Thermodynamic Stability and Physicochemical Characterization of Ligand (4*S*)-4-Benzyl-3,6,10-tris(carboxymethyl)-3,6,10-triazadodecanedioic Acid (H_5[(*S*)-4-Bz-ttda]) and Its Complexes Formed with Lanthanides, Calcium(II), Zinc(II), and Copper(II) Ions

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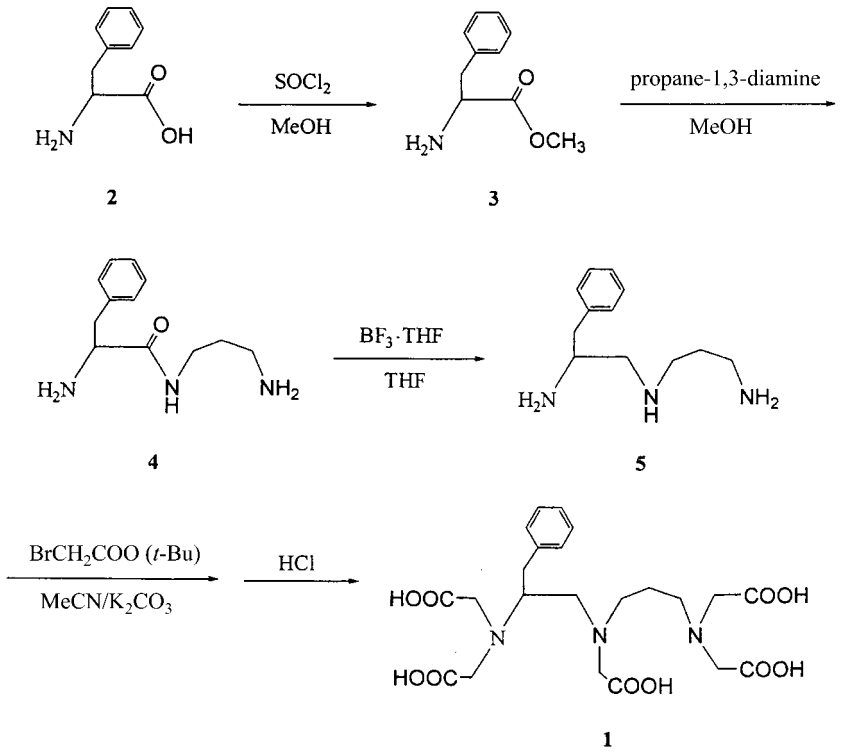
A derivative of H_5ttda (= 3,6,10-tris(carboxymethyl)-3,6,10-triazadodecanedioic acid = *N*-{2-[bis(carboxymethyl)amino]ethyl}-*N*-{3-[bis(carboxymethyl)amino]propyl}glycine), H_5[(*S*)-4-Bz-ttda] (=(4*S*)-4-benzyl-3,6,10-tris(carboxymethyl)-3,6,10-triazadodecanedioic acid = *N*-{(2*S*)-2-[bis(carboxymethyl)amino]-3-phenylpropyl}-*N*-{3-[bis(carboxymethyl)amino]propyl}glycine; **1**) carrying a benzyl group was synthesized and characterized. The stability constants of the complexes formed with Ca^{2+}, Zn^{2+}, Cu^{2+}, and Gd^{3+} were determined by potentiometric methods at 25.0 ± 0.1° and 0.1M ionic strength in Me_4NNO_3. The observed water proton relaxivity value of [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ was constant with respect to pH changes over the range pH 4.5–12.0. From the ^{17}O-NMR chemical shift of H_2O induced by [Dy{(*S*)-4-Bz-ttda}]$^{2-}$ at pH 6.80, the presence of 0.9 inner-sphere water molecules was deduced. The water proton spin-lattice relaxation rate for [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ at 37.0 ± 0.1° and 20 MHz was 4.90 ± 0.05 mM^{-1} s^{-1}. The EPR transverse electronic relaxation rate and ^{17}O-NMR transverse-relaxation time for the exchange lifetime of the coordinated H_2O molecule (τ_M), and ^{2}H-NMR longitudinal-relaxation rate of the deuterated diamagnetic lanthanum complex for the rotational correlation time (τ_R) were thoroughly investigated, and the results were compared with those previously reported for the other lanthanide(III) complexes. The exchange lifetime (τ_M) for [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ (2.3 ± 1.3 ns) was significantly shorter than that of the [Gd(dtpa)(H_2O)]$^{2-}$ complex (dtpa = diethylenetriaminepentaacetic acid). The rotational correlation time τ_R for [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ (70 ± 6 ps) was slightly longer than that of the [Gd(dtpa)(H_2O)]$^{2-}$ complex. The marked increase of relaxivity of [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ mainly resulted from its longer rotational time rather than from its fast water-exchange rate. The noncovalent interaction between human serum albumin (HSA) and the [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ complex containing the hydrophobic substituent was investigated by measuring the solvent proton relaxation rate of the aqueous solutions. The association constant (K_A) was less than 100 M^{-1}, indicating a weaker interaction of [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ with HSA.

Introduction. – The use of paramagnetic metal complexes as contrast agents for magnetic resonance imaging (MRI) has been under intense scrutiny [1–3]. A major class of contrast agents are the paramagnetic substances that accelerate magnetic relaxation processes of H_2O protons. Among the paramagnetic contrast agents, Gd^{III} complexes are of particular interest. The search for analogous complexes that would enter hepatocytes or provide a strong noncovalent bonding interaction with the blood plasma protein human serum albumin (HSA) led to a series of Gd^{III} complexes. They are prepared from poly(aminocarboxylic) chelating ligands with various lipophilic substituents. Representative examples are MS-325 (= [(4*R*)-4-({[(4,4-diphenylcyclo-

hexyl)phosphono]oxy}methyl)-3,6,9-tris(carboxymethyl)-3,6,9-triazaundecanedioato(6−)]gadolinate(3−)) [4], [Gd(bopta)]$^{2-}$ (= [4-carboxy-5,8,11-tris(carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatridecan-13-oato(5−)]gadolinate(2−)) [5], [Gd(eob-dtpy)]$^{2-}$ (= [3,6,9-tris(carboxymethyl)-4-(4-ethoxybenzyl)-3,6,9-triazaundecanedioato-(5−)]gadolinate(2−)) [6], and MP-2269 (= (N^2,N^2-bis{2-[bis(carboxymethyl)amino]-ethyl}-N^6-{*N*-[(4-pentylbicyclo[2.2.2]oct-1-yl)carbonyl]-L-α-aspartyl-L-α-aspartyl}-L-lysinato(7−))gadolinate(4−) [7].

The presence of an aromatic group in the ligand seems to lead to some tissue specificity of the complex as well as interaction through noncovalent binding with serum albumin [5–7]. To extend these observations, we synthesized H_5[(*S*)-4-Bz-ttda] (= (4*S*)-4-benzyl-3,6,10-tricarboxymethyl-3,6,10-triazadodecanoic acid = *N*-{(2*S*)-2-[bis(carboxymethyl)amino]-3-phenylpropyl-*N*-{3-[bis(carboxymethyl)amino]propyl}-glycine; **1**), a derivative of H_5ttda [8] bearing a benzyl group (*Scheme*). The protonation, thermodynamic, and conditional stability constants of its complexes with Gd^{3+}, Cu^{2+}, Zn^{2+}, and Ca^{2+} were also determined. Furthermore, its selectivity for Gd^{3+} over endogenously available metal ions was determined. The ^{1}H-NMR for complex [La{(*S*)-4-Bz-ttda}]$^{2-}$ in solution was also studied. The number of inner-sphere water molecules of [Dy{(*S*)-4-Bz-ttda}]$^{2-}$ was determined by the ^{17}O-NMR chemical shift of the water as a function of the Dy^{3+} concentration. The water proton spin-lattice relaxivity r_1 of the [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ complex *vs.* temperature and pH was

Scheme. *Synthesis of (*S*)-4-Bz-TTDA* (**1**)



determined. The EPR and ^{17}O-NMR transverse-relaxation-rate data were analyzed together in a simultaneous multiple-parameter least-squares fitting procedure to determine the water residence lifetime. The ^{2}H-NMR was used to determine the rotational correlation time. In the experiment of albumin binding ability, the value of K_A for ([Gd{(*S*)-4-Bz-ttda}]$^{2-}$)·HSA was also determined.

Results and Discussion. – *Protonation Constants. Table 1* summarizes the protonation constants of H_5[(*S*)-4-Bz-ttda], H_5[(*S*)-4-eob-dtpa], the ligand of MS-325, H_5dtpa, and H_5ttda [4][8–10]. The titration curve of H_5[(*S*)-4-Bz-ttda] shows an increase at pH 8.7–5.7 at $a = 3$ (a = mol base/mol ligand present). This is due to the large difference between the second (log K_2) and third (log K_3) protonation constants, *i.e.*, 8.91 and 5.10. The protonation constant of H_5[(*S*)-4-Bz-ttda] is significantly higher than those of H_5[(*S*)-4-eob-dtpa] and H_5dtpa and is similar to those of H_5ttda and the ligand of MS-325. This is due to higher basicity in the skeleton of H_5ttda as compared to H_5dtpa. However, the benzyl-substituent at the ethylene backbone in H_5[(*S*)-4-Bz-ttda] does not alter the protonation constant significantly as compared to H_5ttda. The difference of the protonation constant of H_5[(*S*)-4-Bz-ttda] and H_5ttda is similar to that of H_5[(*S*)-4-eob-dtpa] and H_5dtpa.

Thermodynamic Constants. For the potentiometric titration curves of the complexes of Ca^{2+}, Cu^{2+}, and Zn^{2+} with H_5[(*S*)-4-Bz-ttda], see *Appendix* (*Fig. 1S*). The thermodynamic stability constants of metal chelates with [(*S*)-4-Bz-ttda], [(*S*)-4-eob-dtpa]$^{5-}$, the ligand of MS-325, dtpa^{5-}, and ttda^{5-} are shown in *Table 2* [4][8–10]. The stability constant of the Gd^{3+} complex with [(*S*)-4-Bz-ttda]$^{5-}$ is similar to those with [(*S*)-4-eob-dtba]$^{5-}$ and the ligand of MS-325 but slightly higher than those with ttba^{5-} and dtpa^{5-}. This interesting result might be attributed to the lipophilic substituent at the ethylene backbone of [(*S*)-4-Bz-ttda]$^{5-}$, [(*S*)-4-eob-dtpa]$^{5-}$, and the ligand of MS-325. It has been reported that Gd^{3+} complexes with poly(aminocarboxylates) display a hydrophilic region (the metal ion and the carboxylates) and a hydrophobic region (the ligand backbone) [11]. With the lipophilic substituent at the ethylene backbone, the ligands tend to separate more compared to those without any substituent or with a hydrophilic substituent, resulting in less steric hindrance as they coordinate to the Gd^{3+} ion. The order of the conditional stability constants of Gd^{3+} complexes (log K_{GdL}) at pH 7.4 is [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ ≈ [Gd{(*S*)-4-eob-dtpa}]$^{2-}$ > MS-325 ≈ [Gd(dtpa)]$^{2-}$ ≈ [Gd(ttda)]$^{2-}$, as shown in *Table 2*. The results of pH dependence of the conditional stability constants for [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ and [Gd(ttda)]$^{2-}$ are very similar (see

Table 1. *Protonation Constants of the Ligands at 25.0 ± 0.1°* [a])

	H_5[(*S*)-4-Bz-ttda]	H_5[(*S*)-4-eob-dtba] [b])	Ligand of MS-325 [c])	H_5dtpa [d])	H_5ttda [e])
[HL]/[L][H]	10.70(0.02)	10.91	11.15	10.49	10.60
[H_2L]/[HL][H]	8.91(0.06)	8.63	8.62	8.60	8.92
[H_3L]/[H_2L][H]	5.10(0.04)	4.26	4.51	4.28	5.12
[H_4L]/[H_3L][H]	3.37(0.03)	2.73	2.96	2.64	2.80
ΣpK_a	28.08	26.53	27.24	26.01	27.44

[a]) All data were obtained in aqueous Me_4NNO_3 solution, $I = 0.1$M, except for [b]) and [c]). [b]) From [10] (KCl, $I = 0.1$M). [c]) From [4] (Me_4NCl, $I = 0.1$M). [d]) From [9]. [e]) From [8].

Table 2. *Stability Constants, Selectivity Constants, and Modified Selectivity Constants for Gd^{3+}, Zn^{2+}, Ca^{2+}, and Cu^{2+} Complexes at 25.0 ± 0.1°*[a])

	[(*S*)-4-Bz-ttda]$^{5-}$	[(*S*)-4-eob-dtba]$^{5-}$ [b])	Ligand of MS-325[c])	dtpa^{5-} [d])	ttda^{5-} [e])
[GdL]/[Gd][L]	23.79(0.04)	23.46	23.2	22.46	22.77
log $K_{GdL'}$ (pH 7.4)	18.97	18.70	18.20	18.14	18.04
log ([CaL]/[Ca][L])	15.95(0.03)	11.74	10.45	10.75	14.45
log β_{CaHL}	5.24(0.04)	5.75	5.66	6.11	–
log $K_{CaL'}$ (pH 7.4)	11.13	6.98	5.45	6.43	9.72
log [CuL]/[Cu][L]	19.70(0.01)	–	21.3	21.38	19.31
log β_{CuHL}	3.89(0.02)	–	5.16	4.81	–
log $K_{CuL'}$ (pH 7.4)	14.88	–	16.30	17.0	14.58
log [ZnL]/[Zn][L]	18.61 (0.02)	–	17.82	18.70	18.59
log β_{ZnHL}	8.63(0.02)	–	5.60	5.60	–
log $K_{ZnL'}$ (pH 7.4)	13.79	–	12.82	14.38	13.86
selectivity[log *K*(Gd/Zn)]	5.18	–	5.38	3.76	4.18
selectivity[log *K*(Gd/Ca)]	7.84	11.72	12.75	11.71	8.32
selectivity[log *K*(Gd/Cu)]	4.09	–	1.9	1.08	3.46
log $K_{sel'}$	9.35	–	7.89	7.06	8.44

[a]) All data were obtained in aqueous Me_4NNO_3, $I = 0.1$M, except for [b]) and [c]). [b]) From [10] (KCl, $I = 0.1$M). [c]) From [4] (Me_4NCl, $I = 0.1$M). [d]) From [9]. [e]) From [8].

Fig. 2S) in the *Appendix*. The conditional stability constants at pH > 11 for [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ and [Gd(ttda)]$^{2-}$ differ by a factor of 10.5, similar to the value obtained at pH 7.4 (8.5). Thus the stability constant of [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ is slightly higher than that of [Gd(ttda)]$^{2-}$ at all pH values.

The species-distribution curve of [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ (see *Fig. 3S* in the *Appendix*) indicates that there is still some free Gd^{3+} at pH 3 but the complex is fully formed above pH 4.53. The pM values [12] for Gd^{3+}, Zn^{2+}, Ca^{2+}, and Cu^{2+} complexes with [(*S*)-4-Bz-ttda]$^{5-}$, [(*S*)-4-eob-dtpa]$^{5-}$, the ligand of MS-325, dtpa^{5-}, and ttda^{5-} are given in the *Appendix* (*Table 1S*). The pGd value of [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ (17.97) resembles that of [Gd{(*S*)-4-eob-dtpa}]$^{2-}$ (17.70) and is slightly larger than those of [Gd(ttda)]$^{2-}$ (17.03) and [Gd(dtpa)]$^{2-}$ (17.14). The stability constant of [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ is slightly larger than those of [Gd(dtpa)]$^{2-}$ and [Gd(ttda)]$^{2-}$, resulting in an increased pGd value for [Gd{(*S*)-4-Bz-ttda}]$^{2-}$. However, the pGd value for [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ is closed to that of [Gd{(*S*)-4-eob-dtpa}]$^{2-}$ due to their similar stability. The pM value of [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ is larger than those of [Zn{(*S*)-4-Bz-ttda}]$^{2-}$, [Cu{(*S*)-4-Bz-ttda}]$^{2-}$, and [Ca{(*S*)-4-Bz-ttda}]$^{2-}$, indicating that Zn^{2+}, Ca^{2+}, and Cu^{2+} cannot replace Gd^{3+} in the [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ complex at physiological pH.

The logarithmic selectivity constants for [(*S*)-4-Bz-ttda]$^{5-}$, [(*S*)-4-eob-dtpa]$^{5-}$, the ligand of MS-325, dtpa^{5-}, and ttda^{5-} are also shown in *Table 2*. The log *K*(Gd/Ca) value of [(*S*)-4-Bz-ttda]$^{5-}$ (7.84) is similar to that of ttda^{5-} (8.32) but lower than those of [(*S*)-4-eob-dtpa]$^{5-}$, the ligand of MS-325, and dtpa^{5-}. This result shows that ligands of type ttda^{5-} form relatively more-stable complexes with Ca^{2+} than ligands of type dtpa^{5-}. This trend is also similar to that previously established for *N*-substituted [Ca(dtpa-py)]$^{2-}$ (= [3,9-bis(carboxymethyl)-6-(pyridylmethyl)-3,6,9-triazadodecanedioato(4−)]calciate(2−)) (log *K* = 8.35) [13] and [Ca(ttda-py)]$^{2-}$ (= [3,10-bis(carboxymethyl)-6-(pyridylmethyl)-3,6,10-triazadodecanedioato(4−)calciate(2−)) (log *K* = 12.02) [14]. The log

K(Gd/Cu) and log K(Gd/Zn) values of $[(S)$-4-Bz-ttda$]^{5-}$ are significantly higher than those of $dtpa^{5-}$ and $ttda^{5-}$, indicating that it exerts a more favorable selectivity toward Gd^{3+} over Zn^{2+} and Cu^{2+}. The modified selectivity constants (K_{sel}) [9] values of $[(S)$-4-Bz-ttda$]^{5-}$, the ligand of MS-325, $dtpa^{5-}$, and $ttda^{5-}$ at pH 7.4 were also determined (*Table 2*). The log K_{sel} of $[(S)$-4-Bz-ttda$]^{5-}$ is higher than those of the ligand of MS-325, $dtpa^{5-}$, and $ttda^{5-}$. Thus, $[(S)$-4-Bz-ttda$]^{5-}$ forms a Gd^{3+} complex that is more stable than MS-325, $[Gd(dtpa)]^{2-}$, and $[Gd(ttda)]^{2-}$ toward transmetallation with Ca^{2+}, Zn^{2+}, and Cu^{2+} metal ions at pH 7.4. This implies that the toxicity of $[Gd\{(S)$-4-Bz-ttda$\}]^{2-}$ may be less than that of MS-325, $[Gd(dtpa)]^{2-}$, or $[Gd(ttda)]^{2-}$.

Dysprosium(III)-Induced Water ^{17}O-NMR Shifts. Fig. 1 shows the dysprosium(III)-induced water ^{17}O-NMR shifts against Dy^{3+} chelate concentration for solutions of $DyCl_3$ and $[Dy\{(S)$-4-Bz-ttda$\}]^{2-}$ in D_2O at 25°. The slope obtained for $[Dy\{(S)$-4-Bz-ttda$\}]^{2-}$ at pH 7.00 is -44.1 ppm mM^{-1} ($r^2 = 0.998$). On the other hand, the slope for $DyCl_3$ is -382.8 ppm mM^{-1} ($r^2 = 0.999$), and the hydration number eight has been proposed for the Dy^{3+} ion [15–17]. Therefore, the $[Dy\{(S)$-4-Bz-ttda$\}]^{2-}$ complex at pH 6.8 contains 0.9 inner-sphere H_2O molecules per Dy^{3+} ion. The number of Ln^{3+}-bound H_2O molecules in the metal complex provides information on the coordination mode of the ligand. The coordination sites of the Gd^{3+} ion in $[Gd\{(S)$-4-Bz-ttda$)]^{2-}$ are

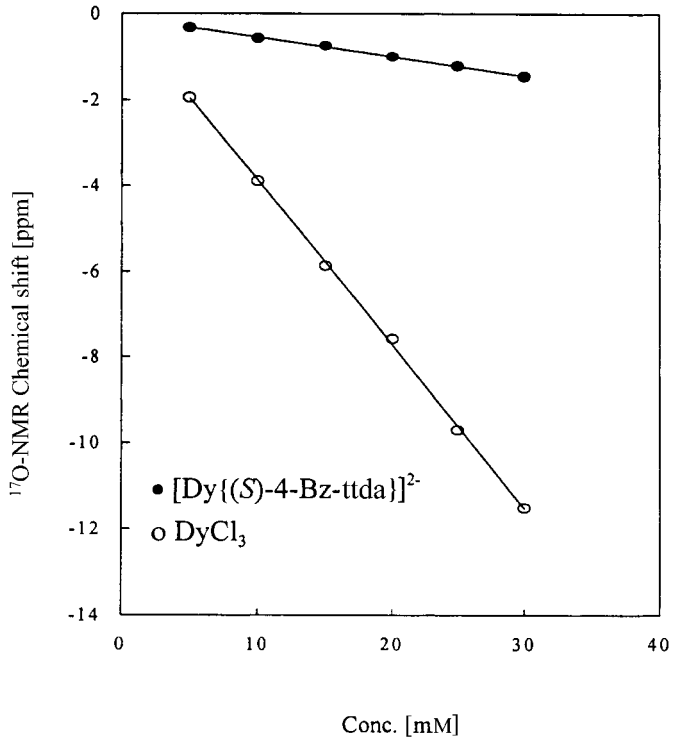


Fig. 1. *Dysprosium(III)-induced water ^{17}O-NMR shift* vs. *Dy^{3+} chelate concentration in D_2O at 25.0 ± 0.1°*

occupied by one H_2O molecule while eight sites are available for the $[(S)$-4-Bz-ttda$]^{5-}$ ligand.

Solution NMR of Lanthanum(III) Chelate. The solution ^{1}H-NMR spectrum of 0.1M [La{(*S*)-4-Bz-ttda}]$^{2-}$ in D_2O at pD 7.0 and $25 \pm 0.1°$ is shown in *Fig. 2*. The peak assignments were also confirmed by a 2D COSY spectrum. The CH_2 protons of the terminal acetate groups (H_a) give four *AB* patterns at δ 3.58 ($J = 40$ Hz), 3.56 ($J =$ 41 Hz), 3.25 ($J = 41$ Hz), and 3.04 ($J = 40$ Hz), respectively. The lack of the fifth *AB* pattern indicates that there are only four carboxylate groups bound to the La^{3+} ion. This result is similar to that obtained from the solution NMR study of [La(dtpa)]$^{2-}$ [18][19]. In the case of [La(dtpa)]$^{2-}$, there are two different *AB* patterns for the four terminal acetate groups and a *s* for the central acetate, indicating that the central carboxylate is not coordinate in the La^{3+} complex.

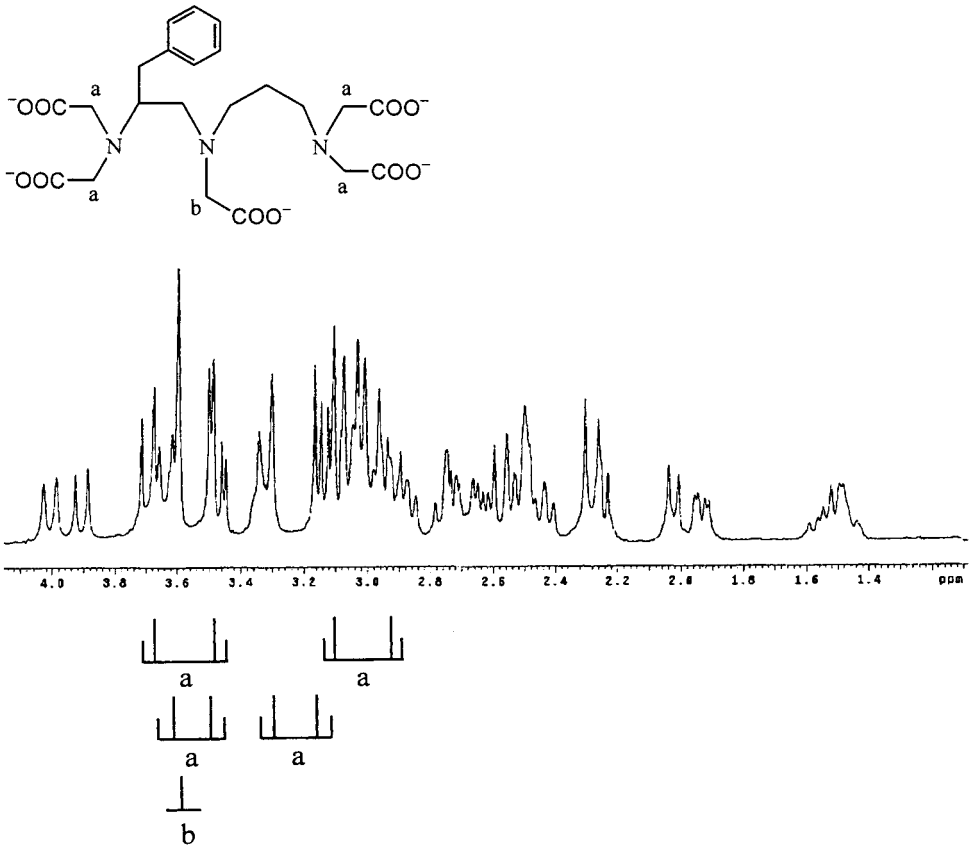


Fig. 2. *^{1}H-NMR Spectrum of [La[(S)-4-Bz-ttda]]$^{2-}$ at $25 \pm 0.1°$*

Relaxometric Studies of the Gadolinium(III) Complexes. The relaxivity r_1 values for [Gd{(*S*)-4-Bz-ttda}]$^{2-}$, measured at various pH values (*Fig. 3*), exhibit no pH dependence over the pH range 4.5 – 12.0, indicating that no free Gd^{3+} ion dissociates from the [Gd{(*S*)-4-Bz-ttda}]$^{2-}$ complex in this pH range of the solution, consistent

with the species distribution curve of $[Gd\{(S)\text{-4-Bz-ttda}\}]^{2-}$ (see *Appendix*, *Fig. 3S*). Thus, the hydration number of $[Gd\{(S)\text{-4-Bz-ttda}\}]^{2-}$ remains constant in this pH range. When the pH is below 4.5, the $[Gd\{(S)\text{-4-Bz-ttda}\}]^{2-}$ complex may undergo partial dissociation and result in a higher relaxivity. The longitudinal relaxivity r_1 value of $[Gd\{(S)\text{-4-Bz-ttda}\}(H_2O)]^{2-}$ is 4.90 ± 0.05 mM^{-1} s^{-1} at pH 6.8, $37.0 \pm 0.1°$, and 20 MHz. The value is significantly higher than those of $[Gd(ttda)(H_2O)]^{2-}$ (3.85 mM^{-1} s^{-1}) [8] and $[Gd(dtpa)(H_2O)]^{2-}$ (3.89 mM^{-1} s^{-1}) [9].

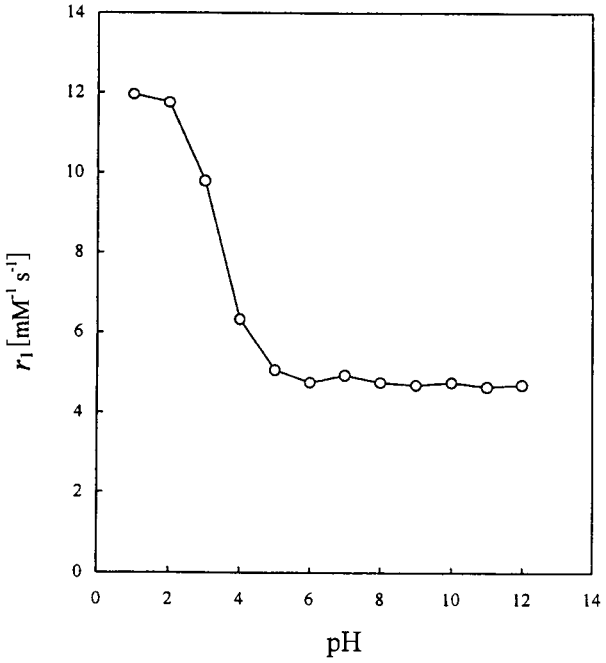


Fig. 3. *pH Dependence of the relaxivity* r_1 *for* $[Gd\{(S)\text{-4-Bz-ttda}\}]^{2-}$ *at* $37 \pm 0.1°$ *and 20 MHz*

The paramagnetic contribution of the solvent longitudinal relaxivity is obtained by *Eqn. 1* [20], where C is the molar concentration of the gadolinium(III) complex, q the number of water molecules bound to metal ion, T_{1M} the longitudinal relaxation time of the bound water protons, and τ_M the residence lifetime of the bound water. Because of the opposite temperature dependencies of T_{1M} and τ_M, two cases can be considered: 1) fast water exchange ($T_{1M} \gg \tau_M$), r_{1p}^{is} increases while temperature decreases; 2) slow water exchange ($T_{1M} \ll \tau_M$), r_{1p}^{is} decreases while temperature decreases. *Fig. 4* shows the temperature dependence of the relaxivity (r_1) for the $[Gd\{(S)\text{-4-Bz-ttda}\}]^{2-}$ complex at pH 6.8 and 20 MHz in the temperature range 278–343 K, *i.e.* a monoexponential decrease of r_1 while temperature increases. This is characteristic of the fast chemical exchange behavior occurring when the τ_M of the coordinated H_2O molecule is much shorter than T_{1M} of the bound water proton.

$$r_{1p}^{is} = Cq/[55.6\ (T_{1M} + \tau_M)] \quad (1)$$

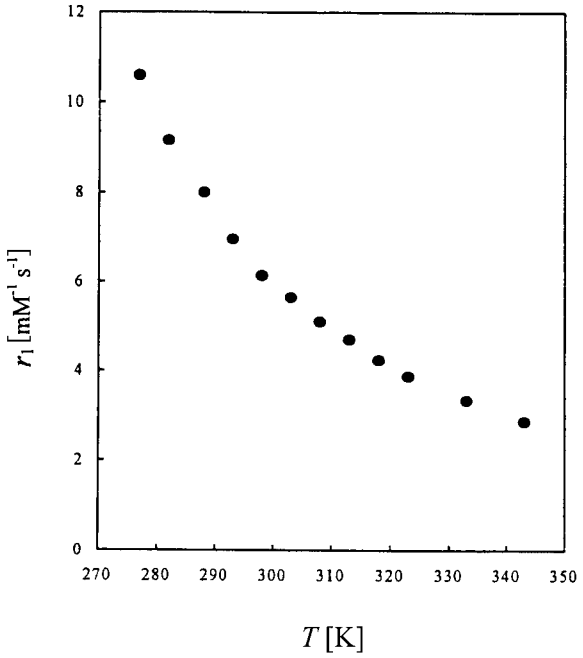


Fig. 4. *Temperature dependence of the relaxivity* r_1 *for the [Gd{(*S*)-4-Bz-ttda}]*$^{2-}$ *at pH 7.0 and 20 MHz*

In fact, T_{1M} can be expressed by *Eqn. 2*, where S is the electron-spin quantum number (7/2 for Gd^{3+}), γ_H the proton nuclear magnetogyric ratio, β the *Bohr* magneton, g the *Landé* factor for the free electron, and r_H the distance between the metal ion and the bound water protons; ω_H and ω_S are the proton and electron *Larmor* frequencies, respectively, and τ_{Ci} $(i=1, 2)$ is the correlation time of the modulation of the dipolar electron-proton coupling. The overall correlation time τ_{Ci} consists of contributions from τ_M, τ_R, and τ_S (the electronic relaxation time of the metal ion) (*Eqn. 3*). To realize how τ_M and τ_R have influences on r_1 of [Gd{(*S*)-4-Bz-ttda}(H_2O)]$^{2-}$, ^{17}O- and ^{2}H-NMR were used to determine the values of τ_M and τ_R, respectively.

$$\frac{1}{T_{1M}} = \frac{2}{15}\,\frac{\gamma_H^2 g^2 S(S+1)\beta^2}{r_H^6}\left[\frac{3\tau_{C1}}{1+\omega_H^2\tau_{C1}^2}+\frac{7\tau_{C2}}{1+\omega_S^2\tau_{C2}^2}\right] \tag{2}$$

$$\frac{1}{\tau_{Ci}} = \frac{1}{\tau_R}+\frac{1}{\tau_M}+\frac{1}{\tau_S} \tag{3}$$

Water-Exchange-Lifetime Studies of Gadolinium(III) Complexes. The measured peak-to-peak line widths ΔH_{pp} of the derivative spectrum can be related to the overall transverse-electronic-relaxation rate $1/T_{2e}$ *via Eqn. 4*, where g_L is the isotropic *Landé g* factor ($g_L = 2.0$ for Gd^{3+}) [21].

$$\frac{1}{T_{2e}} = \frac{g_L \mu B \pi \sqrt{3}}{h} \Delta H_{pp} \tag{4}$$

The temperature dependence of transverse-electronic-relaxation rates at the X-band (0.34 T) at pH 7 for a 50 mM solution of $[Gd\{(S)\text{-4-Bz-ttda}\}(H_2O)]^{2-}$ is given in the *Appendix* (*Fig. 4S*). The data were fitted simultaneously with the following results of ^{17}O-NMR measurements. Analysis of the temperature dependence of the transverse relaxation rate for the ^{17}O water nuclei is the most accurate method for evaluating the exchange lifetime of the water molecules directly coordinated to the metal in a paramagnetic Gd^{3+} chelate [22]. According to the theory of *Swift* and *Connick* [20], the paramagnetic contribution (R^O_{2p}) to the observed transverse relaxation rate is given by *Eqn. 5*, where R^O_{2M} represents the ^{17}O transverse relaxation rate of the coordinated water molecule and $\Delta\omega^O_M$ the chemical-shift difference between the coordinated and bulk water ^{17}O-NMR resonances. R^O_{2M} is expressed by *Eqns. 6* and *7*, where S is the electronic spin quantum number (7/2 for Gd(III)) and $A/\hbar$ the Gd,^{17}O scalar coupling constant; τ_{ei} ($i = 1, 2$) represents the correlation time of the processes modulating the scalar interaction. This modulation may occur through both the longitudinal and the transverse average electronic relaxation times (T_{1e} and T_{2e}) and the mean residence lifetime (τ^O_M) of the water molecule at the paramagnetic site.

$$R^O_{2p} = \frac{Cq}{55.6} (\tau^O_M)^{-1} \frac{R^O_{2M} + (\tau^O_M)^{-1} R^O_{2M} + \Delta\omega^{O2}_M}{(R^O_{2M} + (\tau^O_M)^{-1})2 + \Delta^{O2}_M} \tag{5}$$

$$R^O_{2M} = \frac{1}{3}\left(\frac{A}{\hbar}\right)^2 S(S+1)\left(\tau_{e1} + \frac{\tau_{e2}}{1 + \omega_S^2 \tau_{e2}^2}\right) \tag{6}$$

$$\tau_{ei}^{-1} = \tau_M^{O-1} + T_{ei}^{-1} \tag{7}$$

The temperature dependence of R^O_{2M} is determined by the temperature effect on τ^O_M, τ_V, and $\Delta\omega^O_M$ according to *Eqns. 8* and *9* where the subscript j refers to the different correlation times, ΔH_j is the activation enthalpy for the corresponding dynamic process, B the applied magnetic field strength, and k_B the *Boltzmann* constant.

$$(\tau_j)_T^{-1} = \frac{(\tau_j^{-1})^{298.15} T}{298.15} \exp\left[\frac{\Delta H_j}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \tag{8}$$

$$\Delta\omega^O_M = \frac{g_L \mu_B S(S+1) B}{3 k_B T} \frac{A}{\hbar} \tag{9}$$

The water-exchange rate for $[Gd\{(S)\text{-4-Bz-ttda}\}(H_2O)]^{2-}$ was obtained by measuring the ^{17}O-NMR transverse-relaxation rate (R^0_{2p}) as a function of temperature [21][23]. The data and its best simulation according to *Eqns. 4–9* is shown in *Fig. 5*. As there are a large number of parameters to be determined in the quantitative analysis of the ^{17}O-NMR transverse-relaxation rate (R^O_{2p}) *vs. T* profiles, it is convenient to fix some of them. On this basis, besides the value of q and $A/\hbar$ ($-3.8 \cdot 10^6$ rad s^{-1}), the value of

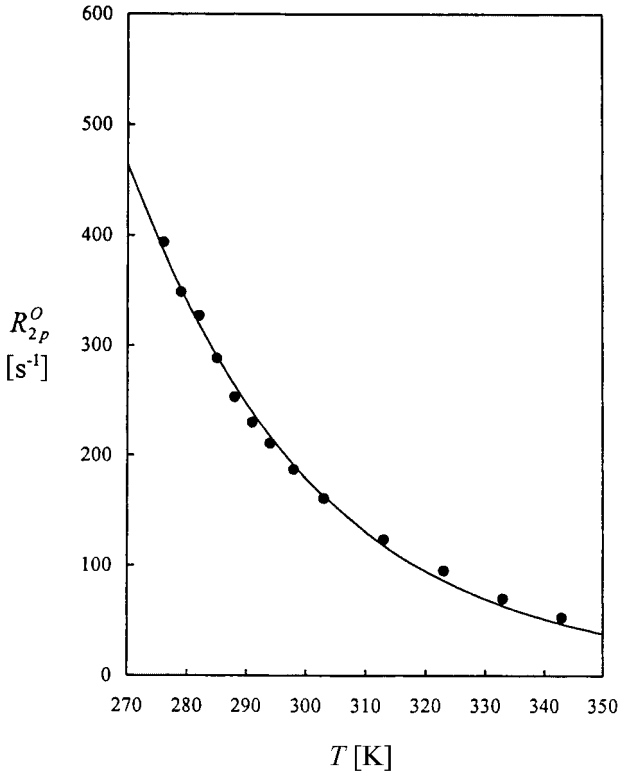


Fig. 5. *Temperature dependence of the ^{17}O-NMR water transverse-relaxation rate at 7.05 T and pH 7 for a 50 mM solution of [Gd{(S)-4-Bz-ttda}(H$_2$O)]$^{2-}$*. The line represents the simultaneous least-squares fit to all data points as described in the test.

Table 3. *Kinetic and NMR Parameters Obtained from the Simultaneous Fit of ^{17}O-NMR and EPR Data*

	Δ^2 [s$^{-2}\cdot 10^{19}$]	τ_v^{298}[ps]	τ_M^{310}[ns]	τ_R^{310}[ps][a])	ΔH_v[kJ mol^{-1}]
[Gd{(*S*)-4-Bz-ttda}(H$_2$O)]$^{2-}$	15 ± 1.2	3.2 ± 0.3	2.3 ± 1.3	70 ± 6	8.8 ± 2.7
[Gd{(*S*)-4-Bz-dtpa}(H$_2$O)]$^{2-}$[b])	–	23 ± 1	87 ± 25	65	8.6 ± 8.0
[Gd{(*S*)-4-eob-dtpa}(H$_2$O)]$^{2-}$[b])	23 ± 0.1	4.0 ± 0.2	82 ± 21	64	1.7 ± 0.9
[Gd(ttda)(H$_2$O)]$^{2-}$[c])	4.6 ± 0.2	25 ± 1	6.3 ± 2.5	57 ± 4	1.6 ± 1.0
[Gd(dtpa)(H$_2$O)]$^{2-}$[b])	4.6 ± 0.2	25 ± 1	143 ± 26	58	1.6 ± 1.8

[a]) The values were obtained by ^{2}H-NMR. [b]) From [33]. [c]) From [16].

ΔH_M is fixed at 30 kJ/mol [24]. The parameters providing the best fit of the data for [Gd{(*S*)-4-Bz-ttda}(H$_2$O)]$^{2-}$ are listed in *Table 3*. The obtained τ_M^O value of 2.3 ns at 310 K is very close to the optimal range for the attainment of the high relaxivity expected when the molecular reorientational time of the complex is lengthened to the nanosecond range [25]. By varying the temperature over a wide range, R_{2p}^O is dominated by $1/\tau_M$ in the slow kinetic region at low temperature and is dominated by $1/\tau_{ei}$ in the fast kinetic region at high temperature. The maximum in the profile of ^{17}O-

NMR transverse-relaxation rate (R_{2p}^{O}) *vs.* T corresponds to the translation from the slow to the fast kinetic regions and was not found for $[Gd\{(S)\text{-4-Bz-ttda}\}(H_2O)]^{2-}$, indicating the fast water-exchange rate for the complex.

As shown in *Table 3*, the water-exchange lifetime of $[Gd\{(S)\text{-4-Bz-ttda}\}(H_2O)]^{2-}$ (2.3 ± 1.3 ns) is similar to that of $[Gd(ttda)(H_2O)]^{2-}$ (6.3 ± 2.5 ns) but lower than those of $[Gd\{(S)\text{-4-Bz-dtpa}\}(H_2O)]^{2-}$ (87 ± 25 ns), $[Gd\{(S)\text{-4-eob-dtpa}(H_2O)]^{2-}$ (82 ± 21 ns), and $[Gd(dtpa)(H_2O)]^{2-}$ (143 ± 26 ns) [26]. The smaller water-exchange lifetime for $[Gd\{(S)\text{-4-Bz-ttda}\}(H_2O)]^{2-}$ and $[Gd(ttda)(H_2O)]^{2-}$ is perhaps due to the longer backbone in the $ttda^{5-}$ ligand, which might be pulled tightly into the first coordination sphere and lead to the highly steric constraint on the water binding site [27]. Analogous considerations can be made for $[Gd(egta)(H_2O)]^{-}$ (=aqua[3,12-bis(carboxymethyl)-6,9-dioxa-3,12-diazatetradecanedioato-(4−)]gadolinate(1−)) [28]. Two coordinating O-atoms in this complex are linked by an ethylenic group, which induces severe constraints on the atoms around the site occupied by the water molecule, thus favoring the water-exchange process.

Since the τ_M value of $[Gd\{(S)\text{-4-Bz-ttda}\}(H_2O)]^{2-}$ is similar to that of $[Gd(ttda)(H_2O)]^{2-}$, the relaxivity r_1 of $[Gd\{(S)\text{-4-Bz-ttda})(H_2O)]^{2-}$ should be similar to that of $[Gd(ttda)(H_2O)]^{2-}$. However, the r_1 of $[Gd\{(S)\text{-4-Bz-ttda})(H_2O)]^{2-}$ is significantly higher than that of $[Gd(ttda)(H_2O)]^{2-}$. On the other hand, the τ_M value of $[Gd(ttda)(H_2O)]^{2-}$ is significantly lower than that of $[Gd(dtpa)(H_2O)]^{2-}$, but both have similar r_1-values. It can be concluded that for small Gd^{3+} chelates, the mean residence lifetime τ_M does not contribute significantly to the overall correlation time for the dipolar interaction, except for Gd^{3+} complexes of $dtpa^{5-}$ bis(amide).

Determination of τ_R by ^2H-NMR. In diamagnetic molecules, the relaxation rate of the ^2H-nucleus is predominantly determined by the quadrupolar mechanism [29], which is strictly intramolecular and solely modulated by rotation of the molecule. For fast-tumbling systems, the relaxation rate R_1 is thus directly related to the rotational correlation time τ_R (*Eqn. 10*), where the quadrupolar coupling constant (e^2qQ/h) depends on the hybridization state of the C-atom carrying the ^2H-atom; its value is *ca.* 170 kHz in the case of an sp^3 C-atom.

$$R_1 = \frac{1}{T_1} = \frac{3}{8}\left(\frac{e^2qQ}{h}\right)^2 \tau_R \tag{10}$$

The measurement of τ_R was performed on the diamagnetic lanthanum(III) complexes deuterated in the α-position relative to the carboxylate groups. At 310 K, the values of τ_R for La^{3+} complexes with $[(S)\text{-4-Bz-ttda}]^{5-}$, $[(S)\text{-4-Bz-dtpa}]^{5-}$, $[(S)\text{-4-eob-dtpa}]^{5-}$, $ttda^{5-}$, and $dtpa^{5-}$ are shown in *Table 3*. The τ_R value of $[La\{(S)\text{-4-Bz-}(^2H_{10})ttda\}]^{2-}$ (70 ± 6 ps) is similar to that of $[La\{(S)\text{-4-Bz-}(^2H_{10})dtpa\}]^{2-}$ (65 ps) [26]. Thus, the change of the C-backbone length does not alter τ_R significantly. On the other hand, the higher τ_R values for $[La\{(S)\text{-4-Bz-}(^2H_{10})ttda\}]^{2-}$, $[La\{(S)\text{-4-eob-}(^2H_{10})dtpa\}]^{2-}$, and $[La\{(S)\text{-4-Bz-}(^2H_{10})dtpa\}]^{2-}$ compared to those of $[La\{(^2H_{10})dtpa\}]^{2-}$ and $[La\{(^2H_{10})ttda\}]^{2-}$ indicate that a benzyl or an ethoxybenzyl group at the C-backbone increases the τ_R value and causes the higher relaxivity of the Gd^{3+} complexes.

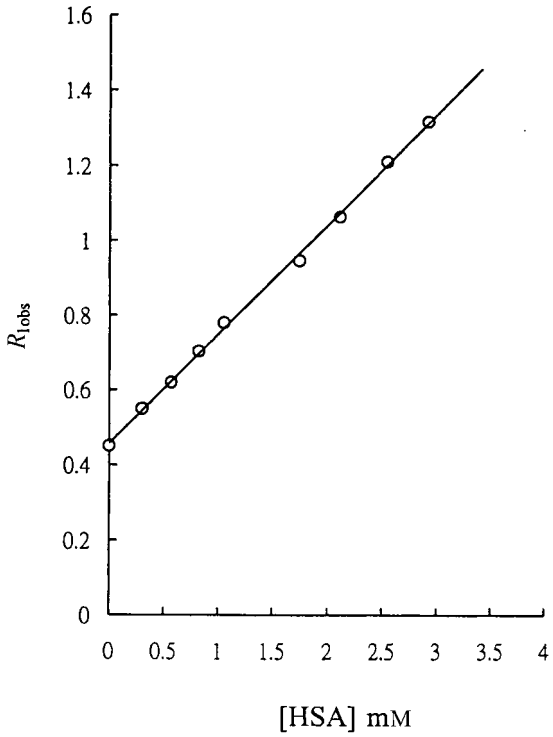


Fig. 6. *Plot of the water proton relaxation rate of a 0.07 mM [Gd{(S)-4-Bz-ttda}(H_2O)]$^{2-}$ solution as a function of the HSA concentration.* pH 7.5, 50 mM phosphate buffer, 20 MHz, 25 ± 0.1°.

Binding of Gadolinium(III) Chelate to Human Serum Albumin (HSA). Binding of a contrast agent to HSA is highly interesting because the formed macromolecular adduct compared to the free complex has a higher relaxivity and a longer retention time in blood, which would qualify it as a contrast agent for angiographic applications [30]. The characterization of the binding parameters was carried out by measuring the relaxation rates of a [Gd{(*S*)-4-Bz-ttda)(H_2O)]$^{2-}$ solution in the presence of different concentrations of HSA at 20 MHz and 25° [31], as shown in *Fig. 6*. Assuming that there are two binding sites with similar affinity in albumin, as suggested by previous studies, the value of $K_A < 1 \cdot 10^2$ M^{-1} for the adduct ([Gd{(*S*)-4-Bz-ttda](H_2O)]$^{2-}$)·HSA was obtained, which is smaller than those of ([Gd{(*S*)-4-eob-dtpa}(H_2O)]$^{2-}$)·HSA (772 ± 195M^{-1}) [6] and ([Gd(bopta)(H_2O)]$^{2-}$)·HSA (290 ± 60M^{-1}) [5] bearing hydrophobic substituents in the ligands. However, the value of K_A for adducts ([Gd{(*S*)-4-Bz-ttda}(H_2O)]$^{2-}$)·HSA, ([Gd{(*S*)-4-eob-dtpa}(H_2O)]$^{2-}$)·HSA, and ([Gd(bopta)-(H_2O)]$^{2-}$)·HSA are significantly lower than that of (MS-325)·HSA ($3.0 \pm 0.2 \cdot 10^4$ M^{-1}) [32], which possesses one diphenylcyclohexyl substituent. Thus, only less-hydrophobic substituents at the surface of the ligand can lead to a smaller association constant K_A.

In conclusion, the linear poly(aminocarboxylate) ligand [(*S*)-4-Bz-ttda]$^{5-}$ forms a thermodynamically stable complex with the trivalent gadolinium cation. This complex

does not dissociate under physiological conditions (pH 7.4) and does not exchange with Ca^{2+}, Cu^{2+}, or Zn^{2+} to an appreciable extent. From analysis of the ^{17}O-NMR relaxometric properties, the replacement of the ethane-1,2-diyl backbone with a propane-1,3-diyl backbone moiety and the introduction of a hydrophobic substituent at the backbone increase the steric constraints at the water binding site and, therefore, enhance the water-exchange rate. For small gadolinium complexes like those studied in this work, τ_R is the determinant correlation time at high fields and physiological temperature. An increased τ_R results, thus, in a higher relaxivity. $[Gd\{(S)\text{-4-Bz-ttda}\}(H_2O)]^{2-}$ possesses a higher relaxivity, a higher thermodynamic stability constant, an optimal water-exchange rate, and a longer rotational correlation time, which might result in a novel type of contrast agent for MRI. However, the relatively weak binding between $[Gd\{(S)\text{-4-Bz-ttda}\}(H_2O)]^{2-}$ and HSA reduces its suitability for potential use as a contrast agent in MR angiography. With the advantages of a short τ_M and long τ_R of $[Gd\{(S)\text{-4-Bz-ttda}\}]^{2-}$, other $ttda^{5-}$ derivatives and their Gd^{3+} complexes are under study in our laboratory. We expect to obtain a novel contrast agent for MR angiography by tuning the lipophilicity of complexes with different pendant hydrophobic substituents at the backbone of the $ttda^{5-}$ ligand.

Experimental Part

1. *General.* $GdCl_3 \cdot 6\,H_2O$ (99.9%), $DyCl_3 \cdot 6\,H_2O$ (99.9%), and $LaCl_3 \cdot 7\,H_2O$ (99.9%) were obtained from *Aldrich* and used without further purification. The concentration of Gd^{3+}, Dy^{3+}, and La^{3+} were determined by chelatrometric titration with H_4edta, and xylenol orange was used as an indicator. All other reagents used for the synthesis of the ligand were purchased from commercial sources unless otherwise noted. ^1H- and ^{13}C-NMR spectra and elemental analyses were used to confirm the composition of the products. ^{17}O-Enriched water (10.5%) was purchased from *Isotec Inc.* HSA was purchased from *Sigma* (St. Louis, Mo., USA) and used without any further purification; the molecular mass was assumed to be 69 kDa [33]. The concentration of the aq. solns. of the Gd^{3+} complexes was obtained from the known millimolar relaxivity of the paramagnetic complex (R_{1p}^F) by measuring their longitudinal water proton relaxation rates (R_{1obs}) at 20 MHz and 25° (*Eqn. 11*) [34], where 0.38 s^{-1} is the relaxation rate of pure water at this temperature and magnetic field strength.

$$R_{1obs} = R_{1p}^F[GdL] + 0.38 \tag{18}$$

2. *Synthesis.* L-*Phenylalanine Methyl Ester Hydrochloride* (**3**·HCl). To L-phenylalanine (**2**; 10.0 g, 60.62 mmol) and anh. MeOH (50 ml), thionyl chloride (7.57 g, 63.61 mmol) was added below 5° (ice bath). After 30 min, the mixture was brought to r.t. for another 19 h. The soln. was evaporated and the white precipitated product collected in a *Büchner* funnel and dried *in vacuo:* **3**·HCl (9.3 g, 85.6%). ^1H-NMR (D_2O): 7.33–7.16 (*m*, 5 arom. H); 4.31 (*t*, H–C(2)); 3.72 (*s*, MeO); 3.24–3.13 (*m*, 2 H–C(3)). ^{13}C-NMR (D_2O): 173.1; 136.8; 132.5; 132.3; 131.2; 57.0; 56.5; 38.4. Anal. calc. for $C_{10}H_{13}NO_2 \cdot HCl$ (215.68): C 55.69, H 6.45, N 6.49; found: C 55.32, H 6.54, N 6.35.

N′-*(3-Aminopropyl)*-L-*phenylalaninamide Trihydrochloride* (**4**·3 HCl). A soln. of **3** (8 g, 44.63 mmol) in anh. MeOH (50 ml) was added to propane-1,3-diamine (25 ml) at r.t.. After 15 h at r.t., the mixture was evaporated. The pale crude yellow oil was purified by cation exchange (*AG 50W* × *8* column (200–400 mesh, H^+ form, 100 ml of resin), 4.2-cm column diameter; elution with aq. HCl soln.). The product was eluted with 4.5–5M HCl, while the earlier fractions contained inorg. salts (NaCl) and impurities: **4**·3 HCl (3.44 g, 34.8%). Amorphous, hygroscopic, yellow powder. ^1H-NMR (D_2O): 7.42–7.27 (*m*, 5 arom. H); 4.18 (*t*, H–C(2)); 3.29–3.04 (*m*, $NCH_2CH_2CH_2N$); 2.68 (*t*, 2 H–C(3)); 1.68 (*quint.*, $NCH_2CH_2CH_2NH$). ^{13}C-NMR (D_2O): 172.2; 137.1; 132.5; 132.2; 131.0; 57.4; 39.8; 39.7; 39.2; 29.0. Anal. calc. for $C_{12}H_{14}N_3O \cdot 3\,HCl$ (330.68): C 66.65, H 6.53, N 19.43; found: C 66.41, H 6.40, N 19.25.

N-*[(2*S*)-2-Amino-3-phenylpropyl]propane-1,3-diamine Trihydrochloride* (**5**·3 HCl). To **4** (3 g, 13.55 mmol) under N_2, anh. THF (50 ml) was added by syringe. The amide only partially dissolved in the

THF. The mixture was cooled to -5 to $0°$, 1M $BH_3 \cdot THF$ (50 ml) added by syringe, and then the mixture gradually warmed up and brought to reflux for 18 h. Then the soln. was evaporated, the residue dissolved in EtOH (100 ml) and 6M HCl (10 ml), and the resulting soln. refluxed for 12 h. The soln. was evaporated, the residue dissolved in dist. H_2O, and the pH of the soln. adjusted to 1.5–2.0 with 6M HCl. The crude product was purified by cation exchange (*AG 50W* × *8* column (200–400 mesh, H^+ form, 100 ml of resin); 4.2-cm column diameter; elution with aq. HCl soln.). The product was eluted with 5.5–6M HCl: **5** · 3 HCl (2.63 g, 79.9%). Amorphous, hygroscopic, yellow powder. ^{1}H-NMR (D_2O): 7.43–7.32 (*m*, 5 arom. H); 3.95 (*quint.*, H–C(2′)); 3.48, 3.35 (*m*, 2 H–C(1′)); 3.17 (*t*, $HNCH_2CH_2CH_2NH_2$); 3.06 (*t*, $HNCH_2CH_2CH_2NH_2$); 3.19–2.98 (*m*, 2 H–C(3′)); 2.10–2.06 (*quint.*, $HNCH_2CH_2CH_2NH_2$). ^{13}C-NMR (D_2O): 133.9; 129.6; 129.5; 128.4; 50.2; 48.9; 45.8; 36.6; 36.5; 23.9. Anal. calc. for $C_{12}H_{16}N_3 \cdot 3HCl$ (316.70): C 71.25, H 7.97, N 20.77; found: C 71.12, H 7.85, N 20.55.

*(4*S*)-4-Benzyl-3,6,10-tris(carboxymethyl)-3,6,10-triazadodecanedioic Acid* (= N-*{(2*S*)-2-[Bis(carboxymethyl)amino]-3-phenylpropyl}*-N-*{3-[bis(carboxymethyl)amino]propyl}glycine*; H_5[(*S*)-4-Bz-ttda]; **1**). To a soln. of **5** (2.5 g, 12.05 mmol) and K_2CO_3 (15 g, 108.5 mmol) in MeCN (250 ml), *tert*-butyl bromoacetate (8.9 ml, 60.29 mmol) was added. The mixture was heated and refluxed for 24 h. The K_2CO_3 was removed by filtration through a *Büchner* funnel and washed with MeCN (40 ml). The filtrate was evaporated and the residue taken up in H_2O and extracted with $CHCl_3$ (3 × 50 ml). The extract was evaporated, the residue dissolved in 2M HCl (50 ml), and the soln. stirred for 4 h at r.t. and then evaporated. The acid treatment was repeated three times (^{1}H-NMR: complete removal of *tert*-butyl group). The residue was dissolved in dist. H_2O (50 ml) and the soln. alkalinized with aq. ammonia to pH 11.0 and submitted to anion exchange (*AG1* × *8* column (200–400 mesh, HCO_2^- form, 60 ml of resin); 3.0-cm column diameter; elution with aq. HCO_2H soln.). The product was eluted with 0.7M HCO_2H. After evaporation, the residue was coevaporated 5 times with H_2O (200 ml) to remove HCO_2H. The residue was dried *in vacuo:* **1** (2.27 g, 37.89%). Pale yellow hygroscopic powder. ^{1}H-NMR (D_2O): 7.33–7.25 (*m*, 5 arom. H); 3.82 (*s*, 8 H, terminal NCH_2COOH); 3.48 (*s*, central NCH_2COOH); 3.33–3.01 (*m*, $NCH_2CH_2CH_2N$, $NCHCH_2N$); 3.05, 2.62 (2*q*, $PhCH_2$); 2.03 (*quint.*, $NCH_2CH_2CH_2$). ^{13}C-NMR (D_2O): 174.6; 173.0; 172.6; 136.7; 129.1; 128.4; 126.9; 63.0; 59.3; 56.9; 56.4; 54.7; 55.9; 54.9; 38.9; 26.1. Anal. calc. for $C_{22}H_{31}N_3O_{10}$ (497.50): C 53.11, H 6.28, N 8.44; found: C 52.93, H 6.14, N 8.29.

3. *Deuteration*. Deuteration of ligands (or of their lanthanum complexes) at the α-position with respect to the carboxylate groups were performed by the procedure described in [35].

4. *Complexation*. The La^{3+}, Dy^{3+}, and Gd^{3+} complexes were prepared by mixing equimolar amounts of hydrated $LnCl_3$ and ligand. The pH was maintained at 7.5 with 1M NaOH. At r.t., complex formation was instantaneous. The pH of the soln. was then increased to 8–9 by adding 1M NaOH to precipitate the excess of uncomplexed Ln^{3+} ions. The soln. was then evaporated and the residue dried overnight at 60°. The synthesis of the Gd^{3+} complex for the HSA experiment was carried out by the procedure described in [34].

5. *Potentiometric Measurements.* pH Titration measurements of H_5[(*S*)-Bz-ttda] in the absence and presence of Gd^{3+}, Ca^{2+}, Zn^{2+}, and Cu^{2+} were performed with a *Metrohm* pH meter equipped with a *PT-100* combination pH electrode. The pH electrode was calibrated with three standard buffer solns., and all calibrations and titrations were carried out under CO_2-free N_2 (to avoid any contact with CO_2) in a glass-jacketed vessel (20 ml) thermostatted at 25.0 ± 0.1°, and 0.1M ionic strength in Me_4NNO_3. The autotitration system consisted of a *702-SM* titroprocessor and a *728* stirrer. CO_2-Free 0.10M KOH was used as the titrant to minimize ionic strength change during the titration. N_2 was bubbled through the solns. to exclude CO_2. Each titration was performed at least 3 times.

For the potentiometric equilibrium studies, solns. of [(*S*)-4-Bz-ttda]$^{5-}$ in the absence of metal ion and then in the presence of each metal ion were used in which the M/L ratio was 1:1. The δ values were obtained after addition of 0.005-ml increments of standard 0.100 mM KOH, and after stabilization from this side of the pH scale equilibrium was then approached from the other side by adding standard 0.10M acid soln. To determine the stability constant for [Gd{(*S*)-4-Bz-ttda}]$^{2-}$, competition titration with H_4edta was performed. A ratio Gd^{3+} [(*S*)-4-Bz-ttda]$^{5-}$/edta^{4-} of 1:1:1 was employed. Under these conditions, the metal ion was partitioned between [(*S*)-4-Bz-ttda]$^{5-}$ and edta^{4-}, and the rate of transmetallation was fast enough between pH 2–4. However, complexation was usually rapid (3–5 min per point to give a stable pH reading) in the Cu^{2+}, Ca^{2+}, and Zn^{2+} studies. The same values of the stability constants were obtained either by direct or back titration.

6. *Computational Method*. The ligand soln. (1 mM) was titrated with KOH over a pH range from 2 to 11. The titration data was fit to a *Fortran* computer program PKAS [36] written for polyprotonic weak acid equilibrium. Equimolar metal/ligand solns. were titrated with KOH over the pH range 2–11, collecting *ca.* 110 data points per titration, and the stability constants were determined by analysis of the titration curve with the *Fortran* computer program BEST [36]. The average difference between observed and calculated $-\log [H^+]$ was < 0.04

throughout all titrations. A value of 13.78 was employed for pK_w at 25°. The species-distribution diagrams were calculated with the *Fortran* program SPE and SPEPLOT [36].

7. *Relaxation Measurement*. Combining equal molar amounts of the stock $GdCl_3$ and the [(*S*)-4-Bz-ttda]$^{5-}$ ligand soln. gave the Gd^{3+} chelate soln. A slight excess (3%) of the ligand was used, and the soln. was left for at least 2 h at r.t. to ensure completion of the complexation. The Gd^{3+} chelate solns. at various pH values were prepared by combining the buffer soln. with an approximately diluted Gd^{3+} complex soln. in a 1:1 (*v*/*v*) ratio. The following buffer systems (all 0.10M) were used: chloroacetic acid/NaOH (pH 2 and 3), acetic acid/NaOH (pH 4 and 5), PIPES (= piperazine-1,4-diethanesulfonic acid)/NaOH (pH 6.0 and 6.8), and ammonia/HCl (pH 9 and 10) [37]. These buffer solns. were used to maintain constant ionic strength (*i.e.*, 0.10M). The 0.10M buffers were sufficient to maintain the soln. pH within the desired range. The buffered Gd^{3+} chelate solns. were all allowed to equilibrate for at least 2 h. The pH of these solns. was determined immediately before relaxation time T_1 measurements.

Relaxation times T_1 of aq. solns. of [Gd{(*S*)-4-Bz-ttda}] were measured to determine relaxivity r_1. All measurements were made with a *Bruker Minispec-NMS-120* NMR spectrometer operating at 20 MHz as a function of temp. Before each measurement, the spectrometer was tuned and calibrated. The values of T_1 were measured from eight data points generated by an inversion-recovery pulse sequence. The slopes of plots of $1/T_1$ *vs.* the concentration of Gd^{3+} complex give r_1 in $mM^{-1}\ s^{-1}$.

8. *EPR Measurements*. The EPR spectra were recorded at the X-band (0.34 T) with a *Bruker ER-200D-SRC* spectrometer operated in continuous-wave mode, the samples being in 1-mm glass tubes. The cavity temp. was stabilized by electronic temp. control of the gas flowing through the cavity and verified by substituting a thermometer for the sample tube. Measurements were made from 273 up to 363 K. The peak-to-peak linewidth was measured from the recorded spectra by using the instrument software.

9. *NMR Measurements*. *^{17}O-NMR*. The hydration number of [Dy{(*S*)-4-Bz-ttda}]$^{2-}$ was determined by the method of *Alpoim et al*. [38]. The ^{17}O-NMR spectra were recorded by a *Varian-Gemini-400* spectrometer at 25°. The induced ^{17}O shift (d.i.s.) measurements were determined with D_2O as external standard. An equimolar soln. of Dy^{3+} and ligand was prepared, and a stoichiometric amount of standardized NaOH was added so that the complex was fully formed. Six solns. of varying Dy^{3+} concentrations were prepared by serial dilution of the stock soln.

For the measurement of the ^{17}O-NMR transverse-relaxation rate, a *Varian Gemini-300* (7.05 T, 40.65 MHz) spectrometer was used, equipped with a 5-mm probe (external D_2O lock). Experimental settings were: spectral width 10000 Hz, pulse width 7 μs, acquisition time 10 ms, no sample spinning. A *Varian VT-J103* temp. control unit was used to stabilize the temp. The value of the transverse relaxation rate was obtained by evaluating the linewidth at half-height ($\Delta\nu_{1/2}$) of the water ^{17}O signal ($R_2 = \pi\,\Delta\nu_{1/2}$). Solns. containing 2.6% of the ^{17}O isotope were used.

^{2}H-NMR. The measurement was carried out in a 10-mm (o.d.) tube on a *Varian Gemini-400* (9.4 T) spectrometer equipped with a broadband probe. No field frequency lock was used.

Solution NMR. ^{1}H-NMR Spectra were measured with a *Varian Gemini-400* spectrometer in 5-mm (o.d.) tubes at 25°. The La^{3+} complex for ^{1}H-NMR study was prepared by mixing an aliquot of the ligand soln. with an amount of the appropriate La^{3+} soln. to have a 1:1 molar ratio at pD 7.0 (by using NaOD). The final concentration of [La{(*S*)-4-Bz-ttda}]$^{2-}$ was *ca*. 30 mM. The chemical shifts were referenced to D_2O (4.72 ppm).

Data Analysis. The simultaneous least-squares fitting and the binding constant K_A were determined by fitting the experimental data with the program *Scientist*® for *Windows*™ by *Micromath*®, version 2.0.

Appendix:

Table 1S. *pM Values for the Gd^{2+}, Zn^{2+}, Ca^{2+}, and Cu^{2+} Complexes at pH 7.4*[a])

	[(*S*)-4-Bz-ttda]$^{5-}$	[(*S*)-4-eob-dtpa]$^{5-}$ [b])	Ligand of MS-325 [c])	dtpa^{5-} [d])	ttda^{5-} [e])
pGd	17.97	17.70	17.20	17.14	17.03
pCu	13.88	–	15.31	16.06	13.56
pCa	10.13	5.99	4.46	5.45	9.73
pZn	14.04	–	11.83	13.39	13.22

[a]) All data were obtained in aqueous Me_4NNO_3 solution, $I = 0.1$M, except for [b]) and [c]). [b]) From [10] (KCl, $I = 0.1$M). [c]) From [4] (Me_4NCl, $I = 0.1$M). [d]) From [9]. [e]) From [8].

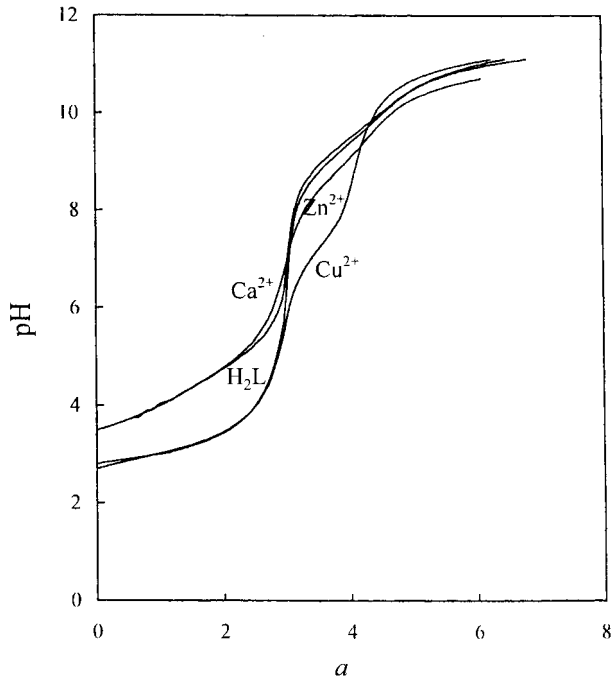


Fig. 1S. *Titration curves for 1:1 ratios of metal ion to [(S)-4-Bz-ttda]$^{5-}$ (= L). μ* = 0.1M Me_4NNO_3; T = 25.0 ± 0.1°; concentrations of ligand and metal ion = $1.0 \cdot 10^{-3}$ M. *a* = mol base/mol ligand.

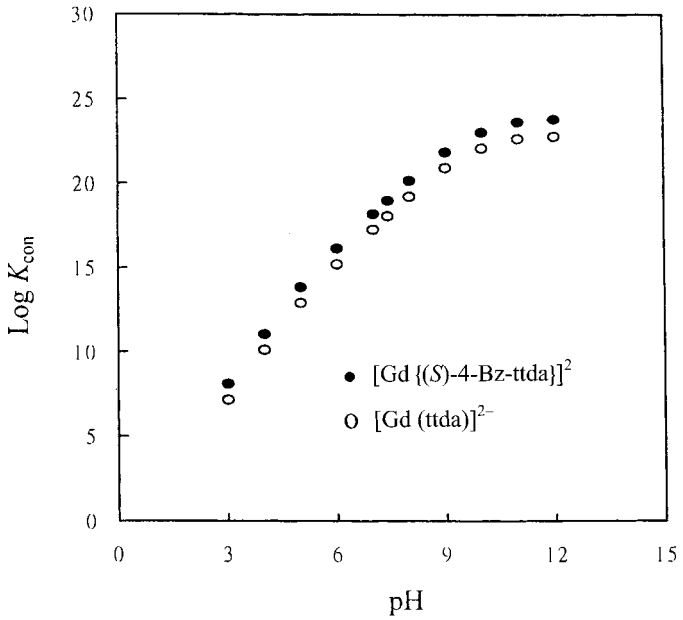


Fig. 2S. *pH Dependencies of conditional stability constants for [Gd{(S)-4-Bz-ttda}]$^{3-}$ and [Gd(ttda)]$^{2-}$*

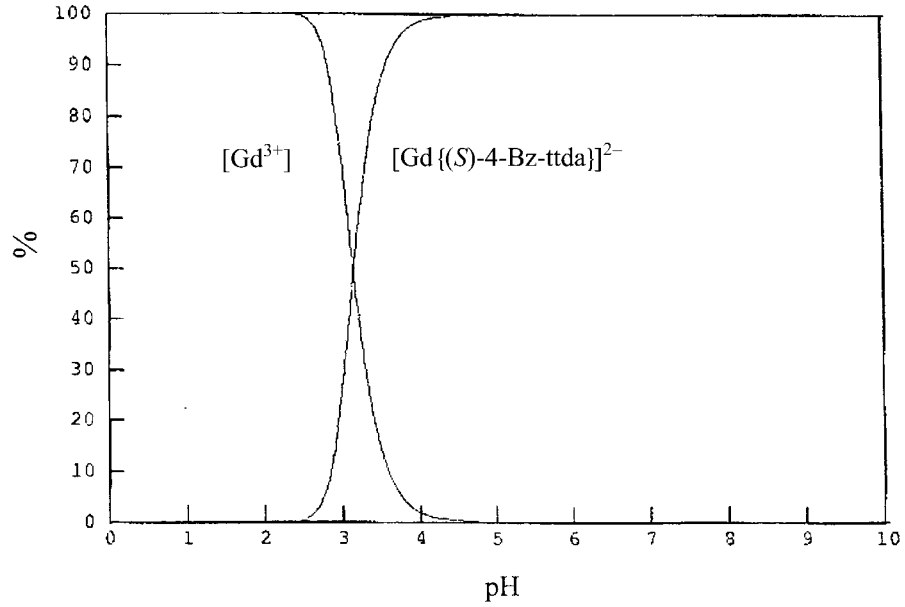


Fig. 3S. *Species distribution curves for the* $1.0 \cdot 10^{-3}$ M *[Gd{(*S*)-4-Bz-ttda}]*$^{2-}$ *system containing a 1:1 molar ratio of Gd37 to ligand.* $T = 25.0 \pm 0.1°$; $\mu = 0.10$M Me_4NNO_3; % = percent relative to $1.0 \cdot 10^{-3}$ M total [(*S*)-4-Bz-ttda]$^{5-}$ species (=100%).

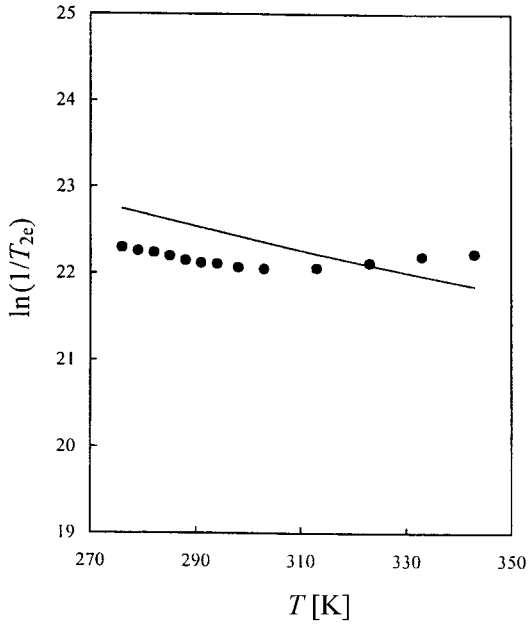


Fig. 4S. *Temperature dependence of transverse electronic relaxation rates at the X-band* (0.34 T) *and pH 7 for 50 m*M *[Gd(*S*)-4-Bz-ttda)(*H_2O*)]*$^{2-}$. The line represents the simultaneous least-squares fit to all data points as described in the text.

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