Amide Functional Group Contribution to the Stability of Gadolinium(III) Complexes: DTPA Derivatives

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Received September 7, 1994[⊗]

The Gd^{3+} complexes of diethylenetriaminepentaacetic acid (DTPA) and several closely related ligands are in use, or being developed, as contrast agents for magnetic resonance imaging (MRI). The bis(amide) derivatives of DTPA are, like the parent DTPA ligand, octadentate complexing agents of gadolinium, replacing two carboxylate coordinating groups with coordinating amide oxygens. Remarkably, this maintains a significant portion of the stability of the gadolinium complex and an increase in the relative selectivity for Gd^{3+} vs Ca^{2+}. The magnitude of the contribution of the amide functional groups to this stability is investigated. A diethylenetriaminetricarboxylic acid bis(amide) derivative in which the two terminal amide groups are replaced by methyl groups (bis(methyl)-diethylenetriaminetriacetic acid, DTTA-BM) is found to have a metal ion affinity at pH 7.4 that is 6.75 log units smaller than the effective stability of the bis-amide DTPA derivatives, corresponding to an average contribution of 3.38 log units to the stability of the complex from each amide functional group. The effective stability of the DTTA-BM Ca^{2+} complex relative to the bis(amide) ligands does not change as much. Hence the amide functional groups contribute significant stability for gadolinium complexation but little or no enhancement of calcium complexation, explaining the relative selectivity of bis(amide) ligands as gadolinium(III) complexing agents.

Introduction

The use of gadolinium(III) compounds as enhancement agents for magnetic resonance imaging (MRI) requires two conflicting properties: both a high relaxivity (exchangeable water protons) and strong complexation of the metal ion (to avoid toxicity).[1−3] The nine-coordinate complex of diethylenetriaminepentaacetate acid (DTPA) with gadolinium, $[Gd(DTPA)(H_2O)]^{2-}$ is in clinical use as a contrast agent. As a dianion it is highly water soluble; there are also problems associated with its high osmolality, since the complex must be injected at relatively high concentration into a patient for imaging.

Uncharged complexes of gadolinium with DTPA bis(amide) ligands have been described.[4] These ligands are prepared by reaction of DTPA dianhydride with the appropriate amines. In this study the bis(ethylamide) derivative of DTPA **1** and the bis(methylamide) **2** were used; these ligands are described as DTPA-BEA and DTPA-BMA, respectively.[5,6] The crystal structure of [Gd(DTPA-BEA)] shows it to be nine-coordinate, utilizing two amide oxygens in place of two carboxylates. The stability constant, log K_{ML} is correspondingly smaller for the neutral complex than for the DTPA complex, but not as small as might be expected. For compound **2**, (DTPA-BMA) log K_{ML} is 16.85 vs 22.46 for the DTPA complex.[6,7] However the relative binding of gadolinium by competing ligands in the presence of physiological ions (Ca^{2+}, etc.) at neutral pH is what correlates with toxicity; hence, it is significant that the relative binding of gadolinium vs some other ions actually improves somewhat from the DTPA to the bis(amide) derivatives.[6]

The crystal structures of the DTPA-BEA complex **1**[5] and the very closely related DTPA-BMA complex[8] show that these are tricapped trigonal prismatic complexes in which the water molecule and two nitrogens occupy capping positions while the carboxylate and amide oxygens occupy the more strongly coordinating apical sites. The Gd−O amide bond is relatively short. This and the relatively high stability of these complexes raises the question of precisely how much amide coordination contributes to the stability of the complex. In order to explore this question a ligand was derived in which two acetate groups (of DTPA) are replaced by non-coordinating alkyl groups. For synthetic ease, ligand **3** was first prepared, but the Gd^{3+} complex is insoluble in water. The addition of acetate groups to *N*,*N*″-dimethyldiethylenetriamine gives a quaternary product (**6**) if a large excess of bromoacetic acid is used. Careful control of the stoichiometry gives ligand **4** (bis(methyl)diethylenetriaminetriacetic acid, DTTA-BM). Its synthesis and the characterization of its solution thermodynamic properties (protonation constants and stability constants with Gd^{3+} and Ca^{2+}) are reported in this paper. Structures of **1**−**4** and **6** are shown in Chart 1.

Experimental Section

Physical Measurement. ^{1}H-NMR measurements were performed on a Brucker AM 500. ^{13}C-NMR measurements were performed on a Brucker AM 400. The spectra obtained in D_2O were referenced to sodium 3-(trimethylsilyl)propionate (^{1}H NMR; 0 ppm) or dioxane (^{13}C NMR; 66.7 ppm). Microanalytical and mass spectral analyses were performed by the analytical facilities of the University of California at Berkeley.

Syntheses and Materials. All chemicals were reagent grade and used as obtained (Aldrich) unless otherwise stated. *N*,*N*-Dimethylformamide was purchased in Aldrich Sure-Seal bottles and was transferred to reaction flasks using syringe techniques.

Diethylenetriamine *N*,*N*′,*N*″-Tritosylamide (Ts$_3$dien) was synthesized as described in the literature.[9]

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[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1995.

(1) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901−927
(2) Tweedle, M. F. In *Lanthanide Probes in Life, Chemical, and Earth Sciences*; Bünzli, J.-C. G.; Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989; Chapter 5
(3) Choppin, G. R. In *Lanthanide Probes in Life, Chemical, and Earth Sciences*; Bünzli, J.-C. G.; Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989; Chapter 1.
(4) Quay, S. C. U.S. Patent 4,687,659, 1987.
(5) Konings, M.; Dow, W.; Love, D.; Raymond, K. N.; Quay, S; Rocklage, S. *Inorg. Chem.* **1990**, *29*, 1488.
(6) Cacheris, W. P.; Quay, S. C., Rocklage, S. M. *Magn. Reson. Imaging* **1990**, *8*, 467−481.
(7) Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum: New York; 1974, 1975, 1976, 1977; Vols. 1−4.
(8) Ehnbom, L.; Pedersen, B. *Acta Chim. Scand.* **1992**, *46*, 126.
(9) Atkins, T. J.; Richman, J. E.; Oettle, W. F. *Org. Synth.* **1979**, *58*, 86.

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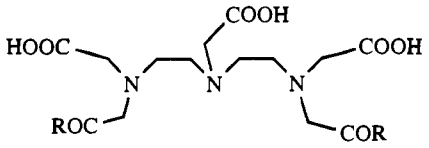
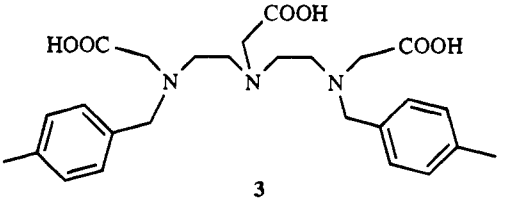
Chart 1



1 R = NHEt, (DTPA-BEA)
2 R = NHMe, (DTPA-BMA)



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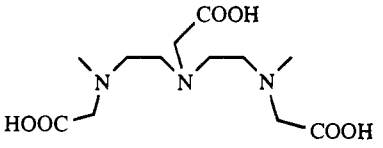
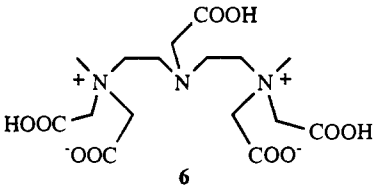


4 (DTTA-BM)



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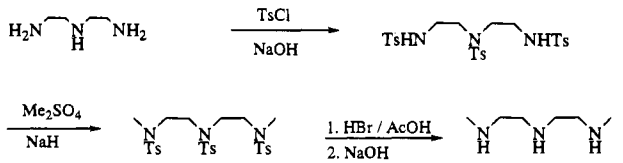
Scheme 1



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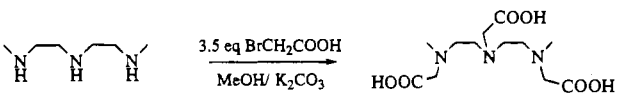
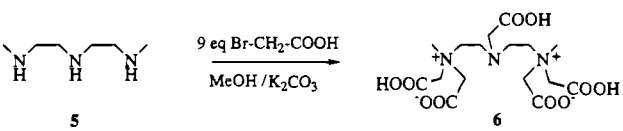
Scheme 2



5 **4 (DTTA-BM)**

Scheme 3



5 **6**

Synthesis of N,N'-dimethyl diethylenetriamine N,N',N''-tritosylamide (Me_2Ts_3dien). A flame-dried 3 L round bottom flask equipped with a mechanical stirrer was charged with 236 g (0.418 mol) of Ts_3dien and 1.5 L of anhydrous DMF. The flask was cooled in ice, and 61.0 g (60% in oil, 1.53 mol) of NaH was added with stirring. When gas evolution subsided, 140.0 mL (186.2 g, 1.48 mol) of dimethyl sulfate was added dropwise over 1.5 h. The mixture was stirred for 1 h at room temperature and 2 h at 70 °C. An aqueous solution of 50 g of ammonium acetate and 50 mL of 27% ammonia in 200 mL of water were added dropwise to quench the excess sodium hydride and dimethyl sulfate. The mixture was stirred for 1 h, and the solvent was removed on a rotary evaporator. One liter of methanol was added, and the thick suspension was thoroughly stirred, and the precipitate was filtered and washed with methanol, water, methanol, and diethyl ether. The white solid was dissolved in 1 L of methylene chloride, and this solution was filtered, concentrated to 500 mL, treated with 800 mL of methanol, reconcentrated to 1 L and treated with 500 mL of methanol. The resulting solution was chilled in ice, filtered, and washed with methanol.

Yield: 201.8 g (81.6%). Mp: 145−147 °C. ^{1}H-NMR ($CDCl_3$): 2.42 (br s, 9H), 2.77 (s, 6H), 3.28 (br s, 8H), 7.32 (d, J = 8.0 Hz, 6H), 7.69 (d, J = 8.0 Hz, 6H).

Synthesis of *N,N'*-Dimethyldiethylenetriamine (5). An HBr/HOAc solution was prepared by slowly adding 900 mL of 48% HBr to an ice-cooled flask containing 500 mL of acetic anhydride. The mixture was stirred overnight. The HBr/HOAc mixture was added to a 3 L flask along with 201.5 g (0.340 mol) of Me_2Ts_3dien. The mixture was heated to reflux for 10 h. The solvent was reduced to 700 mL on a rotary evaporator and the oily residue extracted with 2 × 500 mL of diethyl ether. The aqueous layer was further evaporated to 500 mL and an additional amount of tarry residue was removed by filtration through glass wool. Ethanol (500 mL) was added to induce crystallization. The mixture was cooled in ice, and 1.5 L of diethyl ether was added. The solid was removed by filtration and washed with 2:1 ethanol/ether and then diethyl ether. The solid was mixed with 100 g of crushed NaOH and the resulting thick slurry was extracted with methylene chloride. The solid was removed by filtration and washed with methylene chloride. The filtrate was evaporated to an oil on a rotary evaporator. The liquid was distilled under vacuum from a small piece of sodium metal using a 15 cm Vigreux column; the fraction was collected at 74−76 °C, under 4 mmHg of pressure.

Yield: 118.9 g (93.5%). ^{1}H-NMR ($CDCl_3$): 1.04 (br s, 3H), 2.24 (d, J = 2.4 Hz, 6H), 2.47−2.52 (m, 4H), 2.53−2.58 (m, 4H). FAB-MS (TG/G): 144.1 ([M + Na]$^+$), 132.1 ([M + H]$^+$).

Synthesis of DTTA-BM (4). Bromoacetic acid (1.85 g, 13.35 mmol) in solution in MeOH (50 mL), was added dropwise to a stirred, refluxing mixture of **2** (0.50 g, 3.81 mmol) and K_2CO_3 (5.27 g, 38.16 mmol) in freshly distilled MeOH (100 mL) under nitrogen. Vigorous stirring at reflux was continued for 12 h. The hot mixture was filtered and the filtrate evaporated under vacuum. The white residue (4.3 g) was diluted in water (pH = 9) and the pH was adjusted to 2 with HBr. This aqueous solution was applied to a cation exchange column (Dowex 50WX2-400−NH_4OH loaded, 5 × 12 cm). The column was washed with water (1 L), and the product was eluted with 0.5 M NH_4OH. The solvent was removed by rotary evaporation and the white residue was applied to an anion exchange column (AG 1 × 8-400, formate loaded 5 × 6 cm) and eluted with formic acid solution 0.5 M to give the white hygroscopic free acid **4**.

Yield: 430 mg (34%). Mp: 72 °C. ^{1}H-NMR (D_2O, pD 3): 3.85 (s, 4H, CH_2, side arms), 3.55 (s, 2H, CH_2, middle arm), 3.40 (broad t, 4H, CH_2, backbone), 3.14 (t, J = 6.07 Hz, 4H, CH_2, backbone), 2.99 (s, 6H, $2CH_3$). ^{13}C-NMR (D_2O, pD 3): 176.19 (1C, -CO_2, middle arm), 170.08 (2C, CO_2, side arms), 58.61 (−CH_2), 55.01 (1C, −CO_2, middle arm), 54.23 (−CH_2), 49.95 (−CH_2), 41.83 ($2CH_3$). FAB-MS (TG/G): 328.1 ([M + Na]$^+$), 306.1 ([M + H]$^+$), Anal. Calcd (found) for $C_{12}H_{23}N_3O_6{}^{-3}/_2H_2O$: C, 43.37 (43.34); H, 7.83 (8.31); N, 12.65 (13.08).

Synthesis of Bis(methyl) DTPA (6). This compound resulted when a large excess of bromoacetic acid (9.54 g, 68.70 mmol) in solution in MeOH (100 mL), was added dropwise to a stirred, refluxing mixture of **2** (1.00 g, 7.63 mmol) and K_2CO_3 (15.00 g, 108.54 mmol) in freshly distilled MeOH (200 mL) under nitrogen. Vigorous stirring at reflux was continued for 12 h. The hot mixture was filtered and the filtrate evaporated under vacuum. The residue was suspended in hot EtOH, filtered, and dried. This white powder (6.8 g) was diluted in water (pH = 9), and the pH was adjusted to 2 with HBr. This solution was applied to a cation exchange column (Dowex 50WX2-400−NH_4OH

(10) Franczyk, T. S. Ph.D. Dissertation, University of California at Berkeley, 1991; pp 77−78 and 112.

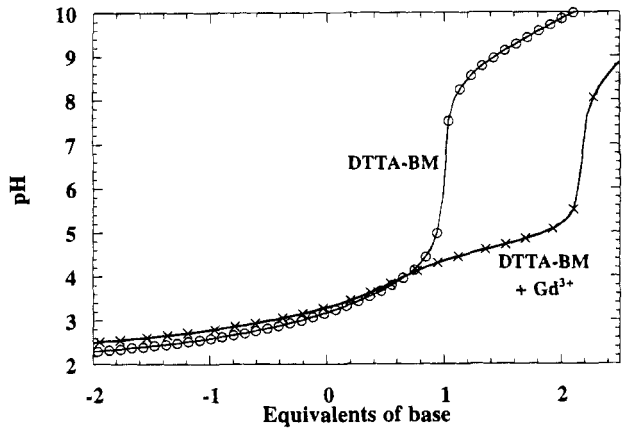


Figure 1. Titration curves for DTTA-BM (0.1249 mmol) and DTTA-BM/Gd^{3+} (0.1400/0.0901 mmol), T = 25 °C, μ = 0.1 M.

loaded, 5 × 12 cm). The column was washed with water (1 L) and the product was eluted with 0.5 M NH_4OH. The solvent was removed by rotary evaporation and the white residue was applied to an anion exchange column (AG 1 × 8-400, formate loaded 5 × 6 cm) and eluted with 0.5 M formic acid to give the white hygroscopic free acid **6**.

Yield: 770 mg (22%). Mp: 128 °C. ^{1}H-NMR (D_2O, pD ~1.5): 4.47 (m, 8H, CH_2, side arms), 3.98 (t, J = 6.3 Hz, 4H, CH_2, backbone), 3.57 (s, 2H, CH_2, middle arm), 3.47 (s, 6H, $2CH_3$), 3.20 (t, J = 6.2 Hz, 4H, CH_2, backbone). ^{13}C-NMR (D_2O, pD ~1.5): 174.17 (1C, $-CO_2$, middle arm), 167.89 (4C, $-CO_2$, side arms), 61.24 (4C, CH_2, side arms), 59.57 (2C, CH_2, backbone), 53.92 (1C, CH_2, middle arm), 50.41 (2C, CH_2, backbone), 47.26 (2C, $2CH_3$). FAB-MS (TG/G): 444.2 ($[M + Na]^+$), 422.2 ($[M + H]^+$), Anal. Calcd (found) for $C_{16}H_{27}N_3O_{10}\cdot{}^1/_2H_2O$: C, 42.83 (43.20); H, 6.74 (6.50); N, 9.37 (9.24).

Potentiometric Titrations. Proton association constants and metal ion complex stability constants were calculated using the BASIC computer program BETA90.[10] This employs a modified Newton-Raphson algorithm which solves the simultaneous nonlinear mass balance equations, refining on $-\log[H^+]$ values. Stability constants of the divalent Ca^{2+} and trivalent Gd^{3+} complexes of ligand **4** were determined by direct titration of equimolar amounts of metal and ligand (1−3 mM) adjusted to low pH (approximately 2) with a known amount of HCl (0.1 M) and then titrated with potassium hydroxide (0.1 M) and back-titrated. All titrations were performed in aqueous solution at constant 0.1 M ionic strength (KCl), at 25 °C.

Results and Discussion

The potentiometric titration curves for DTTA-BM (compound **4**, bis(methyl)diethylenetriaminetriacetic acid) and [Gd(DTTA-BM)] are shown in Figure 1. The DTTA-BM curve shows a

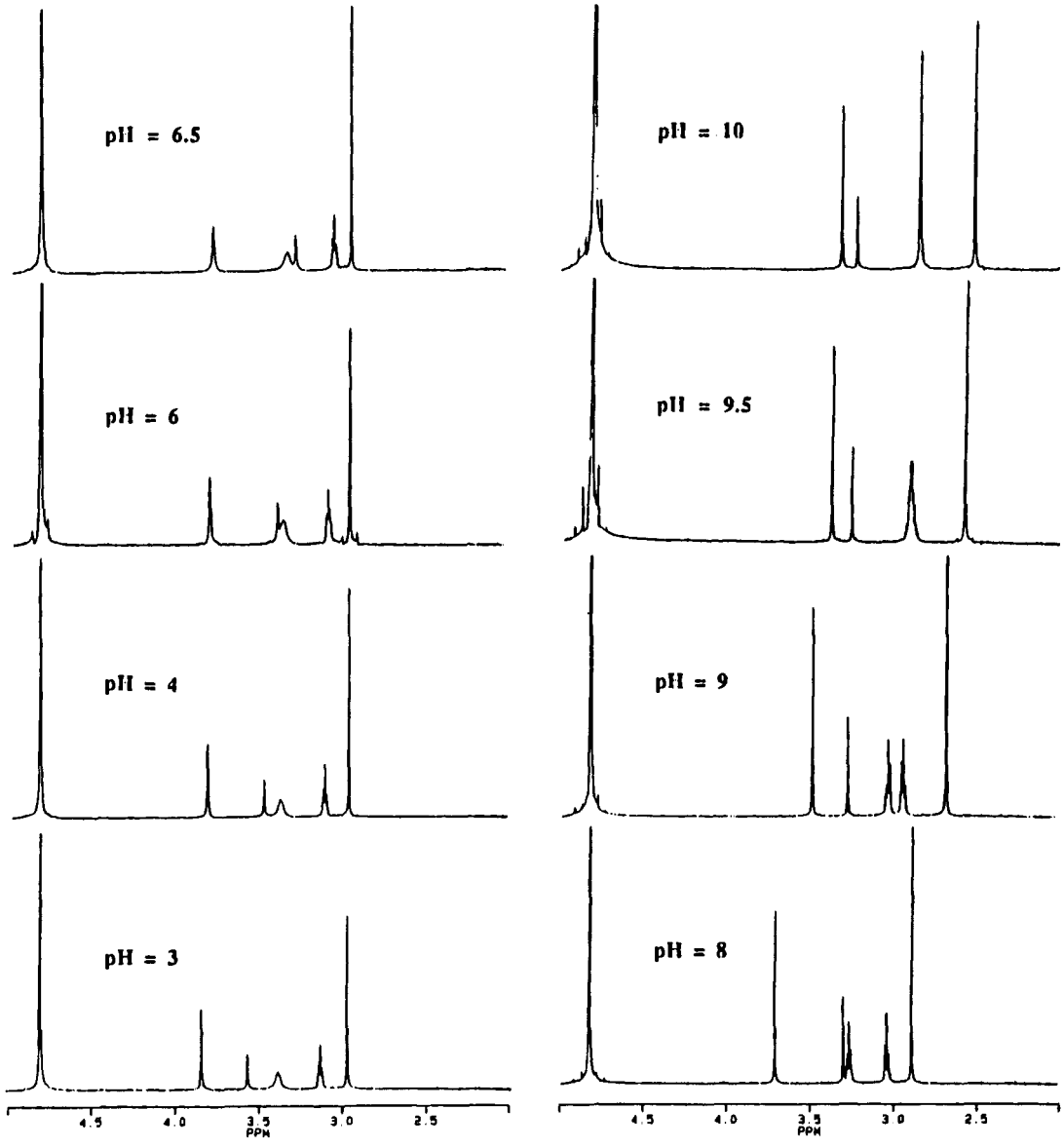


Figure 2. ^{1}H NMR spectra of DTTA-BM as a function of pH.

Table 1. Protonation Constants for DTPA, DTPA-BMA and DTTA-BM

	log K (25 °C, μ = 0.10 M (KCl))		
equilibrium	DTPA[a]	DTPA-BMA[b]	DTTA-BM
[HL]/[L][H]	10.49	9.37	10.70
[H_2L]/[HL][H]	8.60	4.38	9.09
[H_3L]/[H_2L][H]	4.28	3.31	3.59
[H_4L]/[H_3L][H]	2.64	1.43	1.93
[H_5L]/[H_4L][H]	2.00	low	low
ΣpK	28.0	18.5	25.3

[a] From ref 7. [b] From ref 6.

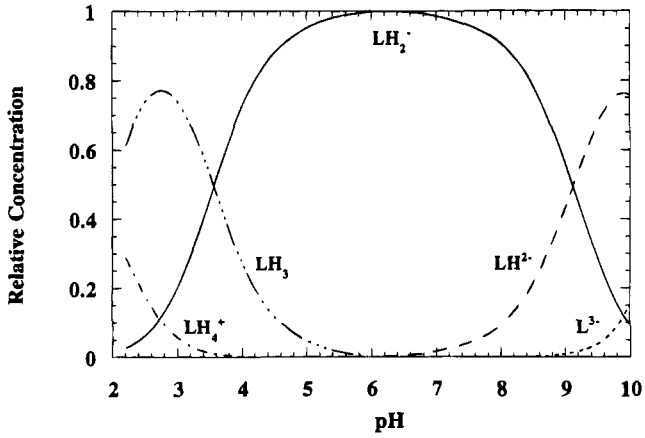


Figure 3. Species distribution plot of DTTA-BM, valid for all concentrations.

very sharp increase between pH 4 and 8. This is due to the large separation of pK_a values of 9.09 and 3.59 (the second and third amine). The first amine of this ligand has a pK_a of 10.70 and the carboxylates all have pK_a values below 2. In Table 1 the potentiometrically determined protonation constants of DTTA-BM (ligand **4**) are compared with values reported earlier for DTPA and DTPA-BMA (diethylenetriaminepentaacetic acid bis(methylamide)). ^{1}H-NMR titration measurements of ligand **4** at various pH values in water (Figure 2) very clearly show that the central amine is less basic; between pH = 3 and 7 only the CH_2 from the middle acetate group is strongly affected by the deprotonation. At higher pH, coalescence of the two triplets corresponding to the four ethylenic groups of the backbone is observed. The two highest pK_a's of DTTA-BM may be identified with protonation of the terminal nitrogens (pK_a = 10.70 and 9.09) followed by the central nitrogen atoms (pK_a = 3.59). The replacement of two terminal amide groups by methyl groups results in an increase of pK_{a_1} (1.33 units), pK_{a_2} (4.71 units), and of pK_{a_3} (0.28 unit). These very significant differences in basicity of the amine groups are primarily due to hydrogen bonding to amide protons in the DTPA-BMA backbone. The assignments for compound **4** are supported by the ^{1}H-NMR data (Figure 2). The species distribution curves for DTTA-BM are shown in Figure 3; between pH 4.5 and 8 the dominant species is the diprotonated ligand (LH_2^-).

When Gd^{3+} is added to the solution, the potentiometric titration curve changes drastically (Figure 1). The species distribution for the complex [Gd(DTTA-BM)] is shown in Figure 4. At pH 6 there is still some free Gd^{3+} but by pH 7 the complex is fully formed.

The potentiometric titration curve for $[Ca(DTTA\text{-}BM)]^-$ is shown in Figure 5, and the species distribution for the calcium complex is shown in Figure 6. Between pH 4 and 8 the main ligand species is LH_2^- and the calcium complex does not form. Only above pH 8 is the Ca^{2+} complex formed, although never quantitatively at these concentrations.

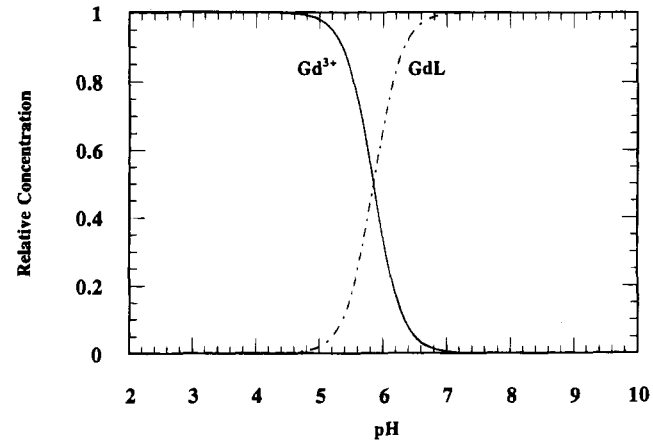


Figure 4. Species distribution plot of the DTTA-BM/Gd^{3+} system (total ligand 10^{-5} M, total Gd^{3+} 10^{-6} M).

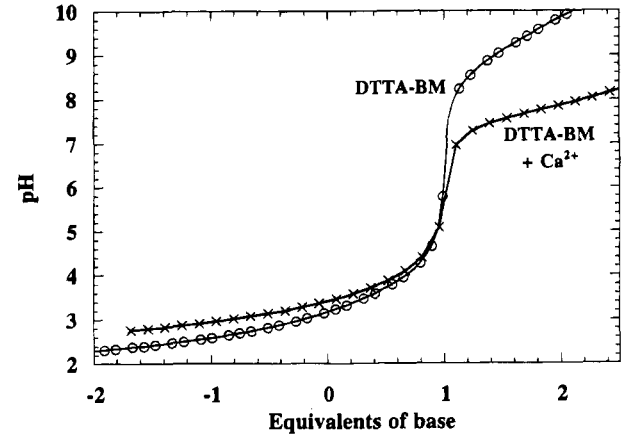


Figure 5. Titration curves for DTTA-BM (0.1249mmol) and DTTA-BM/Ca^{2+} (0.0549/0.0626 mmol), T = 25 °C, μ = 0.1 M.

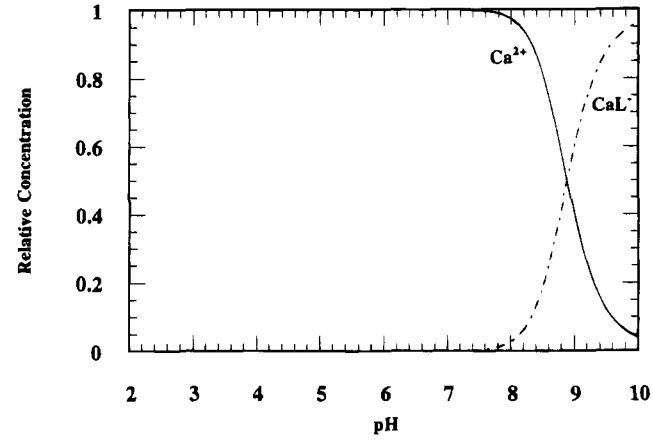


Figure 6. Species distribution of DTTA-BM/Ca^{2+} (total ligand 10^{-5} M, total Ca^{2+} 10^{-6} M).

In Table 2, a comparison of stability constants and the conditional stability at pH 7.4 is presented for the three polyprotic ligands: DTPA, DTPA-BMA (**2**), and DTTA-BM (**4**). The thermodynamic stability constant (K_{ML}) is defined[7] as M + L = ML, K_{ML} = [ML]/[M][L]. The pM value of a complex specifies the degree of metal chelation at a given pH, and is defined as −log[free metal ion] under stated conditions of total ligand and metal.[11] The pM value allows a direct comparison of the ligands' metal ion affinity, with protonation

(11) Harris, Wesley R.; Raymond, Kenneth N.; Weitl, Frederick L. *J. Am. Chem. Soc.* **1981**, *103*, 2667−2675.

Table 2. Stability Constants for DTPA, DTPA-BMA, and DTTA-BM Gd^{3+} and Ca^{2+} Complexes[a]

	DTPA[b]		DTPA-BMA[c]		DTTA-BM	
	log K_{ML}	pM[d]	log K_{ML}	pM	log K_{ML}	pM
Gd^{3+}	22.46	19.10	16.85	15.83	13.12	9.08
Ca^{2+}	10.75	7.43	7.17	6.39	7.25	6.00
selectivity	11.71	11.66	9.68	9.44	5.87	3.06

[a] Measured potentiometricaly with $\mu = 0.10$ M at 25 °C. [b] From ref 7. [c] From ref 6. [d] Calculated for pH 7.4 with [L] = 10^{-5} M, [M] = 10^{-6} M.

competition accounted for. In Table 2, the pM values are given at physiological pH 7.4 and compared with K_{ML}. At this pH [Gd(DTPA)] presents a very high pM value of 19.10. As expected the [Gd(DTPA-BMA)] pM value is higher (15.83) than that of [Gd(DTTA-BM)] (9.08). The change in ligand structure upon going from the bis(amide) DTPA-BMA ligand to the bis-(methyl) ligand DTTA-BM creates a large change in proton basicity as well as metal ion complex formation constant. Since the issue is the degree to which the complexation of the metal ion is due to amide coordination, it is the decrease in metal ion free energy (measured here as pM value) that is significant, not the formation constant. The decrease of the free Gd^{3+} ion concentration of 6.75 log units upon loss of the two amide groups is a measure of their contribution to the complex stability, about 3.4 log units per amide.

The stability constant of the Ca^{2+} complex $[Ca(DTTA-BM)]^-$ (log K 7.25, pM 6.00 [i.e., uncomplexed Ca^{2+}]) is approximately the same as for that formed with compound **2** (log K 7.15, pM 6.39). Hence there is a selectivity for Gd^{3+} over Ca^{2+} produced by the presence of amide groups in the ligand. This is a highly significant feature for future ligand design of gadolinium MRI agents.

Acknowledgment. This research was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division, U.S. Department of Energy, under Contract No. DE-AC03−76SF00098. C.P.-R. thanks the "Ligue Nationale Contre Le Cancer" for support. We acknowledge the earlier efforts of Drs. M. Konings and D. L. Jameson, and we particularly thank Mr. C. J. Sunderland for assisting in the assembling of the manuscript.

IC9410553