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Physicochemical Properties of the High-Field MRI-Relevant $[Gd(DTTA\text{-}Me)(H_2O)_2]^-$ Complex

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To study the physicochemical properties of the DTTA chelating moiety (H_4DTTA = diethylenetriaminetetraacetic acid = *N*,*N*′-[iminobis(ethane-2,1-diyl)]bis[*N*-(carboxymethyl)glycine]), used in several compounds proposed as magnetic resonance imaging (MRI) contrast agents, the methylated derivative H_4DTTA-Me (*N*,*N*′-[(methylimino)-bis(ethane-2,1-diyl)]bis[*N*-(carboxymethyl)glycine]) was synthesized. Protonation constants of the ligand were determined in an aqueous solution by potentimetry and ^{1}H NMR pH titration and compared to various DTTA derivatives. Stability constants were measured for the chelates formed with Gd^{3+} (log $K_{GdL} = 18.60 \pm 0.10$) and Zn^{2+} (log $K_{ZnL} = 17.69 \pm 0.10$). A novel approach of determining the relative conditional stability constant of two paramagnetic complexes in a direct way by ^{1}H NMR relaxometry is presented and was used for the Gd^{3+} complexes $[Gd(DTTA\text{-}Me)(H_2O)_2]^-$ (L_1) and $[Gd(DTPA\text{-}BMA)(H_2O)]$ (L_2) [K_{L_1/L_2}*(at pH 8.3, 25 °C) = 6.4 ± 0.3]. The transmetalation reaction of the Gd^{3+} complex with Zn^{2+} in a phosphate buffer solution (pH 7.0) was measured to be twice as fast for $[Gd(DTTA\text{-}Me)(H_2O)_2]^-$ in comparison to that for $[Gd(DTPA\text{-}BMA)(H_2O)]$. This can be rationalized by the higher affinity of Zn^{2+} toward $DTTA\text{-}Me^{4-}$ if compared to $DTPA\text{-}BMA^{3-}$. The formation of a ternary complex with L-lactate, which is common for DO3A-based heptadentate complexes, has not been observed for $[Gd(DTTA\text{-}Me)(H_2O)_2]^-$ as monitored by ^{1}H NMR relaxometric titrations. From the results, it was concluded that the heptadentate $DTTA\text{-}Me^{4-}$ behaves similarly to the commercial octadentate $DTPA\text{-}BMA^{3-}$ with respect to stability. The use of $[Gd(DTTA\text{-}Me)(H_2O)_2]^-$ as an MRI contrast agent in vitro and in animal studies is conceivable, mainly at high magnetic fields, where an increase of the inner-sphere-coordination water actually seems to be the most certain way to increase the relaxivity.

Introduction

The use of paramagnetic gadolinium chelates as contrast agents for medical magnetic resonance imaging (MRI) has increased considerably during the last 2 decades. A great effort has been spent in the same time period to develop new compounds giving better contrast.[1−3] The parameters that can be tuned in order to obtain more efficient agents, meaning to increase the water proton relaxivity, are the rotational tumbling time of the complex, the exchange rate of water molecules from the first coordination sphere of Gd^{3+}, and the number of water molecules in the first sphere. A stringent condition for all new chelating ligands is that the complex formed with the gadolinium ion is thermodynamically and kinetically very stable, and therefore all commercial MRI contrast agents have only one water molecule in the first coordination sphere. The safety of gadolinium-based contrast agents became an important issue because it has become the leading suspect for nephrogenic systemic fibrosis (NSF), a disease with high morbidity and mortality.[4−7] Besides their application in medical diagnostics, MRI contrast agents are also used in medical research and

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pharmacological studies. These studies, in general performed in vitro or in animals, depend on the development of new agents delivering good contrast at high magnetic fields. Restrictions concerning high complex stability are, however, often less severe than those in the case of clinical use.

A common approach to achieving high relaxivity is to load a rigid macromolecule like a dendrimer with many chelate units binding Gd^{3+}.[8−11] Relaxivities up to ∼30 mM^{-1} s^{-1} (at 20 MHz) have been achieved in this way. The relaxivity values are, however, strongly magnetic-field-dependent, and at fields of 3 T and above, the relaxivity drops rapidly even below those of small commercial contrast agents. In contrast to that field-dependent boost in efficiency, raising the number of inner-sphere water molecules leads to an increase in the relaxivity that is proportional to the number of water molecules independent of the magnetic field. Therefore, several research groups devoted their efforts to the synthesis of chelates allowing the presence of more than one water molecule in the first coordination sphere of the metal.[12−15]

Because the stability of the chelate complexes is a major concern, macrocyclic ligands based on the DO3A unit have often been chosen.[16−20] Gadolinium complexes of these ligands are thermodynamically and kinetically relatively stable[21] and have two inner-sphere water molecules. However, it has been found that these compounds can form ternary complexes with anionic metabolites present in serum.[18,20,22] Bidentate anions like lactate or carbonate bind directly to the paramagnetic center and replace the two inner-sphere water molecules. As a consequence, the relaxivity drops by about 60%, leading, on the one hand, to a loss in efficiency as a contrast agent but allowing, on the other hand, eventual monitoring in the presence of such anions.

Our group recently developed small to medium sized molecules binding two to six Gd^{3+} ions with heptadentate acyclic DTTA chelating groups (H_4DTTA = diethylenetriaminetetraacetic acid = *N*,*N*′-[iminobis(ethane-2,1-diyl)]bis[*N*-(carboxymethyl)glycine]).[23−27] In these complexes, each Gd^{3+} binds two water molecules that exchange relatively rapidly with the bulk, leading to moderately high relaxivities. Interestingly, relaxivities at magnetic fields of 3 T and above are still relatively high, leading to better contrast than that of commercial contrast agents.[25] Measurements of stability constants on complexes with $DTTA^{4-}$ showed that complex stability constants, K_{ML}, vary strongly depending on the group bound to the central nitrogen.[26]

In this paper, we present a study of complexes with DTTA-Me^{4-} (H_4DTTA-Me = *N*,*N*′-[(methylimino)bis(ethane-2,1-diyl)]bis[*N*-(carboxymethyl)glycine]), which mimics the DTTA chelating unit linked by a methylene group to organic backbones. Already in 1957, Schwarzenbach et al. presented protonation constants, K_i, and complex stability constants, K_{ML}, for some divalent ion complexes of the DTTA-Me^{4-} ligand.[28] Here we report the protonation and stability constants of DTTA-Me^{4-} and its Gd^{3+} (Na[Gd(DTTA-Me)$(H_2O)_2$] = sodium{[*N*,*N*′-[(methylimino-κ*N*)bis(ethane-2,1-diyl)]bis[*N*-(carboxy-κ*O*)methyl]glycinato-κ*N*,κ*O*]](4−)}gadolinite(1−)) and Zn^{2+} complexes, respectively, determined by potentiometry and ^{1}H NMR pH titrations. NMR relaxometry is used to determine the conditional relative stability constant, K_{L_1/L_2}*, of [Gd(DTTA-Me)$(H_2O)_2$]$^-$ with respect to the commercial contrast agent Omniscan [(Gd(DTPA-BMA)(H_2O)] to measure transmetalation toward endogenous Zn^{2+} ions and to assess a possible formation of ternary complexes with the bidentate ligand L-lactate.

Experimental Section

Synthesis of H_4DTTA-Me. All commercial reagents were used as received unless otherwise noted.

***N*,*N*′-[(Methylimino)bis(ethane-2,1-diyl)]bis[*N*-(carboxymethyl)glycine] Tetrakis(1,1-dimethylethyl) Ester.** *N*-Methyl-2,2′-diaminodiethylamine (1.0 g) was dissolved in 24 mL of dry *N*,*N*-dimethylformamide in the presence of 4.1 equiv of K_2CO_3 (4.84 g). A total of 4.1 equiv of *tert*-butyl bromoacetate (6.8 g) was added dropwise. The solution was stirred overnight at room temperature under an argon atmosphere. The solvent was evaporated, and water and dichloromethane (60 and 40 mL, respectively) were added to the residue. The crude product was extracted three times with dichloromethane (40 mL). The combined organic phases were dried with sodium sulfate, filtered, and evaporated. The product was

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purified by silica gel chromatography (eluant 1:3 ethyl acetate/hexane), and 1.3 g of *N*,*N*′-[(methylimino)bis(ethane-2,1-diyl)]-bis[*N*-(carboxymethyl)glycine] tetrakis(1,1-dimethylethyl) ester (yellow oil) was obtained (yield 27%). ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 1.45 (s, 36 H); 2.24 (s, 3 H); 2.52 (m, 4 H); 2.83 (m, 4 H); 3.45 (s, 8 H). MS (ESI): *m*/*z*: 574 [MH]$^+$.

***N*,*N*′-[(Methylimino)bis(ethane-2,1-diyl)]bis[*N*-(carboxymethyl)glycine].** The intermediate *N*,*N*′-[(methylimino)bis(ethane-2,1-diyl)]bis[*N*-(carboxymethyl)glycine] tetrakis(1,1-dimethylethyl) ester was deprotected by stirring overnight in 150 mL of boiling 6 M HCl. After evaporation, the crude product was washed three times in water by dissolution and evaporation and then purified by ion-exchange chromatography on a Bio-Rad AG 50W-8X resin eluted with a gradient of HCl (1−5 M) and then aqueous NH_3. The pure fractions were evaporated and washed three times with water; the resulting white solid *N*,*N*′-[(methylimino)bis(ethane-2,1-diyl)]bis[*N*-(carboxymethyl)glycine] was dried under vacuum (yield 50%). ^{1}H NMR (400 MHz, D_2O, δ in ppm at pH ∼2): 2.98 (s, 3 H); 3.45 (m, 4 H); 3.50 (m, 4 H); 3.88 (s, 8 H). MS (ESI): *m*/*z*: 350 [MH]$^+$. Elem anal. Calcd for H_4DTTA-Me(HCl)$_3$(H_2O)$_4$: C, 29.41; H, 6.21; N, 7.91. Found: C, 29.56; H,5.85; N, 7.65.

Protonation and Stability Constants by Potentiometric Measurements. Stock solutions of $GdCl_3$ (97.7 mM) and $ZnCl_2$ (89.5 mM) were prepared with double-distilled water and standardized by titration with a Na_2H_2EDTA solution in a urotropine buffer at pH 5.8 using xylenol orange as the indicator. A stock solution of the ligand H_4DTTA-Me (13.4 mM) was prepared and titrated with a Na_2H_2EDTA solution in a urotropin buffer at pH 5.8 using xylenol orange as the indicator in the presence of an excess of Gd^{3+}. The concentration of the ligand solution was confirmed by potentiometry on the basis of titration curves obtained in the absence and in the presence of a 50-fold excess of $CaCl_2$.

The protonation constants of the ligand DTTA-Me^{4-} and the stability constants of its complexes with Gd^{3+} and Zn^{2+} (C_L = 3 mM, C_M = 3 mM; I = 0.1 M KCl, titrated with 50 mM KOH) were determined by pH potentiometric titrations. The titrations were carried out using 3 mL sample volumes in a thermostatted glass-jacketed vessel (25 ± 0.2 °C) with a magnetic stirrer (under a dinitrogen atmosphere to avoid the effects of CO_2) and dosed with a Metrohm Dosimat 665 automatic burette. A combined glass electrode (C14/02-SC, reference electrode Ag/AgCl in 3 M KCl, Moeller Scientific Glass Instruments, Switzerland) connected to a Metrohm 692 pH/ion meter was used to measure the pH. The hydrogen ion concentration was calculated from the measured pH values by using a correction term, obtained as the difference between the measured and calculated pH values in a titration of HCl (0.1 M) with standardized KOH, as suggested by Irving et al.[29] The potentiometric data (about 160 points collected over the pH range 2−12) were refined with the *Hyperquad 2000* program.[30,31]

Deuteration Constants by ^{1}H NMR Measurements. An aqueous stock solution of H_4DTTA-Me (3 mM) was prepared in D_2O (99.8%), with I = 0.1 M NaCl. DCl or NaOD solutions were added to 5 mL of this solution, placed into a glass-jacketed vessel (25 ± 0.2 °C) with a magnetic stirrer (in a dinitrogen atmosphere to avoid the effects of CO_2). The pH was measured as described above. For NMR titration, ∼400 μL of the solution was placed into a 5 mm NMR tube and ^{1}H NMR spectra were recorded on a Bruker Avance-400 (25 ± 0.2 °C, measured by a substitution technique[32]). After the NMR measurement, the solution was poured back into the thermostatted vessel. The chemical shifts were recorded using *tert*-butyl alcohol as an internal standard (1.24 ppm). The chemical shift data (about 40 points collected over the pD range 2−12) were refined with the Visualizeur/Optimiseur programs running on a Matlab platform.[33]

Conditional Relative Stability Constant K_{L_1/L_2}* of [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ Relative to [Gd(DTPA-BMA)(H$_2$O)]. H_3DTPA-BMA was synthesized according to a procedure described by Geraldes et al.[34] Aqueous solutions of the complexes [Gd(DTTA-Me)(H_2O)$_2$]$^-$ (**sol L$_1$**) and [(Gd(DTPA-BMA)(H_2O)] (**sol L$_2$**), each containing a 3-fold excess of ligand, were prepared, and the pH set was to 8.4 (**sol L$_1$**, 4 mM $GdCl_3$ and 12 mM H_4DTTA-Me; **sol L$_2$**, 4 mM $GdCl_3$ and 12 mM H_3DTPA-BMA). Seven samples (m = 1−7) containing 1.40 mL of different amounts of **sol L$_1$** and **sol L$_2$** (0, 16, 33, 50, 67, 84, and 100% of **sol L$_2$**) were prepared in 2 mL flasks. Through the addition of ∼0.60 mL (exactly weighted) of water containing NaCl, final concentrations for each sample were C^{Gd} = 3 mM, $C_m^{L_1} + C_m^{L_2}$ = 9 mM, and I = 0.1 M NaCl. Measurements of the water proton longitudinal relaxation rates, $R_1 = 1/T_1$, were performed on a Bruker Minispec mq60 (60 MHz) at 25 ± 0.2 °C (measured by a substitution technique[32]). NMR tubes were left 15 min inside the thermostatted probe before measurement. Relaxivities and pHs of the samples were checked 2 weeks later to confirm that systems had reached equilibrium.

Transmetalation of [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ with Zn^{2+}. Transmetalation reactions with Zn^{2+} were studied as described by Laurent et al.[35] Equimolar amounts of $ZnCl_2$ were added to [Gd(DTTA-Me)(H_2O)$_2$]$^-$ and to [(Gd(DTPA-BMA)(H_2O)] solutions (2.5 mM each) containing a phosphate buffer (pH 7, [KH_2PO_4] = 26 mM, [Na_2HPO_4] = 41 mM). The samples were vigorously stirred and the water proton longitudinal relaxation rates measured on a Bruker Minispec mq60 (60 MHz, 37 °C). The measurements were carried out over 3 days using an automatic measurement routine.

Search for Ternary Adduct Formation between [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ and L-Lactate. The formation of ternary adducts can be studied by measuring the water proton relaxivity.[20] Solutions of 1 mM [Gd(DTTA-Me)(H_2O)$_2$]$^-$ containing increasing amounts of lactate (0, 0.24, 0.97, 1.49, 1.95, 7.57, 12.2, 20.1, 30.3, 40.1, and 50.0 mM) were prepared at pH 7.0. Measurements of the water proton longitudinal relaxation rates, R_1, were performed with a Stelar fast-field cycling relaxometer at 10 MHz and 25 ± 0.2 °C (measured by a substitution technique[32]).

Results and Discussion

Potentiometry. Protonation constants of DTTA-Me^{4-} were determined by potentiometry (Figure 1). From literature

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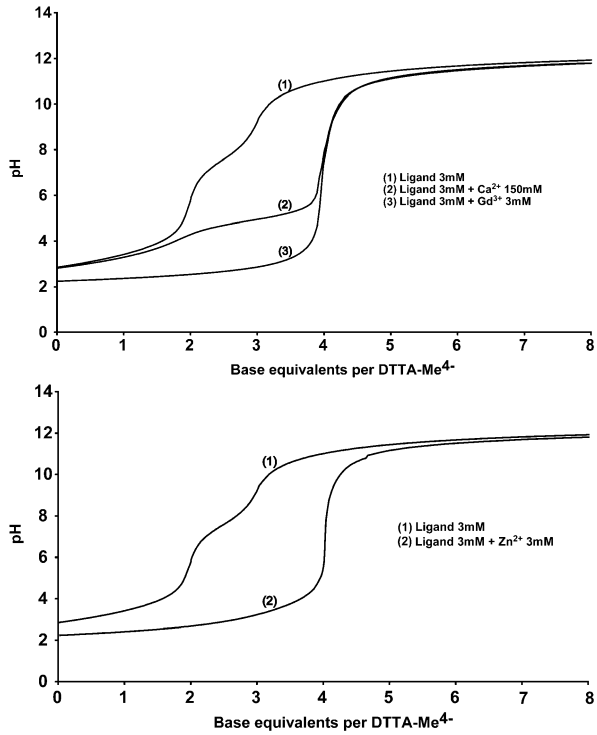


Figure 1. Titration curves at 25 °C and $I = 0.1$ M KCl of the DTTA-Me^{4-} ligand and its Ca^{2+}, Zn^{2+}, and Gd^{3+} complexes.

data on acyclic poly(aminocarboxylate) molecules, four protonation constants can be expected over the pH range 2−12 for DTTA-Me^{4-}.[23,26] The stepwise protonation constants K_i (eq 1) were obtained by fitting of the potentiometric data (Table 1).

$$K_i = \frac{[H_iL]}{[H_{i-1}L][H^+]} \quad \text{with } i > 0 \tag{1}$$

An analysis of the potentiometric data (Figure 1) using five protonation constants has been performed because of a significant improvement of the quality of fit. The protonation constant for the first protonation step, K_1, of DTTA-Me^{4-} is similar to that of $DTPA^{4-}$ and $TTAHA^{6-}$. However, the ligand tpy-$DTTA^{4-}$ (Scheme 1) has a K_1, which is about 2 orders of magnitude lower.[23,24] These results suggest that for all of these ligands the first protonation occurs on the central nitrogen. The loss of basicity of the central nitrogen for tpy-$DTTA^{4-}$ with respect to DTTA-Me^{4-}, $DTPA^{4-}$, and $TTAHA^{6-}$ is a consequence of the direct binding of the terpyridine moiety to the central nitrogen instead of a methylene group. Costa et al.[23,24] investigated ligands where two DTTA chelators are linked in para and meta positions to a xylene core via a methylene group. The first protonation constants for $pX(DTTA)_2^{8-}$ and $mX(DTTA)_2^{8-}$ (Scheme 1) are intermediate between the values of $TTAHA^{6-}$, $DTPA^{5-}$, and DTTA-Me^{4-} and that of tpy-$DTTA^{4-}$. In this case, the methylene group is intercalated between the aromatic ring and poly(aminocarboxylate) and quenches partially inductive or mesomeric effects, affecting the protonation constants of the DTTA entities. The decrease from log K_4 to log K_5 is larger for DTTA-Me^{4-} than expected by a statistical approach (0.3). The protonation constant log K_6 for DTTA-Me^{4-} could not be determined because it occurs below pH 2.

^{1}H NMR in D_2O. ^{1}H NMR chemical shifts of DTTA-Me^{4-} were assigned on the basis of signal multiplicities. Samples were prepared in D_2O, and pD values have been obtained from the equation $pD = pH_{apparent} + 0.44$,[41] where $pH_{apparent}$ is the measured pH of the sample. The pD dependences of the chemical shifts, $\delta_X^{obs}(D^+)$, of the doublets d1 and d2 and the singlets s1 and s2 (Scheme 2) due to successive deuteration of DTTA-Me^{4-} can be expressed as in eq 2:

$$\delta_X^{obs}(D^+) = \sum_{i=0}^{5} P_i \delta_X^{D_iL^{i-4}} \tag{2}$$

where $\delta_X{}^{D_iL^{i-4}}$ are the intrinsic chemical shifts of protons X (X = s1, s2, d1, or d2) of the D_iL^{i-4} species ($i = 0-5$) and P_i are the fractional populations of species L^{4-}, DL^{3-}, D_2L^{2-}, D_3L^-, D_4L, and D_5L^+, respectively:

$$P_i(D^+) = \frac{[D_iL^{i-4}]}{L_{Total}} = \beta_i^D P_0 [D^+]^i \quad \text{with } \beta_i^D = \frac{[D_iL^{i-4}]}{[L^{4-}][D^+]^i} \tag{3}$$

L_{Total} is the total concentration of the ligand L, β_i^D are the cumulative deuteration constants, $[D^+]$ is the deuteron concentration in mol/L calculated as 10^{-pD}, and P_0, the fractional population of species L^{4-}, is given by eq 4:

$$P_0 = \frac{1}{1 + \sum_{i=1}^{5} \beta_i^D [D^+]^i} \tag{4}$$

The stepwise deuteration constants K_i^D are expressed following eq 5:

$$K_i^D = \frac{\beta_i^D}{\beta_{i-1}^D} \tag{5}$$

The log K_i^D values of DTTA-Me^{4-} in D_2O were calculated from least-squares refinement of the chemical shifts measured as a function of pD (Figure 2). The deuteration constants found, log $K_1^D = 11.40$, log $K_2^D = 8.10$, log $K_3^D = 4.10$, log $K_4^D = 3.54$, and log $K_5^D = 2.53$, are higher than those obtained in H_2O by potentiometry because the deuteron interacts more strongly with oxygen and nitrogen atoms than the proton does. Corrected log K_i^H values were calculated from log K_i^D according to log $K_i^D = 0.32 + 1.044$ log K_i^H:[42,43] log $K_1^H = 10.61$, log $K_2^H = 7.45$, log $K_3^H = 3.62$, log $K_4^H = 3.09$, and log $K_5^H = 2.12$. Taking into account that $pH_{apparent}$ had to be converted to pD and that an empirical correction has been used to calculate log K_i^H, the protonation constants as obtained from the deuteration constants are in good accordance with potentiometric values (Table 1).

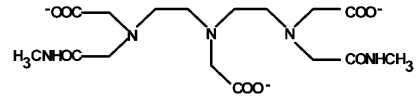
(41) Mikkelsen, K.; Nielsen, S. O. *J. Phys. Chem.* **1960**, *64*, 632–637.
(42) Perrin, D. D.; Dempsey, B. *Buffers for pH and Metal Ions Control*; Chapman and Hall: London, 1974.
(43) Pierre, V. C.; Melchior, M.; Doble, D. M. J.; Raymond, K. N. *Inorg. Chem.* **2004**, *43*, 8520–8525.

Table 1. Thermodynamic Protonation and Stability Constants for Various Ligands and Their Gd^{3+} and Zn^{2+} Complexes (T = 25 °C; I = 0.1 M)

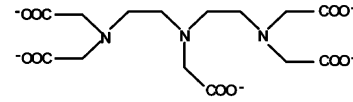
			p,mX(DTTA)$_2^{8-c}$						
	DTTA-Me^{4-a}	bpy(DTTA)$_2^{8-b}$	para	meta	tpy-DTTA^{4-d}	TTAHA^{6-e}	DTPA^{5-f}	EDTA^{4-}	DTPA-BMA^{3-g}
log K_1	10.75(0.03)[h]	9.87[i]	9.84	9.45	8.65	10.66	10.41	10.08[j]	9.37
log K_2	7.56(0.03)[h]	9.16[i]	8.80	8.12	7.63	8.56	8.37	6.42[j]	4.38
log K_3	3.76(0.05)[h]	3.09[i]	3.52	3.97	5.25	8.38	4.09	3.11[j]	3.31
log K_4	2.74(0.05)[h]	1.5[i]	2.40	2.70	3.30	2.92	2.51	2.33[j]	1.43
log K_5	1.90(0.15)					2.39	2.04		
log K_6						2.0			
log K_{GdL}	18.60(0.10)	18.2	19.1	17.0	10.87	19.0	22.5	17.7[j]	16.85
log K_{GdHL}	2.12(0.24)		2.1	3.2	3.73	8.3	1.8		
log K_{ZnL}	17.69(0.10)	18.0	17.94	16.19		18.91	18.29	16.4[k]	12.04
log K_{ZnHL}	3.73(0.10)	3.4	3.76	4.24		8.01	5.6		4.04
log K_{ZnH2L}						3.68			
pGd[l]	15.8(0.1)	14.9	16.2	15.1	10.6	15.5	19.2	15.9	15.8

[a] I = 0.1 M KCl; this work; numbers in parentheses correspond to 2 times the standard deviation. [b] I = 0.1 M $(CH_3)_4NCl$; from ref 26. [c] I = 0.1 M $(CH_3)_4NCl$; from ref 23. [d] I = 0.1 M KCl; from ref 24. [e] I = 0.1 M KCl; from ref 36. [f] I = 0.1 M KCl; from ref 37. [g] I = 0.1 M KCl; from ref 38. [h] From ref 28 at T = 20 °C, I = 0.1 M KCl: log K_1 = 10.89, log K_2 = 7.39, log K_3 = 3.65, log K_4 = 2.8. [i] Protonation constants of the poly(aminocarboxylate) moiety. [j] I = 0.1 M KCl; from ref 39. [k] From ref 40. [l] pM values of GdL complexes under physiologically relevant conditions (pH 7.4; $[Gd]_{total}$ = 1 μM; $[L]_{total}$ = 10 μM) (see the text).

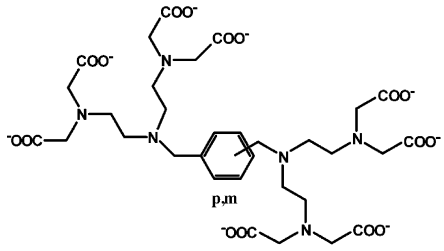
Scheme 1



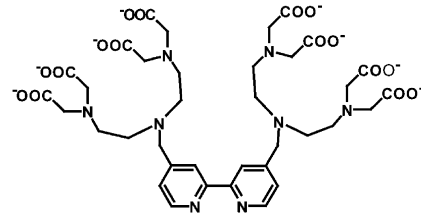
DTPA-BMA^{3-}



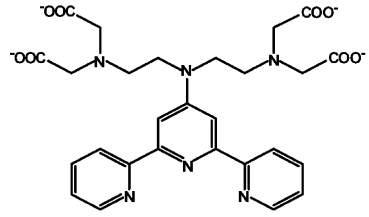
DTPA^{5-}



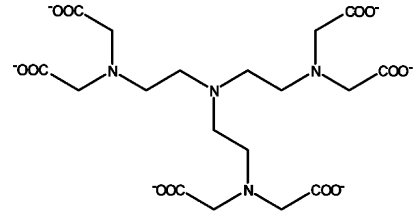
pX(DTTA)$_2^{8-}$ and mX(DTTA)$_2^{8-}$



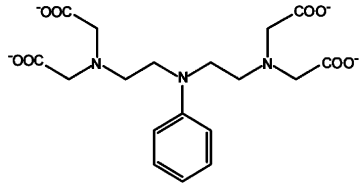
bpy(DTTA)$_2^{8-}$



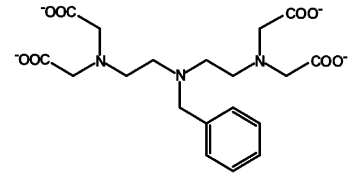
tpy-DTTA^{4-}



TTAHA^{6-}



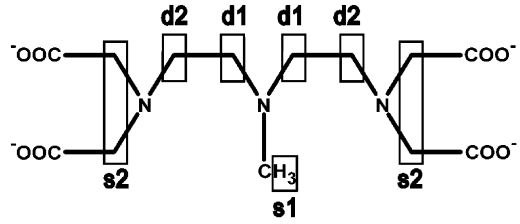
BEATA^{4-}



BEBTA^{4-}

The microscopic titration scheme obtained by ^{1}H NMR spectroscopy is indicative of the deuteration sites. The deuteration of a basic site leads to a deshielding of adjacent protons. Following Figure 2, by lowering pD, the protons d1 and s1 become first strongly deshielded while d2 and s2 are much less affected. It is, therefore, concluded that the first deuteration step, described by K_1, occurs mainly on the central nitrogen. Between pD 9.5 and ~6, protons d1 and s1 become again more shielded and protons d2 and s2 become deshielded. In the second deuteration step, both

Scheme 2



terminal nitrogen atoms are deuterated and the deuterium on the central nitrogen is released. The bisdeuterated ligand undergoes stabilization by the formation of five-membered rings due to hydrogen-bond formation between the deuteron on the terminal nitrogen and an oxygen atom of the carboxylate group, lowering strongly the log K_5^D value. A similar behavior has been already found by ^{1}H NMR titration on DTPA^{5-} and EPTPA.[37,44,45] The three subsequent log K_i^D values, corresponding to a repeated deuteration of the central nitrogen (log K_3^D) and the deuteration of the first and second carboxylate (log K_4^D and log K_5^D), are confirmed by the chemical shift changes of the d1/s1 and d2/s2 protons, respectively.

Stability Constants by Potentiometry. The thermodynamic stability of a metal M with charge *m* complexed to a chelate L with charge *n* is given by the stability constant K_{ML} (eq 6):

$$K_{ML} = \frac{[ML^{m+n}]}{[M^m][L^n]} \quad (6)$$

$[M^m]$, $[L^n]$, and $[ML^{m+n}]$ are the equilibrium concentrations of the metal ion, the deprotonated ligand, and the complex, respectively. At low pH, protonation of the complexes occurs, which is characterized by the complex protonation constants K_{MH_iL} (eq 7):

$$K_{MH_iL} = \frac{[MH_iL^{m+n+i}]}{[MH_{i-1}L^{m+n+i-1}][H^+]} \quad \text{for } i = 1, 2,... \quad (7)$$

The stability constants K_{ML} and K_{MHL} of the complexes $[M^m(DTTA\text{-}Me)]^{m-4}$ ($M^m = Gd^{3+}$, Zn^{2+}) were obtained by fitting potentiometric data measured over the pH range 2−12 (Figure 1). The thermodynamic stability of $[Gd(DTTA\text{-}Me)(H_2O)_2]^-$ (Table 1) is comparable to those of $[Gd(TTAHA)(H_2O)_2]^{3-}$, $[Gd_2(pX(DTTA)_2)(H_2O)_4]^{4-}$, and $[Gd_2(mX(DTTA)_2)(H_2O)_4]^{4-}$. All of these compounds bind the metal via a heptadentate DTTA^{4-} unit with a methylene group on the central nitrogen. Replacing this nonionic methylene group by a metal binding acetate group results in an increase of the stability constant by 3−4 orders of magnitude, as was found for the octadentate DTPA^{5-} (Table 1). Replacing the methylene group on the central nitrogen by a tpy group leads, however, to a decrease in K_{GdL} of nearly 8 orders of magnitude. Tse and Powell observed a much smaller decrease of log K_{GdL} from 17.50 to 15.42 on going from BEBTA^{4-} to BEATA^{4-} (Scheme 1), where an electron-withdrawing phenyl group is replaced by an electron-donating benzyl.[46]

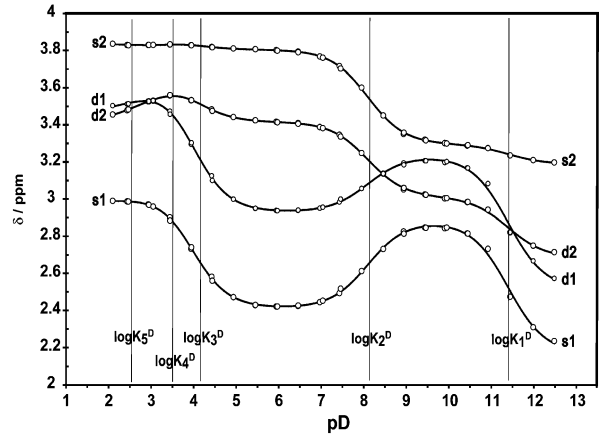


Figure 2. ^{1}H NMR (400 MHz) DTTA-Me^{4-} chemical shifts versus pD in D_2O at room temperature and $I = 0.1$ M KCl. Lines were calculated using the fit parameters from eqs 2−6 describing the first five deuteration steps of DTTA-Me^{4-}.

The pGd value (eq 8), usually calculated for pH 7.4, $[Gd]_{total} = [ML] = 1\ \mu M$, and $[L]_{total} = 10\ \mu M$,[47] expresses the influence of the ligand H_iL basicity and the protonation of the complex. A higher pGd value means higher complex stability under these conditions, with α_L (eq 9) being the inverse of the $[L^{4-}]$ population fraction for an H_5L ligand.

$$pGd = -\log [Gd^{3+}]_{free} = -\log \frac{[ML]}{K_{ML}[L^{4-}]} = -\log \frac{[Gd]_{total}\alpha_L}{K_{ML}([L]_{total} - [Gd]_{total})} \quad (8)$$

$$\alpha_L = \frac{[L]_{total}}{[L^{4-}]} = 1 + \sum_{i=1}^{5} \beta_i [H^+]^i \quad (9)$$

Table 1 shows pGd values for several poly(aminocarboxylate) Gd^{3+} complexes. The pGd of $[Gd(DTTA\text{-}Me)(H_2O)_2]^-$ (pGd = 15.8) is much smaller than those of DTPA and DOTA (pGd = 19.2) complexes, but it is about the same as those of the DTPA-BMA and EDTA complexes.

Conditional Relative Stability Constant K_{L_1/L_2}* of $[Gd(DTTA\text{-}Me)(H_2O)_2]^-$ Relative to $[Gd(DTPA\text{-}BMA)(H_2O)]$. The conditional relative stability constant K_{L_1/L_2}* (eq 10) is defined by the ratio between the two conditional stability constants K_{GdL_1}* and K_{GdL_2}* of the Gd^{3+} complexes $[Gd(DTTA\text{-}Me)(H_2O)_2]^-$ and $[Gd(DTPA\text{-}BMA)(H_2O)]$, respectively. These two constants, depending on the pH, are equal to their corresponding thermodynamic stability constants K_{GdL_1} and K_{GdL_2} divided by the inverse of their nonprotonated ligand fractions α_{L_1} and α_{L_2}, respectively (eq 10).

(44) Tóth, É.; Pubanz, D.; Vauthey, S.; Helm, L.; Merbach, A. E. *Chem.—Eur. J.* **1996**, *2*, 1607–1615.
(45) Wang, Y.-M.; Lee, C.-H.; Liu, G.-C.; Sheu, R.-S. *J. Chem. Soc., Dalton Trans.* **1998**, 4113–4118.
(46) Tse, P.-K.; Powell, J. E. *Inorg. Chem.* **1985**, *24*, 2727–2730.
(47) Paul-Roth, C.; Raymond, K. N. *Inorg. Chem.* **1995**, *34*, 1408–1412.

$$K_{L_1/L_2}{}^* = \frac{K_{GdL_1}{}^*}{K_{GdL_2}{}^*} = \frac{\alpha_{L_2} K_{GdL_1}}{\alpha_{L_1} K_{GdL_2}} = \frac{\alpha_{L_2}}{\alpha_{L_1}} K_{L_1/L_2}$$

$$\text{with } \alpha_{L_1} = 1 + \sum_{i=1}^{5} \beta_i(L_1)\,[H^+]^i$$

$$\text{and} \quad \alpha_{L_2} = 1 + \sum_{i=1}^{4} \beta_i(L_2)\,[H^+]^i \quad (10)$$

The conditional relative stability constant $K_{L_1/L_2}{}^*$ of gadolinium complexes can be measured via ^{1}H NMR relaxivity measurements provided the relaxivities of the two complexes, $r_1^{GdL_1}$ and $r_1^{GdL_2}$, are sufficiently different. The relaxivity of a paramagnetic compound in an aqueous solution is commonly expressed by eq 11:[48]

$$r_1 = \left(\frac{1}{T_1} - \frac{1}{T_{1,H_2O}}\right)\frac{1}{c_{Gd^{3+}}} \quad [s^{-1}\,mM^{-1}] \qquad (11)$$

$1/T_1$ and $1/T_{1,H_2}O$ are the measured longitudinal relaxation rates of water protons of solutions with and without the paramagnetic ion, respectively. The relaxivity, r_1^m, of a sample composed of a mixture of **sol L$_1$** and **sol L$_2$** (see the Experimental Section) is given by the relative concentrations [GdL$_1$] and [GdL$_2$] at equilibrium with respect to the total concentration of gadolinium in the mixture, C_m^{Gd} (eq 12):

$$r_1^m = r_1^{GdL_1}\left(1 - \frac{[GdL_2]}{C_m^{Gd}}\right) + r_1^{GdL_2}\left(\frac{[GdL_2]}{C_m^{Gd}}\right) \qquad (12)$$

If the total concentrations of the two ligands in the mixture, $C_m^{L_1}$ and $C_m^{L_2}$, are known, the conditional relative stability constant $K_{L_1/L_2}{}^*$ can be calculated with eq 13 (see the Supporting Information):

$$K_{L_1/L_2}{}^* = \frac{\left(\frac{r_1^m - r_1^{GdL_2}}{r_1^{GdL_1} - r_1^{GdL_2}}\right)\left(\frac{C_m^{L_2}}{C_m^{Gd}} - 1 + \left(\frac{r_1^m - r_1^{GdL_2}}{r_1^{GdL_1} - r_1^{GdL_2}}\right)\right)}{\left(1 - \left(\frac{r_1^m - r_1^{GdL_2}}{r_1^{GdL_1} - r_1^{GdL_2}}\right)\right)\left(\frac{C_m^{L_1}}{C_m^{Gd}} - \left(\frac{r_1^m - r_1^{GdL_2}}{r_1^{GdL_1} - r_1^{GdL_2}}\right)\right)} \qquad (13)$$

From the compositions of the individual samples, we can calculate relaxivities r_1^m, using $K_{L_1/L_2}{}^*$, $r_1^{GdL_1}$, and $r_1^{GdL_2}$ as parameters (see the Supporting Information). Experimental data can be fitted by this function, yielding the conditional relative stability constant $K_{L_1/L_2}{}^* = 6.4 \pm 0.3$ at pH 8.3 and 25 °C (Figure 3). At that pH, both ligands exist mainly in the monoprotonated form in solution and the [H$^+$] concentration is not altered by the establishment of the equilibrium as defined in eq 14.

$$[Gd(DTPA\text{-}BMA)(H_2O)] + HDTTA\text{-}Me^{3-} \rightleftharpoons^{K_{L_1/L_2}{}^*} [Gd(DTTA\text{-}Me)(H_2O)_2]^- + HDTPA\text{-}BMA^{2-} \quad (14)$$

The thermodynamic relative stability constant K_{L_1/L_2} (eq 10), which is independent from the pH, can be calculated using $\alpha_{L_1} = 332.4$ and $\alpha_{L_2} = 12.7$ (calculated from values in Table 1 and sample compositions) as $K_{L_1/L_2} = 168$. The conditional relative stability constant $K_{L_1/L_2}{}^*$ obtained from the competition experiment by relaxometry can be compared to the values calculated from the stability constants from several potentiometric experiments, log $K_{Gd\text{-}DTTA\text{-}Me} = 18.60$ (this work, Table 1) and log $K_{Gd\text{-}DTPA\text{-}BMA} = 16.85$, reported by Rizkalla et al.[49] Both the directly measured and the calculated $K_{L_1/L_2}{}^*$ show a higher relative thermodynamic stability of [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ compared to [Gd(DTPA-BMA)(H$_2$O)] (Table 2) at pH 8.3. The conditional relative stability constant log $K_{L_1/L_2}{}^*$ as a function of the pH

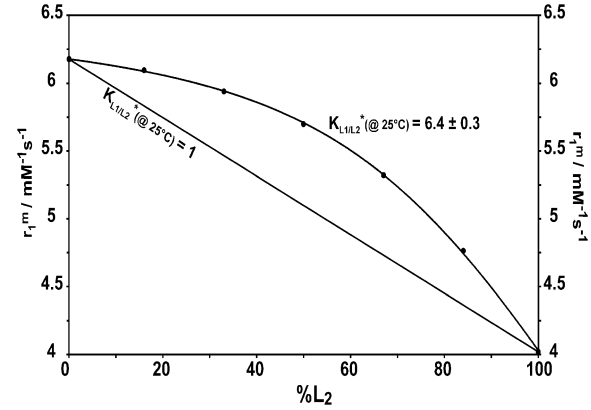


Figure 3. Relaxivities r_1^m of samples containing different concentrations of DTPA-BMA (% L$_2$). The conditional stability constant $K_{L_1/L_2}{}^*$ is calculated from a fit.

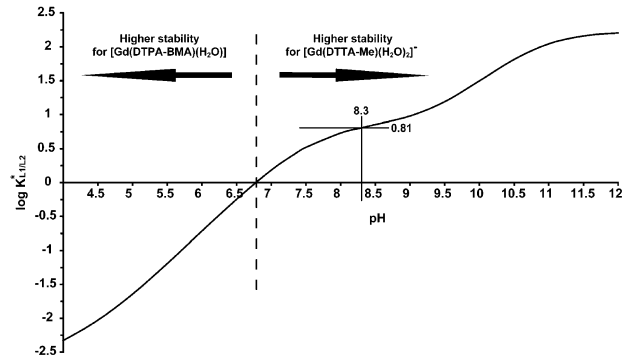


Figure 4. Conditional relative stability constant log $K_{L_1/L_2}{}^*$ as a function of the pH.

Table 2. Conditional, $K_{L_1/L_2}{}^*$, and Thermodynamic, K_{L_1/L_2}, Relative Stability Constants (T = 25 °C, I = 0.1 M) for [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ (GdL$_1$) and [Gd(DTPA-BMA)(H$_2$O)] (GdL$_2$) from Relaxivity and Potentiometric Experiments

	relaxivity[a]	potentiometry[b]
log $K_{L_1/L_2}{}^*$, pH 8.3	0.81 ± 0.02[c]	0.33
log $K_{L_1/L_2}{}^*$, pH 7.4	0.47	−0.01
log K_{L_1/L_2}	2.23	1.75[d]

[a] I = 0.1 M NaCl. [b] I = 0.1 M KCl; logK_{ML} for [(Gd(DTPA-BMA)(H$_2$O)] from ref 38. [c] Measured directly. [d] Calculated from log K_{GdL_1} and log K_{GdL_2}.

(48) Tóth, É.; Helm, L.; Merbach, A. E. Relaxivity of Gadolinium(III) Complexes: Theory and Mechanism. In *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, 1st ed.; Merbach, A. E., Tóth, É., Eds.; John Wiley & Sons: Chichester, U.K., 2001; pp 45−119.

(49) Rizkalla, E. N.; Choppin, G. R.; Cacheris, W. *Inorg. Chem.* **1993**, *32*, 582–586.

is shown in Figure 4. At pH above 6.8, [Gd(DTTA-Me)$(H_2O)_2$]$^-$ is more stable than [Gd(DTPA-BMA)(H_2O)]. Especially at physiological pH 7.4, the gadolinium complex with the heptadentate DTTA-Me^{4-} is about 3 times more stable than the one with the octadentate DTPA-BMA^{3-}, as calculated from the directly measured K_{L_1/L_2}*.

Relative stability constants of very stable complexes are often measured by competition experiments. In the case of complexes of lanthanide cations, the methods mainly used are spectrophotometric competition titration using colored ligands[50,51] or potentiometric competition experiments using, for example, EDTA.[52,53] The measurement of ^{1}H NMR relaxivities at one magnetic field in mixed solutions of gadolinium complexes is a simple method to establishing the relative stabilities of gadolinium complexes. The only necessary condition is that the relaxivities of the two individual complexes have to be sufficiently different with respect to experimental uncertainty. By a proper choice of the magnetic field, such a difference in the relaxivity can always be found and therefore the relative stability constants can be measured directly for any couple of gadolinium complexes. The standard deviation of 5% on K_{L_1/L_2}* obtained in our case is much lower than what can typically be obtained by potentiometric competition experiments.

Transmetalation of [Gd(DTTA-Me)$(H_2O)_2$]$^-$ with Zn^{2+}. Zn^{2+} is one of the most abundant endogenous metal ions with a concentration of ~32 μM in the human plasma.[54] To determine the stability of the [Gd(DTTA-Me)$(H_2O)_2$]$^-$ complex against the presence of Zn^{2+} ions, a transmetalation experiment was carried out in a phosphate buffer (pH 7.0).[35] Replacement of Gd^{3+} in the complex with Zn^{2+} leads to free gadolinium ions, which precipitate in the presence of phosphate as $GdPO_4$. Because Zn^{2+} is diamagnetic, the total amount of paramagnetic species in solution decreases and therefore the measured decrease in relaxation allows one to follow the transmetalation reaction.

In Figure 5, the evolution with time of the ratio of relaxation rates $R_1^m/R_{1,0}$, where R_1^m is the relaxation rate at time t and $R_{1,0}$ is the relaxation rate at time zero (just before the successive addition of phosphate and Zn^{2+}), is shown for [Gd(DTTA-Me)$(H_2O)_2$]$^-$, together with the evolution of [(Gd-DTPA-BMA)(H_2O)] as a comparison. After a small initial increase (Figure 5, inset), the relative relaxation rates of both complexes decrease because of the transmetalation reaction. According to Laurent et al.,[35,55] two characteristic values can be used to describe the behavior of a Gd^{3+} chelate in a transmetalation experiment, i.e., the time to reach $R_1^m/R_{1,0} = 0.8$ (ratio index), which gives information about the kinetics of the reaction, and the $R_1^m/R_{1,0}$ value at very long time, t = ∞ (long time index, considered after 3 days = 4320 min), reflecting the thermodynamic aspect of the system. From the values in Table 3, it can be concluded that transmetalation is twice as fast for [Gd(DTTA-Me)$(H_2O)_2$]$^-$ compared to [(Gd-DTPA-BMA)(H_2O)]. In any case, both are much less stable with respect to transmetalation than the complexes with cyclic ligands like DOTA^{4-} or HPDO3A^{3-}, which are especially kinetically stable.[35,56] The lower relative thermodynamic stability of [Gd(DTTA-Me)$(H_2O)_2$]$^-$ against transmetalation with Zn^{2+} as compared to that of [(Gd-(DTPA-BMA)(H_2O)] can be understood by comparing the stability constants K_{ML} for Gd^{3+} and Zn^{2+} (Table 1). Even if [Gd(DTTA-Me)$(H_2O)_2$]$^-$ has a higher stability than [(Gd(DTPA-BMA)(H_2O)], the relative stability with respect to Zn^{2+} is lower as a consequences of the high stability of the zinc complex of DTTA-Me^{4-}: $\log(K_{Gd\text{-}DTTA\text{-}Me}/K_{Zn\text{-}DTTA\text{-}Me}) = 0.91$ with respect to $\log(K_{Gd\text{-}DTPA\text{-}BMA}/K_{Zn\text{-}DTPA\text{-}BMA}) = 4.81$.[38]

To confirm that the presence of Zn^{2+} ions is provoking transmetalation, we performed a blank experiment (Figure 5). A sample containing the [Gd(DTTA-Me)$(H_2O)_2$]$^-$ complex only with a phosphate buffer but without Zn^{2+} ions shows no change in relaxivity over more than 3 days, confirming that phosphate at the considered concentration and pH is unable to extract Gd^{3+} from the chelate.

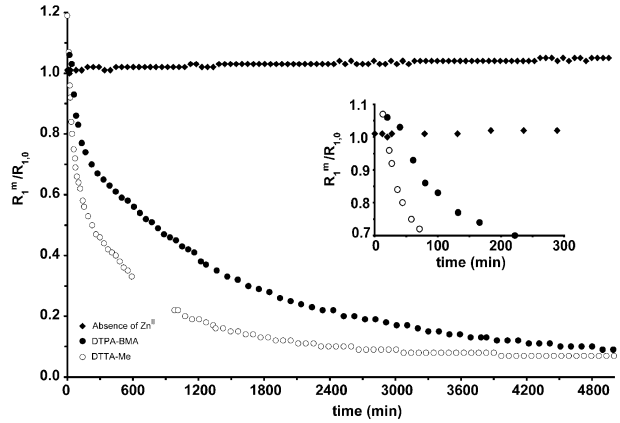


Figure 5. Evolution of $R_1^m/R_{1,0}$ (T = 310 K; B = 1.41 T, 60 MHz; pH 7.0) versus time for (○) [Gd(DTTA-Me)$(H_2O)_2$]− and (●) [Gd(DTPA-BMA)(H_2O)] complexes in the presence of equimolar amounts of Zn^{2+} ions in a phosphate buffer solution. Evolution of (◆) [Gd(DTTA-Me)$(H_2O)_2$]$^-$ in a phosphate buffer solution without Zn^{2+} ions.

Table 3. Transmetalation with Zn^{2+}: Time Required To Reach the Ratio $R_1^m(t)/R_{1,0} = 0.8$ (Ratio Index[55]) and Value of $R_1^m(t{=}4320)/R_{1,0}$ (Long Time Index[55]) for [Gd(DTTA-Me)$(H_2O)_2$]$^-$ and [Gd(DTPA-BMA)(H_2O)] (37 °C, pH 7.0)

	t for $R_1^m(t)/R_{1,0} = 0.8$ [min]	$R_1^m(t = 4320)/R_{1,0}$	
[Gd(DTTA-Me)$(H_2O)_2$]$^-$	44	0.07	this work
[Gd(DTPA-BMA)(H_2O)]	100/100	0.11/0.10	this work and ref 55
[Gd(DTPA)(H_2O)]$^{2-}$	250	0.49	55
[Gd(DOTA)(H_2O)]$^-$	>5000	0.99	55
[Gd(HPDO3A)(H_2O)]$^-$	>5000	0.99	55

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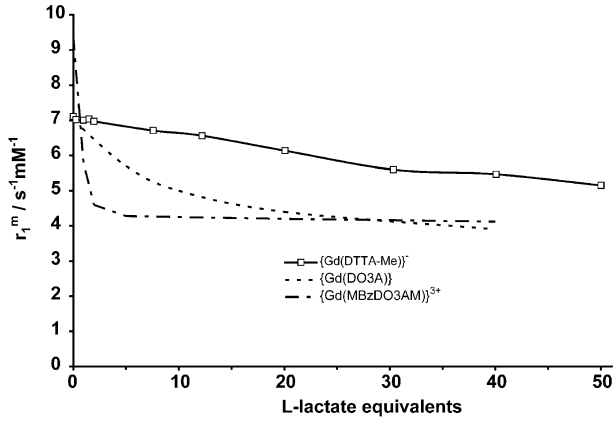

Figure 6. Relaxivity r_1^p ($T = 298$ K; $B = 0.235$ T, 10 MHz; pH 7.0) versus L-lactate equivalents for 1 mM solutions of [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ (□, this work) and [Gd(DO3A)(H$_2$O)$_2$] (- - -) and [Gd(MBzDO3AM)(H$_2$O)$_2$]$^{3-}$ (− · −) from ref 20.

Search for Adduct Formation between [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ and L-lactate. It has been shown by NMR[18,20] and by luminescence emission studies[57,58] that lanthanide complexes with heptadentate DO3A-based ligands form ternary adducts with bidentate anions like carbonate, acetate, phosphate, malonate, and lactate. The anions replace the two water molecules from the first coordination sphere of the lanthanide, leading in the case of gadolinium complexes to a substantial decrease in relaxivity. Like DO3A-based ligands, DTTA-Me^{4-} is heptadentate and its Gd^{3+} complex has also two water molecules in the first coordination sphere. Therefore, it could be prone to bind bidentate anions. We studied the eventual complex formation of [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ with L-lactate anions, which are present in human blood at ∼2 mM; concentrations up to 25 mM can be found under stress or shock.[59,60] Following Terreno et al.,[20] we used ^{1}H NMR relaxivity, r_1, to detect the replacement of first-sphere water molecules by a lactate anion.

The change in relaxivity upon titration of [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ with L-lactate is shown in Figure 6. The addition of 10 equiv of lactate to a 1 mM solution of [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ leads only to a slight decrease in r_1 of 6.5%. This decrease is much smaller than that observed on complexes based on cyclic DO3A derivatives (Figure 5). Even with 50 equiv of lactate, relaxivity decreases only by about 2 s^{-1} mM^{-1}. Possible explanations for the small decrease of r_1 are (1) the formation of a small amount of the ternary adduct [Gd(DTTA-Me)(C$_3$O$_3$H$_5$)]$^{2-}$, (2) the replacement of only one water molecule on some of the Gd^{3+} complexes, and (3) the formation of an outer-sphere complex, leading to a decrease of outer-sphere relaxation.

The difference in the formation of ternary complexes with lactate between compounds with cyclic ligands (DO3A-based) and the acyclic ligand (DTTA-Me^{4-}) is probably due to the arrangement of the two first-shell water molecules. In DO3A-type complexes, these water molecules are adjacent, allowing a bidentate binding of the anion. In the [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ complex, the two water molecules are probably not next to each other in the coordination polyhedron and the lactate could only bind via one oxygen to the gadolinium. No effect on relaxivity, by the way, has been observed in the presence of a 10-fold excess of phosphate in solution (see Figure 5), confirming the absence of the formation of ternary complexes for this anion. Furthermore, in contrast to DO3A complexes of gadolinium, the [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ complex is negatively charged and the formation of ternary adducts or outer-sphere complexes with anions is less favorable.

Conclusion

We report the synthesis and physicochemical characterization of the gadolinium complex with DTTA-Me^{4-}, derived from the parent TTAHA^{6-}. As other DTTA-type complexes, used, for example, in the metallostar,[26] [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ has two water molecules directly bound to the paramagnetic center. The complex shows thermodynamic protonation and stability constants similar to DTTA bound to bpy or a xylene via a CH$_2$ group. From ^{1}H NMR, it was concluded that the first protonation occurs on the central nitrogen, followed by protonation of the two terminal nitrogen atoms and deprotonation of the central one. The next protonation steps are a repeated protonation of the central nitrogen followed by the protonation of carboxylic acids. The pGd value, which is indicative of the stability at physiological conditions, is very close to that of [Gd(DTPA-BMA)(H$_2$O)].

To measure the conditional relative stability constant for two gadolinium complexes, we propose a method based on relaxivity measurements. This versatile method allows the determination of the relative stability for any couple of ligands, provided its ^{1}H NMR relaxivities are sufficiently different at an appropriate magnetic field. Using this method, we found that in a slightly basic environment (pH 8.3) the [Gd(DTTA-Me)(H$_2$O)$_2$]$^-$ complex has a higher stability with respect to [Gd(DTPA-BMA)(H$_2$O)], as has been shown by the direct competition experiment using relaxivities as the indicator. Transmetalation with Zn^{2+} is, however, less favorable for the heptadentate DTTA-Me^{4-} compared to the octadentate DTPA-BMA^{3-}. Whereas the long time behavior of both complexes is similar, showing relatively strong transmetalation, the metal exchange from Gd^{3+} to Zn^{2+} is about twice as fast for DTTA-Me^{4-} because of the higher stability of the zinc complex. In contrast to DO3A-based ligands with two water molecules in the first coordination sphere, the formation of ternary complexes with the bidentate lactate anion is very weak. Even at a 10-fold excess of the anion, the measured relaxivity decreases only very slightly.

From the results, it can be concluded that the Gd^{3+} complex of heptadentate DTTA-Me^{4-} behaves similarly to

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the commercial octadentate DTPA-BMA^{3-}. Considering recent suspicions against [Gd(DTPA-BMA)(H_2O)] for being involved in NSF disease, DTTA-type chelates will not be admitted as contrast agents in clinical MRI. However, their use in vitro and in animal studies is absolutely conceivable, mainly at high magnetic fields, where the increase of the inner-sphere-coordination water actually seems to be the most certain way to increase the relaxivity.

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Supporting Information Available: Demonstration of K_{L_1/L_2}* (eq 14) and relaxivities r_1^n as a function of K_{L_1/L_2}* and the total concentrations of Gd^{3+}, L_1, and L_2; concentrations and measured relaxation times as a function of the sample composition for [Gd(DTTA-Me)(H_2O)$_2$]$^-$/[Gd(DTPA-BMA)(H_2O)] mixtures (Table S1); calculated conditional, K_{L_1/L_2}*, and thermodynamic, K_{L_1/L_2}, relative stability constants (Table S2); pH distribution diagrams for DTTA-Me (Figure S1), Zn-DTTA-Me (Figure S2), and Gd-DTTA.Me (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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