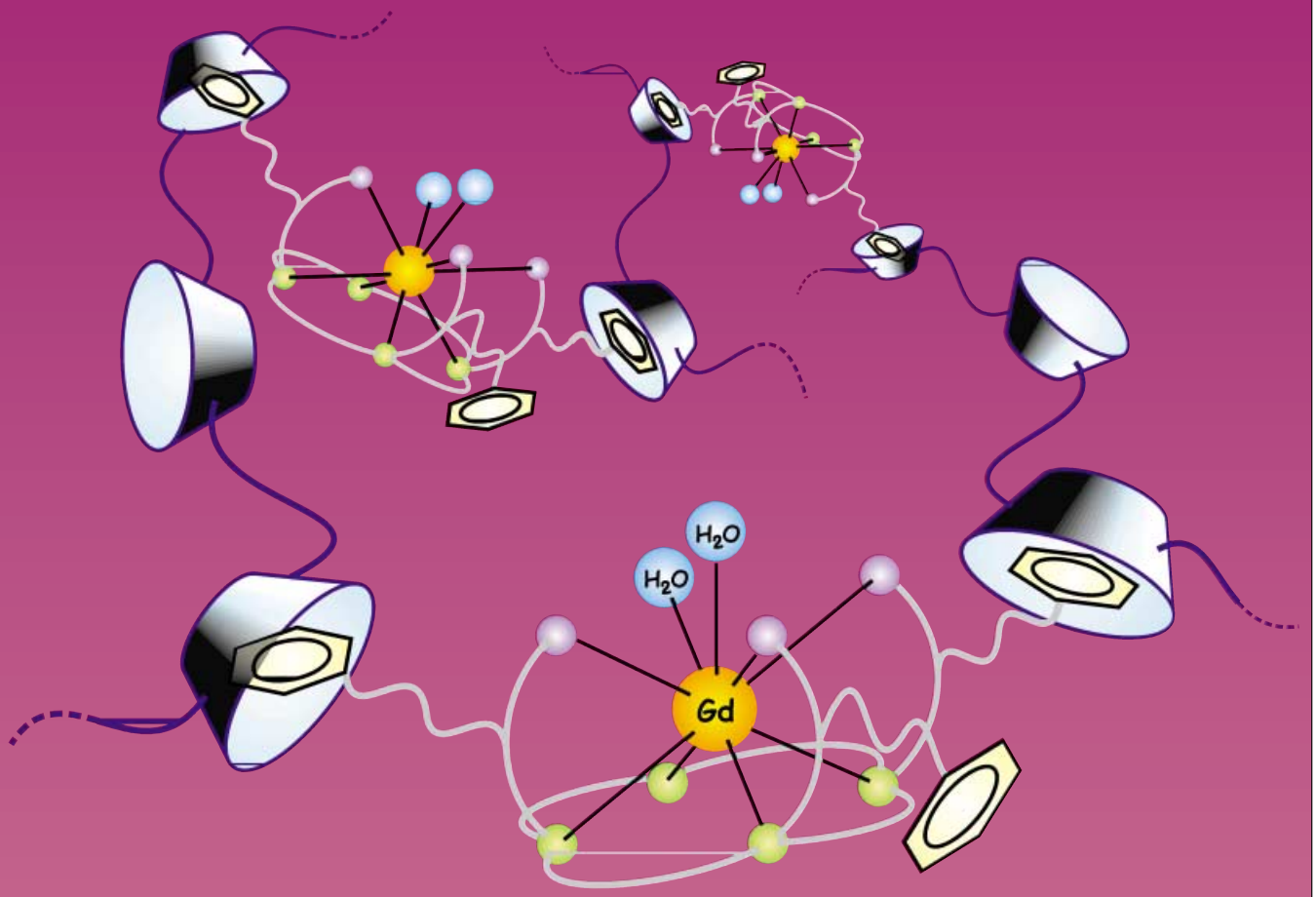
Interesting Candidate for Novel MRI Applications



The possible ways in which multisite interactions between a β-cyclodextrin oligomer and a suitably trifunctionalized Gd^{III}–chelate complex can occur are depicted. This kind of tightly assembled adduct may represent a very interesting candidate for novel MRI applications in which a high number of paramagnetic Gd^{III} ions endowed with high relaxivity are necessary. The relaxivities found for the paramagnetic adducts represent a remarkable step forward in the relaxivity scale. For more information, see the following pages.

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High-Relaxivity Contrast Agents for Magnetic Resonance Imaging Based on Multisite Interactions between a β-Cyclodextrin Oligomer and Suitably Functionalized Gd^{III} Chelates

Silvio Aime,*[a] Mauro Botta,[b] Franco Fedeli,[d] Eliana Gianolio,[a] Enzo Terreno,[a] and Pierlucio Anelli[c]

Abstract: The results reported in this work show that tightly assembled adducts formed by trisubstituted Gd^{III} complexes and a β-CD multimer (Poly-β-CD, d.p. ca. 12) may represent very interesting candidates for novel MRI applications wherein a high number of paramagnetic ions endowed with high relaxivity (per Gd^{III} ion) are necessary. The relaxivities found for the paramagnetic adducts represent a remarkable step forward on the relaxivity scale. However, a detailed investigation of the determinants of the relaxation enhancement in these systems shows that their relaxivities are still limited by a nonoptimal τ_R and a relatively long exchange lifetime of the coordinated water(s). Moreover, the exchange rate of the water molecule(s) coordinated to the Gd^{III} ion further decreases upon binding to the Poly-β-CD. It is suggested that this finding is related to the structural properties of the supramolecule, which brings a high density of hydroxyl groups into the proximity of the "guest" complexes, and this yields an overall reinforcement of the hydrogen-bonding network involving the coordinated water(s). On the other hand, such a tight arrangement appears responsible for an enhanced contribution to the observed relaxivity arising from water molecules in the second coordination sphere of the metal center.

Keywords: chelates · cyclodextrins · high relaxivity · MRI contrast agents · noncovalent interactions

Introduction

It is now well established that the use of contrast agents (CAs) adds relevant information in several applications of Magnetic Resonance Imaging (MRI),[1, 2] and approximately 35 % of the diagnostic protocols currently carried out in clinical practice require their administration. The most important class of CAs for MRI is represented by Gd^{III} chelates, whose high paramagnetism (seven unpaired electrons) can yield a strong enhancement of the water proton relaxation rates.[3, 4] This ability is, at first, assessed in "in vitro" experiments through the measurement of the relaxivity, r_1, which refers to the relaxation enhancement observed in the presence of the paramagnetic complex at 1 mM concentration.[5]

The development of novel applications of MRI and CAs will largely depend on the availability of systems endowed with high relaxivities. With the magnetic fields commonly used in MRI (0.2 – 2.1 T), a relaxivity increase is expected upon an increase of the molecular reorientational time, τ_R, of the chelate.[6] This approach has been extensively exploited either through the synthesis of covalent conjugates with macromolecular systems like proteins,[7–10] dendrimers,[11–13] and polypeptides[14–16] or through noncovalent interactions between a suitably functionalized chelate and proteins (essentially Human Serum Albumin)[17–25] or micelles.[26] These efforts have led to remarkable results in the delineation of blood vessels, in which such macromolecular systems are confined. The next step will deal with the creation of protocols that address the visualization of small targets like plaques,

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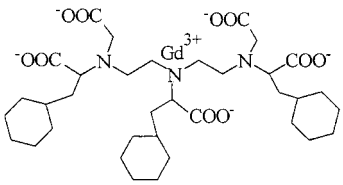
thrombi, receptors over-expressed in the presence of a given pathology, and so on.

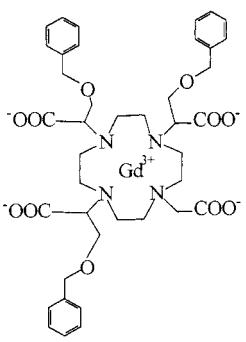
For these purposes, it appears necessary that a significant number of contrast agent molecules, which should have the highest attainable relaxivity,[27] are delivered at the targeting site.

Recently, we showed that a good relaxation enhancement can be obtained upon the formation of a relatively high-molecular-weight adduct between a suitably functionalized Gd^{III} complex and a mixture of oligomers (average M_W of 6 kDa) of β-cyclodextrin (Poly-β-CD).[28] Moreover, it was suggested that much higher relaxation rates could have been obtained if the reorientational motions of the macromolecular adducts were further slowed down, for instance, by increasing the size of the β-CD multimer.

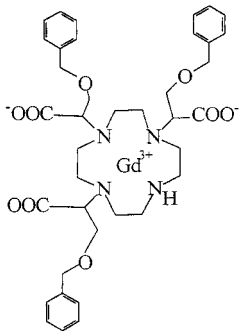
Unfortunately, synthetic attempts to increase the size of the β-CD multimer above 10–15 units were unsuccessful because larger dimensions invariably yielded insoluble products. It was thought that higher M_W adducts could be obtained with the available β-CD oligomers through the use of multifunctionalized chelates, wherein each functionality interacts with a single β-CD cavity, possibly of different oligomers. Whether the binding leads to the formation of an interlocked supramolecular adduct or not, the presence of more interacting moieties on the surface of the complex would improve the overall affinity towards the β-CD oligomer. In turn, we expect that the involvement of multisite interactions with β-CD oligomers will significantly improve the relaxation properties of Gd-based contrast agents. In order to prove it, we have carried out a detailed relaxometric investigation aimed at characterizing the binding interaction between β-CD oligomers and Gd^{III} chelates containing three hydrophobic substituents on their surface.



[Gd{dtpa(cym)$_3$}]



[Gd{dota(bom)$_3$}]



[Gd{do3a(bom)$_3$}]

Results and Discussion

The structures of the three Gd^{III} chelates considered in this work are shown. The relaxometric properties of [Gd{dota-(bom)$_3$}] (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, BOM = benzyloxymethyl) and its noncovalent interactions with human serum albumin (HSA) have been previously investigated in detail.[17] [Gd{dtpa(cym)$_3$}] (DTPA = diethylenetriaminepentaacetic acid) results from the introduction of three cyclohexylmethyl substituents (CyM) on the acetate arms of DTPA. It is strictly analogous to the recently reported [Gd{dpta(bom)$_3$}] complex, which was shown to possess a high affinity binding to HSA.[20] The replacement of the phenyl rings by cyclohexyl moieties is aimed at providing an enhanced affinity towards β-CD cavities. [Gd{do3a(bom)$_3$}] (DO3A = 1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid) is a derivative of [Gd(do3a)], in which three acetic hydrogen atoms have been replaced by benzyloxymethyl substituents. It has been reported that, in aqueous solutions, [Gd(do3a)] is present as a mixture of two isomers with one and two-coordinated water molecules, respectively, with the latter isomer being largely dominant (90%) at ambient temperature.[29] The major drawback of [Gd{do3a(bom)$_3$}] is related to its very poor solubility in water (ca. 0.2 mM at 25 °C), which makes its relaxometric characterization more difficult. However, upon interacting with β-CD or with HSA, the dissolved quantities are sufficient for proton relaxivity investigations.

The relaxivities at 20 MHz, 25 °C, and pH 7 of the three Gd^{III} chelates investigated in this work are reported in Table 1. The r_1 values are significantly higher (of ca. 60–80%) than those measured for the parent compounds [Gd(dota)], [Gd(dtpa)], and [Gd(do3a)].[30] The increase in the relaxivity is mainly due to the increase in τ_R caused by the presence of the three bulky residues on the surface of the metal complexes.

Then, we measured the water proton relaxation rates of solutions (0.2 mM) of each complex in the presence of increasing amounts of β-CD and of its 15 KDa multimer, respectively. The analysis of these titrations is not straightforward because the possibility of multisite interactions leads to a

Table 1. Relaxivities of the free Gd^{III} complexes and parameters related to their interaction with β-CD and Poly-β-CD.

	r_1 [mM^{-1}s^{-1}]	β-CD			Poly-β-CD		
		K_{50}	ε_b	r_1^b [mM^{-1}s^{-1}]	K_{50}	ε_b	r_1^b [mM^{-1}s^{-1}]
[Gd{dtpa(cym)$_3$}]	9.1 ± 0.45	5.7 ± 0.2	2.75 ± 0.14	25.0 ± 1.25	2.2 ± 0.1	5.8 ± 0.29	52.7 ± 2.6
[Gd{dota(bom)$_3$}]	7.5 ± 0.37	15.4 ± 0.8	3.33 ± 0.16	25.0 ± 1.25	4.3 ± 0.2	6.5 ± 0.32	49.0 ± 2.4
[Gd{do3a(bom)$_3$}]	10.0 ± 0.5	39.5 ± 2.0	3.04 ± 0.15	30.4 ± 1.50	7.0 ± 0.3	6.1 ± 0.30	61.0 ± 3.0

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number of simultaneous equilibria that have to be considered. Only in the case of monomeric β-CD, can adducts with different GdL/β-CD stoichiometry (from 1:1 to 1:3) be formed, and the number of species can dramatically increase in the presence of Poly-β-CD. Thus, the presence of several equilibria in these solutions makes it impossible to get K_A values for each adduct.

Therefore, we had to evaluate the overall interaction affinity of these chelates towards β-CD and Poly-β-CD not in terms of individual K_As but through a specific parameter, K_{50}, which was evaluated from graphical analysis of the binding isotherm. By plotting the data as an enhancement factor (ε*) versus the [β-CD]/[GdL] ratio (in Figure 1 the data for [Gd{dtpa(cym)$_3$}] are reported), we found that K_{50} indicates the [β-CD]/[GdL] ratio at which 50% of the overall relaxation enhancement is observed.

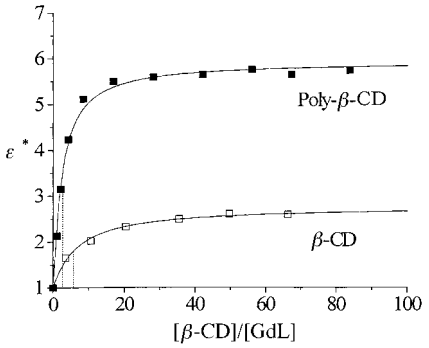

Figure 1. Relaxation enhancement factor (ε*) of a 0.2 mM solution of [Gd{dtpa(cym)$_3$}] as a function of the ratio [β-CD]/[GdL] (lower curve) or [Poly-β-CD]/[GdL] (upper curve) (20 MHz, 25 °C, pH = 7). Dotted lines indicate the K_{50} values for the two systems.

The enhancement factor (ε*) of a solution containing the paramagnetic chelate and the interacting substrate is calculated in Equation (1).

$$\varepsilon^* = \frac{R_{1obs}^{Gd+S} - R_{1obs}^{S}}{R_{1obs}^{Gd} - R_{1obs}^{w}} \quad (1)$$

In the equation, the measured relaxation rates refer to solutions containing the complex and the substrate (Gd + S), the substrate (S), the complex (Gd), and the pure solvent (w).

K_{50} is, therefore, inversely related to the overall affinity showed by the metal complex towards the macromolecular substrate; the smaller K_{50}, the higher the overall binding affinity between the complex and the "host" substrate.

A more detailed inspection of Figure 1 indicates that the enhancement factor ε* achieves a maximum constant value, ε_b, either in the titration with β-CD or in the Poly-β-CD system. This observation suggests that also in the latter case, as for the simpler monomeric β-CD, the presence of an excess of Poly-β-CD leads to the formation of a single bound species for the Gd^{III} complex unless different interaction equilibria with very similar affinity constants are in operation. The results obtained in terms of K_{50}, the maximum relaxivity enhancement (ε_b), and the relaxivities of the "fully bound" adducts ($r_1^b = \varepsilon_b \times r_1$) are summarized in Table 1.

The higher affinity shown by [Gd{dtpa(cym)$_3$}] may be accounted for in terms of the stronger binding of the cyclohexyl moiety with respect to the benzyloxy substituent. The relaxivity enhancements achieved upon the formation of the adducts are significantly higher (ε_b of ca. 3) than the values observed for monofunctionalized Gd^{III} chelates, which interact with β-CD ($\varepsilon_b < 2$).[28, 31] This result confirms that the trifunctionalized Gd^{III} complexes may bind to more than one β-CD cavity, and this leads to the formation of adducts of larger size and consequently longer τ_R and higher relaxivity.

As expected, on going from β-CD to Poly-β-CD, K_{50} decreases, and ε_b and r_1^b increase (Table 1).

Interestingly, the relative binding affinity of the three complexes found for β-CD is maintained in the case of the oligomer, but the K_{50} values are smaller owing to the higher probability of interaction between the Gd^{III} chelates and the β-CD cavities of the multimer.

As far as the relaxivity is concerned, the r_1^b values of the "fully bound" adducts are very high. The increase in relaxivity on passing from β-CD to Poly-β-CD may be accounted for by the longer τ_R of the adducts with the latter substrate. As anticipated above, slowly moving adducts can be formed because: i) several Gd^{III} chelates can bind to the same multimer chain or ii) each Gd^{III} chelate may interact simultaneously with two or three multimeric chains.

Clearly, the relaxivity increase on going from the adducts formed by [Gd{dtpa(cym)$_3$}] and [Gd{dota(bom)$_3$}] to that formed by [Gd{do3a(bom)$_3$}] reflects, as a first approximation, the presence of two inner-sphere water molecules in the [Gd{do3a(bom)$_3$}] complex. Though quite high, these values are still significantly lower than those predicted by theory.[4] Higher relaxivity values can be obtained when the experimental conditions are forced towards the formation of interlocked supramolecular adducts. For instance, by increasing the molar ratio between [Gd{do3a(bom)$_3$}] and the Poly-β-CD, we were able to measure r_1^b values as high as 80 mM^{-1} s^{-1}. This remarkable relaxivity enhancement is expected as a consequence of the increase in the reorientational motion of the chelate upon formation of an interlocked structure between the metal complex and host cavities of different multimeric chains, although other factors that affect the hydration state of the complex may be important (vide infra). Such adducts are the systems that one will pursue in "in vivo" procedures after accumulation of the β-CD multimer units at the site of interest followed by a second step, which involves their recognition by the functionalized complexes. An "in vitro" investigation of the relaxometric properties of these interlocked species appears rather difficult because their formation is favored at high GdL/Poly-β-CD ratios. Under such conditions, the molar fraction of the bound complex is lower and, therefore, it is not straightforward to evaluate the concentration of the Gd^{III} chelate entrapped in the interlocked structure. Thus the evaluation of the factors responsible for the relaxation enhancement has been carried out at lower GdL/Poly-β-CD ratios. We believe that the determinants for the relaxometric enhancements assessed under these experimental conditions well represent those ones responsible for the "in vivo" relaxivity of the interlocked structures.

Exchange lifetime: The residence lifetime of the water molecule(s) coordinated to the metal center (τ_M) is one of

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the key parameters responsible for the relaxivity of chelates immobilized on slowly moving substrates. In fact, the inner-sphere contribution to the observed relaxation rate is given by Equation (2).

$$R_{1p}^{is} = \frac{q[C]}{55.5(T_{1M} + \tau_M)} \quad (2)$$

In the equation, T_{1M} is the relaxation time of the protons of the coordinated water molecule(s). Thus, a "quenching" of the attainable relaxivity occurs when τ_M is longer than T_{1M}, and this condition has been often met when a Gd^{III} chelate is bound to a macromolecular system.[32]

An accurate determination of τ_M can be obtained from the measurement of ^{17}O-R_{2p} as a function of temperature. The observed relaxation rate is dominated by the contact interaction and it is dependent on either τ_M and R_{2M}^O (the transverse relaxation rate of the metal-bound ^{17}O water nucleus) [Eq. (3)].

$$R_{2p}^O = \frac{q[C]}{55.5}\tau_M^{-1}\frac{R_{2M}^{O\,2} + \tau_M^{-1}R_{2M}^O + \Delta\omega_M^{O\,2}}{(R_{2M}^O + \tau_M^{-1})^2 + \Delta\omega_M^{O\,2}} \quad (3)$$

R_{2p}^O values increase as the temperature increases until τ_M becomes short enough with respect to R_{2M}^O, and this causes a decrease in R_{2p}^O with a further increase in temperature. The resulting bell-shaped behavior may be fitted to Equation (3), and the actual τ_M value at any temperature can be obtained. Following this procedure, it has been possible to evaluate the exchange lifetime of the coordinated water molecule in [Gd{dtpa(cym)$_3$}] and [Gd{dota(bom)$_3$}] (Table 2). The low solubility of [Gd{do3a(bom)$_3$}] prevented any ^{17}O-NMR measurement because this experiment requires relatively high concentrations of the paramagnetic complex. We have assumed that its τ_M^{298} may be approximately 90 ns, that is, the value found for the parent [Gd(do3a)].[29] It is likely that this is an upper limit because it has been found that the introduction of bulk substituents on either linear or macrocyclic polyaminocarboxylate ligands usually causes a decrease in the exchange lifetime of the coordinated water.[33]

The τ_M^{298} values measured for [Gd{dtpa(cym)$_3$}] and [Gd{dota(bom)$_3$}] are significantly shorter (ca. one third) than those reported for the reference chelates [Gd(dtpa)] and [Gd(dota)].[34]

The next step dealt with the determination of the exchange lifetimes of the coordinated water(s) in the supramolecular adducts with the Poly-β-CD by using the same ^{17}O-NMR procedure. Now, the improved solubility of [Gd{do3a(bom)$_3$}] in the presence of Poly-β-CD allows the direct comparison of the three complexes. As an example, in Figure 2 the experimental profile obtained for the [Gd{dota(bom)$_3$}]/Poly-β-CD adduct is compared with that of the free complex.

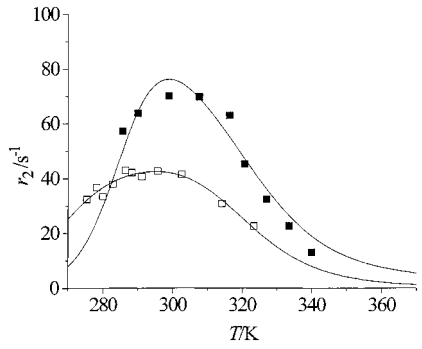

Figure 2. Temperature dependence of the paramagnetic contribution to the ^{17}O transverse relaxation rate of water for [Gd{dota(bom)$_3$}] (4 mM) free (□) and bound to Poly-β-CD ([Poly-β-CD] = 30 mM) (■) (2.1 T, pH 7).

As shown in Table 2, the τ_M values, obtained from the fitting of the ^{17}O-R_{2p} resonances, at least for the [Gd{dtpa(cym)$_3$}] and [Gd{dota(bom)$_3$}] adducts, are longer (of ca. 70–80%) than the corresponding values measured in the absence of Poly-β-CD. Analogous findings were previously obtained for τ_M of free and HSA-bound complexes.[19, 20] On this basis, the exchange process appears to be determined not only by intramolecular properties (e.g. enthalpy of the Gd–O bond) but also by changes in the overall hydrogen-bonding network surrounding the coordinated water(s).

In order to gain direct information about the effect of τ_M on the proton relaxivity of a given complex, it is necessary to analyze the temperature dependence of the ^1H longitudinal relaxation rates of its aqueous solution. The obtained results for the macromolecular adducts between Poly-β-CD and the three complexes considered in this work are reported in Figure 3. In all these systems, the behavior of r_1 in the low-temperature range unambiguously suggests a "quenching" effect of the exchange lifetime on the overall relaxivity. However, this information alone does not allow us to draw any conclusions on the occurrence of a concomitant limiting effect of τ_R (through its effect on T_{1M}) on the attainable relaxivity of these macromolecular adducts. Further insights can be gained through a detailed investigation of the field dependence of the observed relaxivity.

Table 2. Best-fit parameters obtained at 298 K from ^1H-NMRD and ^{17}O-R_{2p} versus T analysis. For [(Gd–L)/Poly-β-CD] adducts, τ^{ss} represents the correlation time associated with the motions (reorientation or exchange) of the second-sphere water molecules (q^{ss}).

	[Gd{dtpa(cym)$_3$}]	[Gd{dtpa(cym)$_3$}]/ Poly-β-CD	[Gd{dota(bom)$_3$}]	[Gd{dota(bom)$_3$}]/ Poly-β-CD	[Gd{do3a(bom)$_3$}]	[Gd{do3a(bom)$_3$}]/ Poly-β-CD
τ_M [ns]	100 ± 4.9	180 ± 9.1	80 ± 4	140 ± 7.3	90[a]	120 ± 6.2
ΔH_M [KJ mol^{-1}]	56 ± 2.8	61 ± 3.1	74 ± 3.7	96 ± 4.6		38 ± 1.9
Δ^2 [$s^{-2} \times 10^{19}$]	1.9 ± 0.095	1.2 ± 0.06	3.4 ± 0.17	1.3 ± 0.06	2.8 ± 0.14	0.8 ± 0.04
τ_v [ps]	42 ± 2.1	27.5 ± 1.4	14 ± 0.7	31.4 ± 1.6	27.6 ± 1.4	44.4 ± 2.2
ΔH_v [KJ mol^{-1}]	10	10	10	10		10
τ_R [ns]	0.15 ± 0.007	7.0 ± 0.35	0.12 ± 0.006	7.0 ± 0.35	0.14 ± 0.007	1.5 ± 0.075
τ^{ss} [ps]		53 ± 2.65		41 ± 2.05		42.3 ± 2.1
q^{ss}		19.6 ± 1		15.0 ± 0.8		16.2 ± 0.85

[a] This value has been assumed as the poor solubility of the complex prevents its determination by ^{17}O measurements.

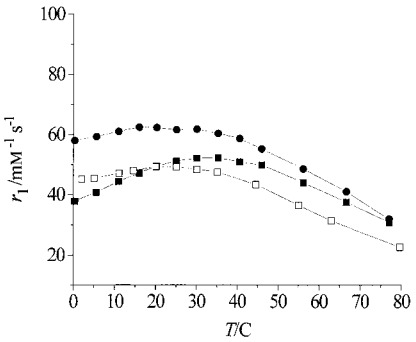

Figure 3. Temperature dependence of the relaxivity of the adducts [Gd(L)/Poly-β-CD] for the three complexes ([Gd(L)] = 0.1 mM; [Poly-β-CD] = 10 mM) (20 MHz, pH = 7). [[Gd{dtpa(cym)$_3$}]/Poly-β-CD] (■); [[Gd{dota(bom)$_3$}]/Poly-β-CD] (□); [[Gd{do3A(bom)$_3$}]/Poly-β-CD] (●).

1/T_1 NMRD profiles: In Figure 4, the NMRD (Nuclear Magnetic Resonance Dispersion) profiles of the three macromolecular adducts are reported. The fitting of the experimental data to Solomon – Bloembergen – Morgan equations (see Appendix) gives the values for the parameters involved in the relaxation process (Table 2). The fitting procedure has been carried out by considering $q = 1$ for [Gd{dtpa(cym)$_3$}] and [Gd{dota(bom)$_3$}] and $q = 2$ for [Gd{do3a(bom)$_3$}]. Furthermore, τ_M values obtained from ^{17}O-NMR studies have been used and kept fixed in the fitting procedure. Of course the analysis yields weighted averages of the values of the different structures present in the supramolecular system.

As the quantitative analysis of the NMRD profiles was not satisfactory by using the simple inner-outer-sphere model, the observed relaxivities have been assumed to also receive contributions from water protons present in the second coordination sphere of the chelate. The origin of the second coordination term (usually not considered in the evaluation of the relaxivity for the free complexes) appears to be related to the tight association of the various moieties within the formed adducts. In fact, one may envisage that the high density of hydroxyl groups on the crowns of the β-CD cavities may yield

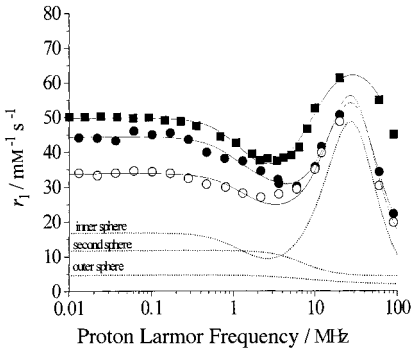

Figure 4. 1/T_1 NMRD profile of [[Gd{dtpa(cym)$_3$}]/Poly-β-CD] (●), [[Gd{dota(bom)$_3$}]/Poly-β-CD] (○), and [[Gd{do3a(bom)$_3$}]/Poly-β-CD] (■) adducts at pH = 7 and 25 °C. The profiles were recorded at 0.1 mM of the Gd complexes and 20 mM of Poly-β-CD. Under these conditions, the complexes are totally bound to the Poly-β-CD substrate. The solid curves through the data points were calculated with the parameters reported in Table 3, whereas the dotted lines refer to the three contributions to the overall relaxivity of [[Gd{dota(bom)$_3$}]/Poly-β-CD].

strong interactions with the water molecules on the surface of the complexes, and this lengthens their lifetime in the proximity of the paramagnetic center. From the fitting of the experimental data, we estimated the presence of approximately fifteen – twenty second-sphere water molecules (see Table 2), at an average distance of 4 Å from each paramagnetic center. This second-sphere contribution was analyzed on the basis of the Solomon – Bloembergen – Morgan model, suitably modified by introducing a generic correlation time (τ^{ss}, see the Appendix Section), which deals with the motion (exchange and/or rotation) of the second coordination sphere water molecules. The τ^{ss} values obtained from the fitting of the experimental NMRD profiles appear to be quite short (Table 2) as expected for the average values of lifetimes for these water molecules at the surface of the paramagnetic complex. Interestingly, it has been recently reported that water molecules hydrogen bonded to a Gd^{III} chelate may have residence times in the range 20 – 60 ps; the times depend on the polar group on the surface of the metal complex.[35]

Further support for the data from the model used for analyzing the NMRD profiles of the adducts with Poly-β-CD has been gained from EPR measurements. In fact, it is well known that the values of the electronic relaxation times (T_{1E} and T_{2E}) of the Gd^{III} ion may be crucial for determining the relaxivity in slowly moving paramagnetic chelates,[6] and it has been reported that a rough estimation of T_{2E} may be obtained from the analysis of the peak-to-peak separation (ΔH_{pp}) of the EPR signal [Eq. (4)].[34]

$$\frac{1}{T_{2E}} = \frac{g_L \mu_B \pi \sqrt{3}}{h} \Delta H_{pp} \qquad (4)$$

In the equation, the symbols have their usual meanings.

The linewidth of the EPR signal is slightly narrower upon the formation of the macromolecular adduct, and this indicates an increase in T_{2E}. Analogous results have been obtained for the [Gd{dtpa(cym)$_3$}] complex, and the T_{2E} values calculated from the experimental ΔH_{pp} are reported in the left column of Table 3.

It is particularly interesting to note that, at least qualitatively, the T_{2E} values obtained from EPR data are in excellent agreement with those calculated, by means of the Bloembergen – Morgan model, from the values of Δ^2 and τ_V derived from the NMRD analysis (right column in Table 3).

The observed increase of the electronic relaxation times of the Gd^{III} chelates upon the formation of the macromolecular adducts with Poly-β-CD is an unexpected finding that

Table 3. Electronic transverse relaxation times at 0.34 T and 25 °C determined from the peak-to-peak distance of the EPR signal ($^{0.34}T_{2E}^{EPR}$) or calculated from the Bloembergen – Morgan model ($^{0.34}T_{2E}^{BM}$) by using the Δ^2 and τ_V values obtained from the fitting of the NMRD profiles (see Table 2).

	$^{0.34}T_{2E}^{EPR}$ [ps]	$^{0.34}T_{2E}^{BM}$ [ps]
[Gd{dtpa(cym)$_3$}]	470 ± 22	280 ± 14
[Gd{dtpa(cym)$_3$}]/Poly-β-CD	490 ± 24.5	560 ± 28
[Gd{dota(bom)$_3$}]	310 ± 15.5	270 ± 13.5
[Gd{dota(bom)$_3$}]/Poly-β-CD	400 ± 20	480 ± 24

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contributes to the attainment of the high relaxivities shown by these systems. Although the τ_R values for the overall reorientation found for these Poly-β-CD adducts indicate that the complexes are tightly locked in the frame of the multimer, one may show that a higher relaxivity enhancement might be achieved by a further increase of τ_R (up to ca 30 ns). Moreover a concomitant shortening of τ_M down to the optimal value of 20 ns[4] (in Figure 5 a simulation for the [Gd{dota-(bom)$_3$}] complex is reported) would maximize the attainable relaxivity at an operating frequency of 20 MHz. This finding outlines again the central role of τ_M in determining the relaxivity of macromolecular Gd^{III} chelates.

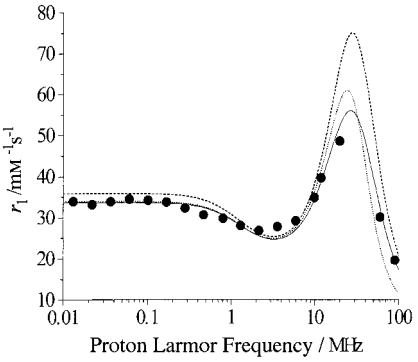

Figure 5. $1/T_1$ NMRD profile of [[Gd{dota(bom)$_3$}]/Poly-β-CD] (●); the other lines represent the simulation of the relaxivity profile using the set of parameters reported in Table 2, but τ_R is increased to 30 ns (dotted), and τ_M shortened to 20 ns (dashed).

Unfortunately, as shown above, the formation of the supramolecular adducts with Poly-β-CD promotes an increase in τ_M in such systems that is likely to be due to the high density of OH groups, which stabilize an extended hydrogen-bonding network. In turn this supramolecular structure interferes with the exchange process of the coordinated water. On the other hand, the same phenomenon has a positive effect and results in a significant contribution from water molecules in the second coordination sphere that balances, in part, the "quenching" effect on the observed relaxivity associated with the relatively slow exchange of the inner-sphere water(s).

Conclusion

The results reported in this work represent an important step along the route to systems endowed with high relaxivities. The relaxivities shown by [Gd^{III}–L/Poly-β-CD] adducts are more than one order of magnitude higher than the characteristic values reported for the extracellular agents currently used in clinical practice. Moreover, they are also higher than the relaxivities shown by Gd^{III} complexes interacting with HSA. It is likely that the described paramagnetic supramolecules may be very useful in the MRI visualization of thrombi, plaques, and even receptors involved in neoangiogenesis processes or overexpressed on the surface of tumor cells, once units of the β-CD multimer were endowed with specific targeting capabilities. One expects that after the initial anchoring of the polymeric chains at the targeting site, progressive growth of the supramolecule will take place, and thus a large number of paramagnetic complexes will accumulate in the region of interest.

Experimental Section

Synthesis of the DTPA(CyM)$_3$, DOTA(BOM)$_3$, and DO3A(BOM)$_3$ ligands and of the corresponding Gd^{III} complexes: DOTA(BOM)$_3$ and DO3A(BOM)$_3$ ligands were synthesized according to previously reported procedures,[36] and the corresponding Gd^{III} complexes were prepared following the General Procedure described in the same paper. The DTPA(CyM)$_3$ ligand was prepared according to a modification of the Rapoport synthesis of DTPA.[37] L-Phenylalanine *tert*-butyl ester was alkylated (DIEA, Diisopropylethylamine MeCN) at first with 2-(2-bromoethoxy)tetrahydropyrane and then with *tert*-butyl bromoacetate. Cleavage of the tetrahydropyranyl protection (aq. HCl) and bromination of the hydroxy group (NBS (*N*-bromosuccinimide), Ph_3P, CH_2Cl_2) gave *N*-(2-bromoethyl)-*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]-L-phenylalanine 1,1-dimethylethyl ester.

Double alkylation of L-phenylalanine 1,1-dimethylethyl ester with such a bromo derivative (MeCN/pH 8 phosphate buffer) gave the pentaester of the corresponding DTPA-like ligand. Hydrogenation of the three aromatic rings (H_2, 5% Rh/C, MeOH), followed by hydrolysis of the *tert*-butyl ester groups (trimethylsilyl iodide, $CHCl_3$) gave DTPA(CyM)$_3$. Full details of the synthetic methodologies for the synthesis of DTPA(CyM)$_3$ are given elsewhere.[38] Characterization of ligand DTPA(CyM)$_3$: m.p. 169 °C (dec.); $[\alpha]_D^{20} = +37.02$ ($c = 2.5$ in 4 N NaOH); ^{13}C NMR (D_2O + KOD, 25 °C): $\delta =$ 28.6, 28.7, 29.0, 35.4, 35.7, 36.2, 37.5, 38.6, 40.7, 50.8, 53.2, 58.0, 66.7, 68.3, 176.9, 178.6, 179.8; MS (ESI): m/z (%): 681 $[M-H]^-$; elemental analysis calcd (%) for $C_{35}H_{59}N_3O_{10}$: C 61.65, H 8.72, N 6.16; found: C 62.11, H 8.76, N 6.23. The Gd^{III} complex of DTPA(CyM)$_3$ was prepared by using $GdCl_3$ and *N*-methylglucamine as the base.

Poly-β-CD multimer: Poly-β-cyclodextrin, consisting of a mixture of oligomers ($M_W = 3-15$ kDa), was purchased from Cyclolab (Budapest, Hungary). According to the producer, adjacent β-CD units are linked through a 1-chloro-2,3-epoxy propane group. The commercial product was purified by size-exclusion HPLC by using NH_4HCO_3 (120 mM) as eluent, on a ÄCTA Explorer 100 (Amersham Pharmacia Biotech, Uppsala, Sweden). Several fractions were collected corresponding to compounds with different polymerization degrees. The β-CD multimer used for the experimental measurements was the one with the higher molecular weight (15 kDa).

Water proton relaxivity measurements: The longitudinal water proton relaxation rate was measured by using a Stelar Spinmaster (Stelar, Mede, Pavia, Italy) spectrometer operating at 20 MHz, by means of the standard inversion-recovery technique (16 experiments, 2 scans). A typical 90° pulse width was 3.5 μs, and the reproducibility of the T_1 data was ±0.5%. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper-constantan thermocouple (uncertainty ±0.1 °C).

The proton $1/T_1$ NMRD profiles were measured over a continuum of magnetic-field strength from 0.00024 to 0.28 T (corresponding to 0.01–12 MHz proton Larmor Frequency) on a Stelar Fast Field-Cycling relaxometer. This relaxometer worked under complete computer control with an absolute uncertainty in $1/T_1$ of ±1%. Data points at 20 MHz, 60 MHz, and 90 MHz were added to the experimental NMRD profiles and were recorded on the Stelar Spinmaster (20–60 MHz) and on a JEOL EX-90 (90 MHz) spectrometer, respectively.

The concentration of the Gd^{III} complexes in the solutions for the relaxometric determinations was obtained by means of ICP analysis.

^{17}O measurements: Variable-temperature ^{17}O NMR measurements were recorded at 2.1 T on the JEOL EX-90 spectrometer, equipped with a 5 mm probe, by using a D_2O external lock. Experimental settings were as follows: spectral width 10000 Hz, 90° pulse (7 μs), acquisition time 10 ms, 1000 scans, and without sample spinning. Aqueous solutions containing 2.6% of ^{17}O isotope (Yeda, Israel) were used. The observed transverse relaxation rates (R_{2obs}^O) were calculated from the signal width at half-height ($\Delta\nu_{1/2}$): $R_{2obs}^O = \pi\Delta\nu_{1/2}$.

EPR measurements: The EPR spectra were measured at room temperature at X-band (0.34 T) on a Bruker EMX 10/12 spectrometer. The sample (1 mM aqueous solution of the Gd^{III} chelate) was inserted into a quartz capillary.

Appendix

^{1}H water relaxation rate: The relaxivity of a Gd^{III} complex can be accounted for by the sum of contributions arising from water molecules in the inner, second, and outer coordination spheres of the metal ion [Eq. (5)].

$$r_1 = r_1^{His} + r_1^{Hss} + r_1^{Hos} \tag{5}$$

The r_1^{His} refers to the contribution from the exchange of the water protons in the first coordination sphere of the paramagnetic metal ion [Eq. (6)].

$$r_1^{His} = \frac{q[C]}{55.6(T_{1M} + \tau_M)} \tag{6}$$

In this equation, q is the hydration number, $[C]$ is the molar concentration of the paramagnetic chelate, T_{1M} is the longitudinal relaxation time of the inner-sphere water protons, and τ_M is their residence lifetime. The Solomon–Bloembergen theory[39] enables one to determine the magnetic-field dependence of T_{1M} [Eq. (7)].

$$\frac{1}{T_{1M}^H} = \frac{2}{15}\frac{\gamma_H^2 g_e^2 \mu_B^2 S(S+1)}{r_H^6}\left[\frac{3\tau_{c1}}{1+\omega_H^2\tau_{c1}^2} + \frac{7\tau_{c2}}{1+\omega_S^2\tau_{c2}^2}\right] \tag{7}$$

In this equation, S is the electron spin quantum number (7/2 for Gd^{III}), γ_H is the proton nuclear magnetogyric ratio, μ_B is the Bohr magneton, g_e is the Landè factor for the free electron, and r_H is the distance between the metal ion and the inner-sphere water protons. ω_H and ω_S are the proton and electron Larmor frequencies ($\omega_S = 658.21\omega_H$), respectively, and $\tau_{ci}(i=1,2)$ are the correlation times related to the modulation of the dipolar electron–proton coupling. Such an interaction may be modulated by the reorientation of the paramagnetic species, τ_R, by the residence lifetime, τ_M, and by the electronic relaxation times, T_{iE} ($i=1,2$) [Eq. (8)].

$$\tau_{ci}^{-1} = \tau_R^{-1} + \tau_M^{-1} + T_{iE}^{-1} \tag{8}$$

Analogously to the nuclear relaxation time, the electronic relaxation time is also magnetic-field dependent. For Gd^{III} complexes, T_{iE} are determined by the modulation of the transient zero field splitting (ZFS_T) of the electronic spin states caused by the dynamic distortions of the ligand field, and, according to the Blombergen–Morgan theory,[39] their magnetic-field dependence is given by the following equations [Eq. (9)] and [Eq. (10)].

$$T_{1E}^{-1} = \frac{1}{25}\Delta^2\tau_V[4S(S+1)-3]\left(\frac{1}{1+\omega_S^2\tau_V^2} + \frac{4}{1+4\omega_S^2\tau_V^2}\right) \tag{9}$$

$$T_{2E}^{-1} = \frac{1}{50}\Delta^2\tau_V[4S(S+1)-3]\left(3 + \frac{5}{1+\omega_S^2\tau_V^2} + \frac{2}{1+4\omega_S^2\tau_V^2}\right) \tag{10}$$

In this equation, Δ^2 is the square of the transient ZFS_T energy, and τ_V is the correlation time related to its modulation.

The second-sphere term, r_1^{Hss}, describes the contribution arising from water molecules held in the proximity of the surface of the metal complex by hydrogen-bonding interactions with polar groups of the ligand. By using the same approach described above for the inner-sphere contribution and by assuming that the residence lifetime of the second-sphere water protons is significantly shorter than their relaxation time, r_1^{Hss} is given by Equation (11).

$$r_1^{Hss} = \frac{q^{SS}[C]}{55.6}\frac{1}{T_{1M}^{Hss}} \tag{11}$$

In this equation, q^{ss} is the number of second-sphere water molecules, and T_{1M}^{Hss} is the longitudinal relaxation time of the second-sphere water protons. Analogously to the inner-sphere contribution, T_{1M}^{Hss} is magnetic-field dependent [Eq. (12)].

$$\frac{1}{T_{1M}^{Hss}} = \frac{2}{15}\frac{\gamma_H^2 g_e^2 \mu_B^2 S(S+1)}{r_{SS}^6}\left[\frac{3\tau_{c1}^{SS}}{1+\omega_H^2(\tau_{c1}^{SS})^2} + \frac{7\tau_{c2}^{SS}}{1+\omega_S^2(\tau_{c2}^{SS})^2}\right] \tag{12}$$

In this equation, r_{SS} is the average distance between the metal ion and the second-sphere water protons, and τ_{ci}^{SS} ($i=1,2$) is the correlation time associated with the motion of the second-sphere water molecules.

For simplicity τ_{ci}^{SS} are given by Equation (13).

$$(\tau_{ci}^{SS})^{-1} = (\tau^{SS})^{-1} + T_{iE}^{-1} \tag{13}$$

In this equation, τ^{ss} is related to the motions (reorientation or exchange) of the second-sphere water protons.

The outer-sphere term, r_1^{Hos}, describes the contribution from water molecules which diffuses around the paramagnetic complex and, according to the model developed by Hwang and Freed,[39] may be related to the minimum distance between the metal and the outer-sphere water protons, a, the relative solute–solvent diffusion coefficient, D, and, again, the electronic relaxation times, T_{iE} [Eq. (14)].

$$r_{1p}^{Hos} = C^{OS}\left(\frac{1}{aD}\right)[7J(\omega_S) + 3J(\omega_H)] \tag{14}$$

In this equation, C^{OS} is a constant ($5.8\times10^{-13}\ s^{-2}M^{-1}$), and the dependence on the electronic relaxation times is expressed in the non-Lorentzian spectral density functions $J(\omega_i)$.

^{17}O water relaxation rate: The exchange lifetime of a metal-bound water molecule in a paramagnetic chelate may be accurately assessed by measuring the temperature dependence of the paramagnetic contribution to the water ^{17}O transverse relaxation rate (R_{2p}^O).

R_{2p}^O is related to τ_M through the values of $\Delta\omega_M^O$ (i.e. the ^{17}O chemical shift difference between coordinated and bulk water molecules) and R_{2M}^O (which, in analogy to T_{1M}, is the transverse relaxation rate of the coordinated water oxygen) according to the Swift and Connick equation [Eq. (15)].[40]

$$R_{2p}^O = \frac{qC}{55.6}\tau_M^{-1}\frac{R_{2M}^{O\,2} + \tau_M^{-1}R_{2M}^O + \Delta\omega_M^{O2}}{(R_{2M}^O + \tau_M^{-1})^2 + \Delta\omega_M^{O2}} \tag{15}$$

The temperature dependence of $\Delta\omega_M^O$ is described by the following equation [Eq. (16)].

$$\Delta\omega_M^O = \frac{g_e\mu_B S(S+1)B_0 A}{3k_B T\ \hbar} \tag{16}$$

In this equation, B_0 is the magnetic-field strength, and $A/\hbar$ is the Gd—^{17}O scalar coupling constant (the value for polyaminocarboxylate Gd^{III} complexes has been fixed to $-3.8\times10^6\ rad\,s^{-1}$).[39]

For relatively small-sized Gd^{III} chelates and at 2.1 T, R_{2M}^O is essentially dominated by the electron–nucleus scalar interaction ([Eqs. (17)] and (18)).

$$R_{2M}^O = \frac{1}{3}\left(\frac{A}{\hbar}\right)^2 S(S+1)\left(\tau_{E1} + \frac{\tau_{E2}}{1+\omega_S^2\tau_{E2}^2}\right) \tag{17}$$

$$\tau_{Ei}^{-1} = T_{iE}^{-1} + \tau_M^{-1} \tag{18}$$

Finally, the temperature dependence of R_{2p}^O is expressed in terms of the Eyring relationship for τ_M and τ_V [Eq. (19)].

$$(\tau_j)_T^{-1} = \frac{(\tau_j^{-1})^{298.15}T}{298.15}\exp\left[\frac{\Delta H_j}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \tag{19}$$

In this equation, j refers to the two different dynamic processes involved ($j=v, M$), and ΔHj is the corresponding activation enthalpy.

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[1] B. L. Engelstad, G. L. Wolf, *Magnetic Resonance Imaging* (Eds.: D. D. Stark, W. G. Bradley, Jr.), V. C. Mosby Company, St. Louis, **1988**, p. 161.
[2] J. A. Peters, J. Huskens, D. J. Raber, *Prog. Nucl. Magn. Reson. Spectrosc.* **1996**, *28*, 283.
[3] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293.
[4] S. Aime, M. Botta, M. Fasano, E. Terreno, *Chem. Soc. Rev.* **1998**, *27*, 19.
[5] S. H. Koenig, R. D. Brown III, *Prog. Nucl. Magn. Reson. Spectrosc.* **1990**, *22*, 487.
[6] R. B. Lauffer, *Chem. Rev.* **1987**, *87*, 901.
[7] R. C. Brasch, *Magn. Reson. Med.* **1991**, *22*, 282.
[8] M. Spanoghe, D. Lanens, R. Dommisse, A. Van der Linden, F. Alderweireldt, *Magn. Reson. Imaging* **1992**, *10*, 913.
[9] H. Paajanen, T. Reisto, I. Hemmilä, M. Komu, P. Niemi, M. Kormano, *Magn. Reson. Med.* **1990**, *13*, 38.
[10] P. Niemi, T. Reisto, I. Hemmilä, M. Kormano, *Invest. Radiol.* **1991**, *26*, 820.
[11] E. C. Wiener, M. W. Brechbiel, H. Brothers, R. L. Magin, O. A. Gansow, D. A. Tomalia, P. C. Lauterbur, *Magn. Reson. Med.* **1994**, *31*, 1.
[12] L. D. Margerum, B. K. Campion, M. Koo, N. Shargill, J.-J. Lai, A. Marumoto, P. C. Sontum, *J. Alloys Compd.* **1997**, *249*, 185.
[13] H. C. Roberts, M. Saeed, T. P. L. Roberts, R. C. Brasch, *Acad Radiol.* **1998**, *5*, S31.
[14] P. F. Sieving, A. D. Watson, S. M. Rocklage, *Bioconjugate Chem.* **1990**, *1*, 65.
[15] S. Aime, M. Botta, S. Geninatti Crich, G. Giovenzana, G. Palmisano, M. Sisti, *Chem. Commun.* **1999**, 1577.
[16] T. Desser, D. Rubin, H. Muller, F. Qing, S. Khodor, Zanazzi, S. Young, D. Ladd, J. Wellons, K. Kellar, J. Toner, R. Snow, *J. Magn. Reson. Imaging* **1994**, *4*, 467.
[17] S. Aime, M. Botta, M. Fasano, S. Geninatti Crich, E. Terreno, *J. Biol. Inorg. Chem.* **1996**, *1*, 312.
[18] S. Aime, M. Botta, S. Geninatti Crich, G. B. Giovenzana, R. Pagliarin, M. Piccinini, M. Sisti, E. Terreno, *J. Biol. Inorg. Chem.* **1997**, *2*, 470.
[19] E. Toth, F. Connac, L. Helm, K. Adzamli, A. E. Merbach, *J. Biol. Inorg. Chem.* **1998**, *3*, 606.
[20] S. Aime, M. Chiaussa, G. Digilio, E. Gianolio, E. Terreno, *J. Biol. Inorg. Chem.* **1999**, *4*, 766.
[21] R. B. Lauffer, D. J. Parmalee, S. U. Dunham, H. S. Ouellet, R. P. Dolan, S. Witte, T. J. McMurry, R. C. Walowitch, *Radiology* **1998**, *207*, 529.
[22] C. F. G. C. Geraldes, A. D. Sherry, I. Lázár, A. Miseta, P. Bogner, B. Berenyi, B. Sumegi, G. E. Kiefer, K. McMillan, F. Maton, R. N. Muller, *Magn. Reson. Med.* **1993**, *30*, 696.
[23] S. Aime, A. S. Batsanov, M. Botta, J. A. K. Howard, D. Parker, K. Senanayake, G. Williams, *Inorg. Chem.* **1994**, *33*, 4696.
[24] L. Van der Elst, F. Maton, S. Laurent, F. Seghi, F. Chapelle, R. N. Muller, *Magn. Reson. Med.* **1997**, *38*, 604.
[25] C. F. G. C. Geraldes, A. D. Sherry, P. Vallet, F. Maton, R. N. Muller, T. D. Mody, G. Hemmi, J. L. Sessler, *J. Magn. Reson. Imaging* **1995**, *5*, 725.
[26] S. Aime, L. Barbero, M. Botta, *Magn. Reson. Imaging* **1991**, *9*, 843.
[27] A. D. Nunn, K. E. Linder, M. F. Tweedle, *J. Nucl. Med.* **1997**, *41*, 155.
[28] S. Aime, M. Botta, L. Frullano, S. Geninatti Crich, G. B. Giovenzana, R. Pagliarin, G. Palmisano, M. Sisti, *Chem. Eur. J.* **1999**, *5*, 1253.
[29] E. Tóth, O. M. Ni Dhubhghaill, G. Besson, L. Helm, A. E. Merbach, *Magn. Reson. Chem.* **1999**, *37*, 701.
[30] S. Aime, M. Botta, S. Geninatti Crich, G. B. Giovenzana, R. Pagliarin, M. Sisti, E. Terreno, *Magn. Reson. Chem.* **1998**, *36*, S200.
[31] S. Aime, S. Geninatti Crich, E. Gianolio, E. Terreno, A. Beltrami, F. Uggeri, *Eur. J. Inorg. Chem.* **1998**, 1283.
[32] S. Aime, M. Botta, M. Fasano, E. Terreno, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging* (Eds.: A. E. Merbach, E. Tóth), Wiley, Chichester, **2001**, Chapter 5, p. 193.
[33] S. Aime, M. Botta, M. Fasano, E. Terreno, *Acc. Chem. Res.* **1999**, *32*, 941.
[34] 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.
[35] A. Borel, L. Helm, A. E. Merbach, *Proceedings of the COST D8/D18 European Workshop*, Prague, **2000**, p. 18.
[36] S. Aime, M. Botta, G. Ermondi, F. Fedeli, F. Uggeri, *Inorg. Chem.* **1992**, *31*, 1100.
[37] M. A. Williams, H. Rapoport, *J. Org. Chem.* **1993**, *56*, 1151.
[38] L. Calabi, A. Maiocchi, M. Lolli, F. Rebasti, PCT WO 98/05625, **1998**; [*Chem. Abstr.* **1998**, *128*, 192927p].
[39] L. Banci, I. Bertini, C. Luchinat, *Nuclear and Electronic Relaxation*, VCH, Weinheim, **1991**, p. 91.
[40] T. J. Swift, R. E. J. Connick, *J. Chem. Phys.* **1962**, *37*, 307.

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