Complexes of Ga^{3+} and In^{3+} with the *N,N''*-Bis(butylamide) Derivative of Diethylenetriaminepentaacetic Acid: Stability Constants and Nuclear Magnetic Resonance Studies in Aqueous Solution

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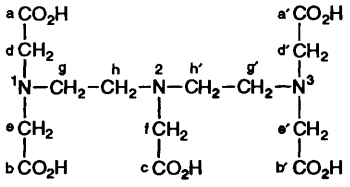
Potentiometric titrations showed that the sum of the first three protonation constants of diethylenetriaminepentaacetate *N,N''*-bis(butylamide) (L^1) decreases by 6.6 units when compared to the diethylenetriaminepentaacetate (dtpa), and that the complexes of Ga^{3+} and In^{3+} of L^1 have stability constants 6.1–6.3 log units lower than those of dtpa. The decreased basicity of the bis(amide) derivative thus correlates with the lower stability of its complexes. However, at pH 7.4 the lower overall basicity of L^1 compared to dtpa partly compensates the lower stability of its complexes, thus causing their conditional stability constants to be comparable to those of the dtpa complexes. The efficacy of L^1 in binding Ga^{3+} and In^{3+} under physiological conditions is discussed by comparing pM values at pH 7.4, showing that this compound can withstand the competition of transferrin for In^{3+} in biological media relevant to medical applications. The ^{13}C and ^1H NMR shifts were measured for the complexes of Al^{3+}, Ga^{3+}, In^{3+} and Y^{3+} with L^1 and compared with ^{13}C shifts for the dtpa complexes. The complexes of Al^{3+} and Ga^{3+} are hexadentate, possibly of octahedral geometry, and form various structural isomers, whereas those of In^{3+} and Y^{3+}, with a single structural isomer of eight-co-ordination, are very similar to the lanthanide complexes. The complexes of Al^{3+} and Ga^{3+} of L^1 have considerable populations of isomers with bound amide groups, whereas the single structural isomer of the complexes of In^{3+} and Y^{3+} with both amide groups co-ordinated yield a variety of enantiomers in solution.

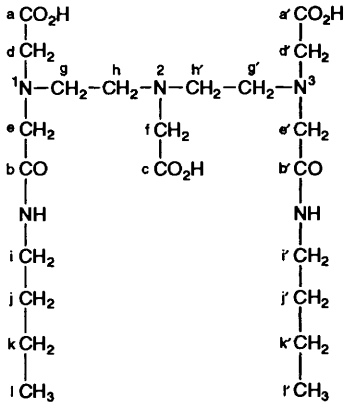
Among the chelating agents which have found applications in biology and medicine, diethylenetriaminepentaacetic acid (H_5dtpa) remains one of the most used because (*i*) it forms stable complexes with many polyvalent metal cations, and (*ii*) it possesses enough functional groups to allow the preparation of bifunctional chelating derivatives. The simplest and most commonly used bifunctional derivative is the dianhydride form.[1] Synthesis of other forms with different functional groups and with different locations on the dtpa backbone have been reported.[2–5] The presence of a second functional group not involved in the chelating process allows covalent linkage to proteins and synthetic macromolecules.[6,7] These macromolecule–dtpa conjugates are of considerable interest as carriers of polyvalent metal ions *in vivo* for diagnosis or therapy purposes with the hope of overcoming the limitations of small-molecular-weight complexes.[6–9] Two major benefits are expected, an enhancement of the blood-pool stability and a better tissue targeting.

As a part of a research program involving low-density lipoprotein drug delivery, we needed to label those with a radioelement that would remain associated *in vivo* and could be located by non-invasive techniques. We used the bis(octadecylamide) of dtpa for labelling low-density lipoproteins with indium-111.[10] This derivative has the advantage of possessing two lipophilic long chains for entrapping the chelating agent in the phospholipidic monolayer of the low-density lipoprotein, as previously proposed for labelling of lipidic vesicles.[3,11–14] Its disadvantage is the sacrifice of two carboxylate groups for linking the two hydrocarbon chains which can result in an *in vivo* loss of chelate stability relative to dtpa.

X-Ray structural determinations have been reported for the complex of Nd^{III} with dtpa[15] and those of Gd^{III} and Dy^{III} with bis(amides) of dtpa.[16,17] In both cases the ligands are octadentate. Studies of the solution structures and dynamics of lanthanide(III) complexes of the same ligands confirm that they are also octadentate in solution,[18–24] but heptadentate modes have also been proposed.[25–27] Additional information on the dynamics of the systems obtained from variable-temperature ^1H and ^{13}C NMR spectra and relaxation measurements shows that these complexes occur in several isomeric forms in solution.[22,23] The measured thermodynamic stability constants of the gadolinium complexes of the bis(propylamide) and bis(propyl ester) derivatives of dtpa as well as their conditional stability constants at pH 7.4 were smaller than those of $[Gd(dtpa)]^{2-}$ by about 6 log units.[28,29] The crystal structure of the $Na_2[In(dtpa)]$ complex shows that the ligand is octadentate;[30] NMR studies of this complex confirm that the eight-co-ordination is maintained in solution.[30] However, neither structural determinations in the solid state nor solution structures have been reported so far for complexes of bis(amide) derivatives of dtpa with Group 13 trivalent metal ions. Thus the determination of the thermodynamic stability constants and the solution structures of such complexes with the bis(octadecylamide) derivative are required to evaluate its potential chelating properties for *in vivo* applications. In order to carry out such studies we have prepared the bis(butylamide) of H_5dtpa, H_3L^1, which for solubility reasons is more appropriate to aqueous solution studies than is the bis(octadecylamide). This report describes the synthesis and characterization of H_3L^1, the determination of its protonation constants, of its stability constants for complexes of Ga^{III} and In^{III}, as well as multinuclear magnetic resonance studies of those complexes in aqueous solution. We also performed solution NMR studies on its complexes of Al^{3+} and



H_5dtpa



H_3L^1

Y^{3+} and all the NMR results obtained were compared with studies on complexes of La^{3+} and Lu^{3+} with the corresponding bis(propylamide) H_3L^2,[22,23] as well as with our studies and literature data on complexes of Al^{3+}, Ga^{3+}, In^{3+}, Y^{3+}, La^{3+} and Lu^{3+} with dtpa.[19,25,30]

Experimental

Reagents.—All chemicals were of analytical grade, used as received unless specifically noted, and obtained from the following sources: diethylenetriaminepentacetic acid dianhydride, pentasodium and di-sodium or -potassium salts of H_5dtpa (Sigma); butylamine, aluminium nitrate hydrate, gallium nitrate hydrate, indium nitrate pentahydrate, yttrium chloride (Aldrich); solvents (Prolabo). Other materials are cited under specific sections.

Synthesis and Characterization of the N,N″-*Bis(butylamide) of* H_5dtpa, H_3L^1.—Butylamine (0.327 cm^3, 3 mmol) was added dropwise with stirring to a solution of diethylenetriaminepentaacetic acid dianhydride (0.536 g, 1.5 mmol) in dry dimethylformamide (50 cm^3, 40 °C). The colourless reaction mixture was stirred (40 °C, 1 h) and filtered. Chloroform (50 cm^3) was added and, after cooling at 4 °C for 2 h, the white solid was filtered off, washed with diethyl ether (100 cm^3) and dried at 40 °C overnight. The compound was recrystallized, from hot ethanol–hexane (110:110 cm^3), after cooling at 4 °C overnight. The white crystals were filtered off, washed with acetone (10 cm^3) and diethyl ether (10 cm^3) and dried overnight at 40 °C. Yield 43%. Soluble at 25 °C in water, ethanol, dimethylformamide, and dimethyl sulfoxide; insoluble in diethyl ether, acetone, chloroform, and hexane; m.p. 105 °C (Found: C, 48.75; H, 8.3; N, 12.9. $C_{22}H_{41}N_5O_8 \cdot 2H_2O$ requires C, 48.9; H, 8.40; N, 13.0%). Fast atom bombardment mass spectrum: *m/z* 504.1 (M^+); calc. 504.3. IR (Perkin-Elmer 554 spectrometer, KBr): 3350, 3270 (N–H, amide); 2950, 2920, 2860 (C–H, alkyl); 1740, 1710 (C=O, acid); 1640 (C=O, amide); 1530 (N–H, amide); 730 (C–H, alkyl). NMR (Bruker AC 250 MHz spectrometer): δ_H(250 MHz, solvent and standard CF_3CO_2D at δ 11.30, 25 °C) 0.64 (6 H, t, 7.2, $2CH_3$), 1.08 [4 H, m, $2CH_2$(k)], 1.27 [4 H, q, 7.1, $2CH_2$(j)], 3.07 [4 H, t, 7.3, $2CH_2$(i)], 3.47 [4 H, t, 4.9, $2CH_2$(h)], 3.76 [4 H, t, 5.3, $2CH_2$(g)], 3.81 [2 H, s, CH_2(f)], 4.19 [4 H, s, $2CH_2$(d)], and 4.22 [4 H, s, $2CH_2$(e)]; (solvent D_2O, standard HDO at δ 4.75, pH 3.45) 0.83 (6 H, t, 7.3, $2CH_3$), 1.25 [4 H, sxt, 7.8, $2CH_2$(k)], 1.44 [4 H, q, 7.0, $2CH_2$(j)], 3.18 [4 H, t, 6.8, $2CH_2$(i)], 3.23 [4 H, t, 6.0, $2CH_2$(h)], 3.33 [4 H, t, 5.05, $2CH_2$(g)], 3.64 [2 H, 2s, CH_2(f)], 3.70 [2 H, s, $2CH_2$(d)], and 3.83 [2 H, s, $2CH_2$(e)]; assignments confirmed by ^1H–^1H correlation (COSY) spectra; δ_C(62.9 MHz, solvent D_2O, standard $SiMe_4$, pH 3.45, 60 °C) 15.70 [(q, 124.6, CH_3), 22.18 [t, 126.8, CH_2(k)], 33.32 [t, 126.3, CH_2(j)], 42.12 [t, 143, CH_2(i)], 54.32 [t, 142, CH_2(h)], 54.48 [t, 142, CH_2(g)], 57.71 [t, 140, CH_2(f)], 59.59 [t, 141, CH_2(d)], 59.94 [t, 141 Hz, CH_2(e)], 171.52 [s, CO_2H(c)], 174.61 [s, CO(b)], and 175.08 [s, CO_2H(a)].

Potentiometric Measurements.—Reagents and solutions. Metal-ion solutions were prepared at about 0.025 mol dm^{-3} from the nitrate salts with demineralized water (obtained by a Millipore/Milli-Q system) and were standardized by titration with Na_2H_2edta (disodium salt of ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid).[31] For Ga^{3+} a back titration with a standard solution of $ZnSO_4$ was made. The solutions of Ga^{3+} and In^{3+} were kept in an excess of nitric acid in order to prevent hydrolysis. The exact amount of acid was checked by titration with KOH solutions of 1:1 ratios of those metal ions and edta. The amount of KOH consumed in excess of the amount needed to neutralize all of the ligand protons represents the excess of HNO_3. Carbonate-free solutions of the titrant, KOH, were prepared by dilution of a commercial ampoule of Titrisol (Merck) analytical concentrate with demineralized water under a stream of purified nitrogen gas. The solutions were standardized by titration with nitric acid and discarded when the percentage of carbonate was about 0.5% of the KOH present.

Equipment. An Orion 720 measuring instrument was used together with a 91-01 glass electrode, 90-05 Ag–AgCl reference electrode and a Wilhelm-type salt bridge containing 0.10 mol dm^{-3} KNO_3 solution. A glass-jacketed titration cell (100 cm^3) completely sealed from the atmosphere was used and the temperature was controlled using a Grant W6 thermostat (25.0 ± 0.1 °C) by circulation of thermostatted water through the jacketed cell. Atmospheric CO_2 was excluded from the cell during the titration by passing purified N_2 across the top of the experimental solution and the standard base (or acid) was added through a capillary tip at the surface of the solution by a Metrohm Dosimat 665 burette. The ionic strength of the solutions was kept at 0.10 mol dm^{-3} with KNO_3.

Measurements. 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 pH range of the titration. The term pH is defined as $-\log[H^+]$. The liquid-junction potential, E_j, was found to be negligible under the experimental conditions used. The value of $K_W = [H^+][OH^-]$ was determined from data obtained in the alkaline range of the titration, considering E'° and Q valid for the entire pH range, and found equal to $10^{-13.78}$ mol^2 dm^{-6}.

The potentiometric equilibrium measurements were made on 20.00 cm^3 of approximately 2.5×10^{-3} mol dm^{-3} ligand solutions diluted to a final volume of 30.00 cm^3, first in the absence of metal ions and then in the presence of each metal ion for which the $c_L : c_M$ ratios were 1:1. The E data were taken after additions of 0.025 or 0.050 cm^3 increments of a standard 0.110 mol dm^{-3} KOH solution, and after stabilization in this direction, equilibrium was then approached from the other direction by adding 0.100 mol dm^{-3} standard acid.

In the case of the two metal ions studied (Ga^{3+} and In^{3+}), as the extent of formation of the complexes, even at low pH, was too high for the use of the direct potentiometric method, ligand–ligand competition titrations were performed. The

ligand used for the competition in the case of In^{3+} was the dianion of K_2H_2edta. The ratios $c_L{}^1:c_L{}':c_M$ 1:1:1 and 1:0.4:1 were used [H_3L^1 being the neutral form of the bis(butylamide), the ligand for which the stability constant of the metal complex was to be determined, and H_4L' the neutral form of edta, the reference ligand for which the stability constant of the complex of the same metal is known to be accurately determined]. The second ratio led to a better competition reaction, which means that all the complexed species existed in solution at least at 30% of the total metal-ion concentrations. The competition reaction can be written in terms of equilibrium (1).

$$[ML']^- + [H_4L^1]^+ \rightleftharpoons [ML^1] + 2H^+ + [H_2L']^{2-} \quad (1)$$

Potentiometric studies of the complexes of Ga^{3+} and the stability constants were calculated at pH values above 7 by relying on the competition or displacement reaction[32] (2). At

$$[GaL^1(OH)]^- + 3OH^- \rightleftharpoons [Ga(OH)_4]^- + [L^1]^{3-} \quad (2)$$

this pH range (7–11) the constant corresponding to the formation of the species $[GaL^1(OH)]^-$ was determined and used, as a constant, in other parts of the titration curves (at low pH values) to obtain the constants of the other equilibrium reactions.

In the cases of the competition reactions the equilibria were slow to attain, but in spite of this it was possible to perform the measurements by automated titrations and no batch method was needed. It was necessary to wait 10–15 min at each point of the titration in the pH region where the competition reaction was performed. The same values of the stability constants were obtained whether using the direct titration curve (with KOH) or the back-titration curves (with HNO_3).

Calculation of Equilibrium Constants.—Protonation constants $K_i^H = [H_iL]/[H_{i-1}L][H]^i$ were calculated by fitting the potentiometric data by use of the SUPERQUAD program.[33] The stability constants of the various species formed in aqueous solution were also obtained with the aid of this program. The initial computations were in the form of overall stability constants or $\beta_{M_mL_lH_h}$ values: $([M_mL_lH_h]/[M]^m[L]^l[H]^h)$. The species found in this work are ML, M(HL) and ML – H, where $\beta_{ML-H} = \beta_{ML(OH)}K_W$. Differences, in log units, between the values $\beta_{M(HL)}$ (or β_{ML-H}) and β_{ML} provide the stepwise protonation constants. The species introduced were limited to those which can be justified by established principles of co-ordination chemistry. Species distribution curves were generated with the aid of the program SPE.[34] The errors quoted are the standard deviations of the overall stability constants given directly by the program. In the case of the stepwise formation and protonation constants the standard deviations were determined by the normal propagation rules and do not represent the total experimental errors.

Hydrolysis Species of Ga^{3+} *and* In^{3+}.—The ions Ga^{3+} and In^{3+} form several hydrolytic species in aqueous solution, the stability constants of which show some discrepancies in the literature. We have used the values listed in Table 1 (in log units), considered reliable.[35–37]

NMR Measurements.—Proton and ^{13}C NMR spectra were recorded on Varian XL-200 (200.053 and 50.300 MHz, respectively) and Varian Unity 500 (499.843 and 125.697 MHz, respectively) spectrometers, ^{27}Al, ^{71}Ga and ^{115}In NMR spectra on a Varian Unity 500 spectrometer at 130.263, 152.426 and 109.545 MHz, respectively. Proton chemical shifts were referenced to sodium 3-trimethylsilyl[$^2H_4$]propionate and ^{13}C shifts to *tert*-butyl alcohol, which was used as internal standard (methyl signal at δ 31.2). Solutions of the complexes for these measurements (0.01 mol dm^{-3} for ^1H, 0.10 mol dm^{-3} for ^{13}C) were made up in D_2O by mixing equimolar solutions of H_3L^1 and hydrated nitrates of Al^{3+}, Ga^{3+}, and In^{3+} or the chloride of Y^{3+} followed by adjustment of the pH with solutions of NaOD or DCl in D_2O. The pH of the solutions was measured at room temperature on a Metrohm E520 pH-meter equipped with an Ingold 405-M3A glass electrode, and was uncorrected for the D_2O content of the solution.

Results and Discussion

Potentiometric Studies.—Protonation constants. Table 2 summarizes the protonation constants determined for H_3L^1 and the H_5dtpa values taken from the literature.[38] The present compound has six basic centres, from which we have obtained only three protonation constants: a high value (9.36 in log units) for the first protonation and two lower values (4.44 and 3.31 in log units). The other constants being very low were impossible to determine by potentiometric measurements. The overall basicity of H_3L^1 is about 6.6 log units lower than that of H_5dtpa. As previously observed by Sherry *et al.*,[28] the formation of two amide linkages in the dtpa structure generally results in a drop of log K_1 of 0.8–1.0 units and of log K_2 of 3.8–4.2 units relative to H_5dtpa and was attributed to the greater electron-withdrawing ability of an amide over that of a carboxylate group. The acid–base behaviour of H_3L^1 is very similar to that of the bis(methylamide) and the bis(propylamide) derivatives studied before[24,28] which exhibit very similar protonation constants [log K_1 = 9.37, log K_2 = 4.38, log K_3 = 3.31 and log K_4 = 1.43 for the bis(methylamide) in 0.1 mol dm^{-3} $NaClO_4$; log K_1 = 9.4, log K_2 = 4.5, log K_3 = 3.4 for the bis(propylamide) in 0.1 mol dm^{-3} NaCl], in spite of the differences in the ionic medium.

It has been suggested, based on protonation and NMR measurements, that the first protonation step in dtpa[39–41] predominantly occurs at the central nitrogen and that the second one involves a terminal nitrogen with the concerted migration of the proton on the central nitrogen to the other

Table 1 Stability constants of the hydrolytic species of Ga^{3+} and In^{3+} in aqueous solution

Quotient	Ga^{3+}	In^{3+}
$[M(OH)]/[M][OH]$	10.87	9.5
$[M(OH)_2]/[M][OH]^2$	20.95	18.2
$[M(OH)_3]/[M][OH]^3$	30.33	—
$[M(OH)_4]/[M][OH]^4$	38.34	33.9
$[M_4(OH)_4]/[M]^4[OH]^4$	—	47.8

Table 2 Protonation (log K_i^H) constants of the ligand and stability constants (log $K_{M_mL_lH_h}$) of the complexes of Ga^{3+} and In^{3+} (25.0 °C, I = 0.10 mol dm^{-3} KNO_3)

	Equilibrium quotient (log units)	
	H_3L^1	H_5dtpa *
$[HL]/[L][H]$	9.36(1)	10.71
$[H_2L]/[HL][H]$	4.44(2)	8.64
$[H_3L]/[H_2L][H]^2$	3.31(2)	4.28
$[H_4L]/[H_3L][H]^3$	< 2	2.64
$[H_5L]/[H_4L][H]^4$	< 2	2.0
$[GaL]/[Ga][L]$	18.18(5)	24.3
$[Ga(HL)]/[GaL][H]$	3.20(6)	4.16
$[GaL]/[GaL(OH)][H]$	4.57(3)	7.51
$[InL]/[In][L]$	22.7(1)	29.0
$[In(HL)]/[InL][H]$	1.9(1)	—
$[InL]/[InL(OH)][H]$	10.2(2)	11.72

* 25.0 °C, I = 0.10 mol dm^{-3}.[38]

terminal nitrogen atom. In the case of the bis(amide) derivatives of dtpa the low value of the second protonation constant could, in principle, be assigned to a carboxylate group or to a terminal nitrogen atom. The pK_a of acetic acid is 4.8 and the protonation of acetate groups linked to non-protonated nitrogen atoms gives log K values slightly lower (of the order of 4), which become about 2 or less when the protonation occurs at acetate groups linked to a tertiary amine which is already protonated, due to the effect of the charge of the ammonium group and the simultaneous disruption of the possible hydrogen bonds in which the carboxylate groups could be involved.[42,43] The protonation of a second nitrogen atom will also yield a value of log K of about 4, as this protonation will be affected by the electrostatic effect of the nearby positively charged nitrogen atom already protonated. However, in general the second protonation of polyaminopolycarboxylic acids occurs at a second nitrogen atom; the few cases where the second protonation occurs at the acetate group have some special structural features such as in the case of the small cyclic compound 1-oxa-4,7-diazacyclononane-4,7-diacetic acid.[44] In the present cases and with similar bis(amide) derivatives, the second protonation occurs at a nitrogen atom, as predicted by hydrogen-bonding schemes[21] and by theoretical studies[45] and shown by the quantitative microscopic protonation scheme of a series of these compounds including H_3L^1.[46] The large decrease of log K_2 for H_3L^1 by 4.0 units relative to H_5dtpa cannot be explained by differences in electron-withdrawing ability, as the measured Hammett σ constants of 0.28 and 0.30 respectively for the CO_2H and $CONH_2$ groups are almost equal.[47] It is rather attributed[21] to differences in the hydrogen-bonding networks formed by the various protonated species of dtpa and L^1. For dtpa the second protonation is favoured by the formation of very strong hydrogen-bonded ring systems between each of the two protonated terminal nitrogen atoms and the two adjacent carboxylate groups, the negative charge of which stabilizes the new positive charges on nitrogen atoms. For L^1 a hydrogen-bonding system already exists in the unprotonated molecule, involving the amide nitrogen, the backbone nitrogen and the carboxylate group, which must be disrupted when the terminal nitrogen atoms are protonated. These protonations are therefore less favoured, resulting in a lower log K_2 value. Therefore, the increased acidity of the nitrogen atoms of the L^1 derivative relative to dtpa is not due to inductive effects but to charge and hydrogen-bonding effects.[21] Fosshein[45] using molecular-modelling techniques, and based also on inductive effects and differences in hydrogen-bonding patterns, suggested that the large drop in the log K_2 value of a bis(methylamide) derivative of dtpa is explained by the fact that protonation of the second nitrogen will involve movement of a larger fraction of protons from the central nitrogen to the unprotonated terminal nitrogen compared to dtpa and such movement is less favoured energetically in the bis(amide) derivative than in dtpa.

In conclusion the replacement of two carboxylate groups of H_5dtpa by butylamide substituents leads to a significant decrease in the overall basicity of H_3L^1 which will have important implications for its metal complexing behaviour.

Metal Complexation Studies.—The stability constants of the complexes formed by H_3L^1 with Ga^{3+} and In^{3+} are also collected in Table 2 together with corresponding values for the similar complexes of H_5dtpa taken from the literature.[38] Only ML, M(HL) and ML(OH) species were found according to the best model given by the SUPERQUAD program. The constants for ML and ML(OH) were obtained by competition reactions with edta (for the complexes of In^{3+}) or with OH^- (for those of Ga^{3+}). The values of the constants for the M(HL) species were obtained by direct titrations (1 : 1 ratio) considering as constants the values for ML and ML(OH) first determined by the competition procedures. We took advantage of the very strong tendency of Ga^{3+} to undergo hydrolysis to $[Ga(OH)_4]^-$ to determine its formation constants with H_3L^1, which could not be determined by a direct technique. At high pH the metal complex formed dissociates with concomitant formation of a more stable ion[32,48] $[Ga(OH)_4]^-$. However the final constants will be affected by the uncertainties of the hydrolysis constants especially the overall constant, β_4^{hydr}, but the best value in the literature has been employed in the calculations.[35]

The indium complex of H_3L^1 has a stability constant of about 4.5 log units higher than that of the gallium one, as has been found for other polyaminopolycarboxylates.[37,49,50] The reverse trend in these constants would be expected because the interactions involved in these complexes are mostly electrostatic, the In^{3+} ion having the larger effective ionic radius,[51] as is found for complexes involving 'harder' ligands (like some ligands containing phenol derivatives).[37,49] The trend observed for polyaminopolycarboxylates is attributed in part to the fact that carboxylate is less hard than phenolate for co-ordination,[37,49,50] but also to higher co-ordination numbers of In^{3+} relative to Ga^{3+} (six for Ga^{3+}, eight for In^{3+}, see NMR studies below).

The value of the hydrolysis constant obtained for the indium complex of L^1 [log $K_{InL(OH)}$] is only slightly lower (10.2, see Table 2) than the corresponding constant for dtpa (11.72). However, the value for the gallium complex of L^1 (4.57) seems very low when compared with the corresponding hydrolysis constant for dtpa (7.51). One explanation for this can be found in the different co-ordination spheres of Ga^{3+} and In^{3+} in the two complexes. The NMR studies (see below) indicate that for In^{3+} all the carboxylate and amide groups are bound, in an eight-co-ordinate fashion. By contrast, the gallium complex, which has an hexadentate co-ordination, contains both free and metal-bound carboxylate and amide carbonyl groups. This means that the positive charge around the Ga^{3+} cation cannot be neutralized by the negative donor atoms of the ligand directly involved in the co-ordination, contrary to what happens with the indium complex. Then the co-ordination of a water molecule and its dissociation to OH^- ion could be easier around Ga^{3+} than around In^{3+}. It is interesting that the value found for the gallium complex with H_3L^1 is exactly what could be predicted by the correlation of log $K_{GaL(OH)}$ *versus* log K_{ML} observed by Martell and co-workers[32,48] for a large series of ligands. The facile formation of the monohydroxo complex $[GaL(OH)]^-$ points to the involvement of a water molecule directly co-ordinated to the metal ion. Table 2 also shows that the protonation constants of the metal complexes of both ligands are quite different for Ga^{3+} and In^{3+}. The values in the range 3.2–4.2 for the gallium complexes indicate the presence of free carboxylate group(s), whereas the much lower value for the indium complexes indicates that all carboxylate groups are bound. This is confirmed by NMR studies (see below).

The data of Table 2 also indicate that the formation of two amide bonds in dtpa remarkably decreases the stability constants, by 6.12 for gallium complexes and 6.3 for indium complexes (in log units). As we have found before, the formation of the bis(amide) derivative leads to a decrease in the overall basicity of the ligand (about 6.56 log units) which parallels the decrease in stability constants. Sherry *et al.*[28] have verified a similar drop in the stability constant of the gadolinium complex of the bis(propylamide) derivative of dtpa (a decrease of 6.03 log units) and Rizkalla *et al.*[24] have found for the complexes of the bis(methylamide) derivative with some other lanthanides (La^{3+}, Sm^{3+}, Dy^{3+} and Lu^{3+}) values 4.9–5.7 lower than those of dtpa. These results suggest that the replacement of two carboxylate groups of dtpa by corresponding lipophilic amide derivatives (at least for short chains) does not lead to important entropy configurational losses. The differences in free-energy changes of the complexes are probably mainly enthalpic as the carbonyl function is considerably less basic than the amide moiety.

For *in vivo* applications, however, the behaviour of the complexes of Ga^{3+} and In^{3+} with L^1 and dtpa cannot be

evaluated by a simple comparison of the stability constant values. In the blood stream (pH 7.4) the efficacy of a complexing agent is usually reduced by competing ions present in the plasma, where calcium (1.25 mmol dm^{-3}), zinc ($\approx 10^{-2}$ mmol dm^{-3}), hydrogen (39.8 nmol dm^{-3}), and hydroxide (250 nmol dm^{-3}) ions are the most important interferences. Also, the metal ions need to form highly selective complexes with the ligand to prevent transfer of the metal to other competitors present *in vivo* such as transferrin and other proteins. A more reliable guide for ligand efficacy is the pM value ($= -\log[M^{3+}]$) calculated from the stability constants of the complexes formed and the protonation constants of the ligand, taken at physiological pH 7.4, the total ligand concentration being twice that of the total metal.[49,52] The larger the pM values, the more effective is the ligand. Table 3 shows the pM values for the complexes of dtpa and L^1; values for the transferrin complexes, taken from the literature,[52] are also included for comparison. The higher affinity of Ga^{3+} than In^{3+} for the transferrin Fe^{3+} sites results from the similarities in ionic radii[51] of Ga^{3+} and Fe^{3+} and other chemical properties. Owing to the dependence of pM on ligand protonation constants, very different predictions about *in vivo* stability can result if log K or pM values are compared.

As can be observed in Tables 2 and 3, although the complexes of Ga^{3+} and In^{3+} with L^1 have lower stability constants than those of dtpa, the former ligand is also less basic, so the lower stability of the complex is partly compensated for by the lower overall basicity of the ligand. At physiological conditions the pGa for L^1 is slightly lower than that of dtpa (the difference is about 0.9) and also lower than the value for transferrin, so L^1 cannot compete for Ga^{3+} with this protein in the plasma. By contrast, in the case of the indium complexes a different situation occurs. Although the pIn for L^1 is 3.65 lower than that of dtpa, it still is about 1.8 units higher than that of transferrin. Therefore, we would predict a stable complex of L^1 relative to transferrin at physiological conditions and so, from the standpoint of conditional stability, this ligand can be as good as dtpa for biological applications.

NMR Studies.—The structures of the trivalent metal complexes $[M(dtpa)]^{2-}$ ($M = Al^{3+}$, Ga^{3+}, In^{3+}, Y^{3+}, La^{3+} or Lu^{3+}) were studied by comparing the ^{13}C NMR spectra of their aqueous solutions at pH 7.0, formed by mixing stoichiometric amounts of the metal ion and H_5dtpa. The spectra of the complexes of Ga^{3+}, In^{3+} and Y^{3+} have been studied previously,[30] as have those of La^{3+} and Lu^{3+}.[19,25] Table 4 summarizes the ^{13}C shift data from our work and the literature. As described previously,[30] the pattern of carboxylate signals directly gives the number of free and metal-bound carboxylate groups in the complexes. Table 4 indicates that the complexes of In^{3+}, Y^{3+}, La^{3+} and Lu^{3+}, with three carboxylate signals in the δ 179–182 region of approximate relative intensities 2:2:1, all have five metal-bound CO_2^- groups in an eight-co-ordinate geometry possibly of square-antiprismatic structure.[19,30] The complexes of Al^{3+} and Ga^{3+} have very similar ^{13}C NMR spectra, but quite different from those of the other trivalent metal ions. In the δ 176–182 ppm region, $[Al(dtpa)]^{2-}$ has two CO_2^- signals with relative intensities 2:1, and $[Ga(dtpa)]^{2-}$ has three CO_2^- signals with relative intensities 1:1:1. They correspond to three co-ordinated CO_2^- groups in each case.[30] Both complexes have another signal at about δ 172–173, corresponding to two free carboxylate groups. Therefore Al^{3+} and Ga^{3+} form octahedral complexes of co-ordination number six.[30]

The full assignment of the ^{13}C NMR shifts in Table 4 and their unambiguous interpretation in terms of chelate structures and dynamics is a more difficult task. This has been done in the literature for the complexes of La^{3+} and Lu^{3+},[19] and our indium and yttrium assignments were based on the similarities of the ^{13}C shifts of those complexes and of the crystal structures of $[Nd(dtpa)]^{2-}$ and $[In(dtpa)]^{2-}$, which define a square-antiprismatic co-ordination geometry for both metal ions.[15,30] The ^{13}C shifts of $[Al(dtpa)]^{2-}$ and $[Ga(dtpa)]^{2-}$ are also very similar to each other and could in principle arise from one of the three possible structural isomers of an octahedral complex of dtpa with two free carboxylate groups: a free carboxylate on each terminus (*e.g.* b,b'), two free carboxylates on one terminus (*e.g.* a,b), or one central and one terminal free carboxylate (*e.g.* c,b). Each of these structural isomers has more than one geometrical isomer, all of which define asymmetrical structures. The free carboxylate resonance corresponding to two equivalent carbons was assigned in Table 4 to two terminal carboxylates, one on each end of the ligand dtpa (b,b') on the basis of their equivalence. Such a structure has the most symmetrical distribution of free carboxylates and minimizes best the repulsions of their negative charges. However it is not

Table 3 The log K_{ML} and pM values for complexes of Ga^{3+} and In^{3+} with L^1 and dtpa

	L^1		dtpa		Transferrin[a]	
Metal ion	log K_{ML}	pM[b]	log K_{ML}	pM[b]	log K_{ML}	pM
Ga^{3+}	18.18	19.09	24.3	19.94	20.3	20.4
In^{3+}	22.70	20.74	29.0	24.39	19.2	18.9

[a] Values obtained from ref. 52. [b] Values calculated for 100% excess of free L under physiological conditions, pH 7.4; $c_M = 10$, $c_L = 20$ μmol dm^{-3}. The calculations were made with the SPE program.[34]

Table 4 Carbon-13 NMR shifts[a] of $[M(dtpa)]^{2-}$ complexes in D_2O[b] at pH 7.0 and 25 °C

M	C(a,a')	C(b,b')	C(c)	C(d,d')	C(e,e')	C(f)	C(g,g')	C(h,h')
H	172.19	172.19	179.93	58.61	58.61	57.27	54.48	50.79
Al[c]	177.85	172.43	176.99	59.02	64.18	59.59	56.08	56.08
					61.86		55.03	51.20
Ga[c,d]	177.83	173.10	176.74	59.29	63.69	58.96	56.25	55.62
	177.33				61.10		55.32	51.26
In[c,d]	179.25	179.74	179.03	64.05	64.65	59.63	58.21	57.78
Y[c,d]	182.09	183.19	182.20	64.89	65.42	67.90	59.82	57.86
La[e]	181.60	182.32	182.12	64.87	64.87	66.57	59.11	57.71
Lu[e]	181.86	182.46	181.48	65.01	65.01	65.69	59.61	57.70

[a] In ppm; internal standard Bu^tOH (CH_3, δ 31.20). [b] Measured from samples obtained upon adding metal(III) nitrate and Na_5(dtpa) solutions in D_2O, metal to ligand ratio = 0.9:1, final complex concentration 0.10 mol dm^{-3}. [c] Present work. [d] Ref. 30. [e] Refs. 19 (data at 73 °C) and 25.

possible to define the geometrical isomer present in solution for these complexes.

The complexes of Al^{3+}, Ga^{3+} and In^{3+} with L^1 were first investigated by ^{27}Al, ^{71}Ga and ^{115}In NMR spectroscopy in aqueous solution. The relative width factors of these quadrupolar nuclei, which define the nuclear contributions to quadrupole relaxation, $Q^2(2I + 3)/I^2(2I - 1)$, where Q and I are the quadrupole moment and nuclear spin quantum number, respectively, are 1.0:2.34:14.0.[53] For the nitrates of Al^{3+}, Ga^{3+} and In^{3+} in 0.1 mol dm^{-3} aqueous solutions at pH 3.0 and 20 °C the linewidths were 25, 1040 and 1250 Hz, respectively. For the last two ions these values are larger than the literature values of 252 and 375 Hz, respectively, reported for the aqueous ions,[53,54] perhaps due to the binding of nitrates under our conditions. With regard to metal NMR studies, among the three complexes of H_3L^1 only the ^{27}Al NMR resonance could be observed, as the ^{71}Ga and ^{115}In signals were too broad, in agreement with previous observations on hydroxy- and polyamino-polycarboxylic acids.[53,55] Aluminium NMR studies of complexes of hydroxycarboxylates[53,54,56,57] and polyaminopolycarboxylates[53,57,58] indicate that the ^{27}Al chemical shift and linewidth may yield very useful information on the co-ordination geometry and symmetry of the metal ion in the complex. The ^{27}Al NMR signal for a 0.1 mol dm^{-3} [AlL^1] aqueous solution at pH 7.0, 20 °C had a chemical shift of δ +40.3 relative to $[Al(H_2O)_6]^{3+}$ and a linewidth of 2600 Hz. These values are comparable with the δ +37.2 and 1835 Hz for the $[Al(dtpa)]^{2-}$ complex.[58] Taking into consideration the observed linear relationship of ^{27}Al shifts of polyaminopolycarboxylate complexes with the ligand denticity,[58] the shift value for [AlL^1] suggests that the complex has octahedral geometry and the ligand is hexadentate. The ^{27}Al linewidth for this complex is only slightly larger than that of $[Al(dtpa)]^{2-}$, indicating similar symmetries and arrangements of the two ligands around the $^{27}Al^{3+}$ nucleus in the two complexes, with a faster quadrupolar relaxation for the former heavier complex, presumably due to its longer rotational correlation time. The ^{139}La NMR spectrum of $[La(dtpa)]^{2-}$ has previously been observed, with a linewidth of 6300 Hz[59] (^{139}La has a width factor of 0.85 relative to ^{27}Al[53]).

The ^{13}C NMR spectra of the [ML^1] (M = Al, Ga, In or Y) complexes were obtained in aqueous solution at 0.1 mol dm^{-3} concentration (see Fig. 1). The chemical shifts of the carboxylate and amide carbonyl resonances (a, a', b, b', c) and of the alkyl group resonances (i, i', j, j', k, k', l, l') observed for these complexes are listed in Table 4 and compared with corresponding data previously obtained for the complexes of La^{3+} and Lu^{3+}.[23,60] The ^{13}C NMR spectra of the La^{3+}- and Lu^{3+}-L^1 complexes in D_2O at 25 °C indicated the presence of various species in solution, as previously observed for the bis(propylamide) analogues, [LaL^2] and [LuL^2][23] and the bis(methylamide) analogues [LaL^3] and [LuL^3].[24,29] Both complexes displayed various sets of signals for the butyl group, in particular for the α-CH_2 (i, i') carbons. The complex of La^{3+} showed five carboxylate and two amide carbonyl signals, whereas that of Lu^{3+} had seven carboxylate and four amide carbonyl signals.[23] The region between δ 56 and 68 was very crowded with the resonances from the d, d', e, e', f, g, g', h and h' carbons, which were not assigned. It has been shown that these signals correspond to multiple species, which result from the formation of a maximum of eight enantiomers of the complexes, present in four diastereomeric pairs. This is due to the octadentate co-ordination of the ligand to the Ln^{3+} ions *via* the three N atoms of the diethylenetriamine backbone, three carboxylate oxygens and the two amide oxygens, which makes the three nitrogens chiral centres.[23,24,29]

The ^{13}C NMR spectra of the complexes of Y^{3+} and In^{3+} with H_3L^1 (Table 5) were quite similar to those of La^{3+} and Lu^{3+}. They displayed various signals for the carboxylate groups and the amide carbonyls, separated in each group by a maximum of 1.2 and 0.4 ppm for the resonances of Y^{3+} and In^{3+}, respectively. Various sets of signals were also present for the butyl group, with small maximum separations of 1.4 (i, i' carbons), 0.4 (j, j' carbons), 0.2 (k, k' carbons) and 0.1 ppm (l, l') carbons. This indicates that for the complexes of Y^{3+} and In^{3+} all the carboxylate and amide groups of L^1 bind to the cation to give eight-co-ordination, and that the binding of the carbonyls originates a variety of enantiomers at the terminal nitrogen chiral centres.

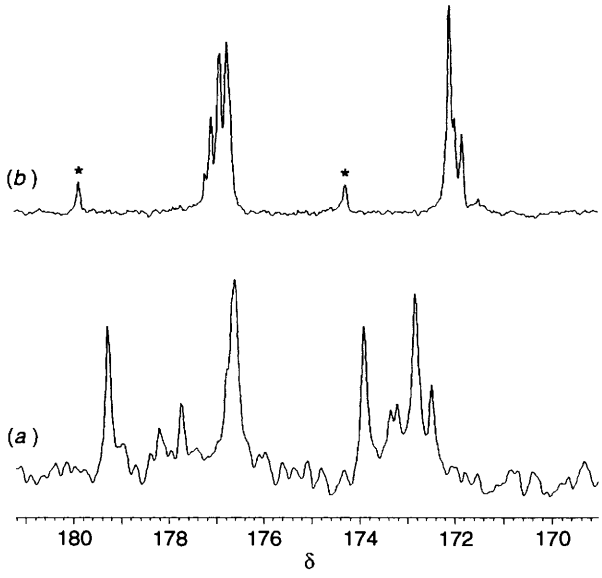


Fig. 1 Carboxylate carbonyl region of the ^{13}C NMR spectra of [ML^1] complexes, 0.1 mol dm^{-3} in D_2O, pH 7.0, 25 °C: M = Ga (*a*) or In (*b*). The asterisk indicates resonances from free H_3L^1

The ^{13}C NMR spectra of the complexes of Al^{3+} and Ga^{3+} are quite different (Table 5). They show a variety of signals for the carboxylate and carbonyl carbons, but which are not clustered around two different shift values, as for the complexes of In^{3+} and Y^{3+}. We could not assign these resonances to types of carbon atoms, but this spread of shifts (180–171 ppm for Al^{3+}, 179–172 ppm for Ga^{3+}) strongly indicates that the complexes contain both free and metal-bound carboxylate and amide carbonyl groups. These should result from six structural isomers of overall six-co-ordinate octahedral geometries, which have the following free groups: (a', b'), (c, b'), (c, a'), (b, b'), (a', b), and (a, a'). All these structural isomers may originate geometrical isomers which have asymmetric binding of dtpa moieties to the metal ion. The complexes with bound amide carbonyl groups also originate enantiomers at the corresponding nitrogens. The multiple resonances present for the butyl carbons (Table 5) should result from a combination of all types of isomerism present in these complexes. However, a detailed analysis of the ^{13}C NMR spectra is not feasible.

We tried to obtain more detailed information on the complexes of L^1 using proton NMR spectroscopy. The spectra of the complexes of Al^{3+}, Ga^{3+} and In^{3+} are shown in Fig. 2. They show some similarities with the spectra reported for the complexes of La^{3+} and Lu^{3+} with H_3L^3,[24] which at 5 °C are sharp and contain various AB patterns corresponding to the bound terminal (d, d') and central (f) acetate methylene protons, as well as to the amide methylene protons (e, e'), with long metal–nitrogen and short metal–oxygen bond lifetimes.[61] The AB patterns of the spectra of the trivalent cations shown in Fig. 2 and also of the yttrium complex were defined using two-dimensional proton-shift correlated (COSY) spectra (see Fig. 3).

The proton spectrum of the yttrium complex (data not shown) was quite broad at 25 °C, possibly due to a rapid rate of exchange between various isomers in solution. At 5 °C it was quite similar to those of the complexes of La^{3+} and Lu^{3+} reported in the literature.[24]

The proton spectrum of the indium complex [Fig. 2(*c*)] is

Table 5 Carbon-13 NMR shifts of [ML^1] complexes in D_2O at pH 7.0 and 25 °C

	Carboxylates	Amide carbonyls	Alkyl groups			
M	C(a,a'), C(c)	C(b,b')	C(i,i')	C(j,j')	C(k,k')	C(l,l')
H	180.13, 171.80	174.60	40.23	31.85	20.79	14.35
Al*	180.26, 180.20,	175.32; 174.70	41.63	31.90	20.84	14.40
	177.37	174.31; 171.72	40.25	31.50		
Ga*	179.29, 178.22,	173.92; 173.36	42.54	32.08	21.04	14.58
	177.76, 176.62	173.22; 172.83	42.35	31.37		
		172.51	40.55	31.27		
In	177.13, 177.04,	172.06	42.90	31.64	20.93	14.48
	176.87, 176.71	171.96; 171.80	42.11	31.20		
			41.74			
			41.50			
Y	181.98, 181.93,	177.29; 177.08	41.64	32.07	21.01	14.57
	181.80, 181.35,	176.01	41.49	31.75	20.95	14.54
	181.15		41.13		20.83	
			40.44			
La	181.64, 180.82,	176.91	43.19	34.04	23.75	11.87
	181.60, 180.78,	176.17	43.12	33.20	22.83	11.80
	180.90		43.02			
			43.21			
Lu	182.96, 182.19,	177.87	43.66	34.34	23.21	12.20
	182.55, 181.82,	177.64	43.63	33.62	23.17	12.11
	182.29, 181.58,	176.55	43.27	33.50	23.14	
	182.23	176.45				

* It was not possible to assign the resonances to types of carboxylate and amide carbonyl groups.

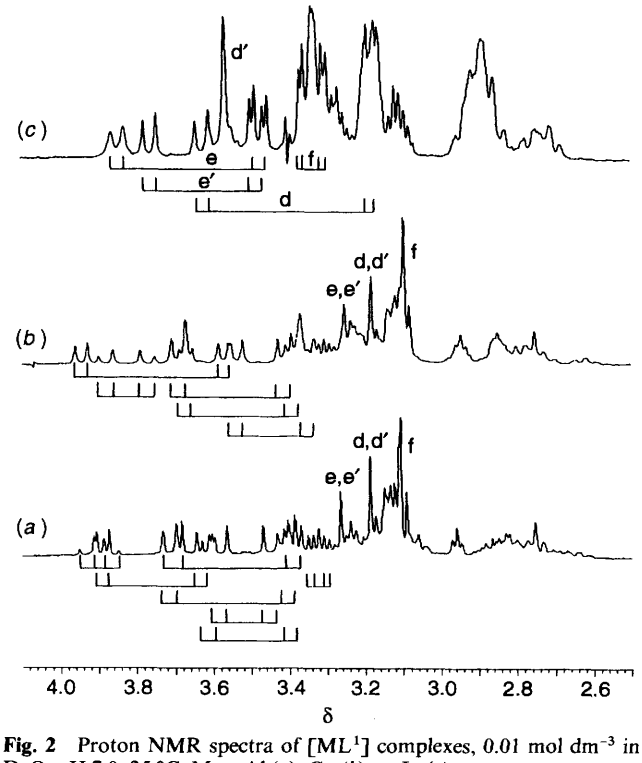


Fig. 2 Proton NMR spectra of [ML^1] complexes, *0.01* mol dm^{-3} in D_2O, pH 7.0, 25 °C: M = Al (*a*); Ga (*b*); or In (*c*)

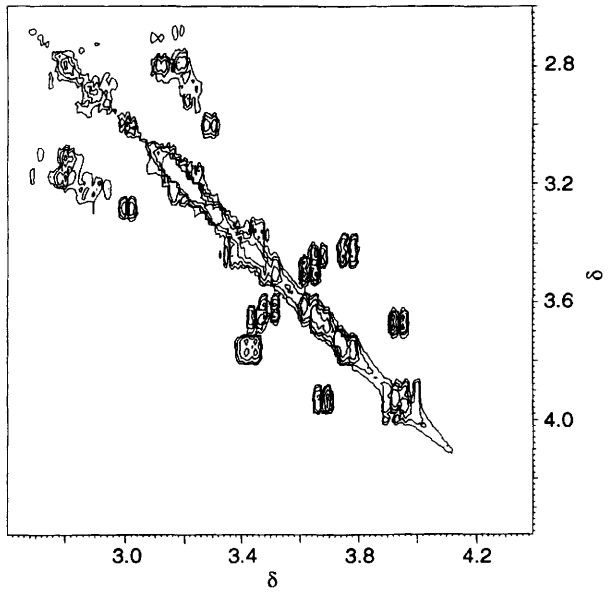


Fig. 3 The COSY spectrum of 0.01 mol dm^{-3} [AlL^1] in D_2O, pH 7.0, 25 °C

sharp at 25 °C, and the splittings broaden and collapse at 60 °C, indicating that the conformer interconversion barriers are higher for this complex than for those of Ln^{3+}. This could be related to the fact that the effective ionic radius of In^{3+} (0.92 Å for co-ordination number eight) is smaller than that of Lu^{3+} (0.98 Å),[51] which is the smallest of the lanthanides. This leads to a more compact and rigid structure which also has a higher stability constant than any of the lanthanide complexes (see Table 2 and ref. 46). The spectrum displays four AB doublet patterns and one singlet in the δ 3.9–3.2 region, all with the same relative intensity, corresponding to the bound acetate and amide methylene groups of H_3L^1. The protons of the terminal acetate groups give an AB pattern with a baricentre at δ 3.41 (d) and a singlet at δ 3.56 (d'), the central acetate group gives one AB quartet at δ 3.45 (f), whereas the protons of the amide methylenes show two AB patterns centred at δ 3.66 (e) and 3.62 (e'). No singlets are present at the frequencies corresponding to acetate and amide groups with unco-ordinated oxygens (see below and ref. 24), indicating clearly that the indium is eight-co-ordinate. This single structural isomer originates various enantiomers at the terminal nitrogens due to amide and oxygen binding, as indicated by the observed split of all the butyl side-chain methylene and methyl protons into at least three sets of signals, corresponding to different environments of the various conformers present in slow intermolecular exchange.[23,24]

The proton NMR spectra of the complexes of Al^{3+} and Ga^{3+} [see Fig. 2(*a*) and 2(*b*), respectively] are more complicated than

that of the indium complex discussed above. They show again sharp resonances at 25 °C which broaden above 60 °C, indicating high interconversion barriers among different conformers. The aluminium complex shows seven AB doublet patterns of very different relative intensities in the δ 4.0–3.3 region, centred at δ 3.90, 3.75, 3.54, 3.53, 3.51, 3.50 and 3.32, corresponding to bound amide (possibly the first two) and terminal and central acetate methylenes. The gallium complex shows five AB doublet patterns of very different relative intensities in the same region centred at δ 3.82, 3.76, 3.55, 3.53 and 3.44, also corresponding to methylene protons of bound amides (possibly the first two) and acetates. In contrast to the indium complex, those of Al^{3+} and Ga^{3+} display singlet resonances at δ 3.25, 3.18 and 3.09 corresponding, respectively, to free amide (e, e'), terminal acetate (d, d') and central acetate (f) methylene groups. This clearly shows the presence of a variety of octahedral complexes which are structural isomers (see before). No detailed characterization of this mixture is possible, but the relative intensities of the three singlets corresponding to the unbound groups (7:9:10 for Al^{3+}, 3:5:7 for Ga^{3+}) indicates that Al^{3+} and Ga^{3+} prefer binding to the amide carbonyl groups and have the least preference for binding the central acetate group. Again the resonances of the butyl side-chain methylene and methyl protons are split into at least three sets of signals, resulting from different environments, which now correspond to different structural, geometric and enantiomeric isomers in slow exchange. A comparison of the maximum splitting for the α-CH_2 (i, i') and β-CH_2 (j, j') protons respectively of the complexes of Al^{3+} (0.24, 0.20), Ga^{3+} (0.26, 0.08) and In^{3+} (0.14, 0.07 ppm) shows that these are substantially larger for the α-CH_2 of the complexes of Al^{3+} and Ga^{3+} than for the indium complex. This could result in part from charge-density effects,[24] but also reflects the presence of structural isomers with very different environments in the first two complexes, as provided by metal-bound and free amide groups.

Conclusion

Potentiometric titrations have shown (see Table 2) that the stability constants, log K_{ML}, of the 1:1 complexes of Ga^{3+} and In^{3+} with dtpa decrease by 6.1–6.3 units when two terminal acetates are modified to give the bis(butylamide) derivative, L^1. This decreased stability of the complexes is linearly correlated with the sum of the macroscopic protonation constants of the ligands, when the structures of the complexes compared do not change significantly.[62] The present ^{13}C and ^1H NMR structural study has shown that this is a good approximation for the system studied. The sum of the first three protonation constants, which in this case corresponds to protonation of the nitrogen donors,[46] Σ log K_N^H, is 17.11 for H_3L^1 and 23.63 for H_5dtpa. The decreased basicity of the backbone nitrogen atoms brought about by formation of two amide groups reflects a decreased contribution to the enthalpy of complexation from the metal–nitrogen bonding, as shown for lanthanide complexes.[63]

Potentiometric studies also have shown (see Table 2) that the stability constants of the complexes of both dtpa and L^1 increase by 4.5–4.7 units when going from Ga^{3+} to In^{3+}. The differences in complex stabilities for these two cations are correlated with the observed differences in their structures in solution, as shown by ^{13}C and ^1H NMR studies. The indium complexes have similar structures to those formed by the lanthanides,[16–24,30] yielding octadentate complexes with both ligands. In the case of the L^1 complex, various enantiomers are present in solution due to different configurations at the two terminal nitrogens, which are chiral centres. The complexes of Ga^{3+} and Al^{3+} have hexadentate co-ordination,[30,58] as has been proposed for divalent cations Ca^{2+}, Cu^{2+} and Zn^{2+} with H_3L^3.[24] The two M^{3+} octahedral complexes have several structural isomers in solution, where the metal ion binds carboxylate as well as amide groups, in contrast to what has been suggested for the M^{2+} octahedral complexes.[24] Thus, the stronger binding of In^{3+} relative to Ga^{3+} to the two ligands results from an increased co-ordination number (eight *versus* six) in the former complexes.

It should be noted that the values of the stability constants in Table 2 for the complexes of Ga^{3+} and In^{3+} do not fit well the linear empirical correlation of log K_{ML} as a function of the cationic charge density, z/r (cationic charge divided by the ionic radius), found by Rizkalla *et al.*[24] for a variety of complexes of divalent metal ions and of La^{3+} and Lu^{3+}. The Shannon radii[51] were used to calculate z/r, with co-ordination numbers of six for Ga^{3+} and eight for In^{3+}. The log K_{ML} values of In^{3+} lie above the correlation line for both ligands whereas those for Ga^{3+} lie below that line. The higher stability of the indium complexes than expected on the basis of z/r values indicates the importance of covalent interactions for this ion, especially with softer ligands.

Finally, we can conclude that derivatization of two carboxylate groups of dtpa to give L^1 or other similar lipophilic derivatives, although resulting in a loss of complex stability relative to dtpa does not necessarily preclude the *in vivo* applications of such complexes. The pM values calculated under physiological conditions give a better prediction of their utility.[49,52] In this study such a comparison, of pM values for the complexes of Ga^{3+} and In^{3+} of the two ligands in water at physiological pH with those for the transferrin complexes,[49] shows that from the standpoint of conditional stability the indium bis(amide) complex is promising for *in vivo* studies. It should be mentioned that the gadolinium complexes of H_5dtpa and H_3L^3 which are not more stable than these indium complexes are in use as contrast agents for clinical magnetic resonance imaging.[64,65] Thus long chain bis(amide) derivatives of dtpa seem to be promising bifunctional ligands for lipophilic labelling with indium-111 of naturally occurring lipidic vesicles such as low-density lipoproteins.[10]

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