Synthesis and Characterization of Various Benzyl Diethylenetriaminepentaacetic Acids (dtpa) and Their Paramagnetic Complexes, Potential Contrast Agents for Magnetic Resonance Imaging

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Four derivatives of diethylenetriaminepentaacetic acid (= 3,6,9-tris(carboxymethyl)-3,6,9-triazaundecanedioic acid (H_5dtpa)), potential contrast agents for magnetic resonance imaging (MRI), carrying benzyl groups at various positions of the parent structure were synthesized and characterized by a thorough multinuclear NMR study, *i.e.*, the (*S*)- and (*R*)-stereoisomers **1a** and **1b** of 4-benzyl-3,6,9-tris(carboxymethyl)-3,6,9-triazaundecanedioic acid (H_5[(*S*)-(4-Bz)dtpa] and H_5[(*R*)-(4-Bz)dtpa], the diamide derivative *N*,*N*′′-bis[(benzylcarbamoyl)methyl]diethylenetriamine-*N*,*N*′,*N*′′-triacetic acid (= 3,9-bis[2-(benzylamino)-2-oxoethyl]-6-(carboxymethyl)-3,6,9-triazaundecanedioic acid; H_3[dtpa($BzA)_2$]; **2**), and the diester derivative *N*,*N*′′-bis{[(benzyloxy)carbonyl]methyl}diethylenetriamine-*N*,*N*′,*N*′′-triacetic acid (= 3,9-bis[2-(benzyloxy)-2-oxoethyl]-6-(carboxymethyl)-3,6,9-triazaundecanedioic acid; H_3[dtpa($BzE)_2$]; **3**). From the ^{17}O-NMR chemical shift of H_2O induced by their dysprosium complexes with ligands **1**–**3**, it was concluded that only one H_2O molecule is contained in the first coordination sphere of these lanthanide complexes. The rotational correlation times (τ_R) of the complexes were estimated from the ^{2}H-NMR longitudinal relaxation rate of the deuterated diamagnetic lanthanum complexes. The exchange time of the coordinated H_2O molecule (τ_M) was studied through the temperature dependence of the ^{17}O-NMR transverse relaxation rate. As compared to $[Gd(dtpa)]^{2-}$, the H_2O-exchange rate is faster for $[Gd\{(S)\text{-}(4\text{-}Bz)dtpa\}]^{2-}$ and $[Gd\{(R)\text{-}(4\text{-}Bz)dtpa\}]^{2-}$-, slower for $[Gd\{dtpa(BzA)_2\}]$, and almost identical for $[Gd\{dtpa(BzE)_2\}]$. The analysis of the ^{1}H-relaxivity of the gadolinium complexes recorded from 0.02 to 300 MHz established that *i*) the relaxivity of $[Gd\{dtpa(BzE)_2\}]$ is similar to that of $[Gd(dtpa)]^{2-}$, *ii*) the slightly slower molecular rotation of $[Gd\{dtpa(BzA)_2\}]$ induces a mild enhancement of its relaxivity, and *iii*) the marked increase of relaxivity of $[Gd\{(S)\text{-}(4\text{-}Bz)dtpa\}]^{2-}$ and $[Gd\{(R)\text{-}(4\text{-}Bz)dtpa\}]^{2-}$ mainly results from an apparently shorter distance between the gadolinium ion and the H_2O protons of the coordinated H_2O molecule.

Introduction. – The efficiency of paramagnetic complexes as potential contrast agents for magnetic resonance imaging (MRI) is related to their ability to enhance the proton relaxation rate of water tissues. This property, the relaxivity, depends on molecular factors like the molecular mobility, the water dynamics, and the noncovalent binding of the complex to endogenous proteins. All these parameters are likely to be affected by the nature and the location of the substituents. Commercial formulations of the paramagnetic gadolinium complexes widely used in MRI, *e.g.*, *Magnevist*®, *Omniscan*®, *Dotarem*®, and *ProHance*®, contain complexes of diethylenetriaminepentaacetic acid (H_5(dtpa); 3,6,9-tris(carboxymethyl)-3,6,9-triazaundecanedioic acid), H_3[dtpa($MeA)_2$] (3,9-bis[2-(methylamino)-2-oxoethyl]-6-(carboxymethyl)-3,6,9-triazaundecanedioic acid), H_4(dota) (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), and H_3[hpdo($A)_3$] (10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) (*Fig. 1*). All these small hydrophilic complexes are nonspecific extracellular contrast agents that do not interact with blood proteins and are excreted through the kidneys. On the contrary, amphiphilic derivatives like *Eovist*® ([Gd{(*S*)-(4-

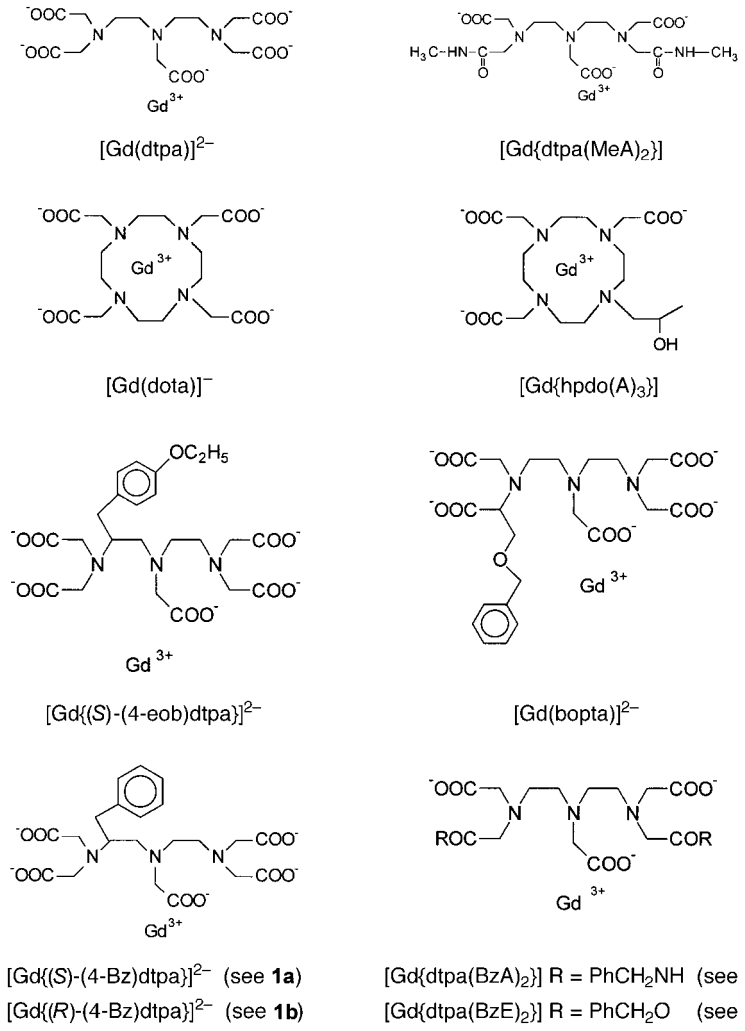


Fig. 1. *Structures of [Gd(dtpa)]$^{2-}$, [Gd{dtpa(MeA)$_2$}], [Gd(dota)]$^{-}$, [Gd{hpdo(A)$_3$}], [Gd{(S)-(4-eob)dtpa}]$^{2-}$, [Gd(bopta)]$^{2-}$, and of the four benzyl derivatives of the dtpa ligand studied in this work*

eob)dtpa}]$^{2-}$; H_5[(4-eob)dtpa] = 3,6,9-tris(carboxymethyl)-4-(4-ethoxybenzyl)-3,6,9-triazaundecanedioic acid) and *MultiHance*® ([Gd(bopta)]$^{2-}$; H_5bopta = 2-[(benzyloxy)-methyl]-3,6,9-tris(carboxymethyl)-3,6,9-triazaundecanedioic acid) (*Fig. 1*) display some hepatobiliary specificity and interact through noncovalent binding with serum albumin [1–9].

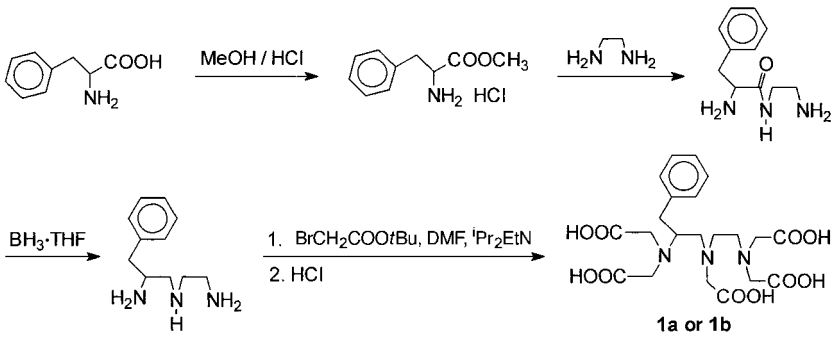
Previous physicochemical characterizations of [Gd(dtpa)]$^{2-}$ [10], [Gd{(*S*)-(4-eob)dtpa}]$^{2-}$ [9], and of some amide derivatives ([Gd{dtpa(EtA)$_2$}] (bis-ethylamide) [11], [Gd{dtpa(PrA)$_2$}] (bis-propylamide) [10][12]) have shown the presence of one H_2O molecule in the first coordination sphere of the gadolinium ion. The relaxivity, defined as the ^{1}H-relaxation-rate enhancement of H_2O induced by 1 mmol of

gadolinium complex per liter, is similar for $[Gd(dtpa)]^{2-}$ and $[Gd\{dtpa(MeA)_2\}]$ at 310 K, whereas the derivatives bearing aromatic substituents like $[Gd(bopta)]^{2-}$ and $[Gd\{(S)\text{-}(4\text{-}eob)dtpa\}]^{2-}$ are characterized by a significant increase of this parameter. This enhancement has been attributed to a slightly reduced mobility of the bulkier complexes and to an apparently shorter mean distance between the protons of the coordinated H_2O molecule and the gadolinium ion [9][13]. In addition, the influence of those latter compounds on the solvent-relaxation rate is enhanced in protein-containing media due to the lengthening of their molecular correlation time subsequent to a noncovalent protein binding.

The presence of an aromatic group seems thus to bring some tissue specificity as well as an increase of relaxivity. To extend these observations, a series of derivatives of H_5dtpa bearing benzyl groups at various positions of the structure were synthesized: both stereoisomers of the structure carrying the substituent at the C-backbone ((*S*)-(4-Bz)dtpa (**1a**) and (*R*)-(4-Bz)dtpa (**1b**)), one bis(benzylamide) ($dtpa(BzA)_2$ (**2**)), and one bis(benzyl ester) ($dtpa(BzE)_2$ (**3**)) (see *Fig. 1*). The *in vitro* physicochemical characterization of their gadolinium complexes involved: *i*) ^{1}H-relaxation-rate measurements over a magnetic-field range extending from $4.7 \cdot 10^{-4}$ to 7.05 T, *ii*) the estimation of their rotational correlation time by ^{2}H-NMR, and *iii*) the determination of the number of coordinated H_2O molecules and of their residence time by ^{17}O-NMR.

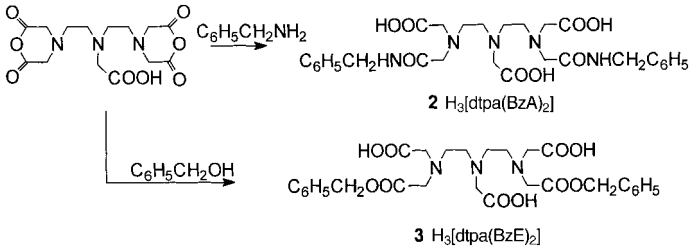
Results and Discussion. – *Synthesis.* Ligands $H_5[(S)$- and $H_5[(R)$-(4-Bz)dtpa] (**1a** and **1b**, resp.) were synthesized as described by *Brechbiel et al.* [14][15] (*Scheme 1*). Thus, commercially available L- or D-phenylalanine was esterified, and the resulting ester hydrochloride was treated with Et_3N and then with ethylenediamine (=ethane-1,2-diamine) to yield the corresponding amide. After reduction with an excess of borane, the crude triamine was purified by recrystallization from MeOH. The polyaminocarboxylate ligands **1a** and **1b** were obtained by alkylation of the (*S*)- and (*R*)-amine, respectively, with *tert*-butyl bromoacetate in DMF and subsequent hydrolysis of the pentaesters [15]. Although (*S*)- and (*R*)-pentacarboxylates **1a** and **1b** are expected to behave identically in H_2O and, therefore, to be characterized by similar physicochemical parameters in this medium, dissimilarities could arise with respect to noncovalent binding to serum proteins to be studied in a future step.

Scheme 1. *Synthetic Scheme for the Preparation of H$_5$[(*S*)-(4-Bz)dtpa]* (**1a**) *and H$_5$[(*R*)-(4-Bz)dtpa]* (**1b**)



The amide and ester derivatives **2** and **3** of H_5dtpa were synthesized by the reaction of benzylamine [11] and benzyl alcohol, respectively, with H_5dtpa bis-anhydride in DMF solution (*Scheme 2*).

Scheme 2. *Synthetic Scheme for the Preparation of Diamide H_3[dtpa(BzA)$_2$]* (**2**) *and Diester H_3[dtpa(BzE)$_2$]* (**3**)



Physicochemical Characterization. The efficacy of a potential contrast agent can be evaluated by its ^{1}H-relaxivity in H_2O expressed in s^{-1} mM^{-1}. *Fig. 2* shows the ^{1}H-nuclear magnetic relaxation dispersion (NMRD) curves which represent the ^{1}H-relaxivities as a function of the ^{1}H-*Larmor* frequency for $[Gd(dtpa)]^{2-}$ and its benzyl derivatives. At 310 K, the ^{1}H-relaxivity of the parent compound and of the diester derivative are comparable, whereas the relaxivity of [Gd{dtpa(BzA)$_2$}] is slightly higher, and those of [Gd{(*S*)-(4-Bz)dtpa}] and [Gd{(*R*)-(4-Bz)dtpa}] are markedly increased. As expected, the ^{1}H-NMRD profiles of these two isomers are similar. At 0.47 T (20 MHz) and 310 K, the ^{1}H-relaxivities of gadolinium complexes of ligands **1a**, **1b**, **2**, and **3** are equal to 4.8, 4.5, 4.2, and 3.8 s^{-1} mM^{-1}, respectively, as compared to 3.9 s^{-1} mM^{-1} for the parent compound.

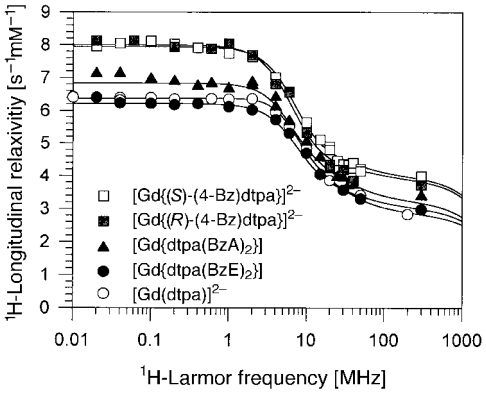


Fig. 2. *^{1}H-NMRD Profiles of the gadolinium complexes of dtpa and of ligands* **1a**, **1b**, **2**, *and* **3**

Classically, the ^{1}H-relaxation-rate enhancement (R_1^p) (p = paramagnetic) induced by the interaction between the electrons of the paramagnetic ion and the ^{1}H-nuclei of H_2O molecules can be divided into: *i*) short-distance interactions, also known as innersphere mechanism (R_1^{is}), and *ii*) long-distance interactions (outersphere mechanism, R_1^{os}; see *Eqn. 1*). The innersphere contribution is related to the exchange of H_2O molecules of the first coordination sphere with bulk H_2O. For gadolinium complexes, this mechanism can be described by the simplified *Solomon-Bloembergen* equations (*Eqns. 2–6*) [16][17].

$$R_1^{\mathrm{p}} = R_1^{\mathrm{is}} + R_1^{\mathrm{os}} \tag{1}$$

$$R_1^{is} = \frac{fq}{T_{1\mathrm{M}} + \tau_{\mathrm{M}}} \tag{2}$$

$$\frac{1}{T_{1M}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 {\gamma_H}^2 {\gamma_S}^2 \hbar^2 S(S+1)\frac{1}{r^6}\left[\frac{7\tau_{\mathrm{c2}}}{1+(\omega_{\mathrm{S}}\tau_{\mathrm{c2}})^2} + \frac{3\tau_{\mathrm{c1}}}{1+(\omega_{\mathrm{H}}\tau_{\mathrm{c1}})^2}\right] \tag{3}$$

$$\frac{1}{\tau_{\mathrm{c}i}} = \frac{1}{\tau_{\mathrm{R}}} + \frac{1}{\tau_{\mathrm{M}}} + \frac{1}{\tau_{\mathrm{S}i}} \tag{4}$$

$$\frac{1}{\tau_{\mathrm{S1}}} = \frac{1}{5\tau_{\mathrm{S0}}}\left[\frac{1}{1+\omega_{\mathrm{S}}{}^2\tau_{\mathrm{V}}{}^2} + \frac{1}{1+4\omega_{\mathrm{S}}{}^2\tau_{\mathrm{V}}{}^2}\right] \tag{5}$$

$$\frac{1}{\tau_{\mathrm{S2}}} = \frac{1}{10\tau_{\mathrm{S0}}}\left[3 + \frac{5}{1+\omega_{\mathrm{S}}{}^2\tau_{\mathrm{V}}{}^2} + \frac{2}{1+4\omega_{\mathrm{S}}{}^2\tau_{\mathrm{V}}{}^2}\right] \tag{6}$$

In *Eqns. 2–6, f* is the relative concentration of the paramagnetic complex and of the H_2O molecules, q is the number of H_2O molecules in the first coordination sphere, τ_{M} is the residence time describing the exchange between coordinated and bulk H_2O, γ_{S} and γ_{H} are the gyromagnetic ratios of the electron (S) and of the proton (H), respectively, ω_{S} and ω_{H} are the *Larmor* angular frequencies of the electron and of the proton, respectively, r is the distance between coordinated H_2O protons and the unpaired electron spin, and τ_{C1} and τ_{C2} are the correlation times modulating the interaction. These correlation times are defined by *Eqn. 4*, where τ_{R} is the rotational correlation time of the hydrated complex, and τ_{S1} and τ_{S2} are the longitudinal and transverse relaxation times of the electron. These latter parameters are field-dependent, as shown in *Eqns. 5* and *6*, where τ_{S0} is the value of τ_{S1} or τ_{S2} at zero field and τ_{V} is the correlation time responsible for the modulation of the magnetic interaction.

Numerous factors can thus influence the innersphere relaxivity of a paramagnetic compound. For small gadolinium complexes like those studied in this work, τ_{R}, τ_{S1} and τ_{S2} are the correlation times responsible for the modulation at low magnetic fields and 310 K, whereas τ_{R} is the determinant correlation time at high fields and physiological temperature. An increased τ_{R} results thus in a higher relaxivity, but this effect can be limited by the chemical exchange when the characteristic time τ_{M} becomes comparable to the relaxation time in the first coordination sphere (see *Eqn. 2*) [18]. On the other hand, the outersphere contribution arises from the translational diffusion of H_2O protons around the chelate and can be represented by the *Freed* model (*Eqns. 7–8*) [19].

$$R_1^{\mathrm{os}} = \frac{6400\pi}{81}\left(\frac{\mu_0}{4\pi}\right)^2 {\gamma_H}^2 {\gamma_S}^2 \hbar^2 S(S+1) NA \frac{[C]}{\mathrm{d}D}\left[7j(\omega_{\mathrm{S}}\tau_{\mathrm{D}}) + 3j(\omega_{\mathrm{H}}\tau_{\mathrm{D}})\right] \tag{7}$$

$$j(\omega\tau_{\mathrm{D}}) = Re\left[\frac{1 + \frac{1}{4}(i\omega\tau_{\mathrm{D}} + \tau_{\mathrm{D}}/\tau_{\mathrm{S1}})^{1/2}}{1 + (i\omega\tau_{\mathrm{D}} + \tau_{\mathrm{D}}/\tau_{\mathrm{S1}})^{1/2} + \frac{4}{9}(i\omega\tau_{\mathrm{D}} + \tau_{\mathrm{D}}/\tau_{\mathrm{S1}}) + \frac{1}{9}(i\omega\tau_{\mathrm{D}} + \tau_{\mathrm{D}}/\tau_{\mathrm{S1}})^{3/2}}\right] \tag{8}$$

In *Eqns. 7* and *8*, d is the distance of closest approach, D is the relative molecular diffusion constant, $[C]$ is the molar concentration of paramagnetic ion, and $\tau_{\mathrm{D}} = d^2/D$ is the translational correlation time.

The outersphere relaxation may be predominant for complexes which have few or no H_2O molecules in the first coordination sphere of their paramagnetic center. For small gadolinium complexes having one H_2O molecule in the first coordination sphere ($[Gd(dtpa)]^{2-}$, $[Gd\{dtpa(MeA)_2\}]$ *etc.*), this relaxation mechanism contributes to about one half of the overall relaxivity.

The evaluation of the parameters defined above is very helpful for the analysis and the understanding of the factors governing the relaxivity of paramagnetic complexes. In a previous work, we have shown that a non-ambiguous analysis of the ^{1}H-NMRD curves is facilitated by an independent evaluation of q, τ_R, and τ_M by specific NMR techniques [9].

Determination of τ_R by ^{2}H-NMR. In diamagnetic molecules, the relaxation rate of the ^{2}H-nucleus is predominantly determined by the quadrupolar mechanism [20–23], which is strictly intramolecular and is modulated by the sole rotation of the molecule. For fast-tumbling systems, the relaxation rate is thus directly related to the rotational correlation time (*Eqn. 9*). The quadrupolar coupling constant ($e^2qQ/\hbar$) depends on the hybridization state of the C-atom carrying the ^{2}H-atom; its value is *ca.* 170 kHz in the case of an sp^3 C-atom.

$$R_1 = \frac{1}{T_1} = \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2 \tau_R \qquad (9)$$

The measurements were performed on the diamagnetic lanthanum complexes deuterated in α-position to the carbonyl groups. At 310 K, the values of τ_R are comparable for $[La\{(S)\text{-}(4\text{-}Bz)(^2H_{10})dtpa\}]^{2-}$ (65 ± 7 ps), $[La\{(R)\text{-}(4\text{-}Bz)(^2H_{10})dtpa\}]^{2-}$ (70 ± 7 ps), $[La\{(^2H_{10})dtpa(BzE)_2\}]$ (65 ± 7 ps), and slightly higher for $[La\{(^2H_8)dtpa(BzA)_2\}]$ (75 ± 8 ps).

Determination of q *by ^{17}O-NMR.* ^{17}O-NMR of H_2O in dysprosium-complex solutions allows the estimation of the number of coordinated H_2O molecules. The chemical shift induced on the O-atom of H_2O depends on the number of H_2O molecules in the first coordination sphere of the dysprosium ion and is independent of the nature of the coordinating groups of the ligand [10]. A simple comparison of the chemical shifts induced by the aqua dysprosium ion, for which q is equal to eight [24], and by the dysprosium complex gives the number of coordinated H_2O molecules. The measurements were performed at 310 K with an excess of ligand to avoid the presence of free dysprosium ions in the solution (*Fig. 3*). As expected for these dtpa derivatives, the number of H_2O molecules in the first coordination sphere of gadolinium complexes was similar and close to one (1.17, 1.25, and 1.12 for the dysprosium complexes of **1a**, **2**, and **3**, resp.). For compound **1b**, the measurement performed at one single dysprosium concentration ($[Dy^{3+}] = 16.5$ mM) gave, as expected, the same value as that obtained for its stereoisomer.

Determination of τ_M by ^{17}O-NMR. The paramagnetic transverse ^{17}O-relaxation rate of H_2O in gadolinium complex solutions is given by *Eqn. 10*, in which T_{2M}, the transverse relaxation rate of the O-atom of the bound H_2O, depends predominantly on the scalar interaction between the unpaired electrons and the O-nucleus (*Eqn. 11*). The chemical shift $\Delta\omega_M$ of the O-atom of this H_2O molecule is given by *Eqn. 12*. In these equations, the outersphere contribution is neglected.

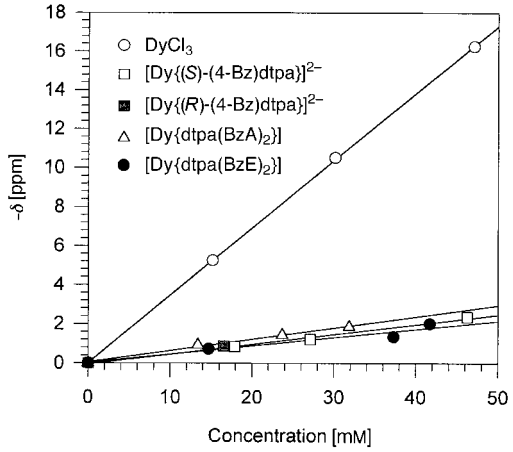


Fig. 3. *^{17}O-NMR chemical shift of water as a function of Dy^{3+} concentration.* ([(*S*)-(4-Bz)dtpa] = 71 mM, [(*R*)-(4-Bz)dtpa] = 53.3 mM, [dtpa(BzA)$_2$] = 51 mM, [dtpa(BzE)$_2$] = 73 mM)

$$\frac{1}{T_2{}^{\mathrm{p}}} = f \cdot q \cdot \frac{1}{\tau_{\mathrm{M}}} \frac{T_{2\mathrm{M}}{}^{-2} + \tau_{\mathrm{M}}{}^{-1} T_{2\mathrm{M}}{}^{-1} + \Delta\omega_{\mathrm{M}}{}^{2}}{\tau_{\mathrm{M}}(\tau_{\mathrm{M}}{}^{-1} + T_{2\mathrm{M}}{}^{-2})^{2} + \Delta\omega_{\mathrm{M}}} \tag{10}$$

$$\frac{1}{T_{2\mathrm{M}}} = \frac{S(S+1)}{3} \left(\frac{A}{\hbar}\right)^{2} \left(\tau_{\mathrm{e}1} + \frac{\tau_{\mathrm{e}2}}{1 + \omega_{\mathrm{S}}{}^{2} \tau_{\mathrm{e}2}{}^{2}}\right) \tag{11}$$

$$\Delta\omega_{\mathrm{M}} = \frac{g_{\mathrm{L}} \mu_{\mathrm{B}} S(S+1) B_0}{3 k_{\mathrm{B}} T} \frac{A}{\hbar} \tag{12}$$

$$\frac{1}{\tau_{ei}} = \frac{1}{\tau_{\mathrm{M}}} + \frac{1}{\tau_{si}} \tag{13}$$

$A/\hbar$ is the hyperfine or scalar coupling constant between the O-atom and Gd^{3+}, and τ_{ei} depends on the relative values of τ_{M} and τ_{Si} (*Eqn. 13*). The *Landé* factor, g_{L}, is equal to 2.0 for Gd^{3+}, μ_{B} is the *Bohr* magneton, and B_0 is the external magnetic field.

The modulation of $T_2{}^{\mathrm{p}}$ is thus determined by the electronic relaxation times τ_{Si} and by the exchange time between coordinated and bulk water τ_{M}. Since the dependences of τ_{M}, τ_{V} and τ_{S0} on temperature can be described by *Eqns. 14*, *15*, and *16*, respectively, the analysis of the temperature dependence of the transverse ^{17}O-relaxation rate allows thus the determination of the parameters defining the rate of exchange and the electronic relaxation times [25].

$$\frac{1}{\tau_{\mathrm{M}}} = \frac{k_{\mathrm{B}} T}{h} \exp\left(\frac{\Delta S^{\neq}}{R} - \frac{\Delta H^{\neq}}{RT}\right) \tag{14}$$

$$\tau_{\mathrm{V}} = \tau_{\mathrm{V}}{}^{298} \exp\left(\frac{E_{\mathrm{V}}}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right) \tag{15}$$

$$\tau_{\mathrm{S0}} = \tau_{\mathrm{S0}}{}^{298} \exp\left(\frac{E_{\mathrm{V}}}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right) \tag{16}$$

$\Delta S^{\neq}$ and $\Delta H^{\neq}$ are the entropy and the enthalpy of activation for the exchange process, τ_{S0}^{298} and τ_V^{298} are the electronic relaxation time at low field and the correlation time at 298.15 K, respectively, and E_v is the activation energy for these processes.

The experimental results are shown in *Fig. 4* which represents the normalized paramagnetic transverse relaxation rate ($1/T_2^r = [H_2O]/([\text{Gd complex}] \cdot T_2^p)$) *vs.* the reciprocal of the temperature. On these curves, a slower exchange rate is qualitatively characterized by a shift of the maximum to the left, whereas an increased exchange rate induces a shift of the maximum to the right. For the clarity of the diagram, the experimental data of the gadolinium complex of $H_5[(R)$-(4-Bz)dtpa] are not presented, but the results of the fit are shown in *Table 1*.

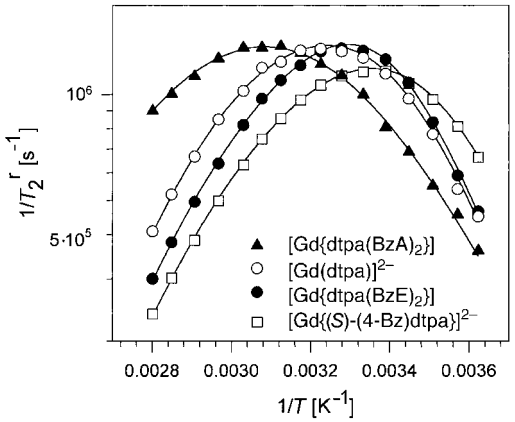


Fig. 4. *Reduced transverse ^{17}O-relaxation rate* ($1/T_2^r$) *as a function of the reciprocal of the temperature.* $(T_2^r)^{-1} = (T_2^p)^{-1} \cdot [H_2O]/[\text{Gd complex}]$.

Table 1. *Values of τ_M and of the Fitted Parameters Obtained from the Transverse ^{17}O-Relaxation-Rate Measurements*

	[Gd(dtpa)]$^{2-}$ [a])	[Gd{(*S*)-(4-eob)dtpa}]$^{2-}$ [b])	[Gd{(*S*)-(4-Bz)dtpa}]$^{2-}$	[Gd{(*R*)-(4-Bz)dtpa}]$^{2-}$	[Gd{dtpa-(BzA)$_2$}]	[Gd{dtpa-(BzE)$_2$}]
τ_M^{310} [ns]	143 ± 26 (132 ± 11)	82 ± 21	87 ± 25	108 ± 14	305 ± 13	134 ± 14
τ_V^{298} [ps]	12 ± 0.3 (25 ± 1)	15 ± 2	23 ± 1	12 ± 1	29 ± 1	26 ± 2
τ_{S0}^{298} [ps]	63 ± 3 (73 ± 6)	63 ± 21	16 ± 1	29 ± 4	15 ± 1	16 ± 2
E_v [kJ/mol]	4.5 ± 4.2 (1.6 ± 1.8)	7.1 ± 5.9	8.6 ± 8.0	7.4 ± 1.7	0.1 ± 8	15.7 ± 4.1
$\Delta H^{\neq}$ [kJ/mol]	51.5 ± 0.3 (51.6 ± 1.4)	53.5 ± 0.3	50.1 ± 0.5	54.6 ± 0.2	40.6 ± 0.1	53.4 ± 0.1
$\Delta S^{\neq}$ [J/mol K]	52.1 ± 0.6 (53.0 ± 4.7)	63.0 ± 1.3	51.7 ± 0.9	64.4 ± 0.5	10.6 ± 0.2	58.6 ± 0.5
$\lvert A/\hbar \rvert$ [$10^6 \cdot$ rad s^{-1}]	3.4 ± 0.1 (3.8 ± 0.2)	4.1 ± 0.5	3.2 ± 0.02	3.0 ± 0.2	3.0 ± 0.2	3.4 ± 0.2

[a]) The values in parentheses are from [26]. [b]) Values obtained by improved fitting of the data of [9].

For $[Gd(dtpa)]^{2-}$, the value of τ_M calculated at 310 K from the data of *Powell et al.* [26] is similar to that obtained from fitting our data to *Eqns. 10–16* (*Table 1*). The other parameters of the fit are also in good agreement with those reported by these authors, except for the parameters describing the electronic relaxation rate [26]. These differences can be related to the fact that *Powell et al.* included two contributions in the expression of the electronic relaxation times (a zero-field-splitting contribution and a spin-rotation contribution), whereas we only take into account the zero-field-splitting contribution. For the diester complex, τ_M is identical to that of $[Gd(dtpa)]^{2-}$, while the diamide derivative is characterized by a τ_M more than two times larger (*Table 1*). On the contrary, the τ_M values of $[Gd\{(S)\text{-}(4\text{-}Bz)dtpa\}]^{2-}$ and $[Gd\{(R)\text{-}(4\text{-}Bz)dtpa\}]^{2-}$ are similar within experimental error and smaller than that of the parent compound. However, they are close to the value previously reported for $\{(S)\text{-}(4\text{-}eob)dtpa\}]^{2-}$ [9], another C(4)-substituted compound (*Table 1*). It is to be noted that all these values of τ_M are too small to have any significant quenching effect on the ^{1}H-relaxivity at 310 K in H_2O.

Temperature Effects on ^{1}H-Relaxivity at 0.47 T. The simultaneous contributions of both innersphere and outersphere mechanisms make the dependence of the ^{1}H-relaxivity on temperature more complex than for transverse ^{17}O-relaxation rates. On the one hand, outersphere relaxivity, which depends on the translational diffusion, increases when temperature decreases. On the other hand, the innersphere relaxivity can be described by two limiting cases: *i*) if the exchange between coordinated and bulk H_2O molecules is fast as compared to T_{1M}, then the innersphere relaxivity depends solely on T_{1M} and increases when temperature is lowered; *ii*) if the exchange time is larger than T_{1M}, then the innersphere relaxivity is proportional to τ_M^{-1} and decreases on reducing the temperature. As a result, when the exchange rate is fast, the overall relaxivity, which is the sum of the innersphere and of the outersphere relaxivities, increases when temperature is decreased, whereas a levelling off or a decrease of the global relaxivity is observed at low temperatures when the exchange rate is slow. As expected from the values of τ_M obtained by ^{17}O-NMR data, the ^{1}H-relaxivities of the parent compound and of the diester and *C*-substituted complexes are characteristic of a fast-exchange region (on the ^{1}H-relaxivity timescale) over the whole temperature range, whereas a limitation of the relaxivity by the exchange rate is clearly observed for the diamide complex (*Fig. 5*). These results are in agreement with those of *Aime et al.* [27] who reported a quenching of the relaxivity of $[Gd\{dtpa(BzA)_2\}]$ at low temperatures and a relaxivity of $4.8\ s^{-1}\ mM^{-1}$ at 298 K, which is comparable to the value of $4.9\ s^{-1}\ mM^{-1}$ measured in the present work.

Fitting of the ^{1}H-NMRD Profiles. Based on the results shown above, the fitting of the NMRD curves was performed according to the classical outersphere and innersphere theories (*Fig. 2*). Some parameters were fixed: 0.36 nm for the distance of closest approach (outersphere mechanism), $3.5 \cdot 10^{-9}\ m^2\ s^{-1}$ for the relative diffusion constant, 1 for the number of coordinated H_2O molecules, and the values of τ_M correspond to those obtained by ^{17}O-NMR. τ_R was allowed to fluctuate (25%) around the value obtained from the analysis of ^{2}H-NMR relaxation rates. The results of the fittings of the ^{1}H-NMRD data are shown in *Table 2*. τ_V ranges between 13 ps and 23 ps for the four benzyl derivatives; values obtained for τ_{S0} are between 63 and 96 ps. τ_V Values obtained by ^{1}H- and ^{17}O-NMR are in relatively good agreement. The larger differences between

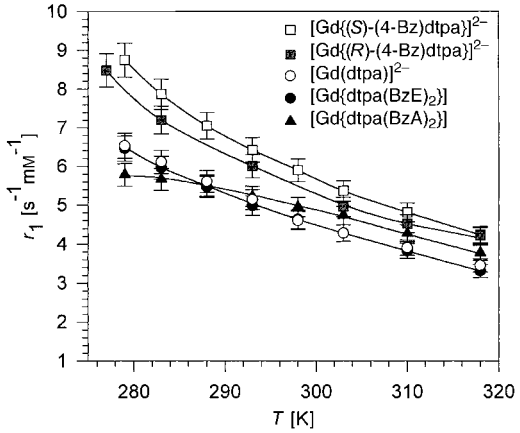


Fig. 5. *Temperature effect on the proton relaxivity of [Gd(dtpa)]$^{2-}$ and of the gadolinium complexes of benzyl derivatives at 0.47* T

τ_{S0} values obtained by both methods can be related to the fact that in the fitting of the NMRD data, τ_{S0} is obtained from the low-field part of the curve, whereas the ^{17}O-NMR measurements are performed at high magnetic fields ($B_0 > 4$ T) where mechanisms other than the zero field splitting contribution could be responsible for the relaxation of the electrons. The small increase of relaxivity of [Gd{dtpa(BzA)$_2$}] appears to result from a slightly longer rotational correlation time, whereas the relaxivity increases for [Gd{(*S*)-(4-Bz)dtpa}]$^{2-}$ and [Gd{(*R*)-(4-Bz)dtpa}]$^{2-}$ can be explained by a shorter mean distance of innersphere interactions: r is equal to 0.292 – 0.294 nm for the C(4)-benzyl isomers, a value smaller than the distance of 0.31 nm calculated for the other compounds. Such an unexpected and apparent shortening of r has already been reported for other *C*-substituted dtpa complexes like [Gd(bopta)]$^{2-}$ [13] or [Gd{(*S*)-(4-eob)dtpa}]$^{2-}$ [9] which carry an aromatic substituent at the C-backbone. This effect can be attributed to a true reduction of the Gd – H distance due to steric hindrance or to a reorganization of a second hydration shell by the hydrophobic group.

Table 2. *Parameters Obtained from Fitting ^{1}H-NMRD Profiles* (T 310 K)

	r [nm]	τ_R [ps][a])	τ_{S0} [ps][c])	τ_V [ps][c])
[Gd(dtpa)]$^{2-}$	0.310	59 (58)	82 (67)	23 (12)
[Gd{(*S*)-(4-eob)dtpa}]$^{2-}$ [b])	0.281	61 (64)	63 (58)	17 (14)
[Gd{(*S*)-(4-Bz)dtpa}]$^{2-}$	0.292	61 (65)	81 (18)	17 (20)
[Gd{(*R*)-(4-Bz)dtpa}]$^{2-}$	0.294	62 (70)	89 (32)	13 (11)
[Gd{dtpa(BzA)$_2$}]	0.310	70 (75)	96 (15)	22 (29)
[Gd{dtpa(BzE)$_2$}]	0.310	58 (65)	79 (21)	16 (21)

[a]) The values in parentheses are those measured by ^{2}H-NMR. [b]) From [9]. [c]) The values in parentheses are those obtained by ^{17}O-NMR.

In conclusion, the ^{1}H-relaxivity of the Gd complexes of the four benzyl derivatives of dtpa studied in this work clearly differs according to the location of the lipophilic substituent and the type of bond. The Gd^{III} complex of the ester derivative **3** behaves

essentially like the parent compound $[Gd(dtpa)]^{2-}$. The Gd^{III} complex of the diamide derivative **2** has a slightly higher ^1H-relaxivity explained by a slower rotation of the compound. The exchange time between bulk and coordinated H_2O molecules (τ_M) is longer for this compound and induces a quenching of the relaxivity at low temperatures. This property, which seems to be characteristic of diamide derivatives [26–30], can limit the relaxivity of dtpa derivatives noncovalently bound to macromolecules like serum proteins or covalently linked to them through amide bonds [31]. In these compounds, the expected increase of the relaxivity due to the lengthening of τ_R will indeed be counteracted by the slow exchange rate between bulk and coordinated H_2O molecules. The behavior of the *C*-substituted compounds $[Gd\{(S)\text{-}(4\text{-}Bz)dtpa\}]^{2-}$ and $[Gd\{(R)\text{-}(4\text{-}Bz)dtpa\}]^{2-}$ is similar: the distance between the protons of the coordinated H_2O molecule and the gadolinium ion seems to be shortened resulting in a more efficient innersphere interaction and, consequently, in an enhancement of the relaxivity. Moreover, their exchange times τ_M are shorter. The decreases of r and τ_M and the increases of r_1, which were already observed for $[Gd\{(S)\text{-}(4\text{-}eob)dtpa\}]^{2-}$ [9], seem to be a common feature of $[Gd(dtpa)]^{2-}$ derivatives substituted by aromatic groups at the ethylene bridge. $[Gd\{(S)\text{-}(4\text{-}eob)dtpa\}]^{2-}$ has also been shown to bind noncovalently to human serum albumin with a subsequent increase of the paramagnetic relaxation rate in albumin solution [9]. Some affinity between both isomers of $[Gd\{(4\text{-}Bz)dtpa\}]^{2-}$ and human serum albumin can thus be expected. The study of the noncovalent binding of these gadolinium complexes in protein-containing media is currently in progress.

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Experimental Part

1. *General*. $[Gd(dtpa)]^{2-}$ was the commercial compound *Magnevist®* (*Schering AG*, Berlin, Germany). All other chemicals were purchased from *Aldrich* or *Fluka* (Bornem, Belgium) and were used without further purification. HPLC (purity control of ligands and complexes): *Waters-600* multisolvent delivery system equipped with a *Rheodyne* injection valve (20-μl loop) and controlled by the 'Millenium' software (*Waters*, Milford, USA); *Novapak-C18* column (4.56 mm × 150 mm); linear gradient of pure 0.05M $(Et_3NH)OAc$ (pH 6) to 100% MeOH, flow rate 1 ml/min for 20 min; UV/diode-array detector for elution monitoring of the ligand or of the complex (254 or 270 nm). Synthesized compounds were characterized by NMR and mass spectrometry. ^1H-NMR Spectra: at 300 MHz; *Bruker-AMX-300* spectrometer (*Bruker*, Karlsruhe, Germany); δ in ppm. Mass spectra (LSI-MS): *VG-Autospec* mass spectrometer (*VG Analytical*, Manchester, UK); samples were dissolved in H_2O and deposited on a glycerol matrix.

2. *^1H-Relaxometry, ^2H- and ^{17}O-NMR.* ^1H-Nuclear magnetic relaxation dispersion (NMRD) profiles were recorded at 310 K on a field-cycling relaxometer (*Field Cycling Systems*, Honesdale, PA) working between 0.02 and 50 MHz. Additional points at 300 MHz were obtained on a *Bruker-AMX-300* spectrometer. Fitting of the ^1H-NMRD curves was performed with a previously described software that uses minimization routines ('Minuit', *CERN* Library) [32][33]. The longitudinal relaxation time T_1 was measured at 20 MHz as a function of temp. on a *Bruker Minispec PC-20* (*Bruker*, Karlsruhe, Germany). The temp. of the sample was maintained by a tetrachloroethene flow.

The ^2H- and ^{17}O-NMR measurements were carried out with 2-ml samples in 10-mm (o.d.) tubes on a *Bruker-AMX-300* spectrometer (*Bruker*, Karlsruhe, Germany) equipped with a broadband probe. No field-frequency lock was used, except for the measurements of the ^{17}O-chemical shifts ($[D_2O] \approx 15\%$). The field

homogeneity was performed on the ^{1}H-free induction decay with the decoupling coil. The temp. was regulated by air or N_2 flow (*Bruker-BVT-2000* unit). Longitudinal ^{2}H-relaxation rates were measured with the inversion-recovery *Fourier* transform sequence with solns. containing the labelled diamagnetic lanthanum complex dissolved in deuterium-depleted water (*Aldrich*, Bornem, Belgium). The experimental data were fitted with a three-parameters minimization routine.

Diamagnetic transverse ^{17}O-relaxation times of H_2O (pH *ca.* 6.5) were measured at natural abundance with a *Carr-Purcell-Meiboom-Gill* sequence and a two-parameters fit of the data. Transverse ^{17}O-relaxation times of H_2O (natural abundance) in solns. containing paramagnetic complexes were calculated from the linewidth. All ^{17}O-NMR spectra were ^{1}H-decoupled.

The concentrations used were close to 1 mM for NMRD measurements (1.2, 1.6, 0.5, and 1.1 mM for Gd complexes of ligands **1a, 1b, 2**, and **3**, resp.) and between 12 and 48 mM for ^{17}O- and ^{2}H-relaxation rate measurements (^{2}H-NMR: 26.2, 24, 48, and 41.7 mM for La complexes of the deuterated analogs of ligands **1a**, **1b**, **2**, and **3** resp.; ^{17}O-NMR: 38.7, 16.6, 12.4, and 24 mM for Gd complexes of ligands **1a**, **1b, 2**, and **3**, resp.).

3. *Synthesis.* 3.1. *(*S*)- and (*R*)-4-Benzyl-3,6,9-tris(carboxymethyl)-3,6,9-triazaundecanedioic Acid* (H_5[(*S*)- and H_5[(*R*)-(4-Bz)dtpa]; **1a** and **1b**, resp.). L- and D-phenylalanine were treated as described [14][15] for the preparation of 4-nitrobenzyl *C*-functionalized dtpa.

L- *and* D-*Phenylalanine Methyl Ester Hydrochloride:* 94 and 97% yield, resp. NMR (D_2O, pH *ca.* 2): L-isomer: 7.4–7.2 (*m*, Ph); 4.3 (*t*, CH); 3.8 (*s*, Me); 3.3–3.1 (2*dd*, CH_2); D-isomer: 7.3–7.1 (*m*, Ph); 4.3 (*t*, CH); 3.7 (*s*, Me); 3.3–3.2 (*dd*, CH_2)

N′*-(2-Aminoethyl)*-L- *and* -D-*phenylalaninamide:* 91 and 89% yield, resp.; oils which were used without further purification. NMR (D_2O, pH *ca.* 2): L-isomer: 7.4–7.1 (*m*, Ph); 3.7 (*t*, CH); 3.5 (*d*, CH_2); 3.3 (*t*, CH_2); 3 (*t*, CH_2); D-isomer: 7.3–7.1 (*m*, Ph); 3.7 (*t*, CH); 3.5 (*d*, CH_2); 3.3 (*t*, CH_2); 3 (*t*, CH_2).

*(*S*)- and (*R*)-N′-(2-Aminoethyl)-1-benzylethane-1,2-diamine Trihydrochloride:* 86 and 88% yield resp.; White solids. NMR (D_2O, pH *ca.* 2): (*S*)-isomer: 7.3–7.2 (*m*, Ph); 3.1–2.4 (*m*, CH, 4 CH_2); (*R*)-isomer: 7.3–7.1 (*m*, Ph); 3.2–2.4 (*m*, CH, 4 CH_2).

*H_5[(*S*)- and H_5[(*R*)-(4-Bz)dtpa]* (**1a** and **1b**, resp.): 55 and 25% yield, resp. HPLC: 12.9 (**1a**) and 12.8 min (**1b**). NMR (D_2O, pH *ca.* 10): (*S*)-isomer: 7.4–7.1 (*m*, Ph); 3.45–2.6 (*m*, 9 CH_2, CH); (*R*)-isomer: 7.3–7.1 (*m*, Ph); 3.5–2.6 (*m*, 9 CH_2, CH). LSI-MS: 484 ($[M+H]^+$ for both isomers).

3.2. N,N″*-Bis[(benzylcarbamoyl)methyl]diethylenetriamine*-N,N′,N″*-triacetic Acid* (= *3,9-Bis[2-(benzylamino)-2-oxoethyl]-6-(carboxymethyl)-3,6,9-triazaundecanedioic Acid*; H_3[dtpa(BzA)$_2$]; **2**). According to [11][28][34], from H_5dtpa bis-anhydride and benzylamine in DMF soln.: 60% yield. HPLC: 12.8 min. NMR (D_2O, pH *ca.* 10): 7.3–7.1 (*m*, 2 Ph); 4.3 (*s*, 2 CH_2); 3.3–2.4 (*m*, 9 CH_2). LSI-MS: 572 ($[M+H]^+$).

3.3. N,N″*-Bis{[(benzyloxy)carbonyl)methyl}diethylenetriamine*-N,N′,N″*-triacetic Acid* (=*3,9-bis[2-(benzyloxy)-2-oxoethyl]-6-(carboxymethyl)-3,6,9-triazaundecanedioic Acid*; H_3[dtpa(BzE)$_2$]; **3**). As described for the bis(benzylamide) [28], with H_5dtpa bis-anhydride and benzyl alcohol in DMF: 45% yield. HPLC: 16.7 min. NMR (D_2O, pH *ca.* 10): 7.4–7.1 (*m*, 2 Ph); 5 (*s*, 2 CH_2); 3.4–2.2 (*m*, 9 CH_2). LSI-MS: 574 ($[M+H]^+$).

3.4. *Deuteration.* Deuteration of ligands (or of their lanthanum complexes) at the α-position with respect to carbonyl groups was performed by the procedure described in [35]: 4 mmol of ligand (or of lanthanum complex) were dissolved in 40 ml of D_2O, the pD was adjusted to 10.6 by addition of K_2CO_3, and the mixture was refluxed under stirring for 24 h. The pD was then adjusted to 2 (ligand soln.) or 6 (lanthanum-complex soln.) with conc. HCl soln., the soln. concentrated to 10 ml, and the solid KCl filtered off. Acetone was added to induce precipitation of the deuterated compound which was recovered by filtration and dissolved in H_2O. The soln. was neutralized with NaOH and the product isolated after lyophilization. The deuteration (> 95%) was confirmed by ^{1}H-NMR.

*H_5[(*S*)-[(4-Bz)($^2H_{10}$)dtpa]*: NMR (D_2O, pH *ca.* 10): 7.4–7.1 (*m*, Ph); 3.45–3.0 (*m*, 1 CH_2, CH); 2.9–2.6 (*m*, 3 CH_2).

*H_5[(*R*)-(4-Bz)($^2H_{10}$)dtpa]*: NMR (D_2O, pH *ca.* 10): 7.3–7.1 (*m*, Ph); 3.5–3.1 (*m*, 1 CH_2, CH); 3.0–2.6 (*m*, 3 CH_2).

H_3[($^2H_8$)dtpa(BzA)$_2$]: NMR (D_2O, pH *ca.* 10): 7.3–7.1 (*m*, 2 Ph); 4.3 (*s*, 2 CH_2); 2.8–2.4 (*m*, 5 CH_2).

H_3[($^2H_{10}$)-dtpa(BzA)$_2$]: NMR (D_2O, pH *ca.* 10): 7.4–7.1 (*m*, 2 Ph); 4.9 (*s*, 2 CH_2); 2.9–2.3 (*m*, 4 CH_2).

3.5. *Complexation.* The La^{III} and Gd^{III} complexes of the ligands were prepared by mixing aq. solns. of equimolar amounts of hydrated $LnCl_3$ and ligand. The pH was adjusted to 6.5–7. The absence of free lanthanide ions in the aq. solns. of the complexes was confirmed by the xylenol orange test [36].

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