±27	44.2±0.1	15.8±0.2	−3.0±0.1	2.56±0.08	35.9±1.2
14	$[Gd(DTPA\text{-}(R,S)BHPA)(H_2O)]$	4.5	1128±95	43.2±0.1	8.1±0.3	−2.8±1.1	1.58±0.15	26.7±3.2

[a] See ref.[15] [b] The reduced ^{17}O transverse relaxation rates measured at variable temperature have been refitted with the equations used in this study.

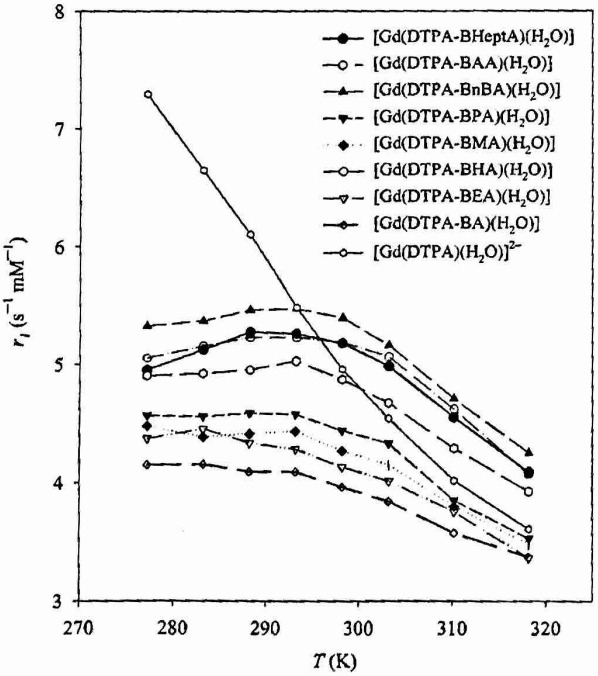


Figure 1. Temperature dependence of the proton relaxivity of linear bis(amide) complexes of $[Gd(DTPA)(H_2O)]^{2-}$ and of the parent compounds at 20 MHz

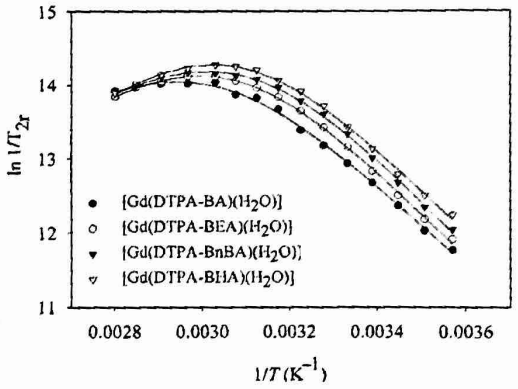


Figure 2. Temperature dependence of the reduced transverse ^{17}O relaxation rate for four linear bis(amide) complexes at 7.05 T; the straight lines represent the fitted curves

less reliable. Nevertheless, this is not a problem for the determination of the water residence time, which is nearly unaffected by the value of the electronic parameters. Table 2 indicates that the substituent size (or the substituent mass in Figure 3) has an effect on the water residence time: the larger the substituent, the faster the water exchange rate. The energy difference between the initial state and the transition state of the exchange seems thus to be reduced for a larger substituent. Steric hindrance can be invoked since it could reduce the energy necessary to reach the transition state assumed to have identical energy whatever the complex.

The ^{17}O transverse relaxation rate of the paramagnetic solutions, $1/T_2^r$, and of the acidified water reference, $1/T_2^{dia}$, were also measured at high pressure. This allows the calculation of the reduced transverse relaxation rate as described above in the variable temperature section. For the fitting of the high pressure data, T_{2M} was then fixed to the value calculated at 400 MHz with the parameters obtained at 300 MHz in the variable temperature study using Equations (7)−(10). The pressure dependence of $1/T_2^r$ at 298.15 K is presented in Figure 4 for five of the studied complexes. The decrease of $1/T_2^r$ as pressure increases, indicates a slowing down of the water exchange process, which has been checked to be reversible for all the studied Gd^{III} chelates. The fitted parameters are given in Table 3. The replacement of two carboxylic groups by two amide groups induces a nearly twofold decrease in the activation volume, as seen by

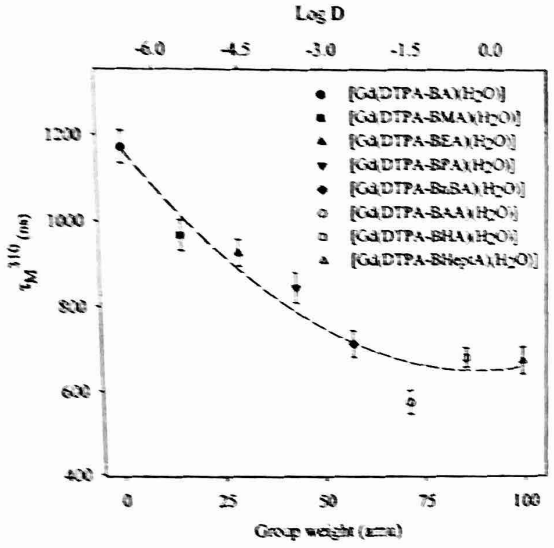


Figure 3. Dependence of the water residence time for linear bis(amide) complexes as a function of the group mass and of the hydrophobicity of the ligand (log D)

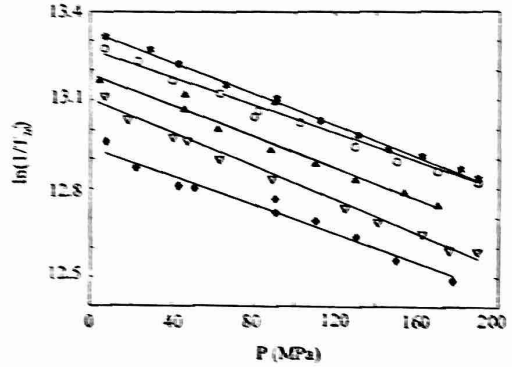


Figure 4. Pressure dependence of the reduced transverse ^{17}O relaxation rate at 298.15 K and 9.4 T for $[Gd(DTPA\text{-}BA)(H_2O)]$ (black diamonds), $[Gd(DTPA\text{-}BEA)(H_2O)]$ (white triangles), $[Gd(DTPA\text{-}BnBA)(H_2O)]$ (black triangles), $[Gd(DTPA\text{-}BAA)(H_2O)]$ (black stars) and $[Gd(DTPA\text{-}BHA)(H_2O)]$ (white circles);the straight lines represent the fitted curves

comparing $[Gd(DTPA\text{-}BA)(H_2O)]$ to $[Gd(DTPA)(H_2O)]^{2-}$. For the $[Gd(DTPA\text{-}BA)(H_2O)]$ complex, the incoming water molecule is therefore more implicated in the water exchange process than it is for the $[Gd(DTPA)(H_2O)]^{2-}$ complex. The decrease of the water exchange rate constant for $[Gd(DTPA\text{-}BA)(H_2O)]$ compared to $[Gd(DTPA)(H_2O)]^{2-}$, by one order of magnitude, therefore is not interpretable in terms of activation volume but could be attributed to the difference between their entropy of activation (see Table 2). The variation of the substituent site, on going from the methyl to the hexyl group, does not affect the value of the activation volume within the experimental errors. The activation volumes are in accordance with the one obtained for the $[Gd(DTPA\text{-}BMA)(H_2O)]$ complex in a previous study ($< 7.5\ cm^3\ mol^{-1}$) and indicates a dissociative interchange I_d mechanism.[4] The activation volume is therefore independent of the nature of the alkyl substituent. This shows in turn that the substituent does not have a major influence on the first coordination sphere nor on its steric crowding.

As stated above, the similar values obtained for the activation volumes for the series have revealed that the inner sphere is not affected by the substitution on the amide group. This result is in agreement with the available crystallographic data of various Gd-DTPA-bis(amide) complexes.[18–21] In fact, it shows that as compared to $[Gd(DTPA)(H_2O)]^{2-}$, the formation of an amide bond does not markedly modify the distances between Gd^{III} and its coordinates (Table 4). A folding of lateral chains in solution, however, is not excluded. The decrease of the residence time when the lateral chain is lengthened could be explained by such a folding due to the hydrophobic repulsion between the complex and the bulk. This phenomenon could disrupt the hydration shell of the complexes and favor the water exchange by modifying the hydrogen bonds between the exchanging water molecule and the hydration shell. For instance, the presence of a hydroxy group in $[Gd(DTPA\text{-}(R,S)BHPA)(H_2O)]$ causes an increase of about 30% of the water residence time (Table 2) as compared to $[Gd(DTPA\text{-}BnPA)(H_2O)]$. Furthermore, this folding occurs outside of the coordination cage and this could thus explain why the

Table 3. Parameters obtained from the fit of the high pressure ^{17}O NMR spectroscopic data; the high pressure study has been performed at 9.4 T and at $T = 298.15$ K if not stated otherwise; the values in parentheses are the water exchange rate constants obtained from the fit of variable temperature ^{17}O NMR spectroscopic data at 298.15 K; T_{2M} was fixed for the fit

Gd complexes	$(k_{ex})^{298}/10^6\ s^{-1}$ variable P	$\Delta V^{\neq}/cm^3mol^{-1}$	$T_{2M}/10^{-7}$ s
$[Gd(DTPA)(H_2O)]^{2-}$ [a]	1.37	+12.5±0.2	–
$[Gd(DTPA\text{-}BA)(H_2O)]$	0.44±0.01 (0.42)	+6.3±0.4	1.30
$[Gd(DTPA\text{-}BMA)(H_2O)]$[20]	0.206	+7.3±0.2	–
$[Gd(DTPA\text{-}BEA)(H_2O)]$	0.52±0.01 (0.49)	+7.3±0.2	1.36
$[Gd(DTPA\text{-}BnBA)(H_2O)]$	0.58±0.01 (0.64)	+7.0±0.4	1.54
$[Gd(DTPA\text{-}BAA)(H_2O)]$	0.68±0.01 (0.78)	+7.1±0.2	1.54
$[Gd(DTPA\text{-}BHA)(H_2O)]$	0.62±0.01 (0.71)	+6.5±0.3	1.14

[a] $T = 291.2$ K.[18] [b] $T = 285.6$ K.[5]

Table 4. Distances obtained from crystallographic data

Average distances [Å] Gd–N	Gd–O_{amide}	Gd–$O_{carboxylate}$	Gd–O_{water}	Complexes
2.64	–	2.40	2.49	$Na_2[Gd(DTPA)(H_2O)]$[a]
2.61–2.74	2.33–2.42		–	[Gd(DTPA-BMA)(H_2O)][b]
2.67	2.44	2.38	2.44	[Gd(DTPA-BBA)(H_2O)][c]
2.70	2.39	2.36	2.42	[Gd(DTPA-BEA)(H_2O)][d]

[a] From ref.[18] [b] From ref.[21] [c] BBA = *Bis*(*benzyl*amide); from ref.[20] [d] From ref.[19]

activation volume is not affected by the nature of the substituent.

In order to characterize the hydrophobicity of our bis(amide) complexes carrying aliphatic linear chains, the log D coefficient of the corresponding ligands was used.[22] At a fixed pH, this log D coefficient reflects the hydrophobicity for the different ligands in solution and takes into account the contribution of ionizable groups. For the calculation of log D, the pH was set to 12 since the ligand structure is similar to the one in the complex (the carboxylate groups and nitrogen atoms are not protonated). The calculated hydrophobicity of bis(amide) ligands is reported in Figure 3. The residence time decreases with increasing hydrophobicity and remains quite similar for larger groups. This plateau could be explained due to the fact that only the first carbon atoms of the chain disturb the hydration shell that contributes to the exchange process.

The influence of the substituent volume on the water residence time was also investigated. The volumes of 4 substituents (R_1 = hydrogen, methyl, isopropyl, *tert*-butyl) were calculated using the van der Waals radii of atoms. The volumes of linear chains were not calculated because their flexibility does not allow a correct description of their volumes. The evolution of the residence times shown in Figure 5 is similar to the one observed in Figure 3. The measured values for [Gd(DTPA-BiPA)(H_2O)] and [Gd(DTPA-BtBA)(H_2O)] (Table 2) confirm a fact previously observed in this study: the only hindrance arising from the first carbon atoms of the substituent leads to a shortening of the residence time.

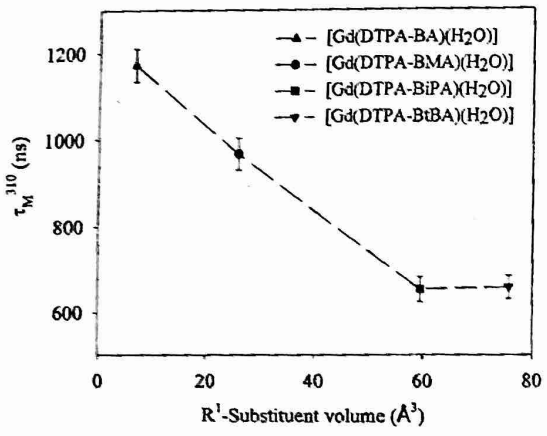


Figure 5. Volume substituent dependence of the residence time for bis(amide) complexes

An alternative explanation can be proposed. The bis(alkylamide) DTPA derivatives present four diastereoisomers in solution.[23] It has been shown for the complex $[Eu(DOTAM)(H_2O)]^{3+}$ that the diastereoisomers have different exchange rates.[24] Recently, the study of interconversion mechanisms between the diastereoisomers proved that the global value of the residence time was mainly fixed by the minor stereoisomer.[25] The exchange rates measured for the bis(alkylamide) derivatives are therefore averages weighted by the molar fractions of the stereoisomers. Like for tetrakis(amide) DOTA derivatives,[26] the alkyl chains could influence the equilibrium between the stereoisomers. According to Figure 3, the lengthening of lateral chains could favor the faster stereoisomer, but this must still be demonstrated.

Study of Tertiary Bis(amide) DTPA Complexes

For the tertiary bis(amides), the evolution of proton relaxivity at varying temperatures also demonstrates a limitation by the residence time (Figure 6). The values of the residence times are similar to those measured for the larger linear bis(amide) complexes [Gd(DTPA-BnBA)(H_2O)], [Gd(DTPA-BAA)(H_2O)], [Gd(DTPA-BHA)(H_2O)], and [Gd(DTPA-BHeptA)(H_2O)] (Table 2). The reduction of the

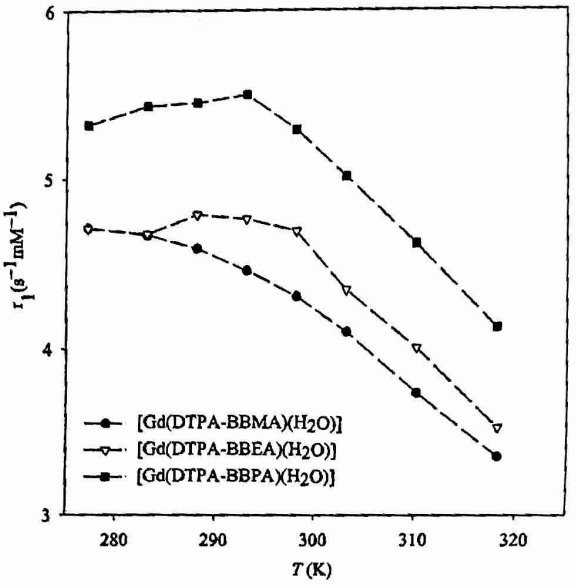


Figure 6. Temperature dependence of the proton relaxivity for tertiary bis(amide) complexes of $[Gd(DTPA)(H_2O)]^{2-}$

residence time of $[Gd(DTPA\text{-}BBMA)(H_2O)]$ compared to $[Gd(DTPA\text{-}BMA)(H_2O)]$, as well as the similarity of the residence time of the tertiary bis(amides), once again confirm the importance of steric hindrance at the level of the nitrogen atom from the amide group.

Conclusions

For the first time an extensive series of Gd-DTPA bis(amide) derivatives has been studied to assess the influence of the amide substituents on the water exchange rate. ^{17}O NMR spectroscopic studies carried out at variable temperatures and high pressures reveal that the substituents, which are close to the first coordination sphere, influence the residence time of bis(amide) DTPA derivatives without modifying the activation volume. However, this influence is relatively weak since the water exchange rate is doubled, at the most, for $[Gd(DTPA\text{-}BA)(H_2O)]$ as compared to $[Gd(DTPA\text{-}BHeptA)(H_2O)]$ and does not reach the exchange rate of $[Gd(DTPA)(H_2O)]^{2-}$. To explain the decrease of τ_M along the series, both the hydrophobicity and the hindrance of the complexes have been addressed, since these complexes are all neutral. The relative influence of these factors depends on the complexes studied. However, another hypothesis is that the lengthening of alkyl substituents favors the stereoisomers with the faster exchange rates and therefore influences the global exchange rate.

Experimental Section

Chemicals: DTPA-BMA was provided by Nycomed (Amersham Health, Amersham, United Kingdom). All chemicals and $GdCl_3{\cdot}6H_2O$ were purchased from Aldrich (Bornem, Belgium).

Ligand Synthesis: The bis(amide) derivatives of DTPA were synthesized by adding an excess of amine to DTPA-bis(anhydride).[19,27] Amine (16.8 mmol) was added to a stirred suspension of DTPA-bis(anhydride) (2 g, 5.6 mmol) in anhydrous DMF (50 mL) and heated at 325 K. After 12 h, the yellow solution was concentrated under reduced pressure. The residue was dissolved in water (50 mL) and the pH of the aqueous phase was adjusted to 7 with saturated $NaHCO_3$ solution. The aqueous phase was extracted with diethyl ether (5 × 30 mL). The organic phase was discarded and the pH of the aqueous solution was adjusted to 2–3 with 3 M HCl and the solvents evaporated once again. The residue was dissolved in a minimum of methanol and filtered. Solvent A (Table 5) was slowly added whilst stirring. The white precipitate was filtered, suspended in solvent B (Table 5) and heated until dissolution. The warm solution was filtered and cooled to 273 K. Cold solvent C (Table 5) was then slowly added whilst stirring to precipitate the ligand. The ligand was filtered, washed with diethyl ether and dried under vacuum. The yields are reported in Table 5. For the synthesis of DTPA-BHeptA, when the pH of the aqueous phase was adjusted to 7 with saturated $NaHCO_3$ solution, the aqueous phase was continuously extracted with diethyl ether for 48 h (to avoid emulsion formation). The purity and structure of the compounds were identified by ^1H, ^{13}C NMR spectroscopy with a Bruker AMX-300 (Bruker, Karlsruhe, Germany) and by LSIMS mass spectrometry (VG Analytical Autospec 6F, Manchester, UK). For this technique, samples were dissolved in water and deposited on a glycerol matrix.

NMR Spectroscopic Data

DTPA-BnPA (4): $C_{20}H_{37}N_5O_8$: 475.54 amu. ^1H NMR (D_2O, 298 K, pH ≈ 6): δ = 0.55 (t, 6 H, 2 × CH_3), 1.2 (sext, 4 H, 2 × CH_2), 2.8 (t, 4 H, 2 × CH_2), 2.9 (t, 4 H, 2 × CH_2), 3.0 (t, 4 H, 2 × CH_2), 3.1 (m, 8 H, 4 × CH_2), 3.55 (s, 2 H, 1 × CH_2) ppm. ^{13}C NMR (D_2O, 298 K, pH ≈ 6, reference pyridine): δ = 10.0, 21.1, 40.1, 49.6, 51.9, 53.6, 58.1, 58.2, 169.5, 172.2, 177.8 ppm.

DTPA-BAA (6): $C_{24}H_{45}N_5O_8$: 531.64 amu. ^1H NMR (D_2O, 298 K, pH ≈ 13): δ = 0.75 (t, 6 H, 2 × CH_3), 1.1–1.3 (m, 8 H, 4 × CH_2), 1.4 (quint, 4 H, 2 × CH_2), 2.3–2.6 (m, 8 H, 4 × CH_2), 2.9–3.2 (m, 14 H, 2 × CH_2 + 5 × CH_2) ppm. ^{13}C NMR (D_2O at 298 K, pH ≈ 13, reference MeOH): δ = 14.0, 22.4, 28.9, 32.6, 39.3, 52.1, 52.6, 58.6, 59.1, 59.4, 173.4, 179.2, 179.6 ppm.

DTPA-BHeptA (8): $C_{28}H_{53}N_5O_8$: 587.75 amu. ^1H NMR (D_2O, 298 K, pH ≈ 11): δ = 0.7 (t, 6 H, 2 × CH_3), 1–1.3 (m, 16 H, 8 × CH_2), 1.3–1.5 (quint, 4 H, 2 × CH_2), 1.8 (m, 4 H, 2 × CH_2), 2.3–2.6 (m, 6 H, 3 × CH_2), 2.9–3.3 (m, 12 H, 6 × CH_2) ppm. ^{13}C NMR (D_2O, 298 K, pH ≈ 11, reference pyridine): δ = 13.5, 22.2, 23.1, 23.2, 26.6, 28.7, 28.8, 31.5, 38.8, 52.1, 52.6, 57.9, 58.3, 58.9, 172.6, 178.5, 181.2 ppm.

DTPA-BBEA (10): $C_{22}H_{41}N_5O_8$: 503.59 amu. ^1H NMR (D_2O, 298 K, pH ≈ 6): δ = 1–1.2 (dt, 12 H, 4 × CH_3), 3.15 (t, 4 H, 2 × CH_2), 3.2–3.4 (m, 8 H, 4 × CH_2), 3.45 (s, 2 H, 1 × CH_2), 3.65 (s, 4 H, 2 × CH_2), 4.05 (s, 4 H, 2 × CH_2) ppm. ^{13}C NMR (D_2O, 298 K, pH ≈ 6, reference pyridine): δ = 14.9, 15.1, 15.9, 16.1, 43.9, 44.7, 53.4, 54.9, 58.2, 58.7, 60.6, 169.8, 176.4, 178.1 ppm.

DTPA-BBPA (11): $C_{26}H_{49}N_5O_8$: 559.70 amu. ^1H NMR (D_2O, 298 K, pH ≈ 1): δ = 0.6 (dt, 12 H, 4 × CH_3), 1.3 (m, 8 H, 4 × CH_2), 2.7–3.1 (m, 10 H, 5 × CH_2), 3.3 (m, 8 H, 4 × CH_2), 4.05 (s, 4 H, 2 × CH_2), 4.25 (s, 4 H, 2 × CH_2) ppm. ^{13}C NMR (D_2O, 298 K, pH ≈ 6, reference pyridine): δ = 6.9, 7.2, 16.6, 17.5, 44.4, 45.1, 45.5, 45.6, 50.0, 52.6, 53.4, 160.9, 165.8, 168.8 ppm.

DTPA-BiPA (13): $C_{20}H_{37}N_5O_8$: 475.54 amu. ^1H NMR (D_2O, 298 K, pH ≈ 3): δ = 0.9 (d, 12 H, 4 × CH_3), 2.95 (t, 4 H, 2 × CH_2), 3.3 (t, 4 H, 2 × CH_2), 3.4 (s, 2 H, 1 × CH_2), 3.7 (2 H, sept,

Table 5. Solvents used and yields for the syntheses of bis(amide) derivatives

Ligands	Solvent A	Solvent B	Solvent C	Yield (%)
DTPA-BnPA	acetone	2-propanol	diethyl ether	69
DTPA-BAA	acetone	ethanol	diethyl ether	51
DTPA-BHeptA	ethyl acetate	ethanol/ethyl acetate (2:2)	acetone	55
DTPA-BBEA	diethyl ether	ethanol	diethyl ether	57
DTPA-BBPA	diethyl ether	ethanol/ethyl acetate (5:5)	diethyl ether	49
DTPA-(R,S)BHPA	acetone	2-propanol	diethyl ether	47
DTPA-BiPA	diethyl ether	2-propanol/ethanol (3:1)	acetone	47

2 × CH), 3.9 (s, 4 H, 2 × CH_2), 4.0 (s, 4 H, 2 × CH_2) ppm. ^{13}C NMR (D_2O, 298 K, pH ≈ 6, reference pyridine): δ = 17.9, 38.7, 47.1, 48.6, 51.0, 53.3, 53.5, 162.7, 168.1, 169.2 ppm.

DTPA-(R,S)BHPA (14): $C_{20}H_{37}N_5O10$: 507.53 amu. ^1H NMR (D_2O, 298 K, pH ≈ 6: δ = 0.9 (d, 6 H, 2 × CH_3), 2.8 (t, 4 H, 2 × CH_2), 2.9–3.0 (m, 8 H, 4 × CH_2), 3.0–3.2 (m, 8 H, 4 × CH_2), 3.55 (s, 2 H, 1 × CH_2), 3.65 (2 H, sext, 2 × CH) ppm. ^{13}C NMR (D_2O, 298 K, pH ≈ 6, reference pyridine): δ = 18.9, 45.3, 49.7, 52.3, 53.8, 58.2, 63.2, 65.4, 169.7, 172.8, 178.0 ppm.

Preparation of the Gadolinium Complexes: The gadolinium complexes were prepared by mixing a slight excess (5%) of ligand with gadolinium chloride. The pH of the solution was adjusted to 6–7. The absence of free gadolinium ions was checked with xylenol orange indicator.[28] In order to eliminate the excess of ligand, the bis(amide) complexes were filtered through a Sep-Pak Vac 6cc cartridge, OH^- form from Waters (Brussels, Belgium) and eluted with demineralized water. The mass was confirmed by ElectroSpray spectrometry (Micromass, Manchester, UK) and the purity of the chelates was checked by HPLC (Waters, Milford, USA), C18 column, inverse phase, pH = 6.5, triethylammonium buffer 0.05 M, flow 1 mL/min, diode array and fluorescence detectors. The concentration of the Gd complex solutions was measured by proton relaxometry after decomplexation of Gd^{3+} and by Induced Coupled Plasma methods (Jobin-Yvon 38+, Jobin-Yvon ISA, Longjumeau, France).

Variable Temperature and High Pressure ^{17}O NMR Spectroscopy: Variable temperature ^{17}O NMR spectroscopic measurements were recorded with a Bruker AMX-300 spectrometer (7.05T, 40.65 MHz). The temperature was controlled by a BVT-2000 unit. ^{17}O NMR spectroscopic measurements at natural abundance were performed in 2-mL samples contained in 10-mm (o.d.) tubes. The concentration of the solutions was lower than 30 mM. ^{17}O diamagnetic transverse relaxation times of water were measured using a Carr-Purcell-Meiboom-Gill sequence (90° and 180° pulse lengths were 25 and 50 μs, respectively). All ^{17}O NMR spectra were proton-decoupled. ^{17}O transverse relaxation times of water in solutions containing Gd complexes were calculated from the spectral linewidth. High pressure ^{17}O NMR spectroscopic measurements were performed up to 200 MPa at 298.15 K and with a Bruker ARX 400 spectrometer (9.4T, 54.2 MHz) equipped with a homebuilt probe head.[29] The Gd^{III} complex solutions were enriched to 3%, using in all cases 10% ^{17}O-enriched water (Yeda R&D Co., Rehovot, Israel). Their Gd^{III} concentration was checked as previously described. The pH of the solutions, measured with a combined glass electrode, was between 4.0 and 6.1. The reference used was 2% ^{17}O-enriched bidistilled water acidified with perchloric acid. The temperature was externally controlled by a circulating flow from an external temperature bath and measured using a built-in Pt resistor. The transverse relaxation times were obtained by the CPMG spin-echo technique.[30]

Proton Relaxometry: The relaxivities were measured at 0.47T with a Bruker Minispec PC-120 thermostatted by a tetrachloroethylene flow at temperatures between 278.15 and 318.15 K. To avoid prototropic exchange, measurements were carried out at pH = 6–7.

Data Analysis: Fitting of variable temperature ^{17}O NMR spectroscopic data was performed with Minuit software (CERN library). Least-squares fits of the ^{17}O high pressure data were performed by Scientist for Windows. The log D coefficients were calculated using log D Suite developed by Advanced Chemistry Development (Toronto, Canada). The volumes of substituents were calculated with the software Molecular Modeling Pro (ChemSW Software, Fairfield, USA). All the equations that are used in the treatment of ^{17}O NMR spectroscopic data and relaxivity measurements are given here.

Relaxivity Measurement: The measured proton relaxivities contain both inner- and outer-sphere contributions [Equation (1)].

$$r_1 = r_1^{is} + r_1^{os} \tag{1}$$

The inner-sphere term is given by Equation (2), where P is the molar fraction of paramagnetic centers and q is the number of coordinated water molecules.

$$\frac{1}{T_1^{is}} = \frac{Pq}{T_{1M} + \tau_M} \tag{2}$$

Variable Temperature ^{17}O NMR Spectroscopy: The residence time of inner-sphere water molecules can be estimated by the analysis of the temperature dependence of the ^{17}O transverse relaxation rate. The observed transverse relaxation rate is the sum of a paramagnetic ($1/T_2^p$) and a diamagnetic ($1/T_2^{dia}$) contribution [Equation (3).]

$$\frac{1}{T_2} = \frac{1}{T_2^p} + \frac{1}{T_2^{dia}} \tag{3}$$

The paramagnetic term itself arises from outer-sphere ($1/T_2^{os}$) and inner-sphere ($1/T_2^{is}$) mechanisms [Equation (4)].

$$\frac{1}{T_2^p} = \frac{1}{T_2^{os}} + \frac{1}{T_2^{is}} \tag{4}$$

The inner-sphere contribution is given by the classical Equation (5), where $\Delta\omega_M$ is the chemical shift difference between bound and bulk water and T_{2M} is the transverse relaxation time of the bound water.

$$\frac{1}{T_2^{is}} = \frac{Pq}{\tau_M} \frac{T_{2M}^{-2} + \tau_M^{-1} T_{2M}^{-1} + \Delta\omega_M^2}{\left(\tau_M^{-1} + T_{2M}^{-1}\right)^2 + \Delta\omega_M^2} \tag{5}$$

It has been shown that for gadolinium complexes the outer-sphere contribution is negligible.[4] $\Delta\omega_M$ is defined by Equation (6), where g_L is the Landé factor (equal to 2 for Gd^{3+}), μ_B is the Bohr magneton, S is the electron spin (3.5 for Gd^{3+}), k_B is the Boltzmann constant, $A/\hbar$ is the hyperfine coupling constant, and T is the temperature.

$$\Delta\omega_M = \frac{g_L \mu_B S(S+1) B_0}{3 k_B T} \frac{A}{\hbar} \tag{6}$$

The transverse relaxation rate of ^{17}O coordinated to the gadolinium ion is predominantly described by the scalar interaction [Equation (7)]. The dipolar and quadrupolar contributions can be neglected since their contribution to the relaxation is 5% at the most.[4] Therefore, T_{2M} is given by Equation (7), where ω_S is the resonance frequency of the electron.

$$\frac{1}{T_{2M}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2 \left(\tau_{e1} + \frac{\tau_{e2}}{1+\omega_S^2 \tau_{e2}^2}\right) \tag{7}$$

The scalar correlation times τ_{ei} are defined by Equation (8).

$$\frac{1}{\tau_{ei}} = \frac{1}{\tau_M} + \frac{1}{\tau_{Si}} \text{ with } i = 1,2 \tag{8}$$

The electronic relaxation times (τ_{Si}) are described by the contribution of the zero-field splitting interaction (no contribution of the spin rotation has been considered) and are expressed by Equation (9) and (10), where τ_v is the correlation time related to the modulation of the zero-field splitting (ZFS) and B is a parameter related to the ZFS and is independent of the temperature.

$$\frac{1}{\tau_{S1}} = B \tau_V \left[\frac{1}{1+\omega_S^2 \tau_V^2} + \frac{4}{1+4\omega_S^2 \tau_V^2}\right] \tag{9}$$

$$\frac{1}{T_{2M}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2 \left[\frac{\beta\tau_v}{2}\left[3+\frac{5}{1+\omega_S{}^2\tau_v{}^2}+\frac{2}{1+4\omega_S{}^2\tau_v{}^2}\right]\right] \tau_{e1} \quad (10)$$

At high magnetic fields, $\omega_S\tau_{e2} >> 1$ and Equation (7) becomes Equation (11).

$$\frac{1}{T_{2M}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2 \tau_{e1} \quad (11)$$

A reduced relaxation rate given by Equation (12) was used in this study, where f is the ratio [paramagnetic complexes]/[water].

$$\frac{1}{T_2'} = \frac{\left(\frac{1}{T_2''}\right)}{f \times q} \quad (12)$$

The reduced transverse relaxation depends thus on the electronic relaxation time τ_{S1}, the hyperfine coupling constant $A/\hbar$, and the water residence time τ_M. The parameters $A/\hbar$ and B are independent of temperature. The temperature dependence of the water residence time and of the correlation time τ_v can be described according to the theories of Eyring and Arrhenius [Equation (13) and (14)], where $\Delta S^{\neq}$ and $\Delta H^{\neq}$ are the entropy and enthalpy of activation, respectively, for the water exchange process and E_v is the activation energy related to τ_v; τ_v^{298} is the correlation time for the modulation of the zero-field splitting interaction at 298.15 K.

$$\frac{1}{\tau_M} = \frac{k_B T}{h}\exp\left(\frac{\Delta S^{\neq}}{R} - \frac{\Delta H^{\neq}}{RT}\right) \quad (13)$$

$$\tau_v = \tau_v^{298}\exp\left(\frac{E_v}{R}\left(\frac{1}{T}-\frac{1}{298.15}\right)\right) \quad (14)$$

The temperature dependence of the ^{17}O reduced transverse relaxation rate, $1/T_2'$, can thus be used to determine the water residence time and the related activation parameters ($\Delta S^{\neq}$ and $\Delta H^{\neq}$).

Variable Pressure ^{17}O NMR Spectroscopy: By neglecting $\Delta\omega_M$ in Equation (5) and using Equation (12), the reduced transverse relaxation rate, $1/T_2'$, can be simplified by Equation (15), where $1/T_{2M}$ is expressed as in Equation (7).[21]

$$\frac{1}{T_2'} = \frac{1}{T_{2M} + \tau_M} \quad (15)$$

Previous studies have shown that the logarithm of the water exchange rate varied almost linearly with pressure, as long as the compressibility coefficient of activation is negligible, as expressed by Equation (16),[31] where $\Delta V^{\neq}$ is the activation volume independent of pressure and $(k_{ex})_0^T$ is the water exchange rate at zero pressure and temperature T; $1/T_{2M}$ is small and therefore can be assumed to be independent of the pressure since a pressure dependence of $1/T_{2M}$ with a $\Delta V_M^{\neq}$ between +5 and −5 cm^{3}mol^{-1} does not affect the value of $\Delta V^{\neq}$ within the experimental errors.

$$\frac{1}{\tau_M} = k_{ex} = (k_{ex})_0^T \exp\{-\frac{\Delta V^{\neq}}{RT}P\} \quad (16)$$

Acknowledgments

We thank the Fonds pour la Recherche dans l'Industrie et l'Agriculture (FRIA) of Belgium. We are also grateful to the Swiss OFES as part of the EU-COST D18 Action and the Swiss National Science Foundation. The authors thank Mrs. Patricia de Francisco for her help in preparing the manuscript.

[1] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293.
[2] *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging* (Eds.: A. E. Merbach, E. Tóth), John Wiley & Sons, Ltd., **2001**, pp. 78.
[3] E. Tóth, A. E. Merbach, *Mod. Chem.* **1998**, *135*, 873.
[4] K. Micskei, L. Helm, E. Brücher, A. E. Merbach, *Inorg. Chem.* **1993**, *32*, 3844.
[5] K. Micskei, D. H. Powell, L. Helm, E. Brücher, A. E. Merbach, *Magn. Reson. Chem.* **1993**, *31*, 1011.
[6] S. Aime, M. Botta, M. Fasano, S. Paoletti, L. Anelli, F. Uggeri, M. Virtuani, *Inorg. Chem.* **1994**, *33*, 4707.
[7] E. Toth, D. Pubanz, S. Vauthey, L. Helm, A. E. Merbach, *Chem. Eur. J.* **1996**, *2*, 1607.
[8] S. W. A. Bligh, A. H. M. S. Chowdhury, D. Kennedy, C. Luchinat, G. Parigi, *Magn. Reson. Med.* **1999**, *41*, 767.
[9] C. F. G. C. Geraldes, A. M. Urbano, M. C. Alpoim, A. D. Sherry, K. T. Kuan, R. Rajagopalan, F. Maton, R. N. Muller, *Mag. Reson. Imag.* **1995**, *13*, 401.
[10] D. Pubanz, G. Gonzalez, D. H. Powell, A. E. Merbach, *Inorg. Chem.* **1995**, *34*, 4447.
[11] S. Aime, A. Barge, M. Botta, D. Parker, A. S. De Sousa, *J. Am. Chem. Soc.* **1997**, *119*, 4767.
[12] S. Aime, M. Botta, S. G. Crich, G. B. Giovenzana, R. Pagliarin, M. Piccinini, M. Sisti, E. Terreno, *J. Biol. Inorg. Chem.* **1997**, *2*, 470.
[13] L. Vander Elst, F. Maton, S. Laurent, F. Seghi, F. Chapelle, R. N. Muller, *Magn. Reson. Med.* **1997**, *38*, 604.
[14] R. N. Muller, B. Radüchel, S. Laurent, J. Platzek, C. Piérart, P. Mareski, L. Vander Elst, *Eur. J. Inorg. Chem.* **1999**, 1949.
[15] I. Solomon, *Phys. Rev.* **1955**, *55*, 559.
[16] N. Bloembergen, *J. Chem. Phys.* **1957**, *27*, 572.
[17] S. Laurent, L. Vander Elst, S. Houzé, N. Guérit, R. N. Muller, *Helv. Chim. Acta* **2000**, *83*, 394.
[18] H. Gries, H. Miklautz, *Physiol. Chem. Phys. Med.* **1984**, *16*, 105.
[19] M. S. Konings, W. C. Dow, D. B. Love, K. N. Raymond, S. C. Quay, S. M. Rocklage, *Inorg. Chem.* **1990**, *29*, 1488.
[20] S. W. A. Bligh, A. H. M. S. Chowdhury, M. McPartlin, I. J. Scowen, *Polyhedron* **1995**, *14*, 567.
[21] G. Gonzalez, H. D. Powell, V. Tissières, A. E. Merbach, *J. Phys. Chem.* **1994**, *98*, 53.
[22] C. Hansch, A. J. Leo, in *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, **1979**.
[23] C. F. G. C. Geraldes, A. M. Urbano, M. A. Hoefnagel, J. A. Peters, *Inorg. Chem.* **1993**, *32*, 2426.
[24] S. Aime, A. Barge, M. Botta, A. S. De Sousa, D. Parker, *Ang. Chem. Int. Ed.* **1998**, *37*, 2673.
[25] F. A. Dunand, S. Aime, A. E. Merbach, *J. Am. Chem. Soc.* **2000**, *122*, 1506.
[26] S. Aime, A. Barge, J. I. Bruce, M. Botta, J. A. K. Howard, J. M. Moloney, D. Parker, A. S. de Sousa, M. Woods, *J. Am. Chem. Soc.* **1999**, *121*, 5762.
[27] S. Laurent, L. Vander Elst, F. Copoix, R. N. Muller, *Invest. Radiol.* **2001**, *36*, 115.
[28] G. Brunisholz, M. Randin, *Helv. Chim. Acta* **1959**, *42*, 1927.
[29] A. Cusanelli, L. Nicula-Dadci, U. Frey, A. E. Merbach, *Inorg. Chem.* **1997**, *36*, 2211.
[30] S. Meiboom, D. Gill, *Rev. Sci. Instrum.* **1958**, *29*, 688.
[31] C. Cossy, L. Helm, A. E. Merbach, *Inorg. Chem.* **1989**, *28*, 2699.

Received February 20, 2002
[I02088]