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Supplementary Material Available: Tables S1-S9, listing atomic positional and thermal parameters and complete bond angles and distances supplied for **3-5** (17 pages); Tables S10-S12, listing observed and calculated structure factor tables for **3-5** (93 pages). Ordering information is given on any current masthead page.

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Synthesis, Equilibrium, and Kinetic Properties of the Gadolinium(III) Complexes of Three Triazacyclodecanetriacetate Ligands

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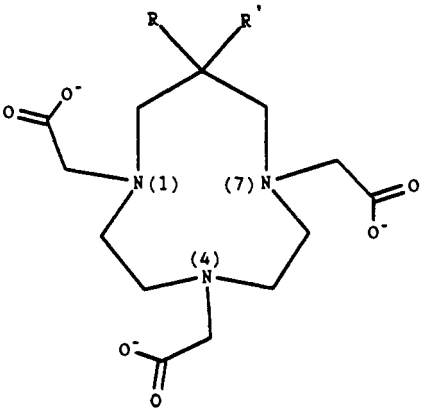
Three new 10-membered-ring triaza tricarboxylate ligands have been synthesized and the formations of their Gd^{3+} complexes examined. The ligands 1,4,7-triazacyclodecane-*N,N',N''*-triacetate ($DETA^{3-}$), 9-methyl-1,4,7-triazacyclodecane-*N,N',N''*-triacetate ($MeDETA^{3-}$), and 9,9-dimethyl-1,4,7-triazacyclodecane-*N,N',N''*-triacetate (Me_2DETA^{3-}) have an unusually high first protonation constant (log $K_1 \approx 14-15$), and evidence is presented for the formation of a strongly hydrogen-bonded proton between the two nitrogens that share the propylene bridge in the monoprotonated forms of these chelates. Exchange of the hydrogen-bonded proton is slow below 0 °C, and its ^1H NMR signal has been detected. Complexation with Gd^{3+} is also slow, preventing determination of metal ion-ligand stabilities by potentiometry. Conditional stability constants for Gd(DETA), Gd(MeDETA), and Gd(Me_2DETA) were measured by using a proton relaxivity technique and compared with similar data for the 9-membered-ring macrocyclic complex Gd(NOTA). The rates of formation of Gd(DETA) and Gd(MeDETA) are similar, while Gd(Me_2DETA) forms more slowly. The rate-determining step of complexation is proton loss and rearrangement of a monoprotonated intermediate, formed in a fast preequilibrium reaction. Dissociation of the complexes takes place both in a spontaneous fashion and through a proton-assisted pathway. The rate of both processes decreases in the order Gd(Me_2DETA) ≫ Gd(NOTA) > Gd(DETA) > Gd(MeDETA). An increase in ring size from 9 to 10 and substitution of a methyl group onto a ring carbon of the ligand lead to increased kinetic stabilities of the complexes. The substitution of two methyl groups onto a ring carbon, however, results in a significant decrease in both the thermodynamic and kinetic stabilities of the resulting Gd^{3+} complex.

Introduction

The development of magnetic resonance imaging (MRI) and the successful use of the complex $Gd(DTPA)^{2-}$ for detecting tumors have resulted in a growing interest in the preparation and study of new Gd^{3+} complexes.[1] The properties of $Gd(DOTA)^-$ ($DOTA^{4-}$ = 1,4,7,10-tetrazacyclododecane-*N,N',N'',N'''*-tetraacetate), particularly its extremely low rate of dissociation, make this complex a very promising candidate for clinical use.[2] However, both $Gd(DTPA)^{2-}$ and $Gd(DOTA)^-$ are charged complexes and the osmolality of the solutions injected intravenously is quite high. In this respect, neutral complexes, formed with the cyclic triaza tricarboxylate ligands would be more favorable. The complex Gd(NOTA) ($NOTA^{3-}$ = 1,4,7-triazacyclononane-*N,N',N''*-triacetate) has a higher water proton relaxivity than $Gd(DTPA)^{2-}$ or $Gd(DOTA)^-$, but both the thermodynamic and kinetic stabilities of Gd(NOTA) are lower than those of the DTPA or DOTA complexes.[3-5] This is likely a consequence of the small size of the cyclononane ring of this chelate.[6]

We have now prepared several new triaza tricarboxylate ligands in an attempt to determine the effect of macrocycle size and rigidity on the complexation properties of these ligands.[7] We report here our data on three new 10-membered-ring chelates, 1,4,7-triazacyclodecane-*N,N',N''*-triacetate ($DETA^{3-}$), 9-methyl-1,4,7-triazacyclodecane-*N,N',N''*-triacetate ($MeDETA^{3-}$), and 9,9-dimethyl-1,4,7-triazacyclodecane-*N,N',N''*-triacetate (Me_2DETA^{3-}) (see I).

The basicity of one nitrogen in $DETA^{3-}$, $MeDETA^{3-}$, and Me_2DETA^{3-} was found to be unusually high. While this work was in progress, the preparation of $DETA^{3-}$ was published,[8] but neither its protonation constants nor its complexation properties were studied in detail.



R = CH_3; R' = H — $MeDETA^{3-}$
R = CH_3; R' = CH_3 — Me_2DETA^{3-}

I

Experimental Section

Synthesis and Characterization of Ligands. 1,4,7-Triazacyclodecane-*N,N',N''*-triacetate (DETA) was prepared as previously described.[9] The

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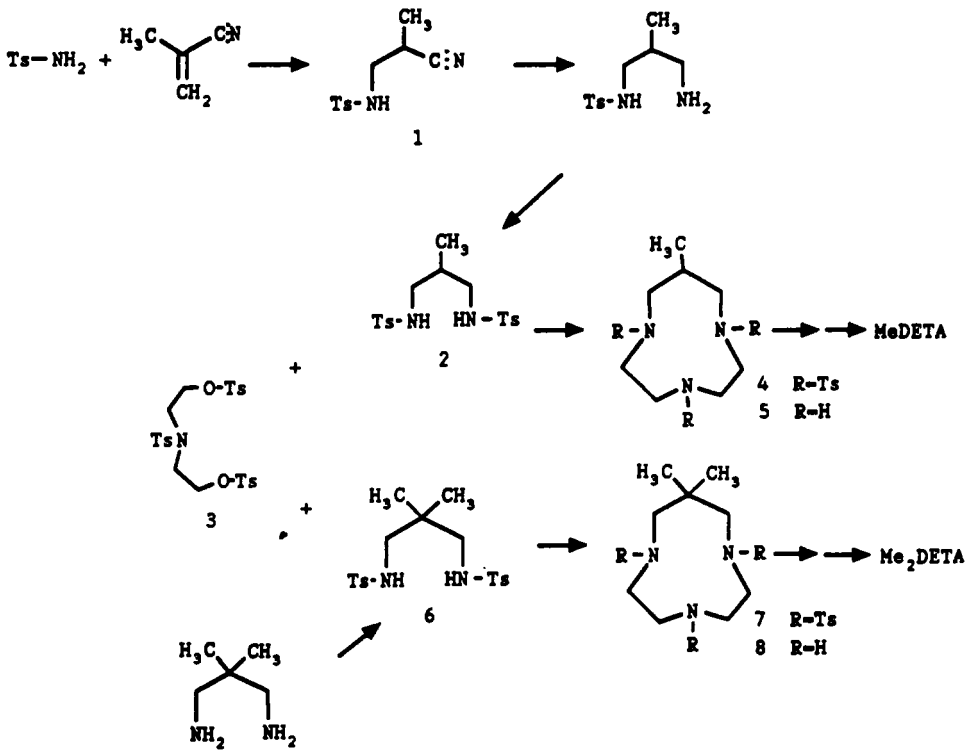
0020-1669/91/1330-2092$02.50/0

Scheme I



methyl-substituted ligands were prepared as outlined in Scheme I.

***N*-(*p*-Tolylsulfonyl)-3-amino-2-methylpropionitrile (1).** *p*-Toluenesulfonamide (20 g, 0.11 mol) and potassium carbonate (32.4 g, 0.23 mol) in 150 mL of dry DMF were stirred at 80 °C while methacrylonitrile (11 g, 0.16 mol) was added dropwise over a period of 30 min. The mixture was stirred for 24 h at 80 °C, followed by removal of solvent under vacuum. The solid was filtered out, washed with water, and recrystallized from hexane–ether to give **1** in 84% yield: mp 87–88 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (d, 3 H), 2.44 (s, 3 H), 2.89 (m, 1 H), 3.11 (t, 2 H), 5.38 (t, 1 H; NH), 7.43–7.35 (m, 4 H).

***N*,*N*′-Bis(*p*-tolylsulfonyl)-2-methyl-1,3-propanediamine (2).** Compound **1** (9.1 g, 38 mmol) was refluxed for 24 h in 130 mL of a 1 M solution of diborane (THF) under a N_2 atmosphere. The mixture was cooled to 10 °C, and THF–water was cautiously added. The solvent was removed, 6 N HCl was added (150 mL), and the solution was refluxed for 2 h. The mixture was concentrated to dryness and the residue allowed to partition between 2 N NaOH and methylene chloride. The organic layer was separated out, dried over anhydrous sodium sulfate, and evaporated to give the free amine. This was dissolved in diethyl ether (38 mL) and NaOH (1.26 g, 31.6 mmol) in 22 mL of water added; to this mixture was added dropwise *p*-toluenesulfonyl chloride (6 g, 32 mmol) in diethyl ether (50 mL) in about 1 h. The organic solvent was evaporated to give a white solid. The crude product was filtered out, washed with water, and crystallized from diethyl ether to afford **2** in 90% yield: mp 118–119 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (d, 3 H), 2.19 (m, 1 H), 2.43 (s, 6 H), 2.84 (m, 4 H), 4.90 (t, 2 H), 7.32–7.74 (m, 8 H).

***N*,*N*-Bis[2-((*p*-tolylsulfonyl)oxy)ethyl]-*p*-toluenesulfonamide (3).** To a solution of *p*-toluenesulfonyl chloride (54.6 g, 0.29 mol) in anhydrous pyridine (100 mL) at 0.5 °C was added dropwise under a N_2 atmosphere a solution of diethanolamine (10 g, 95 mmol) in anhydrous pyridine (50 mL). The mixture was stirred for 2 h at 0 °C. The reaction mixture was poured into ice-cold water and the crude product extracted with methylene chloride. The extracts were washed with a 5 M solution of HCl and then water and were dried over anhydrous sodium sulfate. The solvent was removed, and crystallization from methanol yielded **3** in 71% yield: mp 95–96 °C; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3 H), 2.46 (s, 6 H), 3.37 (t, 4 H), 4.11 (t, 4 H), 7.31–7.78 (m, 12 H).

9-Methyl-1,4,7-tris(*p*-tolylsulfonyl)-1,4,7-triazacyclodecane (4). A solution of compound **2** (8.9 g, 22.4 mmol) and potassium carbonate (7.4 g, 54 mmol) in dry DMF (355 mL) was stirred at 45 °C for 1 h. A solution of compound **3** (16.4 g, 29 mmol) in dry DMF (200 mL) was added dropwise in about 10 h, and the mixture was stirred at 50 °C for 72 h. The solvent was removed under vacuum and ice-cold water added. The white solid was filtered out, washed with water, refluxed in ethanol, and dried in vacuo to give **4** in 60% yield. Analytical sample after chromatography over SiO_2 with methylene chloride as eluent: mp 197–200 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (d, 3 H), 2.43 (s, 6 H), 2.44 (s, 3 H), 2.50 (br m, 1 H), 2.82 (t, 4 H), 3.19 (m, 2 H), 3.42 (br s, 4 H), 3.80 (m, 2 H), 7.34–7.70 (m, 12 H). Anal. Calcd for $C_{29}H_{37}N_3S_3O_6$: C, 56.20; H, 6.02; N, 6.78; O, 15.49; S, 15.52. Found: C, 55.81; H, 6.11; N, 6.63; O, 15.39.

9-Methyl-1,4,7-triazacyclodecane Trihydrochloride (5). Compound **4** was dissolved in 144 mL of 96% sulfuric acid, and the mixture was kept at 100–105 °C for 48 h under a N_2 atmosphere. The reaction was tested at intervals by withdrawing a small aliquot and making it basic with 10% aqueous sodium hydroxide. The absence of cloudiness signified that the reaction was complete. The mixture was then cooled in an ice–acetone bath, and 250 mL of absolute ethanol was cautiously added, followed by 500 mL of anhydrous diethyl ether. The brown solid (hygroscopic) was filtered out under N_2, washed with diethyl ether, and dissolved in water (400 mL); the solution was treated with Norit activated carbon overnight. The mixture was filtered through Celite and concentrated under vacuum to 200 mL; 140 mL of 6 N sodium hydroxide was added, and the resulting mixture was extracted with 10 × 100 mL portions of chloroform. The extracts were dried over anhydrous sodium sulfate, and the solvent was removed under vacuum to give an oily residue. This was dissolved in 340 mL of absolute ethanol, the solution was cooled in an ice–acetone bath, and 100 mL of concentrated (37%) HCl was added dropwise to give a white solid. This was filtered out under N_2, washed with absolute ethanol, and dried under vacuum to yield **5** in 66% yield: mp 234–242 °C dec; ^{1}H NMR (D_2O) δ 0.98 (d, 3 H), 2.30 (m, 1 H), 3.03–3.43 (m, 12 H); ^{13}C NMR (D_2O) δ 16.24, 28.56, 45.15, 45.62, 52.92.

9-Methyl-1,4,7-triazacyclodecane-*N*,*N*′,*N*″-triacetate (MeDETA). The free amine was isolated by dissolving the trihydrochloride salt in 1 M NaOH and extracting with chloroform (5 × 3 mL). The resulting 9-methyl-1,4,7-triazacyclodecane (0.24 g, 1.53 mmol) was dissolved in 5 mL of dichloromethane and 10 mL of diisopropylethylamine. A solution of methyl bromoacetate (0.72 g, 4.68 mmol) in 5 mL of dichloromethane was then added dropwise to the amine solution with stirring at room temperature. The mixture was stirred for an additional 12 h, the solvent evaporated under reduced pressure, and the residue redissolved in chloroform (10 mL). The solution was washed with water (3 × 5 mL), dried over NaOH pellets, and filtered. Evaporation of the chloroform gave the product as a yellow oil (0.48 g, 85% yield), which was used without further purification: ^{13}C NMR ($CDCl_3$) δ 18.19, 33.00, 51.03, 51.08, 52.55, 55.47, 55.98, 56.69, 57.49, 172.40. The resulting trimethyl ester (0.48 g, 1.28 mmol) was mixed with water (20 mL), the pH adjusted to 1 or less by using 1 M HCl, and the mixture refluxed for

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12 h. The solution was then treated with a small amount of charcoal, refluxed for an additional 15 min, cooled to room temperature, filtered, and concentrated to about 5 mL. The pH was adjusted to 7–8 by using 1 M NaOH and the solution loaded onto a 2.5 × 30 cm column of Dowex 1 anion-exchange resin (chloride form, 100–200 mesh). The column was washed with 250 mL of water and the product eluted with a linear gradient of 0.0–0.1 M HCl while the absorbance was monitored at 254 nm. The fraction containing the product was identified by ^{13}C NMR spectroscopy, isolated, concentrated, and freeze-dried to obtain a white powder in 80% yield. Elemental analysis indicated the dihydrochloride salt containing 1.5 waters of hydration; mp 73 °C dec. The ^1H NMR spectrum showed only broad lines at room temperature but became more defined as the temperature was increased: ^1H NMR (D_2O, 80 °C, pH 1, reference HOD at 4.80 ppm) δ 1.47 (d, 3 H), 3.06 (m, 1 H), 3.78 (d, 4 H), 3.98 (br s, 6 H), 4.30–4.60 (m, 8 H); ^{13}C NMR (D_2O, 80 °C, pH 1, reference *p*-dioxane at 67.4 ppm) δ 16.23 (br), 26.89, 52.11, 53.67 (br), 57.42 (br), 63.18 (br), 172.30. Anal. Calcd for $C_{14}H_{25}N_3O_6 \cdot 2HCl \cdot 1.5H_2O$: C, 38.98; H, 7.01; N, 9.74; Cl, 16.44; O, 27.82. Found: C, 38.51; H, 6.90; N, 9.72; Cl, 17.18; O, 27.69 (by difference).

***N,N'*-Bis(*p*-tolylsulfonyl)-2,2-dimethyl-1,3-propanediamine (6).** To a solution of sodium hydroxide (6 g, 0.2 mol) in water (70 mL) and 2,2-dimethyl-1,3-propanediamine (10 g, 98 mmol) in diethyl ether (20 mL) was added dropwise a solution of *p*-toluenesulfonyl chloride (37.3 g, 0.2 mol) in diethyl ether (200 mL) over a period of 1 h at room temperature. The mixture was stirred for 1 h and the organic solvent evaporated. The solid was filtered out, washed with water, and crystallized from acetonitrile–methanol–water to give **6** in 85% yield: mp 128–130 °C; ^1H NMR ($CDCl_3$) δ 0.84 (s, 6 H), 2.42 (s, 6 H), 2.68 (d, 4 H), 5.30 (t, 2 H), 7.30–7.33 (m, 8 H).

9,9-Dimethyl-1,4,7-tris(*p*-tolylsulfonyl)-1,4,7-triazacyclodecane (7). A solution of compound **6** (8.0 g, 20 mmol) and cesium carbonate (15.2 g, 47 mmol) was stirred in dry DMF (330 mL) at 40 °C for 1 h. A solution of compound **3** (12 g, 21 mmol) in dry DMF (170 mL) was added dropwise in about 10 h. The mixture was stirred at 50 °C for 72 h. The solvent was removed under vacuum and ice-cold water added. The white solid was filtered out, washed with water, refluxed in ethanol, and dried in vacuo to give **7** in 46% yield. Analytical sample after chromatography over SiO_2 with methylene chloride as eluent: mp 175–179 °C; ^1H NMR ($CDCl_3$) δ 1.15 (s, 6 H), 2.44 (s, 9 H), 3.10 (s, 4 H), 3.43–3.49 (m, 8 H), 7.31–7.72 (m, 12 H).

9,9-Dimethyl-1,4,7-triazacyclodecane Trihydrochloride (8). Compound **7** was hydrolyzed under the same conditions as compound **4** to afford a white solid in 58% yield: mp 250–257 °C dec; ^1H NMR (D_2O) δ 1.03 (s, 6 H), 3.06 (s, 4 H), 3.24 (m, 4 H), 3.49 (m, 4 H); ^{13}C NMR (D_2O) δ 23.47, 32.53, 45.37, 46.02, 56.53.

9,9-Dimethyl-1,4,7-triazacyclodecane-*N,N',N''*-triacetate (Me_2DETA). This was prepared from compound **8** by using procedures identical with those described for the preparation of MeDETA. Elemental analysis indicated the product isolated from the anion-exchange resin was the dihydrochloride monohydrate salt: mp 105° dec; ^1H NMR (D_2O, pH 1, reference HOD at 4.80 ppm) δ 1.10 (s), 3.28 (br), 3.50 (br), 3.73 (br), 4.05 (br s); ^{13}C NMR (D_2O, pH 1, reference *p*-dioxane at 67.4 ppm) δ 25.46, 35.14, 49.88, 54.43, 55.89, 58.48, 63.29, 170.89, 173.05. Anal. Calcd for $C_{15}H_{27}N_3O_6 \cdot 2HCl \cdot H_2O$: C, 41.29; H, 7.16; N, 9.63; Cl, 16.25; O, 25.67. Found: C, 40.61; H, 7.04; N, 9.46; Cl, 17.12; O, 25.77 (by difference).

Preparation of Solutions. A $GdCl_3$ stock solution was prepared from Gd_2O_3 (Aldrich, 99.9%) by dissolution in concentrated HCl and evaporating the excess acid. The $GdCl_3$ concentrtion was determined by complexometry using xylenol orange as indicator. The concentrations of DETA, MeDETA, and Me_2DETA stock solutions were determined by titration against a standardized $CuCl_2$ solution, using murexide as indicator. The concentrations of standard 0.1 and 1.0 M KOH solutions for pH-potentiometric and ^1H NMR titrations, respectively, were determined by the titration against a 0.01 M potassium hydrogen phthalate solution.

Potentiometric Measurements. pH-potentiometric titrations were performed with a Corning Ion Analyzer 250 pH meter, an Orion 8103 combination electrode, and a Metrohm 665 Dosimat automatic buret. Titrated solutions (5–10 mL) were covered by a cyclohexane layer to exclude CO_2, and the cell was thermostated to 25 ± 1 °C. The ionic strength of the titrated solutions (1–5 mM) was kept constant (0.1 M KCl or 1.0 M KCl + KOH), and the hydrogen ion concentration was obtained from the measured pH values by the method of Irving et al.[10] The protonation constants of the ligands, H_3L, are defined as

$$K_i = [H_iL]/[H_{i-1}][H^+] \quad (1)$$

where i = 1, 2, or 3. The first protonation constant, K_1, of each ligand in this study was found to be too high for accurate assessment by pH potentiometry, so a microscopic constant was estimated from ^1H NMR data. K_2 and K_3 were calculated from potentiometric titration data by using a Simplex/Marquardt algorithm.[4]

Determination of Stability Constants. Complexation of Gd^{3+} by these ligands (eq 2) could not be studied by pH potentiometry because complex

$$Gd^{3+} + H_iL \rightleftharpoons GdL + iH^+ \quad (2)$$

formation was too slow. Since the relaxation rates of water protons measured in the presence of Gd^{3+}_{aq} or any one of the complexes Gd(DETA), Gd(MeDETA), and Gd(Me_2DETA) differ considerably, the concentrations of free Gd^{3+} and GdL can be determined by relaxivity measurements.[7] The relaxivity of water protons (R_{obs}) may be expressed as

$$R_{obs} = R_{Gd}[Gd] + R_{GdL}[GdL] + R_w \quad (3)$$

where R_w is the relaxivity of water protons in the absence of Gd^{3+} and R_{Gd} and R_{GdL} are the relaxivities of Gd^{3+}_{aq} and GdL, respectively. R_{Gd} was determined by measuring the proton relaxation rates of $GdCl_3$ solutions, while R_{GdL} was obtained by measuring the relaxation rate of solutions where complexation was full. Assuming that $[Gd]_t = [Gd] + [GdL]$, the concentration of the complex is obtained directly from the relaxivity data by[8]

$$[GdL] = (R_{Gd}[Gd^{3+}]_t - R')/(R_{Gd} - R_{GdL}) \quad (4)$$

where $R' = R_{obs} - R_w$. One may also assume that the concentration of noncomplexed ligand is given by $[L'] = [L]_t - [GdL]$. Thus, the relaxivity measurements taken over a range of pH values where differing mole fractions of free and ligand-bound gadolinium exist allow an evaluation of a conditional stability constant, K_c ($K_c = [GdL]/[Gd][L']$), at each pH value. Since the first protonation constants of these ligands could not be obtained by potentiometry but only estimated from ^1H NMR–pH titration data, the conditional constants for these three complexes are presented two ways. First, the conditional constants were corrected for competition by the second and third protonations, K_2 and K_3, and reported as K' values.[11] Given the very high values of K_1 for the three ligands, the concentration of nonprotonated chelate [L^{3-}], is essentially zero over the pH range where the relaxivity measurements were taken. Thus, $[L'] = [HL^{2-}] + [H_2L^-] + [H_3L]$ and $[L'] = [HL^{2-}]\alpha_{HL}$, where α_{HL} is given by

$$\alpha_{HL} = 1 + K_2[H^+] + K_2K_3[H^+]^2 \quad (5)$$

It follows that K_c and K' are related by

$$K' = [GdL][H^+]/[Gd][HL] = K_c\alpha_{HL}[H^+] \quad (6)$$

The experimental values of K' allow an accurate estimate of conditional constants at any pH value of interest using $\log K_c = \log K' - \log \alpha_{HL} +$ pH without concern about uncertainties in the $\log K_1$ values. Second, our best estimate of $\log K_{GdL}$ for each Gd chelate is also reported, using the $\log K_1$ values determined by NMR spectroscopy ($\log K_{GdL} = \log K' + \log K_1$), so they may be qualitatively compared with those of other macrocyclic Gd^{3+} complexes.

The stability constants of Gd(DETA) and Gd(MeDETA) were determined by measuring water proton relaxivities for two series of solutions ($[Gd]_t$ = 1 mM, $[L]_t$ = 1.2 or 2.0 mM) at various pH values. A larger ligand excess ($[Gd]_t$ = 1 mM, $[L]_t$ = 5 mM) was used to evaluate the constants for Gd(Me_2DETA). All sample solutions contained 0.1 M KCl and 0.025 M MES or piperazine buffers. The water proton spin–lattice relaxations (T_1) were determined at 40 MHz on a Spin-Lock Ltd. instrument at a single temperature (25 °C).

NMR Spectroscopy. All NMR spectra were recorded on a GN-500 spectrometer in 5-mm tubes. The sample temperatures were controlled by using the GE variable-temperature accessory and are accurate to within ±0.2 °C near room temperature and ±1 °C below 0 °C.

Kinetic Measurements. The formation rates of the complexes were measured in the pH interval 5.4–6.4, where two or three protons are released upon complexation of Gd^{3+} (eq 2 and Table I). Complex formation was followed by spectrophotometry in weakly buffered solutions (MES or piperazine) by monitoring the decrease in pH with bromocresol purple as indicator (588 nm).[12] The concentration of the indicator was 2×10^{-5} M. A suitable buffer concentration (where the pH change was between 0.05 and 0.1 pH unit) was determined experimentally, typically between 5 and 20 mM. The concentration of ligand was 1×10^{-4} M,

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Table I. Protonation Constants (log K_i) of Ligands and Stability Constants (log K' and log K_{GdL}) of the Gd^{3+} Complexes

	log K_1	log K_2	log K_3	log K'	log K_{GdL}
DETA	14.8 ± 0.18	6.12 ± 0.02	3.69 ± 0.02	0.30 ± 0.17	15.1 ± 0.3
MeDETA	15.3 ± 0.08	6.00 ± 0.02	3.65 ± 0.02	−0.64 ● 0.27	14.7 ± 0.3
Me_2DETA	13.9 ± 0.04	6.28 ± 0.02	3.78 ± 0.02	−3.47 ± 0.10	10.4 ± 0.2
NOTA	11.41[a]	5.74[a]	3.16[a]	2.30 ± 0.17	13.7 ± 0.2

[a] From ref 13.

while the concentration of Gd^{3+} was varied between 3×10^{-4} and 4×10^{-3} M. Spectrophotometric measurements were carried out on a Cary 219 spectrophotometer using 1-cm thermostated (25 °C) cells.

The dissociation rates of the complexes were determined from proton relaxivity data in acidic solutions, where the complexes are thermodynamically unstable. The concentration of Gd(DETA) or Gd(MeDETA) was 1×10^{-3} M, and the acid (HCl) concentration was varied between 0.005 and 0.1 M (at pH values >2, a 0.03 M glycine buffer was used). The experiments were started by mixing of equal volumes of complex (2 mM, pH 6.6) and acid (0.2 M KCl) solutions. Dissociation of Gd(Me_2DETA) was studied in 0.03 M glycine buffer between pH 2.4 and 3.2. Equal volumes of the complex (2 mM Gd^{3+}, 10 mM Me_2DETA, pH 7.02) and buffer (0.06 M glycine, 0.2 M KCl) were mixed initially, and the water proton relaxation rate was measured periodically.

Results and Discussion

Protonation of the Ligands. The protonation sequence of the cyclic polyaza polycarboxylate ligands differs from that of the noncyclic amino polycarboxylates, where all nitrogen atoms are typically protonated before the carboxylates are protonated. It has been shown that the first two protonations (log K_1 = 11.41, log K_2 = 5.74[13]) of the 9-membered-ring triaza tricarboxylate ligand $NOTA^{3-}$ occur at nitrogens, while the third occurs at a carboxylate.[14] The 10-membered-ring triaza macrocycle $DETA^{3-}$ has been shown to have a similar protonation sequence.[9]

The potentiometric titration curves of H_3DETA, H_3MeDETA, and H_3Me_2DETA are consistent with the titration of two protons below pH 8. The values of log K_2 and log K_3 evaluated from these curves are summarized in Table I. Between pH 8 and 12.8, the pH values obtained by titration in the absence and presence of the ligand are identical, indicating that deprotonation of a monoprotonated species did not occur below pH 12.8. This shows that the first protonation constants of the 10-membered-ring ligands must be unusually high, much higher than that of the 12-membered-ring ligand DOTRA (log K_1 = 12.8[7]). Further evidence for the existence of monoprotonated forms of these ligands at pH 12.7 was obtained as follows. One equivalent of $GdCl_3$ (0.1 M) was added dropwise to a solution of 0.05 M K_2HDETA and 0.05 M KOH (pH 12.72), whereupon an opalescent precipitate formed immediately. The precipitate dissolved in about 15 min and the pH dropped to 6.04, indicating release of 1 proton equiv. MeDETA behaves similarly. This verifies these ligands exist largely as the HL^{2-} species at pH values near 12.5.

The determination of protonation constants higher than ~13 is difficult, and in many cases, only approximate values can be obtained. Very high protonation constants can be determined by ^{1}H NMR spectroscopy if the chemical shifts of the nonlabile protons of the ligand exhibit a chemical shift change upon deprotonation. The nonlabile proton resonances of DETA, MeDETA, and Me_2DETA shift upfield (lower frequency) with increasing concentrations of KOH. The chemical shifts were determined at different KOH concentrations between 0.01 and 1.0 M with the ionic strength held constant at 1 M by addition of KCl. The results obtained for the acetate proton resonances of all three ligands are shown in Figure 1. The chemical shifts characteristic of the monoprotonated ligand (δ_{HL}) can be obtained from the data measured between pH 8 and 12, where the δ values are constant. The chemical shifts characteristic of the nonprotonated species (δ_L) cannot be obtained experimentally because deprotonation is not full at 1.0 M KOH concentration. Since

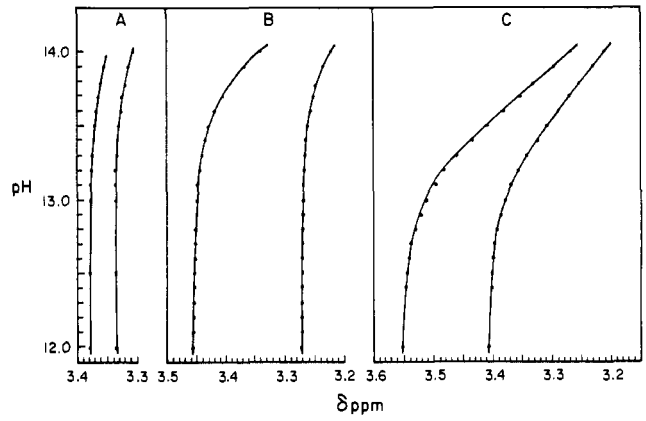

Figure 1. Chemical shifts (δ) of the acetate protons of (A) MeDETA, (B) DETA, and (C) Me_2DETA as a function of pH.

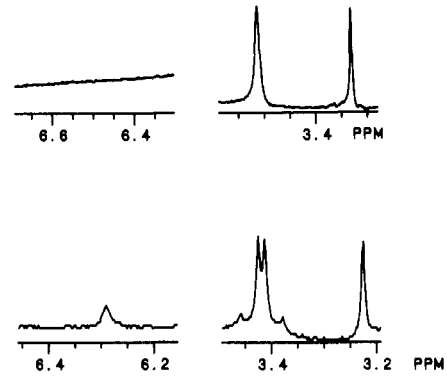

Figure 2. ^{1}H NMR resonances of the acetate protons and the hydrogen-bonded proton on $HDETA^{2-}$ at 25 °C (top) and −5 °C (bottom) in 1:4 H_2O–acetone-d_6. The pH of the sample was 7.98 at room temperature.

proton exchange is fast, the observed chemical shift (δ) may be expressed as the weighted sum of the chemical shifts of the monoprotonated and nonprotonated species

$$\delta = n_{HL}\delta_{HL} + n_L\delta_L \quad (7)$$

where $n_{HL} = [HL]/[L]_t$ and $n_L = [L]/[L]_t$. Given that $n_{HL} + n_L$ must equal 1 over this pH interval, log K_1 was estimated by assuming various values for δ_L and computer fitting the curves to eq 7. For each curve, the sum of the squares of the residuals reached a minimum value as δ_L was incremented through its true value. The value of δ_L at that minimum was accepted as the true value, and in each case this value of δ_L agreed reasonably well with that predicted on the basis of shielding constants (C_N) for similar-type ligands.[9,14] The values of log K_1 reported in Table I represent the average and standard deviation from titration curves for four different protons in DETA (only the acetate proton shifts are shown in Figure 1), five protons in Me_2DETA, but only two protons in MeDETA (the remaining proton resonances in this ligand were broadened over this pH interval). It should be noted that the value of log K_1 for DETA reported in Table I is higher than the value reported previously for this ligand[9] obtained from NMR data taken in the presence of Na^+.

Since the first protonation constant of each 10-membered triaza triacetate ligand examined here is quite high (similar to that of an amide group), we anticipated the exchange rate of these "labile" protons might be quite slow at lower temperatures, as observed

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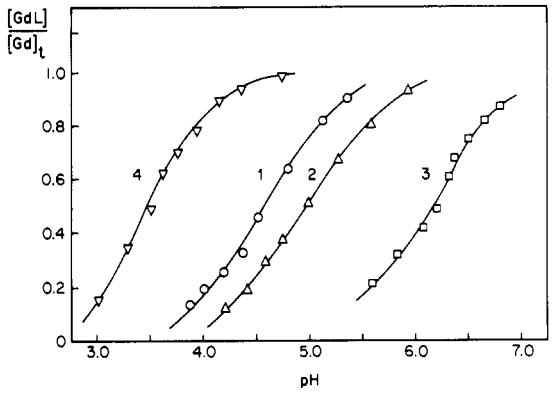

Figure 3. Extent of formation of Gd(DETA) (1), Gd(MeDETA) (2), Gd(Me_2DETA) (3), and Gd(NOTA) (4) as a function of pH. Conditions: $[Gd]_t = 1 \times 10^{-3}$ M; $[L]_t = 1.2 \times 10^{-3}$ M (curves 1, 2, and 4), $[L]_t = 5 \times 10^{-3}$ M (curve 3); 25 °C, 0.1 M KCl.

previously for amide protons.[15] The proton NMR signal of the "labile" proton in DETA could not be observed at room temperature (at pH 7.98, where the monoprotonated species, $HDETA^{2-}$, predominates), but in a 1:4 water–acetone-d_6 mixture at −5 °C, a broad signal appeared at 6.29 ppm, which became narrower at lower temperatures. Figure 2 shows the spectral region of interest, recorded at −5 °C. The integral of the resonance at 6.29 ppm relative to the two sets of acetate protons was 1:4:2, showing that the new signal arises from one proton (and not by a H_3O^+ ion strongly bonded to the nitrogens). Parallel with the appearance of the new signal, the pattern of the acetate proton resonance at high frequency (corresponding to the two acetates attached to nitrogens 1 and 7) is transformed into an AB quartet (Figure 2). This pattern is analogous to that observed for protons of an acetate group attached to a nitrogen atom when the rate of inversion of the nitrogen is slow on the NMR time scale.[16] Since the low-frequency acetate proton resonance remained a singlet at all temperatures down to −25 °C, we may conclude that the slowly exchanging proton observed in the NMR experiment is bonded between atoms 1 and 7 in $HDETA^{2-}$. Therefore, the very high value of the first protonation constant must be a consequence of formation of a strong hydrogen bond between nitrogens 1 and 7 (see I).

The synthesis of a number of unusually strongly basic monocyclic medium-ring diamines, where the nitrogens are separated by three and four (or more) carbon atoms, has been reported by Alder et al.[17-19] They also propose that the very strong basicity of these diamines reflects formation of a strong H-bond between the two nitrogens. A similar observation has been made for the bicyclic derivative of 1,5,9-triazacyclododecane,[21] which is considerably more basic (log $K > 13.5$) than 1,5,9-triazacyclododecane itself.[20] Our direct observation of a hydrogen-bonded proton between nitrogen atoms 1 and 7 of the 10-membered-ring triaza triacetate ligands supports these earlier predictions of Alder et al.[17-19] and Bell et al.[21]

Stability Constants of the Gd^{3+} Complexes. All water proton T_1 relaxation rates were measured on solutions containing Gd^{3+} plus ligand as a function of time to ensure all samples had reached equilibrium. The final results are presented as mole fraction of complex versus pH in Figure 3. Similar data for the 9-membered-ring macrocycle NOTA are also presented for comparison.

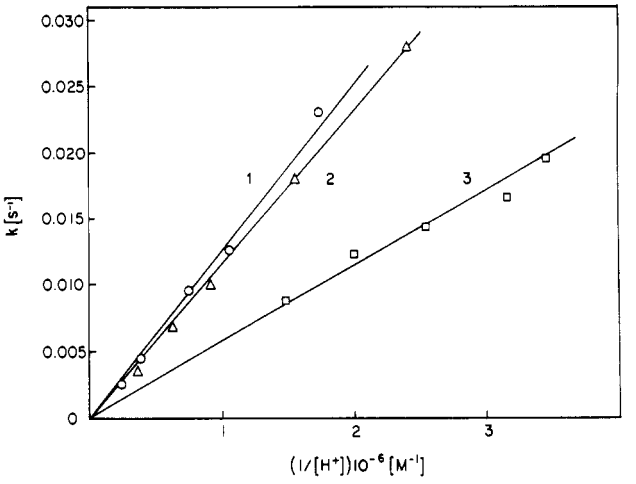

Figure 4. Formation rate constants (k) for Gd(DETA) (1) and Gd(MeDETA) (2) and k_f ($\sim k$) values for Gd(Me_2DETA) (3) versus inverse $[H^+]$.

The stability constants, K' (corrected for proton competition by the second and third protonations only), derived from these data by using eqs 4–6 are summarized in Table I. The values are reported as the average and standard deviation of log K' calculated for each solution that had been preequilibrated at a certain pH value. The data indicate that log K' for Gd(NOTA) is about 2, 3, and 6 orders of magnitude larger than the log K' values for Gd(DETA), Gd(MeDETA), and Gd(Me_2DETA), respectively. This same order is preserved in the position of the mole fraction versus pH curves shown in Figure 3; i.e., the Gd(NOTA) curve lies toward lowest pH values. Given our estimates of the first protonation constants for the three 10-membered-ring chelates from the NMR titrations, one would predict that Gd(DETA) and Gd(MeDETA) may indeed be thermodynamically more stable than Gd(NOTA) (see estimates of log K_{GdL} in Table I), yet Gd(NOTA) has a significantly higher binding constant at physiological pH than any of the 10-membered-ring congeners. This is largely due to the significantly higher first protonation constants of the 10-membered-ring macrocycles, which result in greater competition between protons and the bound Gd^{3+} for these basic nitrogen sites.

Formation Kinetics. The formation rates of the complexes were studied at different pH values as a function of excess $[Gd^{3+}]$ to ensure first-order kinetics. At a given pH, the measured k_f values increased with increasing $[Gd^{3+}]$ and plots of k_f versus $[Gd^{3+}]$ show "saturation" behavior. This is characteristic of rapid formation of a reaction intermediate that rearranges to product in a slow, rate-determining step, similar to that observed previously during formation of Gd(NOTA).[5] The raw data for formation of Gd(DETA), Gd(MeDETA), and Gd(Me_2DETA) were treated identically as in the NOTA study, so the details will not be repeated here (see supplementary material). The reaction intermediate formed in the rapid preequilibrium step is likely a monoprotonated species, GdHL*, on the basis of the following evidence. Upon the mixture of dilute, nonbuffered or weakly buffered solutions of $GdCl_3$ and DETA or MeDETA at pH 6 (where species HL^{2-} and H_2L^- predominate), a rapid pH drop is observed, followed by a further slow decrease in pH. The number of protons released during the slow process is exactly equivalent to the amount of GdL formed, thereby confirming that the intermediate is a monoprotonated species. Furthermore, the number of protons released during the rapid pH drop was consistent with that expected for a mixture of $H_nL^{(3-n)-}$ species reacting with Gd^{3+} to form the intermediate, GdHL*. We propose that the carboxylate groups and perhaps one of the nitrogens in $HDETA^{2-}$ or $HMeDETA^{2-}$ coordinate to the Gd^{3+} ion in the GdHL* species, while the single, very basic nitrogen remains protonated. The formation of a protonated intermediate has been detected by spectrophotometry during the reaction of DOTA with the Ce^{3+} ion.[23] The stability constants of the protonated inter-

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Table II. Formation (k_{OH}) and Dissociation (k_d^0, k_d^1) Rate Constants of the Complexes

	k_{OH}, M^{-1} s^{-1}	k_d^0, s^{-1}	k_d^1, M^{-1} s^{-1}
Gd(DETA)	$(8.2 \pm 2) \times 10^5$	$(5.7 \pm 1) \times 10^{-6}$	$(4.5 \pm 0.6) \times 10^{-3}$
Gd(MeDETA)	$(7.3 \pm 1.6) \times 10^5$	$(3.3 \pm 0.6) \times 10^{-6}$	$(1.6 \pm 0.2) \times 10^{-3}$
Gd(Me_2DETA)	$(3.8 \pm 1) \times 10^4$	$(8.3 \pm 1) \times 10^{-4}$	0.5 ± 0.1
Gd(NOTA)[a]	$(7.1 \pm 1) \times 10^7$	$(8.3 \pm 1) \times 10^{-6}$	$(2.3 \pm 0.3) \times 10^{-2}$

[a] From ref 5.

mediates extracted from the kinetic data (supplementary material) indicate that Gd(HMeDETA)* is somewhat less stable than Gd(HDETA)* (log K_{GdHL}* = 3.13 versus 3.4, respectively) and that this difference in stabilities is comparable to the differences in log K' values for these two complexes (Table I).

The rates of rearrangement of these GdHL* species to the final, thermodynamically stable, fully chelated structures are clearly inversely proportional to pH, as shown in Figure 4. This indicates that the OH^- ion catalyzes proton loss and subsequent rearrangement of the intermediate, and we suggest that this corresponds to a stepping of the Gd^{3+} ion into the central coordination cage of the triaza macrocycle.

$$GdHL^* + OH^- \xrightarrow{k_{OH}} GdL + H_2O \quad (8)$$

Since the straight lines in Figure 4 intersect the abscissa near zero, the rate of spontaneous rearrangement of the intermediate must be very slow. The rate constants, k_{OH}, calculated from the slopes of the lines in Figure 4 (log K_w = 13.80) are summarized in Table II.

The formation rates (k_{OH}) measured for Gd(DETA) and Gd(MeDETA) are identical within experimental error but are about 2 orders of magnitude lower than the value of k_{OH} found previously for Gd(NOTA).[5] This indicates that an increase in the ring size from 9 to 10 has a substantial effect upon the reactivity of these macrocyclic ligands. This likely reflects conformational differences in the GdHL* intermediates resulting from the different steric requirements of the ethylene versus propylene chelate rings. Substitution of two methyl groups onto the same propylene ring carbon in Me_2DETA accentuates this structural feature the most, thereby giving this complex the lowest rate of formation.

Dissociation Kinetics. The dissociation rates of these same complexes were studied at pH values where the complexes are thermodynamically unstable. In order to avoid a change in pH resulting from the protonation of released ligands, a 0.03 M glycine solution was used to buffer all solutions above pH 2. The presence of buffer had no effect on the rate of dissociation of the complexes. Under these conditions, dissociation occurs via a first-order process according to

$$-d[GdL]/dt = k_d[GdL] \quad (9)$$

The rate constants, k_d, were found to be linearly proportional to $[H^+]$, as shown in Figure 5. Since the lines in Figure 5 have positive intercepts, we may assume that dissociation can take place via pH-dependent and -independent pathways

$$k_d = k_{d,0} + k_{d,1}[H^+] \quad (10)$$

where the rate constant $k_{d,0}$ represents spontaneous dissociation of the complex and $k_{d,1}$ reflects the proton-assisted pathway. The values of $k_{d,0}$ and $k_{d,1}$ calculated from the data are presented in Table II. If the slow, rate-determining step during formation of the complexes does indeed involve proton loss from a nitrogen, followed by repositioning of the Gd^{3+} ion into the macrocyclic cavity, then dissociation must occur via reversal of this process. The data are consistent with movement of the Gd^{3+} ion out of the macrocyclic cavity to a position where the carboxylates and

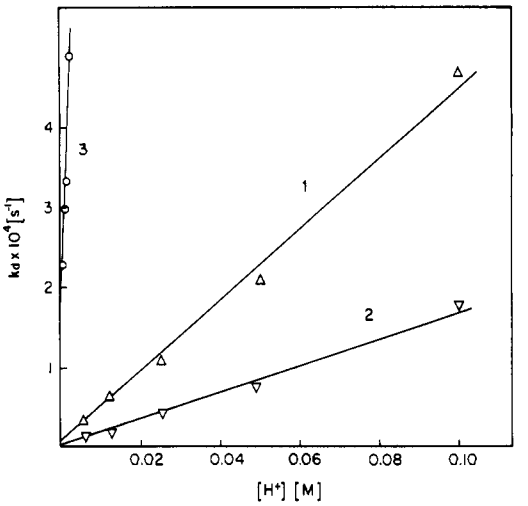

Figure 5. Dissociation rates (k_d) for Gd(DETA) (1), Gd(MeDETA) (2), and Gd(Me_2DETA) (3) as a function of $[H^+]$.

perhaps one or two nitrogens are coordinated, in a slow rate-determining process ($k_{d,0}$). Dissociation of this weakly coordinated Gd^{3+} then occurs very rapidly. Alternatively, one of the nitrogen atoms may become protonated during the rate-determining step ($k_{d,1}$), and this would assist removal of the Gd^{3+} ion from the cavity due to electrostatic repulsion between the protonated nitrogen and the Gd^{3+}. Formation of this protonated complex would also be quite slow, since it would require inversion of a coordinated nitrogen.

A comparison of the rate constants $k_{d,0}$ and $k_{d,1}$ (Table II) indicates that both the spontaneous and proton-assisted dissociation rates decrease in the order Gd(Me_2DETA) $\gg$ Gd(NOTA) > Gd(DETA) > Gd(MeDETA). Both rate constants are significantly affected by ring size and ring substitution. An increase in ring size from 9 (NOTA) to 10 (DETA) leads to an increase in kinetic stability likely because the Gd^{3+} ion fits into a 10-membered-ring macrocycle somewhat better than a 9-membered-ring macrocycle. Substitution of a single methyl group onto one of the propylene chelate rings increases the rigidity of the macrocycle, yielding a complex with even greater kinetic stability [Gd(MeDETA) versus Gd(DETA)]. Methyl substitution in other polyazacycloalkane ligands has also been shown to increase the inertness of their resulting complexes.[24] Substitution of two methyl groups onto a single propylene chelate ring carbon, however, has a detrimental effect upon rates of formation and dissociation and upon the resulting thermodynamic stability of Gd(Me_2DETA). Although our data do not trace the origins of these observations, it is likely that the 6-membered propylene chelate ring conformation in Gd(Me_2DETA) is substantially different from those in Gd(DETA) and Gd(MeDETA) and this conformation allows easier dissociation of one of the macrocyclic nitrogens from the Gd^{3+} coordination sphere.

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Supplementary Material Available: A textual presentation of the formation kinetics of Gd(DETA), Gd(MeDETA), and Gd(Me_2DETA), a table of K* and log K_{GdHL}* values for the reaction intermediates, where L = DETA and MeDETA, and figures showing reciprocal values of formation rates of Gd(DETA) and Gd(MeDETA) as a function of $1/[Gd^{3+}]$ (5 pages). Ordering information is given on any current masthead page.

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