

Bis(phenylethylamide) Derivatives of Gd-DTPA as Potential Receptor-Specific MRI Contrast Agents

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DTPA-bis(amide) derivatives bearing phenyl, phenol or catechol groups that mimic side chains of naturally occurring amino acids, such as phenylalanine, tyrosine or dopamine, were synthesized and characterized by elemental analysis, electrospray mass spectrometry, NMR spectroscopy and IR spectroscopy. The gadolinium(III) complexes of the ligands DTPA-bis(tyramide) [DTPA-(TA)₂], DTPA-bis(3-hydroxytyramide) [DTPA-(HTA)₂] and DTPA-bis(phenylalanine ethyl ester) [DTPA-(PAE)₂], were prepared and then studied in vitro by ¹⁷O NMR spectroscopy and by nuclear magnetic relaxation dispersion (NMRD) measurements. The residence time

of the coordinated water in gadolinium(III) complexes was obtained from ¹⁷O NMR relaxometric *T*₂ measurements. At 310 K, the following τ_M values were obtained: Gd-DTPA-(TA)₂ 582 ns, Gd-DTPA-(HTA)₂ 372 ns and Gd-DTPA-(PAE)₂ 809 ns. As shown by the analysis of the proton NMRD profiles, the larger proton relaxivities of the gadolinium(III) complexes at 310 K relative to that of the parent Gd-DTPA complex are mainly because of the increase in the rotational correlation time.

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Introduction

Magnetic resonance imaging (MRI) is a powerful diagnostic technique which is used for obtaining images of internal organs and tissues.^[1] Nowadays, an increasing number of MRI scans are performed employing contrast agents, which are able to greatly increase the contrast between tissues in magnetic resonance images.^[2] The efficiency of contrast agents is related to their ability to enhance the proton relaxation of water tissues. This property, called *relaxivity*, depends on different factors such as the molecular mobility of the contrast agent, the water dynamics and the noncovalent binding of the contrast agent to endogenous proteins.^[3,4] In recent years, new developments in molecular imaging applications have prompted the development of a novel class of contrast agents characterized by a higher contrasting ability and improved targeting capabilities.

Several strategies have been explored in order to slow down the rotational motion of the gadolinium(III)-based contrast agents and thus to increase their relaxation efficiency. These approaches include (i) the synthesis of covalently or noncovalently bound macromolecular gadolinium(III) chelates such as dendrimers, linear polymers or proteins;^[5–7] (ii) the synthesis of amphiphilic gadolinium(III) complexes which can self-assemble to micelles;^[8,9] (iii) lipophilic gadolinium(III) complexes incorporated in supramolecular systems with a better control of the size such as mixed micelles;^[10–12] and (iv) incorporation of amphipathic gadolinium(III) complexes into the membranes of liposomes.^[13–17]

The properties of the contrast agents that are important for the tissue specificity include molecular size, charge and lipophilicity. Since most of the specific cell-cell interactions or recognitions are regulated by special proteins, receptor targeting can be attained by mimicking signal peptides. For example, adhesive proteins present in extracellular matrices and in blood, such as fibrinogen and collagens, contain the tripeptide arginine–glycine–aspartic acid as the cell recognition site.^[18] Using this binding domain or similar sequences, small peptides can be designed to target receptors.^[19] Additionally, some receptors have an affinity for certain classes of substrates such as amino acids or catechol amines.^[20] These receptors may also bind molecules that resemble the substrate, for example, a derivative of an amino acid that is present in a peptide substrate or an amide derivative of a naturally occurring catechol amine such as dopamine. The coupling of these potential recognition groups to high relaxivity moieties such as [Gd(DTPA)(H₂O)]^{2–} could thus lead to tissue specificity of the contrast agent.

In this paper we report on ligands based on bis(amide) derivatives of DTPA bearing phenyl, phenol or catechol groups that mimic the side chains of naturally occurring

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amino acids such as phenylalanine, tyrosine and dopamine. The gadolinium(III) complexes of these ligands were synthesized and then studied *in vitro*. The residence time of the coordinated water molecule was studied by means of ^{17}O NMR spectroscopy, and nuclear magnetic relaxation dispersion (NMRD) measurements were performed in order to test the efficiency of these complexes as potential MRI contrast agents.

Results and Discussion

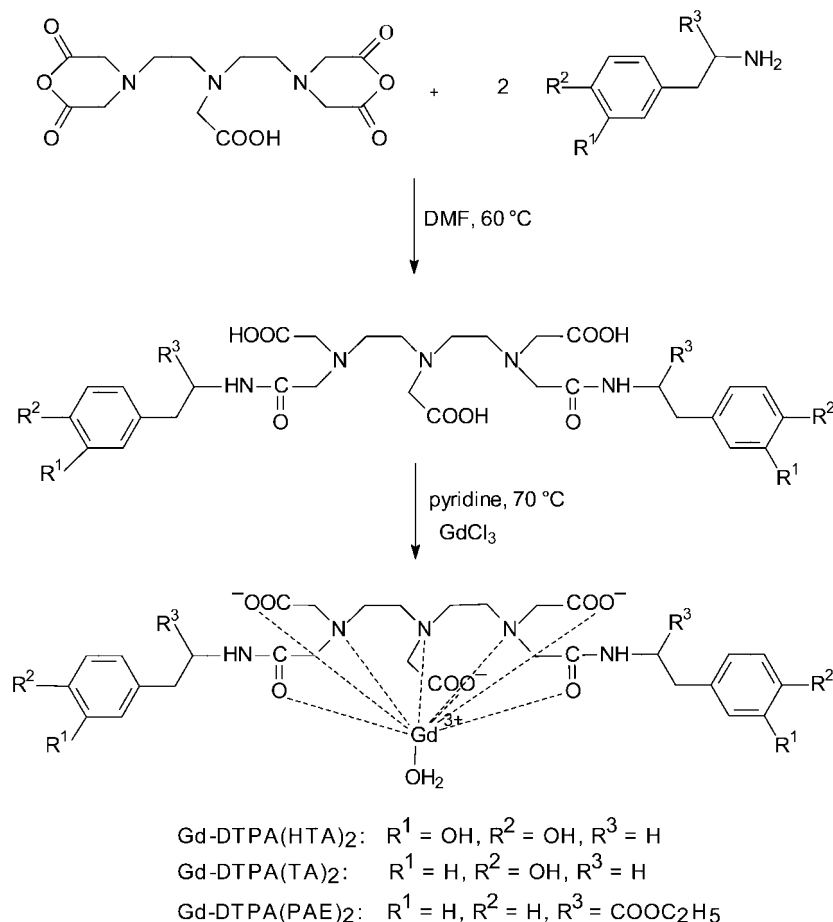
Ligands and Complexes

The ligands DTPA-bis(tyramide) [DTPA-(TA) $_2$], DTPA-bis(3-hydroxytyramide) [DTPA-(HTA) $_2$] and DTPA-bis(phenylalanine ethyl ester) [DTPA-(PAE) $_2$] were synthesized by the reaction between DTPA-bis(anhydride) and the corresponding amine in DMF. In pyridine, the ligands readily formed complexes with gadolinium(III) ions (Scheme 1). Infrared absorption data of all ligands show strong absorptions in the region 1630–1650 cm^{-1} , corresponding to the CO stretching modes.^[21] Shifts of ca. 10 to 20 cm^{-1} to lower wavenumber were observed for the carbonyl stretching frequencies upon complexation. This indicates amide oxygen coordination to the gadolinium(III) ion. These findings are

consistent with previous studies which have shown that DTPA-bis(amide) derivatives coordinate to trivalent lanthanide ions by three acetate oxygen atoms, three nitrogen atoms and two carbonyl oxygen atoms of the amide groups, while the ninth coordination site is occupied by a water molecule.^[22–25] In the case of DTPA-(TA) $_2$ and DTPA-(HTA) $_2$ ligands, no evidence for the coordination of phenol oxygen to gadolinium(III) was observed. Positive-mode electrospray ionization mass spectroscopy (ESI-MS) of the Gd-DTPA-(TA) $_2$, Gd-DTPA-(HTA) $_2$ and Gd-DTPA-(PAE) $_2$ complexes indicate the presence of fully complexed species in the solution (see Experimental Section and Scheme 1).

Estimation of τ_M : ^{17}O NMR and Proton Relaxometric Measurements

The value of the residence time of the coordinated water molecule, τ_M , was classically obtained by analysis of the temperature dependence of the reduced transverse relaxation rate of the water ^{17}O nucleus in solutions containing the gadolinium(III) complexes. The procedure has been described previously.^[26,27] As expected for bis(amide) derivatives of Gd-DTPA,^[28] the curves representing the reduced transverse relaxation rates versus the reciprocal of the tem-



Scheme 1. Synthesis of the ligands and corresponding gadolinium(III) complexes.

perature are closer to the data obtained for Gd-DTPA-BMA than to those of Gd-DTPA (Figure 1). Theoretical adjustment of these experimental data was performed as described previously, assuming the presence of one water molecule in the first coordination sphere.^[26,27] This procedure allows for the determination of (i) $A/\hbar$, the hyperfine coupling constant between the oxygen nucleus of bound water molecules and the gadolinium(III) ion; (ii) the parameters describing the electronic relaxation times of gadolinium(III), that is, the correlation time modulating the electronic relaxation, τ_v , its activation energy, E_v , and a parameter related to the mean-square of the zero-field splitting energy, B ($B = 2.4\Delta^2$); and (iii) parameters related to the water exchange, that is, the enthalpy ($\Delta H^\ddagger$) and entropy ($\Delta S^\ddagger$) of the process. A second fitting was performed with the value of $A/\hbar$ set to $-3.8 \times 10^6 \text{ rad s}^{-1}$. Similar values of τ_M were obtained by both procedures. At 310 K, the water residence time of Gd-DTPA-(PAE)₂ [$\tau_M^{310} = (809 \pm 46)$ or $(811 \pm 72) \text{ ns}$], is quite similar to that reported^[28] for the bis(methylamide) derivative of Gd-DTPA, Gd-DTPA-BMA [$\tau_M^{310} = (967 \pm 36) \text{ ns}$], whereas smaller values are obtained for the two other complexes [$\tau_M^{310} = (582 \pm 149)$ or $(545 \pm 24) \text{ ns}$ for Gd-DTPA-(TA)₂ and $\tau_M^{310} = (372 \pm 18)$

or $(404 \pm 18) \text{ ns}$ for Gd-DTPA-(HTA)₂] (Table 1). The presence of an ethyl ester group on the ethylene chain of the amide substituent seems thus to have a harmful effect on the water exchange rate. This could be related to the steric hindrance and the hydrophobicity of the ethyl chains and/or to the possible formation of hydrogen bonds between the ester function and the coordinated water molecule leading to stabilization of the hydrated state and thus to a slowing down of the water exchange. On the other hand, the presence of two hydroxy groups on the aromatic ring has a beneficial effect on the water exchange rate.

Such values of τ_M are not expected to significantly influence the relaxivity of small complexes at 310 K and 20 MHz, but at lower temperatures the relaxivity should be quenched. This is confirmed by the similarity of the proton relaxivities measured at 298 and 278 K (Figure 2) and their very small variations (less than 10%) observed in the temperature range extending between 310 and 278 K.

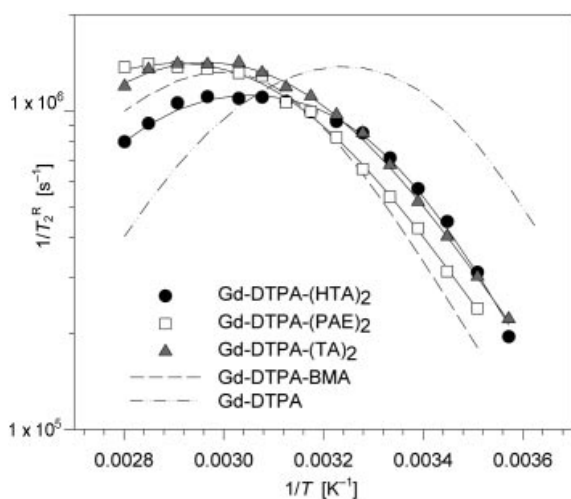


Figure 1. ^{17}O transverse reduced relaxation rate ($1/T_2^R = 55.55/\{T_2^P[\text{Gd complex}]\}$) of aqueous solutions of the gadolinium(III) complexes as a function of the reciprocal of temperature.

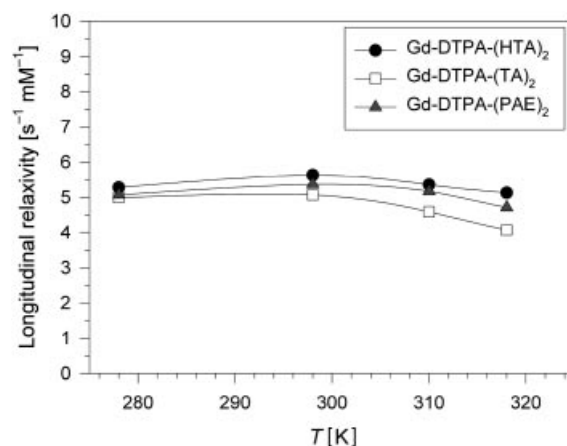


Figure 2. Evolution of the proton longitudinal relaxivity of Gd-DTPA-(TA)₂, Gd-DTPA-(HTA)₂ and Gd-DTPA-(PAE)₂ as a function of the temperature ($B = 0.47 \text{ T}$).

It should be pointed out that such τ_M values are unfavourable in the context of the design of tracers for molecular imaging if covalent coupling of the chelate to a vector is considered.

Table 1. Parameters obtained from the theoretical fitting of the ^{17}O NMR spectroscopic data.^[a]

Parameter	Gd-DTPA-(TA) ₂	Gd-DTPA-(HTA) ₂	Gd-DTPA-(PAE) ₂
$\tau_v^{298} [\text{ps}]$	12.9 ± 2.7 (22.0 ± 0.5)	21.1 ± 0.5 (24.1 ± 0.5)	12.9 ± 0.4 (20.5 ± 1.6)
$B [10^{20} \text{ s}^{-2}]$	2.85 ± 0.57 (6.22 ± 0.15)	1.36 ± 0.03 (9.50 ± 0.2)	3.57 ± 0.10 (4.70 ± 0.40)
$E_v [\text{kJ mol}^{-1}]$	1.8 ± 4.5 (0.1 ± 18.8)	0.1 ± 3.0 (0.1 ± 12.4)	4.8 ± 3.7 (3.8 ± 2.6)
$\tau_{\text{SO}}^{310} [\text{ps}]^{\text{[b]}}$	54 ± 22 (15 ± 1)	70 ± 3 (9 ± 0.4)	43 ± 3 (21 ± 4)
$A/\hbar [10^6 \text{ rad s}^{-1}]$	-3.6 ± 0.7 (-3.8)	-4.5 ± 1.4 (-3.8)	-4.2 ± 0.4 (-3.8)
$\Delta H^\ddagger [\text{kJ mol}^{-1}]$	44.6 ± 0.4 (46.1 ± 0.1)	55.8 ± 0.1 (53.3 ± 0.1)	43.6 ± 0.1 (42.2 ± 0.2)
$\Delta S^\ddagger [\text{J mol}^{-1} \text{ K}^{-1}]$	18.2 ± 1.0 (23.4 ± 0.2)	58.1 ± 0.2 (49.2 ± 0.2)	12.2 ± 0.3 (7.6 ± 0.3)
$\tau_M^{310} [\text{ns}]$	582 ± 149 (545 ± 24)	372 ± 18 (404 ± 18)	809 ± 46 (811 ± 72)

[a] The values in parentheses correspond to the second type of fitting ($A/\hbar$ fixed to $-3.8 \times 10^6 \text{ rad s}^{-1}$), whereas the other values correspond to the first type of fitting ($A/\hbar$ fitted). [b] Values calculated using the equation: $\tau_{\text{SO}} = (5B\tau_v)^{-1}$.

Influence of pH on Proton Relaxivity

In a pH range extending from 4 to 8, no significant change in the proton relaxivity at 20 MHz and 310 K was noticed (r_1 varies by less than 8%). These results show that no decomplexation occurs in this pH range and that the possible acceleration of the exchange rate of the water protons in acidic or basic media induce no or very little change in the proton relaxivity of the complexes. Since the hydrogen bonding is pH dependent, the absence of pH dependence of the Gd-DTPA-(PAE)₂ proton relaxivity could suggest the absence of a hydrogen bond between the ester function and the coordinated water molecule.^[29] However, one can not exclude the existence of this hydrogen bond, since it has been established that the hydrogen bonding between neutral groups is usually insensitive to pH over the wide range in which no protonation or deprotonation of the hydrogen-bond acceptor or donor occurs.^[29]

Proton NMRD Measurements

The proton NMRD relaxivities of the three gadolinium(III) complexes recorded at 310 K are quite similar and 20 to 36% larger than that for Gd-DTPA at 20 MHz (Figure 3, Table 2). The fittings of the proton NMRD profiles were performed as described in the Exp. Sect. and include both inner-sphere and outer-sphere interactions.^[30–32] Some parameters were fixed during the fitting procedure: d , the distance of closest approach for the outer-sphere contribution was set at 0.36 nm, D , the relative diffusion constant was set to $3.3 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$,^[33] τ_M^{310} values were those obtained by ¹⁷O NMR spectroscopy, and the number of water molecules in the first coordination sphere of gadolinium(III) was set to one. τ_V^{310} and τ_{S0}^{310} (the electronic relaxation time at zero field) were optimized for the outer-sphere and the inner-sphere contributions simultaneously. The distance r between the protons of the coordinated water molecule and the gadolinium(III) ion was set to 0.31 nm.

The fitting of the experimental data of the three complexes gave values of τ_{S0} and τ_V close to those of Gd-DTPA. However, the τ_{S0} values calculated from the ¹⁷O NMR spectroscopic data or obtained from the fitting of the proton NMRD profiles are quite different. This can be related to the fact that ¹⁷O data are obtained at high magnetic field where τ_{S1} values are quite large (of the order of 10^{-8} s),

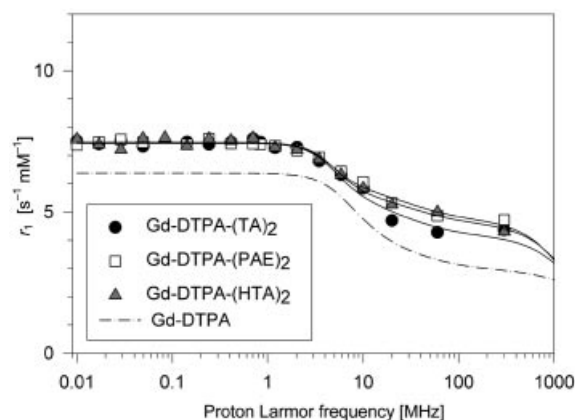


Figure 3. Proton NMRD profiles of the relaxivity of Gd-DTPA-(TA)₂, Gd-DTPA-(HTA)₂ and Gd-DTPA-(PAE)₂ at 37 °C. [Gd-DTPA-(TA)₂] = 3.22 mM, [Gd-DTPA-(HTA)₂] = 3.65 mM and [Gd-DTPA-(PAE)₂] = 2.84 mM. The NMRD profile of Gd-DTPA was added for comparison.^[34]

whereas the τ_{S0} values estimated from the proton relaxometric NMRD data of small complexes are predominantly determined from the low-field data (where τ_{S1} values are of the order of 10^{-10} s). The rotational correlation times τ_R are, as expected, somewhat larger (Table 2) than the value found for Gd-DTPA and agree with the increase in molecular weight on going from Gd-DTPA-(TA)₂ to Gd-DTPA-(PAE)₂.

Transmetalation

The transmetalation by zinc(II) ions was assessed in order to test the stability of the complexes. The procedure is based on the very low solubility of gadolinium(III) ions in phosphate solution and on the subsequent decrease of the proton paramagnetic relaxation rate during the transmetalation process. The data are shown in Figure 4 and are compared to those of Gd-DTPA and Gd-DTPA-BMA. The “ratio index” is defined as the time (t) required to reach a ratio $R_1^P(t)/R_1^P(0) = 80\%$,^[34] and is larger for the three complexes than that of the commercial Gd-DTPA-BMA. After 50 hours, the ratio $R_1^P(t = 3000)/R_1^P(0)$ of Gd-DTPA-(TA)₂ and Gd-DTPA-(PAE)₂ is still better or similar to that of Gd-DTPA-BMA, whereas for Gd-DTPA-(HTA)₂ a

Table 2. Experimental proton longitudinal relaxivity at 20 MHz and 37 °C, and parameters obtained from the theoretical fitting of the proton NMRD data.

Parameter	r_1 at 0.41 T [$\text{s}^{-1} \text{ mM}^{-1}$]	r_1 at 1.4 T [$\text{s}^{-1} \text{ mM}^{-1}$]	τ_R^{310} [ps]	τ_{S0}^{310} [ps]	τ_V^{310} [ps]	τ_M^{310} [ns] ^[c]
Gd-DTPA ^[d]	3.8 ^[a]	3.4 ^[a]	54 ± 1	87 ± 3	25 ± 3	143 ± 25
Gd-DTPA-(TA) ₂	4.8 ^[a] 6.8 ^[b]	4.5 ^[a] 6.0 ^[b]	99 ± 2	96 ± 2	34 ± 4	582 ± 149
Gd-DTPA-(HTA) ₂	5.3 ^[a] 5.4 ^[b]	5.0 ^[a] —	111 ± 2	83 ± 2	31 ± 2	372 ± 18
Gd-DTPA-(PAE) ₂	5.3 ^[a] 6.9 ^[b]	4.8 ^[a] 6.2 ^[b]	127 ± 2	90 ± 1	36 ± 3	809 ± 46

[a] Relaxivity in water. [b] Apparent relaxivity in 4% HSA (i.e., paramagnetic relaxation rate of 1 mM solution of the complex in 4% HSA). [c] Obtained from ¹⁷O relaxometry. [d] From ref.^[34]

lower ratio is obtained. The complexes reported in the present work thus undergo a slower transmetallation than Gd-DTPA-BMA within the first five hours. However, after 50 hours the extent of the transmetallation process is more pronounced for Gd-DTPA-(HTA)₂ (Figure 4).

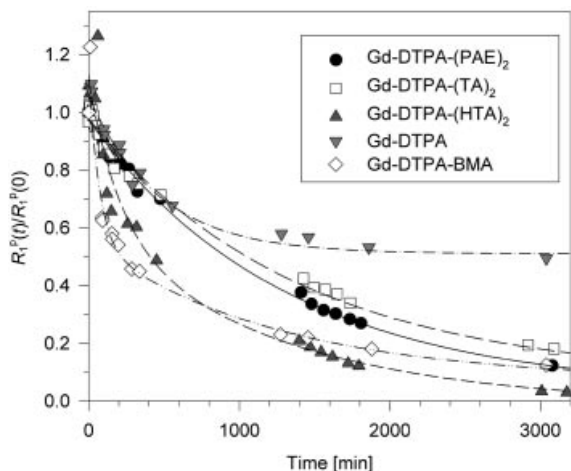


Figure 4. Evolution of $R_1^P(t)/R_1^P(0)$ vs. time for Gd-DTPA-(TA)₂, Gd-DTPA-(HTA)₂, Gd-DTPA-(PAE)₂, Gd-DTPA and Gd-DTPA-BMA (initial concentrations of Gd complexes and ZnCl₂ are 2.5 mM in phosphate buffer pH = 7, $T = 37^\circ\text{C}$, $B = 0.47\text{ T}$).

Interaction with Human Serum Albumin

The interaction of a small gadolinium(III) complex with human serum albumin (HSA) increases its rotational correlation time and subsequently enhances its paramagnetic relaxation rate and its vascular remanence. A quick insight into the strength of the interaction can be obtained by the measurement of the paramagnetic relaxation rate of a solution (1 mM) of the complex in the presence of HSA (4%) at 20 MHz. An increase in the relaxation rate larger than 60% attests to a significant interaction between the gadolinium complex and the protein.^[34] Such experiments performed with all three complexes showed maximum increases of 40% at 0.41 T and smaller values of the paramagnetic relaxation rate at 1.4 T (Table 2), testifying to the absence of a significant binding of these complexes with HSA.

Conclusions

DTPA-bis(amide) derivatives bearing phenyl, phenol or catechol groups were synthesized in high yields and complexed to gadolinium(III) ions. As expected for bis(amide) derivatives of Gd-DTPA, the residence time of the coordinated water, as obtained from ¹⁷O NMR relaxometric T_2 data, is longer than that for the parent Gd-DTPA complex. Surprisingly, a significantly longer residence time of the coordinated water was observed for the complex bearing an ethyl ester substituent. This could be caused by the existence of a hydrogen bond between the ester function and the water molecule, which can result in the stabilization of the hydrated state, or by the steric hindrance and the hydro-

phobicity of the ethyl groups. The larger proton relaxivity of all three complexes relative to that of Gd-DTPA is mainly because of the increase in the rotational correlation time. The stability of these new paramagnetic complexes tested in the presence of zinc(II) ions is better than that of Gd-DTPA-BMA within the first five hours. Taking into account the water residence time and the stability compared with zinc(II) transmetallation, the best candidate as a potential MRI contrast agent is thus Gd-DTPA-(TA)₂. The lack of significant interaction with HSA could be favourable to their use as receptor-specific contrast agents. In a next step, these contrast agents should be tested in the presence of catechol receptors and characterized in vivo in animal models.

Experimental Section

Chemicals: Reagents were obtained from Aldrich Chemical Co. Inc., Acros Organics and Fluka, and were used without further purification. DTPA-bis(anhydride) was prepared by the published procedure.^[35]

Instruments: Elemental analysis was performed with a CE Instruments EA-1110 elemental analyzer. ¹H- and ¹³C NMR spectra were recorded with a Bruker AMX-300 spectrometer, operating at 7.05 T. For ¹³C NMR spectra, methanol was used as the internal reference. IR spectra were measured with a FTIR spectrometer Bruker IFS66, using KBr discs. Mass spectra were recorded with a Q-tof 2 (Micromass, Manchester UK). Samples for the mass spectra were prepared as follows. The complex (2 mg) was dissolved in methanol (1 mL). A sample of this solution (200 μL) was added to a 50:50 water/methanol solution (800 μL). The resulting solution was injected with a flow rate of 5 $\mu\text{L min}^{-1}$.

Synthesis of the Ligands: Ligands DTPA-(PAE)₂, DTPA-(TA)₂ and DTPA-(HTA)₂ were synthesized by the following general procedure. To a solution of DTPA-bis(anhydride) (0.357 g, 1 mmol) in dry DMF (30 mL) was added the corresponding amine (2.5 mmol), and the reaction mixture was heated overnight at 60 $^\circ\text{C}$ under nitrogen.^[35] For the ligand DTPA-(HTA)₂, a small amount of ascorbic acid (50 mg) was added to the mixture to prevent oxidation. After the removal of the solvent, the product was re-dissolved in ethanol and precipitated by addition of diethyl ether. The precipitate was filtered off and dried in vacuo overnight.

Analytical Data for the Ligands

DTPA-(PAE)₂: Yield: 89% (661 mg). C₃₄H₄₉N₅O₁₂ (743.81): calcd. C 58.12, H 6.64, N 9.42; found C 58.55, H 6.61, N 9.40. ¹H NMR ([D₆]dmsO): $\delta = 1.10$ (t, 6 H, CH₃), 2.72 (m, 4 H, 2 $\times$ N-CH₂), 2.81 (m, 4 H, 2 $\times$ N-CH₂), 3.01 (m, 2 H, N-CH₂), 3.25 (s, 4 H, 2 $\times$ N-CH₂), 3.32 (s, 4 H, 2 $\times$ N-CH₂), 3.48 (d, 4 H, 2 $\times$ CH₂-Ph), 4.05 (q, 4 H, CH₂-O), 4.52 (m, 2 H, 2 $\times$ CH), 7.19–7.43 (m, 10 H, Ph), 8.32 (d, 2 H, 2 $\times$ NH) ppm. ¹³C NMR ([D₆]dmsO): $\delta = 172.8, 171.5, 170.7, 168.9, 137.4, 129.3, 128.4, 126.7, 60.8, 56.6, 56.2, 52.2, 48.7, 45.5, 38.9, 35.9, 14.0$ ppm. IR: $\tilde{\nu} = 2972, 2952, 2876$ (CH alkyl), 1733 (CO ester), 1677 (CO acid), 1633 (CO amide I), 1534 (CO amide II) cm⁻¹. ESI-MS(+) (C₃₆H₄₉N₅O₁₂: $M_{\text{calcd.}} = 743$): $m/z = 744$ [M + H]⁺, 766 [M + Na]⁺.

DTPA-(TA)₂: Yield: 85% (536 mg). C₃₀H₄₁N₅O₁₀ (631.68): calcd. C 57.04, H 6.54, N 11.09; found C 57.36, H 6.68, N 11.38. ¹H NMR (D₂O): $\delta = 2.64$ (t, 4 H, 2 $\times$ N-CH₂), 2.97 (t, 4 H, 2 $\times$ N-CH₂), 3.11 (m, 4 H, 2 $\times$ CH₂-NH-CO), 3.35 (m, 4 H, 2 $\times$ CH₂-

Ph), 3.50 (s, 2 H, CH₂–COOH), 3.57 (s, 4 H, 2 × CH₂–COOH), 3.69 (s, 4 H, 2 × CH₂–CO–NH), 6.60–6.92 (AA'BB', 8 H, 2 × Ph) ppm. ¹³C NMR (D₂O): δ = 173.2, 172.1, 169.4, 154.4, 131.2, 130.6, 115.7, 57.2, 54.7, 51.9, 51.4, 51.3, 40.8, 33.9 ppm. IR: ν̃ = 1635 (CO amide I), 1510 (CO amide II) cm⁻¹. ESI-MS(+) (C₃₀H₄₁N₅O₁₀: M_{calcd.} = 631): m/z = 632 [M + H]⁺, 654 [M + Na]⁺.

DTPA-(HTA)₂: Yield: 79% (630 mg). C₃₀H₃₈N₅O₁₂Na₃(H₂O) (747.64): calcd. C 48.18, H 5.39, N 9.37; found C 48.63, H 5.60, N 8.97. ¹H NMR (D₂O): δ = 2.61 (t, 4 H, 2 × N–CH₂), 2.95 (t, 4 H, 2 × N–CH₂), 3.10 (m, 4 H, 2 × CH₂–NH–CO), 3.37 (m, 4 H, 2 × CH₂–Ph), 3.50 (s, 2 H, CH₂–COOH), 3.56 (s, 4 H, 2 × CH₂–COOH), 3.68 (s, 4 H, 2 × CH₂–CO–NH), 6.56–6.82 (m, 6 H, Ph) ppm. ¹³C NMR (D₂O): δ = 179.6, 174.4, 171.4, 152.3, 149.5, 128.9, 117.5, 116.7, 115.7, 59.4, 57.8, 52.1, 48.1, 46.5, 40.9, 35.5 ppm. IR: ν̃ = 1740 (CO acid), 1647 (CO amide I), 1521 (CO amide II) cm⁻¹. ESI-MS(+) (C₃₀H₄₁N₅O₁₂: M_{calcd.} = 663): m/z = 664 [M + H]⁺, 686 [M + Na]⁺.

Synthesis of the Gadolinium(III) Complexes: All complexes were synthesized according to the following general procedure. A solution of hydrated GdCl₃ salt (1 mmol) in H₂O (1 mL) was added to the ligand (1 mmol) dissolved in pyridine (30 mL), and the mixture was heated at 70 °C for 3 h. Because of the basicity of pyridine, the addition of extra base for the deprotonation of the ligand was not necessary. The solvents were evaporated under reduced pressure, and the crude product was then refluxed in ethanol for 1 h. After cooling to room temp., the complex was filtered off and dried in vacuo. The absence of free gadolinium(III) ions was checked with xylenol orange indicator.^[36]

Analytical Data for the Gadolinium(III) Complexes

Gd-DTPA-(PAE)₂: Yield: 35% (315 mg). IR: ν̃ = 1733 (CO ester), 1622 (CO amide I), 1589 (COO⁻ asym. stretch), 1388 (COO⁻ sym. stretch) cm⁻¹. ESI-MS(+) (C₃₆H₄₆GdN₅O₁₂: M_{calcd.} = 897): m/z = 898 [M + H]⁺.

Gd-DTPA-(TA)₂: Yield: 53% (417 mg). IR: ν̃ = 1627 (CO amide I), 1595 (COO⁻ asym. stretch), 1399 (COO⁻ sym. stretch) cm⁻¹. ESI-MS(+) (C₃₀H₃₈GdN₅O₁₀: M_{calcd.} = 785): m/z = 786 [M + H]⁺, 809 [M + Na]⁺.

Gd-DTPA-(HTA)₂: Yield: 95% (770 mg). IR: ν̃ = 2923, 2852 (CH alkyl), 1628 (CO amide I), 1602 (COO⁻ asym. stretch), 1408 (COO⁻ sym. stretch) cm⁻¹. ESI-MS(+) (C₃₀H₃₈GdN₅O₁₂: M_{calcd.} = 817): m/z = 840 [M + Na]⁺.

Sodium and Potassium Ion Content Measurements: The potassium and sodium ion content of the solutions was checked by flame photometry (IL 943, Instrumentation Laboratories, Massachusetts, USA). Na⁺ content (mmol per mol of the complex): Gd-DTPA-(PAE)₂: 0.7; Gd-DTPA-(TA)₂: 0.0; Gd-DTPA-(HTA)₂: 0.0. K⁺ content: no K⁺ could be detected in any of the complexes.

Proton T₁ Measurements: Proton nuclear magnetic relaxation dispersion (NMRD) profiles were recorded at 310 K between 0.24 mT and 0.24 T with a Field Cycling Relaxometer (Stelar Spinmaster FFC-2000, Stelar S. R. L., Mede, Italy) on solutions (0.6 mL) contained in 10-mm o.d. tubes. Proton relaxation rates were also measured at 0.47, 1.5 and 7.05 T on Minispec PC-120, mq-60 and AMX-300 spectrometers (Bruker, Karlsruhe, Germany), respectively. ¹H NMRD data were fitted according to the theoretical inner-sphere model described by Solomon^[30] and Bloembergen^[31] and to the outer-sphere contribution described by Freed.^[32] Calculations were performed with a previously described software.^[26]

¹⁷O NMR Measurements: ¹⁷O NMR measurements of solutions were performed on samples (2 mL) contained in 10-mm external

diameter tubes with a Bruker AMX-300 spectrometer. The temperature was regulated by air or nitrogen flow controlled by a BVT 2000 unit. No field frequency lock was used. ¹⁷O transverse relaxation times of distilled water (pH = 6.5–7) were measured using a CPMG sequence and a subsequent two-parameter fit of the data points. The 90° and 180° pulse lengths were 25 and 50 μs, respectively. ¹⁷O T₂ values of water in complex solution were obtained from line-width measurement. Concentrations of the samples were less than 25 mM {[Gd-DTPA-(TA)₂] = 20.1 mM, [Gd-DTPA-(HTA)₂] = 20.0 mM and [Gd-DTPA-(PAE)₂] = 20.3 mM}. The data were treated as described elsewhere.^[26–28,34]

Transmetalation Kinetics: The technique is based on the measurement of the evolution of the water proton paramagnetic longitudinal relaxation rate (R₁^p) of a buffered solution ([KH₂PO₄] = 0.026 mol L⁻¹, [Na₂HPO₄] = 0.041 mol L⁻¹, pH = 7) containing the gadolinium(III) complex (2.5 mM) and ZnCl₂ (2.5 mM).^[37] The measurements were performed with a spin analyzer Minispec PC-120 (Bruker, Karlsruhe, Germany) at 20 MHz and 310 K. The samples (0.3 mL) were contained in 7-mm o.d. PyrexTM tubes and kept at 310 K in a dry block between measurements (up to 4 d).

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