DALTON
FULL PAPER

Synthesis and complexation of Gd^{3+}, Ca^{2+}, Cu^{2+} and Zn^{2+} by 3,6,10-tri(carboxymethyl)-3,6,10-triazadodecanedioic acid

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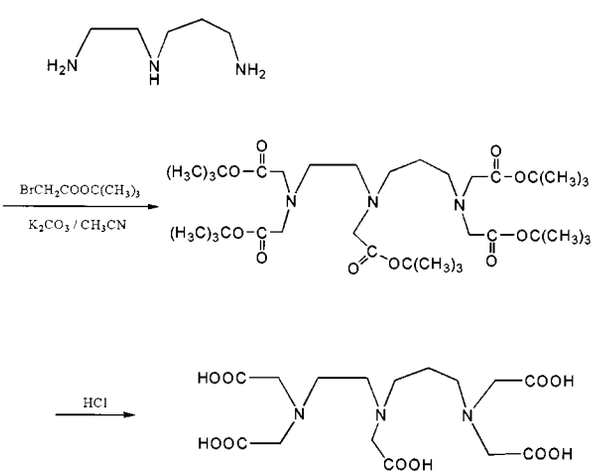
3,6,10-Tri(carboxymethyl)-3,6,10-triazadodecanedioic acid (H_5L), was synthesized and its protonation constants were determined by potentiometric titration in 0.10 mol dm^{-3} Me_4NNO_3 and by NMR pH titration at 25.0 ± 0.1 °C. Stability and selectivity constants have been measured to evaluate the possibility of using the corresponding gadolinium(III) complex as a magnetic resonance imaging contrast agent. The formations of gadolinium(III), copper(II), zinc(II) and calcium(II) complexes were investigated quantitatively by potentiometry. The stability constant for the gadolinium(III) complex is larger than those of Ca^{II}, Zn^{II} and Cu^{II} for this octadentate ligand. The selectivity constants and modified selectivity constants of the ligand for Gd^{3+} over endogenously available metal ions were calculated. The spin–lattice relaxivity R_1 for the gadolinium(III) complex was also determined. It was found to decrease with increasing pH below 4 and became invariant with respect to pH over the range 4–10. Oxygen-17 NMR shifts showed that the $[DyL]^{2-}$ complex had one inner-sphere water molecule. The water proton spin–lattice relaxation rate for the $[GdL]^{2-}$ complex was also consistent with one inner-sphere co-ordination position.

There is a great interest in the synthesis and characterization of new gadolinium(III) complexes of poly(aminocarboxylate) ligands as contrast agents in magnetic resonance imaging (MRI).[1,2] Among paramagnetic contrast agents, stable water soluble gadolinium(III) chelates have the ideal properties of high water relaxivity, chemical stability, and low toxicity *in vivo*. At present, the octa-chelating ligands carboxymethyliminobis(ethylenenitrilo)tetraacetic acid (H_5dtpa), *N,N″*-di(methylcarbamoylmethyl) carboxymethyliminobis(ethylenenitrilo)diacetic acid (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 trivalent gadolinium ion.[3–6] These gadolinium(III) chelates possess sufficient paramagnetism and high stability. The toxic effect of uncomplexed Gd^{3+} and free pro-ligand arising from dissociation of the metal complex is one of the major concerns in MRI.[7–13] The acute toxicity of gadolinium(III) 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(III) chelates.[14,15] The characterization of the complexes of gadolinium(III) with *N,N′*-bis(amide) derivatives of H_5dtpa had been investigated in a series of our studies.[16–18] In the continuing search for chelates for MRI, we have modified the ethylene group of H_5dtpa to introduce a methylene group and explored both the stability and relaxivity of its gadolinium(III) complex. Therefore, this report describes the synthesis of one derivative of H_5dtpa (Scheme 1), *i.e.* H_5L = 3,6,10-tri(carboxymethyl)-3,6,10-triazadodecanedioic acid. Its protonation constants, thermodynamic and conditional stability constants of complexes with Gd^{3+}, Cu^{2+}, Zn^{2+} and Ca^{2+} and its selectivity for Gd^{3+} over endogenously available metal ions are discussed. The ^{17}O NMR shifts of the $[DyL]^{2-}$ complex are investigated. Finally, the spin–lattice relaxivity R_1 of $[GdL]^{2-}$ is also described.



Scheme 1

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 ligand were from commercial sources unless otherwise noted. Proton NMR spectra and elemental analyses were used to confirm the composition of the products.

Preparation of 3,6,10-tri(carboxymethyl)-3,6,10-triazadodecanedioic acid (H_5L)

A suspension of 3.0 g (25.64 mmol) *N*-(2-aminoethyl)propane-1,3-diamine, 25.56 g (136.19 mmol) *tert*-butyl bromoacetate, and 18.41 g (133 mmol) of anhydrous potassium carbonate in 125 cm^3 of acetonitrile was stirred for 20 h. Removal of the solvent at reduced pressure on a rotary evaporator gave a residue which was partitioned between 100 cm^3 of water and 100 cm^3 of chloroform. The aqueous layer was separated and then extracted with two 100 cm^3 portions of chloroform. The chloroform portions were combined and dried over $MgSO_4$. Filtration and evaporation of solvent gave an amber oil, which was purified by chromatography on silica gel using 75% ethyl acetate in methanol as the eluent to give a yellow oil. The oil was then

treated with 45 cm^3 of concentrated aqueous hydrochloric acid (12 mol dm^{-3}) and stirred at room temperature for 6 h. The acid was removed by rotary evaporation and the residue taken up in water (20 cm^3). The solution was loaded onto an AG 50W × 8 cation exchange resin column (200–400 mesh, H^+ form, 3.5 × 20 cm) and washed with distilled water (1 dm^3). The crude product was eluted with 0.5 mol dm^{-3} NH_3(aq). The solution was concentrated by rotary evaporation and the white residue applied to an AG1 × 8 anion exchange resin column (200–400 mesh, HCO_2H form, 3.5 × 20 cm). The column was washed with distilled water and eluted with 0.5 mol dm^{-3} formic acid solution to give the white hygroscopic free acid. Yield: 6.58 g (58%) (Found: C, 40.61; H, 6.84; N, 9.31. $C_{15}H_{25}N_3O_{10}\cdot 2H_2O$ requires C, 40.63; H, 6.59; N, 9.47%); δ_H 3.83 (s, 4 H, NCH_2-COOH), 3.80 (s, 2 H, NCH_2COOH), 3.51 (s, 4 H, NCH_2-CH_2N), 2.28 (t, 4 H, $NCH_2CH_2CH_2N$) and 2.16 (m, 2 H, $NCH_2CH_2CH_2N$). ^{13}C NMR(D_2O): δ 176.52, 175.01, 173.45, 60.46, 60.23, 58.76, 56.37, 55.03, 54.41, 53.56 and 23.04.

General techniques

Solutions of H_5L (0.1 mmol dm^{-3}) for NMR pH titration were made up in D_2O, and the pD was adjusted with DCl or CO_2-free NaOD. Proton NMR spectra were measured in D_2O solution on a Varian Unity Plus 400 spectrometer. The final pD of the ligand solutions was determined with a microelectrode, pD = pH + 0.40.[19] The hydrogen electrode used in the present study allows a reliable and accurate determination of the proton activity over an extended pH range.

The ^{17}O NMR spectra were recorded by a Varian Gemini 300 spectrometer at 21 °C. The induced ^{17}O shift (d. i. s.) measurements were determined with respect to water as external standard. The hydration number of $[DyL]^{2-}$ was determined by the method of Alpoim *et al.*[20] An equimolar solution of Dy^{3+} and ligand was prepared, and a stoichiometric amount of standardized NaOH was added so that the complex was fully formed. Six solutions of differing dysprosium concentrations were prepared by serial dilution of the stock solution.

Solution preparations

Stock solutions of Ca^{2+}, Zn^{2+}, Cu^{2+} and Gd^{3+} were prepared between 0.015 and 0.02 mmol 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 4.65 g reagent grade Na_2H_2edta and diluting it to 250 cm^3 with demineralized water. This was used as a titrant to standardize the solution 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 gadolinium(III) 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 a change from yellow to purple was observed.[21] 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 solution of gadolinium(III) and ligand. A slight excess (2%) of ligand was used to ensure total complexation of gadolinium(III).

Potentiometric measurements

Potentiometric titrations were performed with an automatic titrator system to determine the protonation constants of the ligand and the stability constants of the metal complexes. The autotitrating system consists of a 702 SM Titroprocessor, a 728 stirrer, and a PT-100 combination pH electrode (Metrohm). 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 to avoid any contact with carbon dioxide in a sealed glass vessel (20 cm^3) thermostatted at 25.0 ± 0.1 °C, and an ionic strength of 0.10 mol dm^{-3} Me_4NNO_3. A CO_2-free 0.100 mol dm^{-3} NaOH solution was used as the titrant to minimize ionic strength change during the titration. The purity of the ligand 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. Each titration was performed at least three times. Since the Gd^{3+} chelate is completely or almost completely formed at low pH, its stability constant could not be determined from the normal potentiometric titration method. Therefore, it was evaluated by a ligand–ligand competition potentiometric titration.[22–24] A 1 : 1 : 1 molar ratio of Gd^{3+}, ligand, and a reference ligand with a known metal chelate stability was titrated. A good reference ligand for the Gd^{3+} systems was found to be H_4edta[15] which forms a complex with Gd^{3+} whose stability constant is accurately known.

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-junction potential, E_j, was found to be negligible under the experimental conditions used.

The potentiometric equilibrium studies were made on solutions of ligand, in the absence of metal ions, and then in the presence of each metal ion with the M : L ratio 1 : 1. The E data were obtained after additions of 0.005 cm^3 increments of standard 0.100 mmol dm^{-3} NaOH solution, and after stabilization in this direction equilibrium was then approached from the other direction by adding standard 0.100 mol dm^{-3} acid solution. The equilibria were slow to attain and about 15 min were required for each point of the titration where the ligand–ligand competition took place. However, complexation was usually rapid (1–5 min per point to give a stable pH reading) with Cu^{II}, Ca^{II} and Zn^{II}. The same values of the stability constants were obtained either by using the direct or the back titration.

Computational method

The protonation constants of the ligand were calculated using a FORTRAN computer program PKAS[25] 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.[25] The average difference between observed and calculated $-\log [H^+]$ was <0.04 throughout all titrations. A value of 13.78 was employed for the pK_w at 25 °C. The species distribution diagrams were calculated with the FORTRAN programs SPE and SPEPLOT.[25]

Relaxation time measurement

A gadolinium(III) chelate solution was prepared by combining equimolar amounts of the stock $GdCl_3$ and the ligand solution. A slight excess (3%) of the ligand was used and the solution was allowed to react for at least 2 h at room temperature to ensure completion of the complexation. Gadolinium(III) chelate solutions at various pH values were prepared by combining the buffer solution with an appropriately diluted complex solution in a 1 : 1 (v/v) ratio. The following buffer systems (all 0.10 mol dm^{-3}) were used: chloroacetic acid–NaOH (pH 2 and 3), acetic

Table 1 Thermodynamic data for the successive protonation of H_5L at 25.0 ± 0.1 °C in aqueous Me_4NNO_3 (I = 0.10 mol dm^{-3})

Species		log β			
H	L	H_5L	H_5dtpa[a]	H_3dmdtta[b]	H_4edta[a]
1	1	10.60 (0.02)	10.49	9.37	10.17
2	1	19.52 (0.02)	19.09	13.75	16.28
3	1	24.64 (0.02)	23.37	17.06	18.96
4	1	27.44 (0.03)	26.01		20.01

[a] Data were obtained from ref. 26. [b] Ref. 15.

acid–NaOH (pH 4 and 5), H_2PIPES (piperazine-*N,N*′-bis-(ethane-2-sulfonic acid))–NaOH (pH 6.8), and ammonia–HCl (pH 9 and 10).[21] These buffer solutions were used to maintain constant ionic strength (*i.e.* 0.10 mol dm^{-3}). The 0.10 mol dm^{-3} buffers were sufficient to keep the solution pH within the desired range in most cases. The buffered gadolinium(III) chelate solutions were all allowed to equilibrate for at least 2 h. Their pH was determined immediately before relaxation time T_1 measurements.

Relaxation times T_1 of aqueous solutions of the gadolinium(III) complex of H_5L were measured to determine the relaxivity R_1. All measurements were made using an NMR spectrometer operating at 20 MHz and 37.0 ± 0.1 °C (NMS 120 Minispec, Bruker). Before 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. The slopes of plots of $1/T_1$ *versus* concentration give R_1 in dm^3 mmol^{-1} s^{-1}.

Results and discussion

Protonation constants

The ligand protonation constants are expressed as in eqn. (1).

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

Table 1 summarizes the protonation constants of H_5L, H_3-dmdtta, H_4edta and H_5dtpa measured in the range pH 2–10. The titration curve of H_5L shows one sharp increase between about pH 9.0 and 5.0 (mols of base per mol ligand present = 3). This is due to the large difference between the second (log K_2) and third protonation constant (log K_3), *i.e.* 8.92 and 5.12. The log K_4 (fourth protonation constant) value is 2.80. The first and second protonation constants of H_5L are very similar to those of H_5dtpa (log K_1 = 10.49, log K_2 = 8.60 in 0.1 mol dm^{-3} $NaClO_4$).[26] The third protonation constant decreases in the order H_5L > H_5dtpa. The replacement of the one ethylene group in H_5dtpa by the one trimethylene group results in an increase in log K_2 (*i.e.* 0.31 unit), log K_3 (*i.e.* 0.83 unit), log K_4 (*i.e.* 0.15 unit) and ΣpK_a values (*i.e.* 1.43 unit). This can be explained by considering the chain length between the amino groups. In general, the protonation constant increases with the chain length between the amino groups.[27] The protonation constants of the ligands given in Table 1 decrease in the order H_5L > H_5dtpa > H_4edta > H_3dmdtta.

NMR pH titration

The macroscopic protonation constants of the ligands 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 that 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, and is based on previous observation that the protonation of a basic site of a poly(aminocarboxylate) in acidic solution leads to a deshielding of the adjacent methylene protons.[28] The NMR chemical shifts at

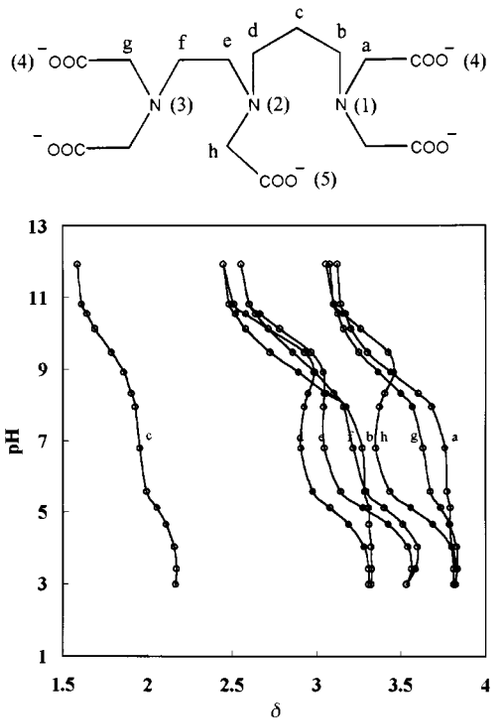


Fig. 1 Proton NMR titration curves for H_5L.

different pH values were assigned on the basis of signal multiplicities and the absence of signal crossovers over the whole pH range. These show that the central nitrogen atom is the most basic. Plots of the chemical shift values (δ) of the methylenic resonance of H_5L as a function of pH are given in Fig. 1. The observed deshielding of the methylene protons of the ligand is correlated with the percentages of protonation of the amino or carboxylate groups.[29–31] The protonation fractions of H_5L (%), f_j, for the nitrogen atoms (f_1, f_2 and f_3) and carboxylate groups (f_4 and f_5) labelled in Fig. 1 were calculated for integer values of n (1–3, number of mols of acid added per mol of polyaminopolycarboxylate). When 1 equivalent of acid is added to the fully unprotonated form of the ligand, the values for H_5L (n = 1, f_1 = 27.4, f_2 = 50.9, f_3 = 19.2) are similar to those for H_5dtpa.[29] These results indicated that the central nitrogen is more strongly basic than the terminal nitrogen atoms.

For n > 1 the protonation fraction values obtained for H_5L are as follows: at n = 2 (f_1 = 89.9, f_2 = 41.0, f_3 = 69.0) and at n = 3 (f_1 = 99.5, f_2 = 93.1, f_3 = 95.1, f_4 = −3.3, f_5 = 25.0). There is a preference for the terminal trimethylene and ethylene nitrogens relative to the central nitrogen for n = 2. The protonated forms with the terminal nitrogen atoms preferentially protonated are stabilized by internal hydrogen bonding between terminal carboxylates and nitrogen atoms, leading to high values of the second and third protonation constants.

Thermodynamic stability constants

The stability of the different gadolinium(III) complexes can be expressed in four ways: (1) the thermodynamic stability constant of the complex, (2) the conditional stability constants at pH 7.4,[15] (3) the selectivity constant, K_{sel} [the difference between the thermodynamic stability constant of the gadolinium complex and that of endogenously available metal ions (K_{ZnL}, K_{CaL} and K_{CuL})],[11] and (4) the modified selectivity constant, $K_{sel'}$ (the stability corrected for competition between the endogenously available metal ion and H^+).[15]

The normal chelate thermodynamic stability constants (K_{ML}) are expressed as in eqn. (2) where M represents the

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

Table 2 Stability constants and selectivity constants of the complexes of Gd^{3+}, Zn^{2+}, Ca^{2+} and Cu^{2+} at 25.0 ± 0.1 °C in aqueous Me_4NNO_3 (I = 0.10 mol dm^{-3})

Parameter	H_5L	H_3dmdtta [a]	H_5dtpa [b]
log ([GdL]/[Gd][L])	22.77 (0.03)	16.85 (0.05)	22.46
log $K_{GdL'}$ (pH 7.4)	18.04	14.84	18.14
log ([CaL]/[Ca][L])	14.45 (0.05)	7.17 (0.04)	10.75
log β_{CaHL}	20.51 (0.05)	11.62	—
log $K_{CaL'}$ (pH 7.4)	9.72	5.11	6.43
log ([CuL]/[Cu][L])	19.31 (0.01)	13.03 (0.03)	21.38
log β_{CuHL}	24.83 (0.01)	16.39	—
log $K_{CuL'}$ (pH 7.4)	14.58	11.06	17.06
log ([ZnL]/[Zn][L])	18.59 (0.03)	12.04 (0.03)	18.70
log β_{ZnHL}	26.36 (0.04)	16.08	—
log $K_{ZnL'}$ (pH 7.4)	13.86	10.02	14.38
Selectivity [log K(Gd/Zn)]	4.18	4.81	3.76
[log K(Gd/Ca)]	8.32	9.73	11.71
[log K(Gd/Cu)]	3.46	3.78	1.08
log $K_{sel'}$	8.44	9.03	7.04

[a] Data were obtained from ref. 15. [b] Refs. 26 and 32.

free, unhydrolysed aquametal ion, L the uncomplexed, totally deprotonated form of the ligand and ML is the normal unprotonated and unhydrolysed complex. All potentiometric titration curves have an inflection point at 5 mol base added per mol ligand. The $[CaL]^{3-}$, $[CuL]^{3-}$ and $[ZnL]^{3-}$ curves increase rapidly from pH 4 to 8, 4 to 10 and 7 to 10, respectively. The stability constant of the complex of Gd^{3+} with H_5L was derived from the competition reaction of H_4edta. In Table 2 the thermodynamic stability constants are presented for the linear poly(aminocarboxylates) H_5L, H_3dmdtta, and H_5dtpa.[15,26,32] The weaker stability of H_3dmdtta chelates when compared to the H_5L and H_5dtpa chelates is assigned to the weaker donor ability of the amide group and the lower basicity of the terminal nitrogen atoms. The higher stability of H_5L chelates when compared to the H_3dmdtta chelates is assigned to the higher basicity of the nitrogen atoms. The thermodynamic stability constants of Ca^{2+} complexes follow the order $[CaL]^{3-}$ (14.45) > $[Ca(dtpa)]^{3-}$ (10.75) > $[Ca(dmdtta)]^{-}$ (7.17). Since the stability constants of calcium(II) complexes with H_5dtpa (log K_{CaL} = 10.75) and H_4edta (log K_{CaL} = 10.61) are similar, it appears that the co-ordination behavior of the H_5dtpa type ligand is similar to that of H_4edta. However, the co-ordination number of calcium(II) in the crystal structure of $[Ca(edta)]^{2-}$ is eight, six donor atoms from H_4edta and two co-ordinated waters.[33] Six-co-ordination of Ca^{2+} with H_3dmdtta, H_5dtpa and H_4edta has been also proposed.[34] In other words, calcium(II) does not take advantage of all donor atoms in the case of H_5dtpa and H_3dmdtta. Therefore, the stability of $[CaL]^{3-}$ complexes is higher than those of $[Ca(dtpa)]^{3-}$ and $[Ca(dmdtta)]^{-}$ due to their lower basicity.

Conditional stability constants and selectivity constants

For biological studies the conditional stability of a metal chelate under physiological conditions (pH 7.4)[11,15] is more important than the thermodynamic stability constant. In Table 2 the conditional stability constants at pH 7.4 are presented for the three poly(aminocarboxylates) H_5L, H_3dmdtta and H_5dtpa. Their order is $[GdL]^{2-} \approx [Gd(dtpa)]^{2-} > [Gd(dmdtta)]$. Fig. 2 shows the pH dependence of the conditional stability for the complexes $[GdL]^{2-}$, $[Gd(dtpa)]^{2-}$ and [Gd(dmdtta)]. The results for $[GdL]^{2-}$ and $[Gd(dtpa)]^{2-}$ are very similar.

Stability constants do not provide directly comparable bases for measuring the total ion sequestering abilities of the ligands under physiological conditions (pH 7.4), and therefore they were used to calculate pM values (pM = −log $[M_f]$), where $[M_f]$ is the concentration of the free aqua metal ion that would be present at equilibrium pH 7.4.[35] The advantage of comparing pM values rather than stability constants of the complexes is that the pM values reflect the influence of ligand basicity and metal chelate protonation. The larger the pM value the higher is the affinity of that ligand for the metal ion under the specified conditions. The relative order of the pM values may change if a different set of conditions (concentration and pH) is used to calculate the pM values. The results given in Table 3 indicate that H_5L is a much stronger gadolinium(III) chelating agent than H_3dmdtta. The pM values of $[GdL]^{2-}$ and $[Gd(dtpa)]^{2-}$ are larger than those of $[CaL]^{3-}$ and $[Ca(dtpa)]^{3-}$, $[CuL]^{3-}$ and $[Cu(dtpa)]^{3-}$, and $[ZnL]^{3-}$ and $[Zn(dtpa)]^{3-}$, respectively. Therefore, the competition among Gd^{3+}, Ca^{2+}, Cu^{2+} and Zn^{2+} with H_5L is seen to favor Gd^{3+} at pH 7.4, indicating that the gadolinium(III) complex should be stable enough to avoid interference by Ca^{2+}, Cu^{2+} and Zn^{2+}. The pGd value for $[GdL]^{2-}$ is slightly higher than that of $[Gd(dtpa)]^{2-}$. Even though the stability constants for H_5L chelates are significantly larger than those of the corresponding H_3dmdtta chelates, the pGd value for $[GdL]^{2-}$ is about 4.0 log units larger than that of [Gd(dmdtta)], because H_5L has high protonation constants, and therefore the formation of its complex is subject to stronger hydrogen ion competition.

Table 3 The pM [a] values of the complexes of Gd^{3+}, Zn^{2+}, Ca^{2+} and Cu^{2+} at pH 7.4

	H_5L	H_5dtpa	H_3dmdtta
pGd	17.03	17.14	13.88
pCu	13.58	16.06	10.05
pCa	9.73	5.45	4.19
pZn	13.22	13.39	9.06

[a] pM = −log $[M]_{free}$ at pH 7.4, $[metal\ ion]_{total}$ = 1 μmol dm^{-3}, and $[ligand]_{total}$ = 1.1 μmol dm^{-3}.

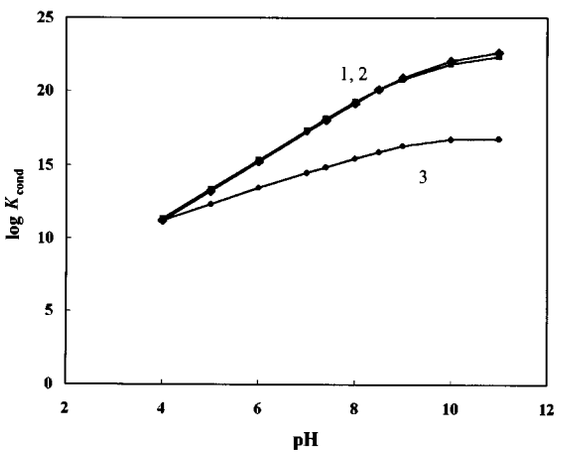


Fig. 2 Variation of the conditional stability constants for $[Gd(dtpa)]^{2-}$ (1), $[GdL]^{2-}$ (2) and [Gd(dmdtta)] (3) with pH.

Species distribution curves of $[GdL]^{2-}$ shown in Fig. 3, generated from the potentiometric data given in Table 2, indicate that there is still some free Gd^{3+} at pH 1 but by pH 3 the complex is fully formed. However, $[GdL]^{2-}$ is the dominant species at physiological pH 7.4.

The logarithmic selectivity constant[11,15] of H_5L, H_5dtpa and H_3dmdtta 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. From these, H_5L shows more favourable selectivity toward Gd^{3+} over Zn^{2+} and Cu^{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 eqn. (3).[15] This equation is

$$K_{sel'} = K_{ML}(a_H{}^{-1} + a_{CaL}{}^{-1} + a_{CuL}{}^{-1} + a_{ZnL}{}^{-1})^{-1} \quad (3)$$

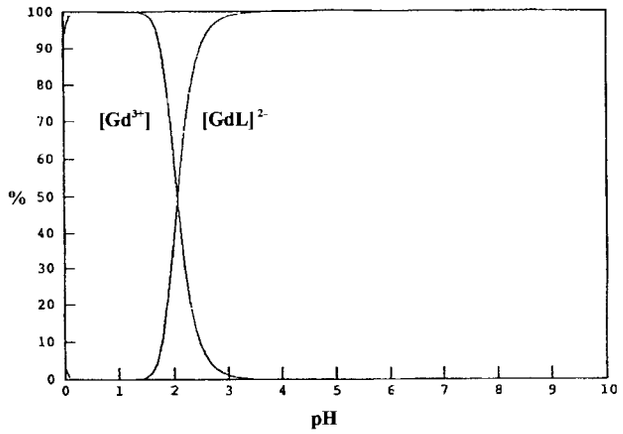


Fig. 3 Species distribution curves for a 7.0×10^{-3} mol dm^{-3} $[GdL]^{2-}$ system containing a 1:1 molar ratio of Gd^{III} to ligand. $T = 25.0 \pm 0.1$ °C; $I = 0.10$ mol dm^{-3} (Me_4NNO_3); % = percentage relative to 7.00×10^{-3} mol dm^{-3} total ligand species = 100%.

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 eqns. (4)–(7).

$$\alpha_H^{-1} = 1 + K_1[H^+] + K_1K_2[H^+]^2 + K_1K_2K_3[H^+]^3 + \ldots . \quad (4)$$

$$\alpha_{CaL}^{-1} = 1 + K_{CaL}[Ca^{2+}] \quad (5)$$

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

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

Table 2 shows the modified selectivity constants of H_5L, H_5dtpa and H_3dmdtta 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.[15] The log $K_{sel'}$ of H_5L (8.44) is higher than that of H_5dtpa (7.04), but slightly lower than that of H_3dmdtta (9.03). The ligands H_5L and H_3dmdtta appear to have comparable selectivity and should have comparable toxicity due to metal ion displacement *in vivo*. Thus, H_5L forms a gadolinium(III) complex that is slightly more stable than [Gd(dtpa)]$^{2-}$ toward transmetallation with endogenous metal ions at pH 7.4.

Dy^{III}-induced ^{17}O water NMR shifts

The Dy^{III}-induced water ^{17}O NMR shifts *versus* chelate concentration for a solution of $DyCl_3$ and $[DyL]^{2-}$ in D_2O at 21 °C are shown in Fig. 4. A hydration number of dysprosium(III) ion of eight has been proposed.[36–38] For the $[DyL]^{2-}$ complex the slope is −48.8 ppm. Under the conditions applied in the present study the slope per Dy^{III}-bound water molecule is −55.70 ppm. It can be concluded that the $[DyL]^{2-}$ complex contains 0.90 inner-sphere water molecule per Dy^{III}. The result is in good agreement with that for H_3dmdtta using the same technique.[20] The number of Ln^{III}-bound water molecules in this complex provides information on the co-ordination mode of the ligand. One co-ordination site of each Ln^{III} is occupied by one water molecule and eight sites are available for the ligand molecule. By binding of three amine nitrogen atoms and five carboxylates, a similar co-ordination mode as found for the previously studied H_5dtpa is attained.[39]

Relaxometric studies of the gadolinium(III) complex

The inner sphere relaxation mechanism could be influenced by the rate of chemical exchange of water from the co-ordination water to the bulk water. The paramagnetic contribution of the solvent longitudinal relaxivity is obtained from eqn. (8),[40] where

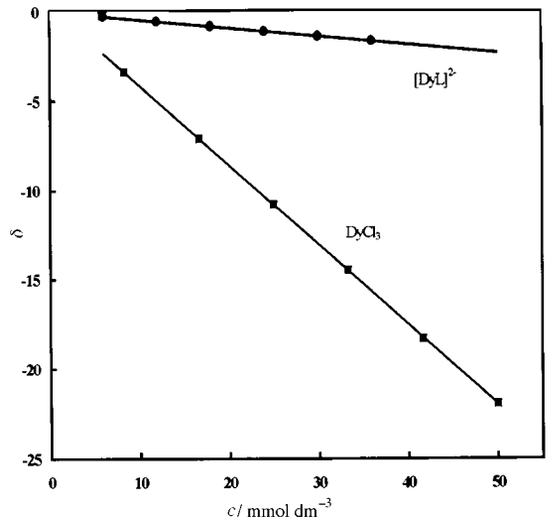


Fig. 4 The Dy^{III}-induced water ^{17}O NMR shift *versus* chelate concentration for solutions of (1) $[DyL]^{2-}$ and (2) $DyCl_3$ in D_2O at 25 °C.

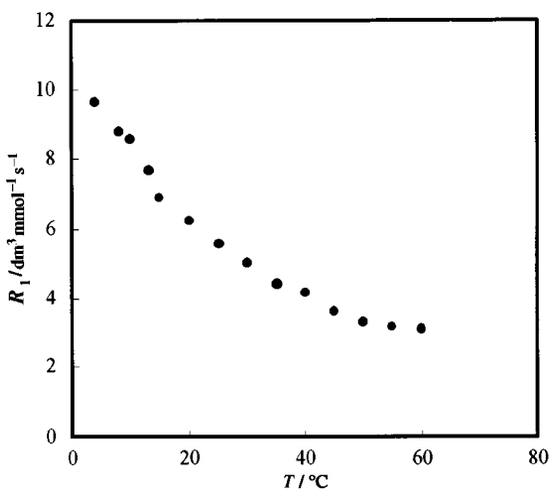


Fig. 5 Temperature dependence of the longitudinal relaxation rate for a 1 mmol dm^{-3} solution of $[GdL]^{2-}$, measured at 20 MHz, pH 6.8.

$$R_1 = Nq/[55.6(T_{1M} + \tau_M)] \quad (8)$$

N is the molar concentration of the gadolinium(III) complex, q the number of water molecules bound per metal ion, T_{1M} the relaxation time of the bound water protons, and τ_M the residence lifetime of the bound water. Owing to the opposite temperature dependences of T_{1M} and τ_M, two cases can be considered: (1) fast chemical exchange ($T_{1M} \gg \tau_M$), R_1 increases with decreasing temperature, and (2) slow chemical exchange ($T_{1M} \ll \tau_M$), R_1 decreases by decreasing the temperature. Fig. 5 shows the temperature dependence of the relaxivity for the complex $[GdL]^{2-}$ at 20 MHz. A monoexponential decrease of the observed relaxivity upon increasing the temperature in the range 5–60 °C was found. This is characteristic of the fast chemical exchange behavior which occurs when the residence lifetime of the co-ordinated water molecule (τ_M) is much shorter than the relaxation time of the bound water proton (T_{1M}).

The spin–lattice relaxivity R_1 of $[GdL]^{2-}$ is given in Table 4. It is similar to those of H_5dtpa and its bis(amide) derivatives under the same experimental conditions.[6,16–18] The relaxivity of a paramagnetic metal complex consists of two components: the inner-sphere and outer-sphere relaxivities. Since the basic skeleton of the ligands studied and the shapes and sizes of the gadolinium(III) complexes are similar, it is assumed that the

Table 4 Relaxivities R_1 of gadolinium(III) complexes at 37.0 ± 0.1 °C and 20 MHz

Complex	pH	Relaxivity R_1[a]/ $dm^3\ mmol^{-1}\ s^{-1}$
$[GdL]^{2-}$	7.5 ± 0.1	3.85 ± 0.03
	limiting[a]	4.50 ± 0.05
$[Gd(dtpa)]^{2-}$	7.6 ± 0.1	3.89 ± 0.03
	limiting[a]	4.65 ± 0.05
[Gd(dmdtta)]	7.5 ± 0.1	3.85 ± 0.03
	limiting[a]	4.60 ± 0.05

[a] Average of relaxivity values over the pH range 3–10.

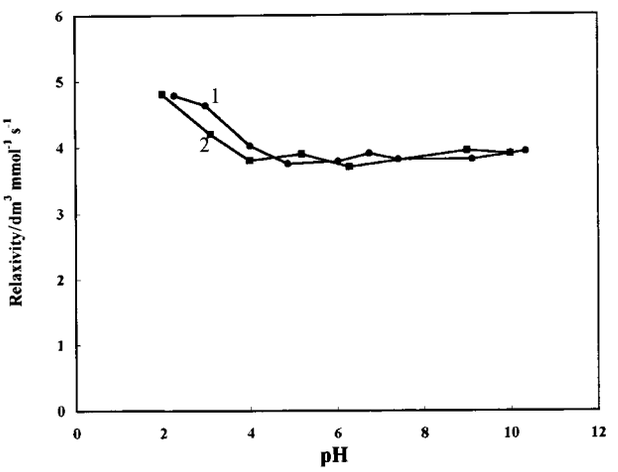


Fig. 6 pH Dependence of the relaxivity for the complexes $[GdL]^{2-}$ (1) and [Gd(dmdtta)] (2), all in 0.10 mol dm^{-3} buffers at 20 MHz and $T = 37.0 \pm 0.1$ °C.

outer-sphere relaxivities are similar. Thus, the observed relaxivity is primarily attributed to the variation in the inner-sphere contribution. The inner-sphere relaxivity is mainly dependent on the hydration number of the gadolinium(III) complex. A larger hydration number leads to a higher relaxivity R_1. However, the q value of the complex of gadolinium(III) with H_5L is the same as those of $[Gd(dtpa)]^{2-}$ and [Gd(dmdtta)][20] leading to almost identical R_1 values. In other words, the similarity in the relaxivity R_1 of $[GdL]^{2-}$, [Gd(dmdtta)] and $[Gd(dtpa)]^{2-}$ confirms that the number of inner sphere water molecules is identical.

The relaxivities R_1 for the complexes $[GdL]^{2-}$ and [Gd(dmdtta)] at various pH values are given in Fig. 6. The relaxivity curve exhibits no pH dependence over the range 4–10. Therefore, no ligand dissociation occurred with this pH range and the hydration number remains constant.

High relaxivity (R_1) and high stability of the paramagnetic metal chelate are the most important prerequisites for a magnetopharmaceutical drug. The fact that the gadolinium(III) complex of H_5L is quite stable in aqueous solution, does not dissociate under physiological conditions (pH 7.4), and does not exchange with Ca^{II}, Cu^{II} and Zn^{II} to an appreciable extent shows that the ionic chelate $[GdL]^{2-}$ may be considered a promising MRI contrast agent.

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