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Synthesis of some *N,N″*-bis(amide) derivatives of diethylenetriaminepentaacetic acid and the stabilities of their complexes with Gd^{3+}, Ca^{2+}, Cu^{2+} and Zn^{2+}†

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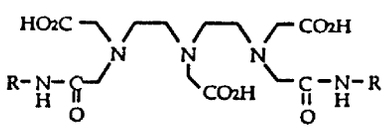
Three *N,N″*-bis(amide) derivatives of H_5dtpa (diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid), H_3L^1 = bis(isopropylamide), H_3L^2 = bis(*tert*-butylamide) and H_3L^3 = bis(benzylamide), were synthesized. Their protonation constants were determined by potentiometric titration in 0.10 mol dm^{-3} KCl and by NMR pH titration at 25 ± 0.1 °C. Stability and selectivity constants were measured to evaluate the possibility of using the corresponding gadolinium(III) complexes as magnetic resonance imaging contrast agents. The formation of the gadolinium(III), copper(II), zinc(II) and calcium(II) complexes were investigated quantitatively by potentiometry. The stability constant determined for Gd^{III} is larger than those for Ca^{II}, Zn^{II} and Cu^{II} for these octadentate ligands. The selectivity constants and modified selectivity constants of the amides for Gd^{3+} over endogenously available metal ions were calculated.

Gadolinium complexes of linear poly(aminocarboxylate) ligands are of considerable interest as contrast agents in magnetic resonance imaging (MRI).[1,2] The octachelating ligands, diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid [(carboxymethyl)iminobis(ethylenenitrilo)tetraacetic acid] (H_5dtpa), *N,N″*-di(methylcarbamoylmethyl)diethylenetriamine *N,N′,N″*-triacetate (H_3dmdtta), 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (H_3hpdotra) and 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (H_4dota) are effective MRI contrast agents when complexed with the trivalent gadolinium ion.[3] These gadolinium chelates possess sufficient paramagnetism and high stability. In order to obtain high contrast for lesion, administration of high doses of contrast agent are sometimes required, especially for non-ionic gadolinium complexes which may be used as low-osmolality contrast agents for MRI.[4] The toxic effects of uncomplexed Gd^{3+} and free pro-ligand arising from dissociation of the metal complex is one of the major concerns in MRI.[5–11] The acute toxicity of gadolinium complexes of the poly(aminocarboxylates) correlates well with the selectivity of the latter for Gd^{3+}. The release of Gd^{3+} is related to the stability constants of the gadolinium complexes.[12,13] This report describes the synthesis of three *N,N″*-bis(amide) derivatives of H_5dtpa, *i.e.* H_3L^1 = the bis(isopropylamide), H_3L^2 = the bis(*tert*-butylamide) and H_3L^3 = the bis(benzylamide). Their protonation constants, thermodynamic and conditional stability constants of complexes with Gd^{3+}, Cu^{2+}, Zn^{2+} and Ca^{2+} and their selectivity for Gd^{3+} over endogenously available metal ions are discussed.

Experimental

Materials

Gadolinium chloride (>99.9%) was obtained from Aldrich Chemical Co. and oven dried at 110 °C for at least 24 h before use. All other reagents used for the synthesis of the amides were from commercial sources unless otherwise noted. Proton NMR spectra and elemental analyses were used to confirm the composition of the products.



	R
H_3L^1	$CH(CH_3)_2$
H_3L^2	$C(CH_3)_3$
H_3L^3	$CH_2C_6H_5$

Preparations

Diethylenetriamine-*N,N′,N″*-triacetic *N,N″*-dianhydride. The anhydride was prepared according to the method of Eckelman *et al.*[14] White solid (33.23 g, 93%); m.p. 183–185 °C; δ_H[200 MHz, solvent $(CD_3)_2SO$, standard $SiMe_4$] 3.71 (8 H, s, terminal NCH_2CO_2), 3.31 (2 H, s, central NCH_2CO_2), 2.75 (4 H, t, NCH_2) and 2.60 (4 H, t, NCH_2).

***N,N″*-Bis(amides) of H_5dtpa: H_3L^1, H_3L^2 and H_3L^3.** The compounds H_3L^1, H_3L^2 and H_3L^3 were synthesized by modification of the procedure of Konings *et al.*[15] The dianhydride (5.0 g, 13.99 mmol) was added in portions over 1 h to ice-cold stirred 99% alkylamine (235 mmol). After 30 min the ice-bath was removed and the reaction mixture stirred at room temperature for 20 h. It was concentrated under reduced pressure to an oil, diluted with water (30 cm^3) and adjusted to pH 1.5 with concentrated HCl. The colourless solid formed was collected and recrystallized from water–acetone to give colourless crystals.

N,N″-*Bis*(*isopropylamide*) H_3L^1. White crystals (3.9 g, 57%), m.p. 192–193 °C (Found: C, 48.8; H, 7.8; N, 14.05. $C_{20}H_{37}N_5O_8 \cdot H_2O$ requires C, 48.65; H, 7.95; N, 14.2%); δ_H(200 MHz, solvent D_2O–NaOD, standard $SiMe_4$) 3.84 [2 H, m, $NCH(CH_3)_2$], 3.08 (4 H, s, NCH_2CON), 3.06 (4 H, s, terminal NCH_2CO_2), 3.02 (2 H, s, central NCH_2CO_2), 2.57 (8 H, s, NCH_2CH_2N) and 1.05 [12 H, d, $NCH(CH_3)_2$].

N,N″-*Bis*(tert-*butylamide*) H_3L^2. White crystals (4.1 g, 56%),

† *Supplementary data available* (No. SUP 57205, 7 pp.): titration curves and NMR spectra. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1997, Issue 1.

m.p. 175–177 °C (Found: C, 50.85; H, 8.2; N, 13.3. $C_{22}H_{41}N_5O_8 \cdot H_2O$ requires C, 50.65; H, 8.3; N, 13.4%); δ_H 3.07 (4 H, s, terminal NCH_2CO_2), 3.04 (2 H, s, central NCH_2CO_2), 3.01 (4 H, s, terminal NCH_2CON), 2.60 (8 H, s, NCH_2CH_2N) and 1.24 (18 H, s, C H_3).

N,N″-*Bis*(*benzylamide*) H_3L^3. White crystals (5.12 g, 60%), m.p. 103–105 °C (Found: C, 55.45; H, 6.85; N, 11.35. $C_{28}H_{37}N_5O_8 \cdot 2H_2O$ requires C, 55.35; H, 6.8; N, 11.55%); δ_H 7.26 (10 H, m, aryl H), 4.30 (4 H, s, $PhCH_2N$), 3.13 (4 H, s, terminal NCH_2CO_2), 3.05 (4 H, s, NCH_2CON), 2.88 (2 H, s, central NCH_2CO_2) and 2.44 (8 H, m, NCH_2CH_2N).

General techniques

Proton NMR spectra were measured in D_2O solution on a Varian XL-200E spectrometer. The pD of the amide solutions were determined with a microelectrode. The final pD was obtained from the equation pD = pH + 0.40.[16]

Solution preparations

Stock solutions of Ca^{2+}, Zn^{2+}, Cu^{2+} and Gd^{3+} were prepared between 0.015 and 0.02 mol dm^{-3} from the nitrate salts with demineralized water (obtained by a Millipore/Milli-Q system) and standardized by titration with Na_2H_2edta (disodium salt of ethylenedinitrilotetraacetic acid) or atomic absorption spectrophotometry. A stock solution was prepared by dissolving reagent grade Na_2H_2edta (4.65 g) and diluting it to 250 cm^3 with demineralized water. This was used as a titrant to standardize the solutions of Gd^{3+} and Ca^{2+}. A weakly acidic gadolinium chloride titrant solution was prepared at pH 5 by using a 0.5 mol dm^{-3} acetate buffer and one drop of pyridine. Six drops of xylenol orange were added as an indicator, followed by titration with Na_2H_2edta solution until the solution changed from purple to yellow. This Gd^{3+}(aq) solution was used to standardize solutions of the linear poly(aminocarboxylates). Titrant solutions of the latter consisted of approximately 2.0–0.6 mmol dm^{-3} solute, to which acetate buffer pH 5 and one drop of pyridine were added. Six drops of indicator solution (xylenol orange) were added followed by titration with stock gadolinium(III) solution until it changed from yellow to purple.[17] Stock gadolinium(III) complex solutions (henceforth identified as GdL and having a concentration range of 1.5–0.5 mmol dm^{-3}) were prepared by mixing equimolar amounts of stock solutions of Gd^{3+} and amide. A slight excess (2%) of amide was used to ensure total complexation of Gd^{3+}.

Potentiometric measurements

Potentiometric titrations were performed with an automatic titrator system to determine the protonation constants of the amides and the stability constants of the metal complexes. The autotitrating system consists of a VIT 90 titrator, a ABU 93 digital autoburette, a SAM 90 sample station and a K601 combination pH electrode (Radiometer). The pH electrode was calibrated using two standard buffer solutions and all calibrations and titrations were carried out under a CO_2-free nitrogen atmosphere in a sealed glass vessel (20 cm^3) thermostatted at 25 ± 0.1 °C and an ionic strength of 0.10 mol dm^{-3} KCl. The concentrations of the metal-ion and amide solutions were maintained between 2.0 and 0.6 mmol dm^{-3}. A CO_2-free 0.100 mol dm^{-3} NaOH solution was used as the titrant to minimize ionic strength changes during the titration. The purity of the amides was also confirmed by potentiometric titration with standard NaOH. Oxygen and carbon dioxide were excluded from the reaction mixtures by maintaining a positive pressure of purified nitrogen in the titration cell. More than 200 data points were collected for each experiment. The electromotive force of the cell is given by $E = E'^{\circ} + Q\log[H^+] + E_j$ and both E'° and Q were determined by titrating a solution of known hydrogen-ion concentration at the same ionic strength, using the acid range of the titration. The liquid-conjuction potential, E_j, was found to be negligible under the experimental conditions used.

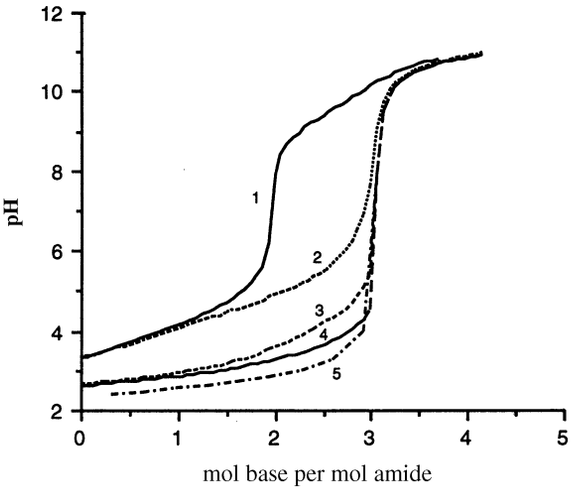


Fig. 1 Potentiometric titration curves for H_3L^1 and 1:1 ratios of various metal nitrates: 1, amide; 2, Ca^{2+}; 3, Zn^{2+}; 4, Cu^{2+}; 5, Gd^{3+}; 25 °C, I = 0.10 mol dm^{-3} (KCl)

Computational method

The protonation constants of the amides were calculated using a FORTRAN computer program PKAS[18] written for polyprotonic weak acid equilibria. The overall stability constants of the various metal complexes formed in aqueous solution were determined from the titration data with the FORTRAN computer program BEST.[18]

The accuracy of this method was verified by measuring the protonation constants and the stability constants for the complexes of Ca^{2+}, Zn^{2+}, Cu^{2+} and Gd^{3+} with the bis(methylamide) derivative of dtpa (H_3dmdtta). The results of our titration technique were compared with literature values.[13] The agreement was excellent, with an average deviation in log K_n and log K_{ML} of 0.13 and 0.15. This is acceptable since literature values for these constants are reported with errors of ± 0.2 log K units.

Relaxation time measurement

Relaxation times T_1 and T_2 of aqueous solutions of gadolinium(III) complexes of linear bis(amide) derivatives of dtpa were measured to determine the relaxivity R_1 and R_2. All measurements were made using a NMR spectrometer operating at 20 MHz and 37 ± 0.1 °C (NMS 100 Minispec, Bruker). Prior to each measurement the spectrometer was tuned and calibrated. The value of T_1 was measured from eight data points generated by an inversion-recovery pulse sequence; T_2 was measured from 10 data points using a Carr–Purcell–Meiboom–Gill pulse sequence with τ = 1 ms. The slopes of plots of $1/T_1$ and $1/T_2$ *versus* concentration give R_1 and R_2 in dm^3 $mmol^{-1}$ s^{-1}.

Results and Discussion

Protonation constants

The amide protonation constants are expressed as in equation (1). The potentiometric titration curve for H_3L^1 is shown in

$$K_n = [H_nL]/[H_{n-1}L][H^+] \quad (1)$$

Fig. 1. Those corresponding to H_3L^2 and H_3L^3 have been deposited (SUP 57205). Table 1 summarizes the protonation constants of the bis(amides) measured in the range pH 3–10. The titration curves of H_3L^1, H_3L^2 and H_3L^3 all show a very sharp increase between pH 9.0 and 4.0 (mol of base per mol amide present = 2). This is due to the large difference between

Table 1 Protonation constants log K_n with uncertainties (σ) in parentheses

	log K_n[25 °C, I = 0.10 mol dm^{-3} (KCl)]				
K_n	H_3L^1	H_3L^2	H_3L^3	H_3dmdtta[a]	H_5dtpa[b]
[HL]/[L][H]	9.39 (0.03)	9.45 (0.03)	9.39 (0.04)	9.37 (0.01)	10.49
[H_2L]/[HL][H]	4.49 (0.01)	4.51 (0.02)	4.57 (0.01)	4.38 (0.01)	8.60
[H_3L]/[H_2L][H]	3.59 (0.01)	3.72 (0.01)	3.54 (0.04)	3.31 (0.04)	4.28
[H_4L]/[H_3L][H]					2.64
ΣpK_a	17.59	18.01	17.50	17.06	26.01

[a]Data were obtained from ref. 13. [b]Ref. 19.

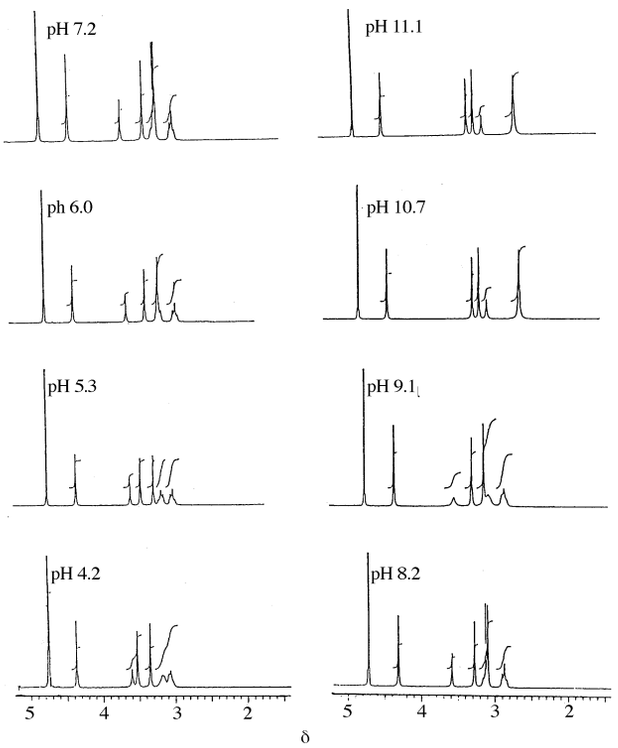


Fig. 2 Proton NMR spectra of H_3L^3 as a function of pH

the first (log K_1) and second protonation constant (log K_2) *i.e.* 9.39 and 4.49, 9.45 and 4.51 and 9.39 and 4.57, respectively. The log K_3 (third protonation constant) values are 3.59, 3.72 and 3.54, respectively. The carboxylates have log K_4 and log K_5 values below 2 (not observed in this study). The present protonation constants are very similar to those of H_3dmdtta (log K_1 = 9.37, log K_2 = 4.38, log K_3 = 3.31 in 0.1 mol dm^{-3} $NaClO_4$).[20] The first protonation is presumably at the central nitrogen. The lower value of the second could be assigned to protonation of one of the terminal nitrogen atoms. The significant difference between log K_1 and log K_2 may result from the disruption of hydrogen bonding between the carboxylate groups and amide proton in the bis(amide) backbone.[20–23] The third protonation constant is assigned to protonation of the remaining terminal nitrogen.

The replacement of the two carboxylate groups in H_5dtpa by the two *N*-isopropyl-, *N*-*tert*-butyl- and *N*-benzyl-amide groups results in a decrease in log K_1 (*i.e.* 1.10, 1.10, 1.04 units), log K_2 (*i.e.* 4.11, 4.09, 3.76 units), log K_3 (*i.e.* 0.69, 0.56, 0.74 units) and ΣpK_a values (*i.e.* 8.42, 8.00, 8.51 units). These significant differences in basicity of the amine groups (ΣpK_a) are presumably due to hydrogen bonding from the terminal nitrogen atom and carboxylate to amide protons in the bis(amides).

NMR pH titration

The macroscopic protonation constants of the amides in Table 1 determined by the potentiometric titration technique do not give a clue to the specific preference of the protonation sites. However, the microscopic protonation scheme which is obtained by NMR spectroscopy coupled with pH titration will. This is constructed by measuring the chemical shifts of the methylenic protons as a function of pH. The protonation of a basic site of a poly(aminocarboxylate) in acidic solution leads to a deshielding of the adjacent methylene protons.[24] The ^{1}H NMR spectra of H_3L^3 as a function of pH are shown in Fig. 2. Those of H_3L^1 and H_3L^2 have been deposited (SUP 57205). These show that the central nitrogen atom is the most basic. Coalescence of the triplets corresponding to the ethylenic group occurs at pH > 9.0 for H_3L^1, H_3L^2 and H_3L^3. Plots of the chemical shift values (δ) of the methylenic resonance of H_3L^3 as a function of pH are given in Fig. 3 (for H_3L^1 and H_3L^2 see SUP 57205). There are three inflections centred at pH about 9.5, 4.5 and 3.5 in each case. The downfield shift of δ in the region pH 4.5–9.5 indicates that the first observed protonation occurs on the central nitrogen atom, which is common to all these *N*-substituted carboxylic systems. At about pH 4.5 a downfield δ shift was also observed for one of the terminal nitrogen atoms which is the second protonation site. These values correlate quite well with the protonation constants of the amides in Table 1 and reflect stepwise protonation of the amino groups of the bis(amides) of dtpa with the formation of the species HL^{2-}, H_2L^- and H_3L.

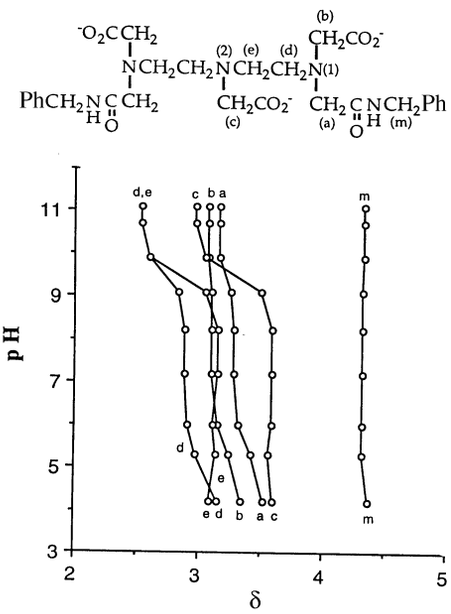


Fig. 3 Proton NMR titration curves for H_3L^3

Thermodynamic stability constants

The stability of the different gadolinium(III) complexes can be expressed in four ways: (1) the thermodynamic stability constant

Table 2 Stability constants and selectivity constants of complexes of Gd^{3+}, Zn^{2+}, Ca^{2+} and Cu^{2+}. Uncertainties (σ) in log *K* values are given in parentheses

	log *K* [25 °C, *I* = 0.10 mol dm^{-3} (KCl)]				
Parameter	H_3L^1	H_3L^2	H_3L^3	H_3dmdtta[a]	H_5dtpa[b]
log *K* ([GdL]/[Gd][L])	17.07 (0.04)	17.15 (0.03)	16.48 (0.05)	16.85 (0.05)	22.46
log K_{GdL}' (pH 7.4)	15.07	15.09	14.48	14.84	18.14
log *K* ([CaL]/[Ca][L])	7.39 (0.05)	7.45 (0.03)	7.13 (0.04)	7.17 (0.04)	10.75
log K_{CaL}' (pH 7.4)	5.39	5.39	5.13	5.11	6.43
log *K* ([CuL]/[Cu][L])	13.38 (0.09)	13.66 (0.13)	12.28 (0.07)	13.03 (0.03)	21.38
log K_{CuL}' (pH 7.4)	11.38	11.60	10.28	11.06	17.06
log *K* ([ZnL]/[Zn][L])	12.31 (0.09)	12.43 (0.12)	11.98 (0.09)	12.04 (0.03)	18.70
log K_{ZnL}' (pH 7.4)	10.31	10.37	9.98	10.02	14.38
Selectivity [log *K* (Gd/Zn)]	4.76	4.72	4.50	4.81	3.76
[log *K* (Gd/Ca)]	9.68	9.70	9.35	9.73	11.71
[log *K* (Gd/Cu)]	3.69	3.49	4.20	3.78	1.08
log K_{sel}'	8.97	8.89	8.78	9.03	7.04

[a]Data were obtained from ref. 13. [b]Refs. 19 and 25.

of gadolinium complex, $K_{GdL(therm)}$ (*i.e.* the stability constant at pH > 11), (2) the conditional stability constants at pH 7.4, $K_{GdL(cond)}$ (*i.e.* the thermodynamic stability constants at pH 7.4),[13] (3) the selectivity constant, K_{sel} {the difference between the thermodynamic stability constant of the gadolinium complex [log $K_{GdL(therm)}$] and that of endogenously available metal ions [$K_{ZnL(therm)}$, $K_{CaL(therm)}$ and $K_{CuL(therm)}$]},[9] and (4) the modified selectivity constant, K_{sel}' (the stability corrected for competition between the endogenously available metal ion and H^+).[13]

The normal chelate thermodynamic stability constants [$K_{ML(therm)}$] are expressed as in equation (2) where M represents

$$K_{ML(therm)} = [ML]/[M][L] \quad (2)$$

the free, unhydrolysed aquametal ion, L the uncomplexed, totally deprotonated form of the ligand and ML is the normal unprotonated and unhydrolysed complex. The potentiometric titration curves for the complexes of Gd^{3+}, Cu^{2+}, Ca^{2+} and Zn^{2+} with H_3L^1 are shown in Fig. 1. Those with H_3L^2 and H_3L^3 have been deposited (SUP 57205). All curves have an inflection point at 3 mol base added per mol amide. The $[CaL^1]^-$, $[CaL^2]^-$ and $[CaL^3]^-$ curves increase rapidly from pH 6 to 10. The titration curves for the complexes of Gd^{3+}, Cu^{2+} and Zn^{2+} with H_3L^1, H_3L^2 and H_3L^3 increase from pH 3.5 to 10. In Table 2 the thermodynamic stability constants are presented for the linear poly(aminocarboxylates) H_3L^1, H_3L^2, H_3L^3, H_3dmdtta and H_5dtpa. The similar stability constants of the complexes of Gd^{3+}, Cu^{2+}, Ca^{2+} and Zn^{2+} with the bis(amides) throughout the series (*i.e.* H_3L^1, H_3L^2, H_3L^3 and H_3dmdtta) may indicate similar ligand basicities. Since the basicity of H_3L^1 ($\Sigma pK_a = 17.59$), H_3L^2 (18.01) and H_3L^3 (17.50) is very similar to that of H_3dmdtta (17.06) the contribution of the enthalpy term to the thermodynamic stability should be similar in the complexes of Gd^{3+}, Ca^{2+}, Cu^{2+} and Zn^{2+}. Thus, the lower stability of the bis(amide) dtpa chelates when compared to the dtpa chelates is assigned to the weaker donor ability of the amide group and the lower basicity of the terminal nitrogen atoms.

Conditional stability constants and selectivity constants

For biological studies the conditional stability of a metal chelate under physiological conditions (pH 7.4) is more important than the thermodynamic stability constant. The former shows the extent of metal chelation at pH 7.4 and can be expressed by equation (3) [9,13] where K_1, K_2, K_3, ... K_n are the stepwise ligand

$$K_{ML(cond)} = K_{ML(therm)}\,(1 + K_1[H^+] + K_1K_2[H^+]^2 + K_1K_2K_3[H^+]^3 + ...)^{-1} \quad (3)$$

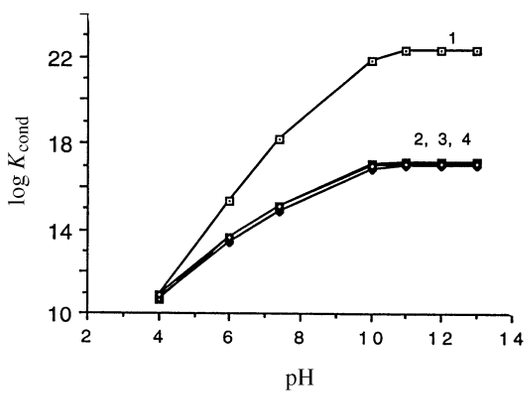


Fig. 4 Variation of the conditional stability constants for $[Gd(dtpa)]^{2-}$ (1), $[GdL^1]$ (2), $[GdL^2]$ (3) and $[GdL^3]$ (4) with pH

protonation constants. In Table 2, the conditional stability constants at pH 7.4 are presented for the five poly(aminocarboxylates) H_3L^1, H_3L^2, H_3L^3, H_3dmdtta and H_5dtpa. Their order is $[Gd(dtpa)]^{2-} > [GdL^1] \approx [GdL^2] > [Gd(dmdtta)] > [GdL^3]$. This suggests the conditional stability constants of the gadolinium complexes are dependent on the ligand basicity.

Fig. 4 shows the pH dependence of the conditional stability for the complexes $[Gd(dtpa)]^{2-}$, $[GdL^1]$, $[GdL^2]$ and $[GdL^3]$. The results for $[GdL^1]$, $[GdL^2]$ and $[GdL^3]$ are very similar. The conditional stability constants at pH > 11 for $[Gd(dtpa)]^{2-}$ and the gadolinium complexes of the bis(amide) derivatives differ by a factor of $10^{5.0}$–$10^{6.0}$ which is higher than that at pH 7.4 ($10^{3.0}$–$10^{3.5}$). This indicates that the stability of $[Gd(dtpa)]^{2-}$ is only slightly higher than those of $[GdL^1]$, $[GdL^2]$ and $[GdL^3]$ at pH 7.4.

The logarithmic selectivity constant [9,13] of H_3L^1, H_3L^2, H_3L^3, H_3dmdtta and H_5dtpa for Gd^{3+} over Zn^{2+}, Ca^{2+} and Cu^{2+} is the difference between log K_{GdL} and log K_{ML} (M = Zn^{2+}, Ca^{2+} or Cu^{2+}) *i.e.* log *K*(Gd/Zn), log *K*(Gd/Ca) and log *K*(Gd/Cu). The selectivity constants are also given in Table 2. Since the basicity of H_3L^1, H_3L^2 and H_3L^3 is very similar to that of H_3dmdtta, the contribution of the stability constant to the selectivity constant should be similar for the complexes of Gd^{3+}, Cu^{2+}, Ca^{2+} and Zn^{2+}. From the selectivity constants, H_3L^1, H_3L^2 and H_3L^3 show a slightly more favourable selectivity toward Gd^{3+} over Zn^{2+} than does H_5dtpa.

The consequences of the selectivity for Gd^{3+} over other endogenous metal ions (Cu^{2+}, Ca^{2+} and Zn^{2+}) and H^+ for a ligand can be calculated by using equation (4).[13] This equation

$$K_{sel}' = K_{ML(therm)}(\alpha_{H^{-1}} + \alpha_{CaL^{-1}} + \alpha_{CuL^{-1}} + \alpha_{ZnL^{-1}})^{-1} \quad (4)$$

Table 3 Relaxivities T_1 and T_2 of gadolinium(III) complexes

Complex	T_1 relaxivity (R_1)/dm^3 mmol^{-1} s^{-1}	T_2 relaxivity (R_2)/dm^3 mmol^{-1} s^{-1}
[GdL^1]	3.99	5.99
[GdL^2]	3.84	5.30
[GdL^3]	4.08	6.06

is obtained by the incorporation of ligand equilibria with Cu^{2+}, Ca^{2+}, Zn^{2+} and H^+ where α is a side reaction coefficient defined as in equations (5)–(8). Iron(III) was not considered

$$\alpha_{H^{-1}} = 1 + K_1[H^+] + K_1K_2[H^+]^2 + K_1K_2K_3[H^+]^3 + ... \quad (5)$$

$$\alpha_{CaL^{-1}} = 1 + K_{CaL}[Ca^{2+}] \quad (6)$$

$$\alpha_{CuL^{-1}} = 1 + K_{CuL}[Cu^{2+}] \quad (7)$$

$$\alpha_{ZnL^{-1}} = 1 + K_{ZnL}[Zn^{2+}] \quad (8)$$

because it is tightly bound by the proteins ferritin and haemosiderin and could not interact with the gadolinium(III) complex. Table 2 shows the modified selectivity constants of H_3L^1, H_3L^2, H_3L^3, H_3dmdtta and H_5dtpa at pH 7.4. The concentrations of Ca^{2+}, Cu^{2+} and Zn^{2+} used were 2.5, 1.0×10^{-3} and 5.0×10^{-2} mmol dm^{-3}, respectively.[13] The log K_{sel}' of H_3L^1 (8.97), H_3L^2 (8.89) and H_3L^3 (8.78) are very similar to that of H_3dmdtta (9.03), but slightly higher than that of H_5dtpa (7.04). Thus, H_3L^1, H_3L^2 and H_3L^3 form gadolinium complexes that are more stable than $[Gd(dtpa)]^{2-}$ toward transmetallation with endogenous metal ions at pH 7.4.

Relaxometry studies

The relaxivities R_1 and R_2 of [GdL^1], [GdL^2] and [GdL^3] are given in Table 3. The R_1 values are similar to the 3.9 dm^3 mmol^{-1} s^{-1} determined for the gadolinium complex of H_3-dmdtta under the same experimental conditions.[4] The relaxivity of a paramagnetic metal complex consists of two components: the inner-sphere and outer-sphere relaxivities. Since all ligands studied have similar functional groups and the final chelate structures and sizes are similar, it is assumed that, to a first approximation, the outer-sphere relaxivities are similar. Thus, the observed relaxivity variation is primarily attributed to the variation in the inner-sphere contribution. The small difference in the relaxivity R_1 of [GdL^1], [GdL^2] and [GdL^3] indicates that the number of inner-sphere water molecules is identical for all three complexes in aqueous solution.

High stability and high R_1 relaxivity of metal chelates are important prerequisites for potential use as magnetopharmaceuticals. The facts that the gadolinium complexes of H_3L^1, H_3L^2 and H_3L^3 are quite stable in aqueous solution, do not dissociate under physiological conditions (pH 7.4) and do not exchange with Ca^{2+}, Cu^{2+} and Zn^{2+} to an appreciable extent show that they may be effective MRI contrast agents. The water solubilities of [GdL^3] and [GdL^2] (1.15 g and 12.08 g in 100 g of water at 25 °C, respectively) are considerably lower than that of [GdL^1] (26.50 g). Thus, the non-ionic chelates [GdL^2] and [GdL^3] have high lipophilicity and may be considered for use as hepatobiliary MRI contrast agents.

Acknowledgements

We are grateful to the National Science Council of the Republic of China for financial support under Contract No. NSC 85-2113-M037-012. Valuable comments on the manuscript from Drs. C. A. Chang and M. Y. Chiang are gratefully acknowledged.

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Received 6*th August* 1996; *Paper* 6/05483H