

# Synthesis, equilibrium and NMR studies of lanthanide(III) complexes of the *N*-mono(methylamide) and *N'*-mono(methylamide) derivatives of diethylenetriamine-*N,N,N',N'',N'''*-pentaacetic acid †

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Two derivative ligands of diethylenetriamine-*N,N,N',N'',N'''*-pentaacetic acid ( $H_5DTPA$ ;  $H_5L^1$ ) were synthesized: the symmetric and asymmetric mono(methylamides)  $DTPA-N'-MA$  ( $H_4L^3$ ) and  $DTPA-N-MA$  ( $H_4L^4$ ). The protonation constants ( $\log K_i^H$ ) of  $L^3$  and  $L^4$  were obtained by pH-potentiometric titration: 10.04, 8.41, 2.73, 1.94 and 10.18, 6.19, 3.55, 2.0, respectively. The protonation constants and the sites of protonation were interpreted on the basis of the pH dependence of the chemical shifts of the non-labile protons. The stability constants ( $K_{LnL}$ ) of the complexes of these ligands with lanthanide(III) ions were determined by direct pH-potentiometry and competition titration. The stability constants decrease in the sequence  $LnL^1 > LnL^3 > LnL^4$ . The  $\log K_{LnL}$  values of the complexes  $LnL^3$  and  $LnL^4$  increase with increasing atomic number of  $Ln^{3+}$  from La to Gd, then remain roughly unchanged up to Er and subsequently decrease slightly to Lu. The  $^1H$  and  $^{13}C$  NMR spectra reveal the presence of 2 isomers for the complexes  $LnL^3$  and 4 isomers for  $LnL^4$  at around 0 °C. The AB multiplets of the acetate methylene protons demonstrate the long lifetime of the  $Ln^{3+}-N$  bonds. The coupling observed between the methyl and methylene protons of the methylamide group in the 2-D COSY spectra indicates partial double bond character of the C–NMe bond.

## 1 Introduction

In recent years the complexes of lanthanides (Ln) with DTPA [ $H_5DTPA = H_5L^1 =$  diethylenetriamine-*N,N,N',N'',N'''*-pentaacetic acid or carboxymethyliminobis(ethylenenitrilo)tetraacetic acid] and with its amide derivatives have attracted considerable interest. This is a consequence of the successful application of  $Gd(DTPA)^{2-}$  as a contrast agent in magnetic resonance imaging for the enhancement of proton relaxation rates.<sup>1–6</sup> In order to eliminate the two negative charges of  $Gd(DTPA)^{2-}$  which are neutralized by two *N*-methylglucamine ( $CH_3NHCH_2(CHOH)_4CH_2OH$ ) cations, a non-ionic compound,  $Gd(DTPA-BMA)$  [ $H_3DTPA-BMA = H_3L^2 = N,N'$ -bis(methylcarbamoylmethyl)-carboxymethyliminobis(ethylenedimino)diacetic acid], was developed.<sup>7,8</sup> The metal chelates used in medical diagnosis or therapy must have high thermodynamic stabilities, which are expressed in the values of the stability constants. The stability constant of  $Gd(DTPA-BMA)$  ( $\log K_{GdL} = 16.85^8$ ) is significantly lower than that of  $Gd(DTPA)^{2-}$  ( $\log K_{GdL} = 22.46^9$ ). However, the difference in the values of the conditional stability constants at physiological pH is lower and the selectivity of the ligand DTPA-BMA for  $Gd^{3+}$  over endogenous ions such as  $Zn^{2+}$  and  $Cu^{2+}$  is more favourable than the selectivity of DTPA<sup>8</sup> (the selectivities are expressed by the ratios of the stability constants).

The practical application of  $Gd(DTPA-BMA)$  and its interesting complexation properties have stimulated vigorous

research on the synthesis and study of new DTPA amide derivative ligands. In the recently synthesized compounds the methyl groups of DTPA-BMA have been replaced by various alkyl or aryl groups.<sup>10–21</sup> Some new macrocyclic DTPA bis(amide) derivatives have also been synthesized.<sup>22–24</sup> X-Ray structural studies of the  $Ln^{3+}$  complexes of some DTPA bis(amide) derivatives have revealed that the ligands are co-ordinated to the  $Ln^{3+}$  via three acetate oxygens, three nitrogen atoms and the two carbonyl oxygens of the amide groups. The ninth co-ordination site is occupied by an  $H_2O$  molecule.<sup>13,17,20,21</sup> The stability constants of the complexes reveal that the selectivities of the various DTPA bis(amide) derivatives for  $Gd^{3+}$  over  $Ca^{2+}$ ,  $Zn^{2+}$  and  $Cu^{2+}$  are higher than those of DTPA itself.<sup>8,14,15,18</sup>

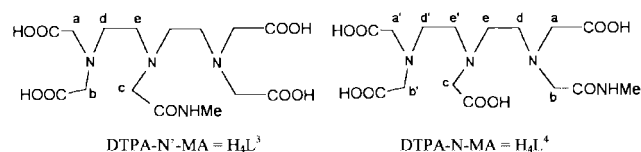
The results of  $^1H$  and  $^{13}C$  NMR studies indicate that the structures of the  $Ln^{3+}$ –DTPA bis(amide) complexes in aqueous solution are similar to their solid-state structures. The multiplicity of the  $^1H$  NMR spectra observed first by Geraldes *et al.*<sup>14</sup> points to the existence of several conformational isomers.<sup>17,24,25</sup> The presence of a  $H_2O$  molecule in the inner sphere was detected for the  $Eu^{3+}$  complexes by means of luminescence lifetime measurements<sup>25,26</sup> and by  $^{17}O$  NMR studies for some  $Dy^{3+}$  complexes.<sup>14</sup>

The natures and numbers of donor atoms in the various recently synthesized DTPA bis(amide) derivatives are the same. The relative positions of the donor atoms are also similar and consequently the stability constants and relaxation effects of the  $Gd^{3+}$  complexes of these ligands are also very similar. The role of the amide groups in complex formation has been studied by Paul-Roth and Raymond, who found that an amide group contributes to the stability constant of the  $Gd^{3+}$  complex by 3.73  $\log K$  units.<sup>15</sup>

† Electronic supplementary information (ESI) available: stability constants of diglycolate complexes, chemical shifts of  $L^4$  as a function of pH. Appendix, various NMR spectra of complexes and of diethylenetriamine protons. See <http://www.rsc.org/suppdata/dt/b0/b0052981/>

In recent years there has been growing interest in the synthesis and study of bifunctional ligands, in which the DTPA (or other ligand) is attached to macromolecules (proteins, monoclonal antibodies, *etc.*). The  $Gd^{3+}$  complexes of the DTPA-conjugated macromolecules are potential intravascular blood pool agents<sup>27–29</sup> or can be targeted to specific organs.<sup>29,30</sup> The  $^{90}Y$  complexes of such bifunctional ligands have been proposed for use in the diagnosis and therapy of certain forms of cancer.<sup>4,31</sup> The ligand DTPA is often bound to macromolecules *via* an amide nitrogen, which makes study of the complexation properties of DTPA monoamide derivatives of great interest.

In the DTPA monoamide derivatives synthesized to date the amide group is attached to a terminal backbone nitrogen, as in the ligand  $L^4$ .<sup>10</sup> In the present work, we report the synthesis and study of a new ligand,  $L^3$ , in which the amide group is attached to the middle nitrogen. Study of the equilibrium and structural properties of the complexes of the two ligands (Scheme 1)



Scheme 1

allows a comparison of the effects of the different sites of the amide groups on the complexation properties of the ligands.

## 2 Experimental

### 2.1 Synthesis of the ligands<sup>32,33</sup>

$H_4L^3$ . To a solution of 20 g (32.43 mmol) of 3,9-bis(*tert*-butoxycarbonylmethyl)-6-carboxymethyl-3,6,9-triazaundecanoic acid di-*tert*-butyl ester in 50 mL of DMF at 0 °C were added 4.12 g (35.67 mmol) of *N*-hydroxysuccinimide and 16.73 g (81.08 mmol) of *N,N'*-dicyclohexylcarbodiimide. After stirring for 3 h at ambient temperature, 15 mL of an aqueous solution (40% w/w) of methylamine were added. After 16 h the deposited solid was removed by filtration and the filtrate evaporated to dryness. The residue was purified by flash chromatography (silica gel, dichloromethane–2-propanol 15:1) to yield 18.43 g of a solid material that was refluxed with 200 mL of *n*-hexane. The insoluble material was removed by filtration and the filtrate evaporated to dryness, to yield 16.51 g of 3,9-bis(*tert*-butoxycarbonylmethyl)-6-methylcarbamoylmethyl-3,6,9-triazaundecanoic acid di-*tert*-butyl ester as a white powder.

To cleave the *tert*-butyl ester groups, a mixture of 16.47 g (26.15 mmol) of this material and 200 mL of trifluoroacetic acid was stirred for 18 h at ambient temperature. The solution was evaporated *in vacuo*, the residue repeatedly dissolved in 2-propanol, and the solvent removed *in vacuo*. The semi-solid residue was dissolved in 300 mL of water, and the pH adjusted to 2.1 by the addition of ion-exchange resin (Amberlite IRA 67,  $OH^-$  form). The solution was evaporated *in vacuo* and the residue crystallized from an ethanol–acetone mixture to yield 5.08 g of  $H_4L^3$  as a white powder. Found (calc.) for  $C_{15}H_{26}N_4O_9$ : C, 44.65 (44.33); H, 6.70 (6.45); N, 13.60 (13.79)%.

$H_4L^4$ . To a solution of 16.14 g (40 mmol) of *N*-(2,6-dioxomorpholinoethyl)-*N'*-(ethoxycarbonylmethyl)-3,6-diaza-octanedioic acid in 150 mL of DMF stirred at 0 °C were added 20.2 g (27.7 mL, 200 mmol) of triethylamine, followed by 1.55 g (50 mmol) of methylamine dissolved in 35 mL of DMF. After stirring for 18 h at room temperature the mixture was filtered and the filtrate evaporated *in vacuo*. The residue was stirred with 1 L of diethyl ether and filtered, and the solid material dissolved in 250 mL of water. To this solution was added 80 mL of 5 M NaOH. After 1 h at room temperature the pH was

adjusted to 7.0 by the addition of an acidic ion-exchange resin (Amberlite IR 120), the resin was separated off by filtration and the solution lyophilized to give the trisodium salt of the desired amide. In order to obtain the free acid the salt was dissolved in 100 mL of water and the solution passed through a column of 100 mL of acidic ion-exchange resin (Amberlite IR 120). The acidic solution was stirred with 1 g of charcoal, filtered and lyophilized, to give 12.08 g of the desired monoamide  $H_4L^4$ . Found (calc.) for  $C_{15}H_{26}N_4O_9$ : C, 44.05 (44.33); H, 6.32 (6.45); N, 13.55 (13.79)%.

### 2.2 Equilibrium measurements

The preparation of the  $LnCl_3$  stock solutions, the determination of the concentrations of the  $LnCl_3$ ,  $H_3L^3$  and  $H_3L^4$  solutions have been carried out as described before.<sup>34</sup>

The protonation constants of the ligands ( $K_i^H = [H_iL]/[H_{i-1}L][H^+]$ ,  $i = 1, 2, 3$  or 4) were determined by pH-potentiometric titration in 0.1 M KCl at 25 °C. The stability constants of the complexes of the Ce-group elements ( $K_{LnL} = [LnL]/[Ln^{3+}][L]$ ) were determined by pH-potentiometry, while in the case of the Gd-group elements a competition method was used. The pH-potentiometric titrations were carried out in the presence of equivalent amounts of  $Ln^{3+}$  and  $H_4L^3$  or  $H_4L^4$  and an excess of diglycolic acid ( $H_2dga$ ). The concentrations of  $Ln^{3+}$  and  $H_4L^3$  or  $H_4L^4$  were  $1 \times 10^{-3}$  or  $2 \times 10^{-3}$  M. In the competition method the concentration of  $dga$  was  $1 \times 10^{-2}$  or  $2 \times 10^{-2}$  M. For determination of the equilibrium constants, 2 or 3 parallel titrations were carried out. The numbers of titration points used to calculate the protonation constants of  $L^3$  and  $L^4$  (obtained by titrating  $2.6 \times 10^{-3}$  M solutions) were 184 and 344, respectively. For the calculation of stability constants from the results of direct titrations the number of data points was 35–60, while in the case of competition titrations 120–200 data points were used.

For calculation of the  $\log K_{LnL}$  values from the competition measurement data the protonation constants of  $dga$  and the stability constants of the complexes  $Ln(dga)^+$ ,  $Ln(dga)_2^-$  and  $Ln(dga)_3^{2-}$  were also determined. In the determination of the protonation constants the concentration of  $H_2dga$  was  $2 \times 10^{-3}$  M. The stability constants were determined on samples in which the  $Ln^{3+}$  and  $H_2dga$  concentrations were  $4 \times 10^{-3}$  and  $2 \times 10^{-3}$  M,  $2 \times 10^{-3}$  and  $1 \times 10^{-2}$  M, or  $2 \times 10^{-3}$  and  $2 \times 10^{-2}$  M, respectively. In the determinations of the stability constants the titration experiments were carried out in 0.1 M KCl at 25 °C.

The pH-potentiometric titrations and the calibration of the electrode system were performed as described before.<sup>34</sup> The ionic product of water ( $-\log K_w$ ) was determined in 0.1 M KCl at 25 °C to be 13.86. For calculation of the equilibrium constants the computer program PSEQUAD was used.<sup>35</sup>

### 2.3 NMR measurements

Solutions of the ligands and complexes were made up in  $D_2O$  (Isotec, 99.9%) and the pD was adjusted with NaOH dissolved in  $D_2O$ .  $^1H$  and broad-band proton-decoupled  $^{13}C$ -NMR spectra were recorded on Bruker AM360 and DRX 500 NMR spectrometers. Proton and  $^{13}C$  chemical shifts were referenced to DSS (sodium 4,4-dimethyl-4-silapentane sulfonate) in the case of the AM 360 or to the solvent in the case of the DRX 500 instrument. The 2-D spectra were recorded with the standard Bruker program with  $z$  gradient. A Eurotherm regulator was used to keep the temperature constant; no temperature calibration was performed. The model calculations were made with Bruker NMRSIM software under Bruker WinNMR.

## 3 Results and discussion

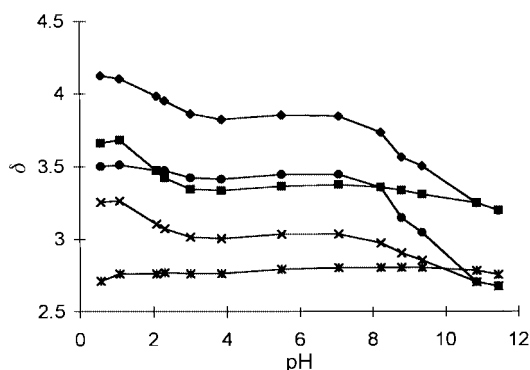
### 3.1 Protonation constants and protonation sequences of $L^3$ and $L^4$

The structures of ligands  $H_4L^3$  and  $H_4L^4$  are similar to that of

**Table 1** Protonation constants ( $\log K_i^H$ ) of DTPA and some DTPA amide derivative ligands (25 °C)

	L <sup>3</sup>	L <sup>4</sup>	L <sup>1a</sup>	L <sup>2b</sup>	L <sup>5c</sup>
$\log K_1^H$	10.04 (0.009)	10.18 (0.010)	10.49	9.37	9.9
$\log K_2^H$	8.41 (0.011)	6.19 (0.012)	8.60	4.38	6.4
$\log K_3^H$	2.73 (0.017)	3.55 (0.014)	4.28	3.31	3.8
$\log K_4^H$	1.94 (0.021)	2.0 (0.019)	2.64	1.43	1.8
$\log K_5^H$	—	—	2.00	—	—
$\Sigma \log K_i^H$	23.12	21.86	28.0	18.49	21.9

<sup>a</sup> Ref. 7. <sup>b</sup> Ref. 6. <sup>c</sup> DTPA *N*-monopropylamide.<sup>8</sup>

**Fig. 1** Chemical shifts of the non-labile protons of L<sup>3</sup> as a function of pH: ◆ a,b; ■ c; ● d; × e; \* Me protons.

H<sub>5</sub>L<sup>1</sup> (Scheme 1), the only difference being that either the middle or a terminal carboxylate group of H<sub>5</sub>L<sup>1</sup> is replaced by a methylamide group. These modifications result in significantly different protonation constants of the ligands. The protonation constants obtained for L<sup>3</sup> and L<sup>4</sup> are compared with those of L<sup>1</sup>, L<sup>2</sup> and L<sup>5</sup> in Table 1; the standard deviations obtained in the calculation are given in parentheses.

The data in Table 1 reveal that the first protonation constants are very similar for all the ligands. This occurs at an amine nitrogen, predominantly the middle nitrogen for L<sup>1</sup> and L<sup>2</sup>.<sup>25,36</sup> The second and third protonation constants of L<sup>2</sup> are very low, because of the formation of hydrogen-bonds between the terminal nitrogens and the amide NH protons, which reduces the basicity of the terminal nitrogens.<sup>25</sup> In the case of L<sup>3</sup>, therefore, it is to be expected that the first proton protonates a terminal nitrogen and that the second is attached to the other terminal nitrogen atom. Since the two terminal nitrogens are well separated from each other the first and second protonation constants of L<sup>3</sup> are relatively high and close to the  $\log K_1^H$  and  $\log K_2^H$  values of L<sup>1</sup>. The third proton presumably protonates L<sup>3</sup> at the middle nitrogen; the third protonation constant is therefore low, because of the hydrogen-bond formation between this nitrogen and the amide NH proton.<sup>25</sup> Further, the  $\log K_3^H$  value is also reduced because of the strong electrostatic repulsion between the middle and the two terminal NH<sup>+</sup> groups. The second protonation constant of L<sup>4</sup> is lower than that for L<sup>3</sup> or L<sup>1</sup> by more than two  $\log K$  units. This drop is very similar to that observed for the protonation of EDTA ( $\log K_1^H = 10.26$  and  $\log K_2^H = 6.16^9$ ) and indicates that the first and second protonations of L<sup>4</sup> occur on the middle and the iminodiacetate nitrogens. The third protonation constant of L<sup>4</sup> is also low, because the third proton is attached to the nitrogen atom bearing the amide group. The fourth protonation constants of L<sup>3</sup> and L<sup>4</sup> are low and are characteristic of the protonation of acetate groups.

These considerations are supported by the results of <sup>1</sup>H NMR titration of the ligands L<sup>3</sup> and L<sup>4</sup>. Figs. 1 and S1 (see ESI material) depict the changes in the chemical shifts of the non-labile protons of L<sup>3</sup> and L<sup>4</sup> as a function of pH. (The pH values reported are pH-meter readings.) Protonation is known to

cause deshielding of the protons which are close to the basic (protonation) sites, resulting in low-field chemical shifts.<sup>36</sup> Fig. 1 reveals dramatic changes in the chemical shifts of protons a, b and d (Scheme 1) of L<sup>3</sup> in the pH range 12–8, which indicates protonation of the terminal nitrogens. The proton signals were identified *via* their integrals, coupling patterns (d and e) and chemical shifts. A smaller low-field change in the chemical shifts of the triplet e and an even smaller one for the singlet c show that the extent of protonation of the central nitrogen is much lower than that of the terminal ones. The changes in the chemical shifts of the protons c, e and a, b in the pH range 4–1 indicate protonation of the central nitrogen and acetate groups, respectively. In this pH range the changes in the chemical shifts of protons d are very small. The signal of the methyl protons does not change its position in the whole range of pH, because the methyl group is distant from the protonation sites.

For the ligand L<sup>4</sup> only the chemical shifts of the acetate methylene protons are presented in Fig. S1, because the signals of the backbone methylene protons are broad and overlap each other. The signal of protons a' and b' was identified as a singlet, with an integral intensity of 4 if the intensity of the signal of the methyl group (at  $\delta$  1.8) was taken as 3. It shows a small downfield shift at around pH  $\approx$  10 and a larger one at around pH  $\approx$  6. However, the signal of protons c is strongly shifted in the pH range 11–8, but exhibits a smaller shift at around pH  $\approx$  6. These observations support the assumption that the first protonation occurs predominantly on the middle nitrogen, while the second involves the iminodiacetate nitrogen in L<sup>4</sup>. The signals of protons a and b were identified on the basis of their behaviour in the acidic range, since their chemical shifts undergo smaller changes at about pH > 6. The peaks of protons b do not reveal a downfield shift at about pH < 3, when the acetate groups are protonated and the signal of protons a is strongly shifted.

### 3.2 Stability constants of complexes of L<sup>3</sup> and L<sup>4</sup>

Direct pH-potentiometric titration can in general not be used to determine the stability constants of the highly stable poly-aminopolycarboxylate complexes. The stability constants of the complexes LnL<sup>1</sup> for instance were determined by competition methods.<sup>37</sup>

For determination of the stability constants of the lanthanoid(III) aminopolycarboxylates by pH-potentiometry we propose the use of diglycolic acid as a competitive ligand. Diglycolate ions form 1:1, 1:2 and 1:3 complexes of medium stability with Ln<sup>3+</sup>, but the conditional stability constants in the pH range 2–4 are relatively high because of the low values of the protonation constants ( $\log K_1^H = 3.87(0.001)$  and  $\log K_2^H = 2.80(0.001)$ ). Since the conditional stability constants of the Ln<sup>3+</sup>-aminopolycarboxylates in the pH range 2–4 are relatively low, diglycolate ions can successfully compete for Ln<sup>3+</sup>. An additional advantage of the use of diglycolic acid is the high rate of dissociation of the Ln<sup>3+</sup>-diglycolate complexes. The use of some aminopolycarboxylic acids as competitive ligands (*e.g.* EDTA) may result in error in the pH-potentiometric titration because the complexes formed with the Ln<sup>3+</sup> ions are relatively inert at pH > 4. However, preliminary pH-potentiometric titrations indicated that the complexation of the lighter Ln<sup>3+</sup> ions, which form complexes with lower stability constants with L<sup>3</sup> and L<sup>4</sup>, could be studied by direct titration. The extent of complexation of these ions at pH 2 is only a few percent, as compared with 30–40% for the ions of the Gd<sup>3+</sup> group. For study of the complexation of the ions La<sup>3+</sup> to Gd<sup>3+</sup>, therefore, direct pH-potentiometry was used, whereas mainly the competition method was applied for the ions Gd<sup>3+</sup> to Lu<sup>3+</sup>. For a few Ln<sup>3+</sup> ions both methods were used in order to compare the efficiencies of the procedures.

In order to obtain reliable data in the competition titrations the stability constants of the complexes Ln(dga)<sup>+</sup>,

**Table 2** Stability constants ( $\log K_{\text{LnL}}$ ) of the complexes formed with the ligands  $L^1$ ,  $L^3$  and  $L^4$  (25 °C, 0.1 M KCl)

Ln	$L^3$	$L^4$	$L^1$ <sup>a</sup>
La	17.62(0.02) <sup>b</sup>	16.43(0.034) <sup>b</sup>	19.48
Ce	18.30(0.006) <sup>c</sup>	17.19(0.005) <sup>c</sup>	20.50
Pr	18.77(0.005) <sup>c</sup>	17.60(0.007) <sup>c</sup>	21.07
Nd	19.10(0.007) <sup>c</sup>	17.90(0.009) <sup>c</sup>	21.60
Sm	19.48(0.011) <sup>c</sup>	18.62(0.007) <sup>c</sup>	22.34
Eu	19.90(0.029) <sup>c</sup>	18.7(0.029) <sup>c</sup>	22.39
Gd	19.9(0.048) <sup>c</sup>	19.4(0.077) <sup>b</sup>	22.46
		19.3(0.20) <sup>c</sup>	
Tb	20.90(0.021) <sup>b</sup>	19.7(0.057) <sup>b</sup>	22.71
Dy	20.5(0.099) <sup>b</sup>	19.3(0.054) <sup>b</sup>	22.82
	20.47(0.028) <sup>c</sup>		
Ho	20.8(0.038) <sup>b</sup>	19.5(0.084) <sup>b</sup>	22.78
		20.0(0.085) <sup>c</sup>	
Er	20.2(0.034) <sup>c</sup>	19.3(0.26) <sup>b</sup>	22.74
		19.3(0.093) <sup>c</sup>	
Tm	20.4 (0.11) <sup>b</sup>	19.1(0.15) <sup>c</sup>	22.72
Yb	20.4(0.20) <sup>b</sup>	19.5(0.20) <sup>c</sup>	22.62
Lu	20.2(0.23) <sup>b</sup>	19.1(0.21) <sup>c</sup>	22.44
	20.2(0.034) <sup>c</sup>		

<sup>a</sup> Ref. 7, 0.1 M KNO<sub>3</sub>. <sup>b</sup> Competition titration. <sup>c</sup> Direct titration.

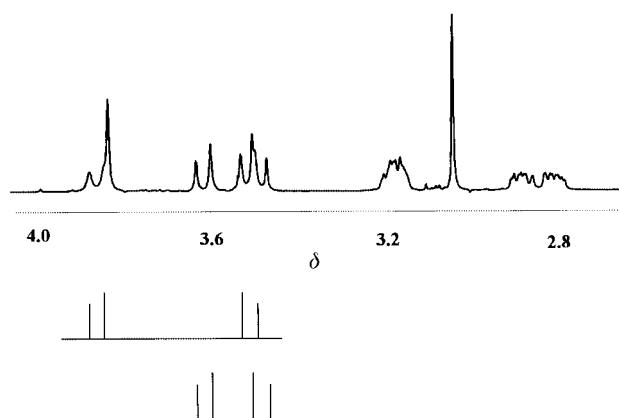
$\text{Ln}(\text{dga})_2^-$  and  $\text{Ln}(\text{dga})_3^{3-}$  were re-determined by pH-potentiometric titration. They are reported in Table S1, where the data of Grenthe and Tobiasson<sup>38</sup> are also presented. The  $\log K_1$ ,  $\log K_2$  and  $\log K_3$  values obtained in 0.1 M KCl in this work are somewhat higher than those determined in 1.0 M KCl.<sup>38</sup>

The stability constants of the complexes of  $L^3$  and  $L^4$  are listed in Table 2, together with the  $\log K_{\text{LnL}}$  values of the complexes  $\text{LnL}^1$ . The standard deviation values calculated<sup>35</sup> are given in parantheses. When three times the standard deviation (generally accepted as the error in the  $\log K$  values) is larger than 0.1, then the  $\log K_{\text{LnL}}$  values in Table 2 are given with one significant figure after the decimal point. A comparison of the stability constants reveals that the sequence of the  $\log K_{\text{LnL}}$  values for the different ligands is  $L^1 > L^3 > L^4$ . This is consistent with the earlier finding that substitution of an amide group for a carboxylate leads to a decrease in the stability constant. The  $\log K_{\text{LnL}}$  values also indicate that the symmetric ligand  $\text{H}_4\text{L}^3$ , containing the amide group on the middle nitrogen, forms more stable complexes than the asymmetric ligand  $\text{H}_4\text{L}^4$ . The difference in  $\log K_{\text{LnL}}$  is around 1.0  $\log K$  unit for the complexes  $\text{LnL}^3$ , probably as a consequence of the higher basicity of  $L^3$ . It is to be seen in Table 1 that  $\Sigma \log K_i^{\text{H}}$  is 1.26  $\log K$  unit larger for  $L^3$  than for  $L^4$ .

The trend in the stability constants with increasing atomic number of the lanthanides (Table 2) is similar for the complexes of  $L^3$ ,  $L^4$  and  $L^1$ . The  $\log K_{\text{LnL}}$  values gradually increase from La to Gd. For the elements Tb to Ho the stability constants are approximately equal, and a weak decrease can then be observed for the heaviest elements. The stability constants of the  $\text{Ln}^{3+}$ -polyaminopolycarboxylate complexes often exhibit a similar trend, if the number of donor atoms is seven or more.<sup>39</sup> To interpret the observed trend we assume that the most important factor is the decrease in ionic size, which may result in a decrease in the co-ordination number of  $\text{Ln}^{3+}$ , or more probably leads to steric hindrance between the co-ordinated functional groups.

### 3.3 NMR spectra of complexes

In view of the similarity in the ligand structures, the structures of the complexes  $\text{LnL}^3$  and  $\text{LnL}^4$  are expected to be similar to those of  $\text{LnL}^1$  and  $\text{LnL}^2$ . The geometry of the complexes  $\text{LnL}^1$  is monocapped square antiprismatic, with a  $\text{H}_2\text{O}$  molecule in the monocapped position.<sup>40-42</sup> The rates of water exchange in the complexes  $\text{GdL}^3$  and  $\text{GdL}^4$ , studied by <sup>17</sup>O NMR spectro-



**Fig. 2** <sup>1</sup>H NMR spectrum of  $\text{LuL}^3$  at 320 K.

scopy, were consistent with the assumed structures of the complexes.<sup>43</sup>

The <sup>1</sup>H NMR spectra of diamagnetic  $\text{LaL}^3$  and  $\text{LuL}^3$  (Fig. 2) are very similar, and therefore only the latter is described here. The methyl protons of the methylamide group of  $\text{LuL}^3$  give rise to a singlet signal at  $\delta$  3.04. The broad multiplet signals at around  $\delta$  2.80–2.82, 2.85–2.9 and 3.17 were identified by means of 2-D COSY (Fig. S2) and selective decoupling experiments as those of the ethylenic protons d and e (Fig. 2). The four acetate methylenic protons give rise to two AB multiplet systems ( $J_{\text{AB}} = 16.3$  and 16.6 Hz) in the range  $\delta$  3.5–3.9 (Fig. 2), which show that the lifetime of the  $\text{Ln}^{3+}$ -N bonds is long on the NMR timescale.<sup>44</sup> The spectra of the complexes  $\text{LaL}^3$  and  $\text{LuL}^3$  are very similar to those of  $\text{LaL}^1$  and  $\text{LuL}^1$ , indicating similar solution structures for these complexes<sup>41,42,45,46</sup> The protons c give a singlet signal for both complexes. Decrease of the temperature from about 70–80 to 0 °C results in a broadening of the signals (except that of protons c), indicating that some dynamic processes are slower in the co-ordinated ligands, as observed for the complexes  $\text{LnL}^1$ .<sup>41,42,45,46</sup>

The broad multiplet signals of the d and e protons of the diethylenetriamine backbone were interpreted in terms of two different ethane conformations, which interconvert by wagging *via* an eclipsed transition state,<sup>14,41,46</sup> but no details of the coupling pattern have been reported. With the assumption of geminal and vicinal couplings between the ethylene protons, we calculated the coupling constants by means of spectrum simulation, from which the conformation of the ethylene group was deduced as close to a staggered state, with the nitrogens in the *gauche* position.<sup>47</sup> The results of the “R” method (developed for six-membered ring ligands)<sup>47</sup> suggest that the dihedral angle between the nitrogens is somewhat larger than 60°, *i.e.* co-ordination makes the conformation of the ethylene groups less “flattened”. (For details of the calculation, see the ESI Material.)

The 2-D COSY spectra of  $\text{LaL}^3$  and  $\text{LuL}^3$  (Fig. S2) proved interesting in that a cross-peak was clearly observed between the signals of the methyl and methylene protons of the amide group. The coupling constant between these protons (separated by a distance of five bonds) is somewhat less than 2 Hz, and thus it is not resolved in the 1-D spectra. Such long-distance proton–proton scalar coupling can be observed in the case of the allyl chain.<sup>47</sup> This finding strongly supports the suggestion that in the amide group the (O)C–N bond has a partial double bond character when the electron density increases on the C=O oxygen, which results in a stronger Ln–O bond. This phenomenon has been assumed in the interpretation of the bonding in several  $\text{Ln}^{3+}$ -DTPA bis(amide) complexes.<sup>14,25</sup> The cross-peaks in the 2-D COSY spectra provide some degree of experimental support for this assumption.

In the <sup>1</sup>H NMR spectrum of the  $\text{NdL}^3$  the paramagnetic ion increases the chemical shifts and results in separate signals for

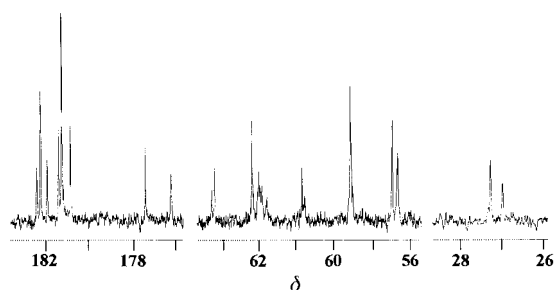


Fig. 3  $^{13}\text{C}\{-^1\text{H}\}$  NMR spectrum of  $\text{LuL}^4$  at 280 K.

all the  $\text{CH}_2$  protons. In this case 10 proton signals may be expected but at 2 °C 19 signals were observed (Fig. S3). Increase of the temperature results in coalescence of the signals and at around 70 °C 10 signals are visible. Similar phenomena were observed for the paramagnetic complexes  $\text{LnL}^1$  where 18 peaks coalesced to 9.<sup>41,42,46</sup> This signal coalescence has been explained as caused by the interconversion of two diastereomeric forms (wrapping isomers) of the complex.<sup>29,41,42,46</sup>

The  $^1\text{H}$  NMR spectrum of  $\text{LuL}^4$  is very complex, consisting of numerous overlapping signals, which indicate the presence of isomeric species. The three nitrogen atoms in  $\text{LnL}^2$  are chiral and the expected 8 isomers have been detected for  $\text{Nd}^{3+}$ -DTPA bis(propylamide).<sup>14</sup> In  $\text{LnL}^4$  the central and one of the terminal nitrogens (to which the amide group is attached) become chiral if the lifetimes of the  $\text{Ln}^{3+}$ -N bonds are long on the NMR timescale. In this case the formation of 4 wrapping isomers can be expected, which would result in a large number of signals. In the  $^1\text{H}$  NMR spectrum of  $\text{LuL}^4$  the methyl group furnishes two peaks,  $\delta$  3.03 and 3.07, in a ratio of 6:4, but the other, overlapping signals have not been identified. In the inverse gated proton-decoupled  $^{13}\text{C}$  NMR spectrum (Fig. 3) 3 peaks appear in the methyl region ( $\delta$  26–28), in an intensity ratio of 1:4:5, instead of the expected four. In the methylene region ( $\delta$  54–66) there are 16–18 overlapping signals. The CO carbon atoms give rise to 8 signals of different intensity in the range  $\delta$  181–183. The spectrum of the paramagnetic complex  $\text{NdL}^4$  is also very rich in signals. The appearance of more than 65 peaks indicates the presence of more than 3 (presumably 4) species.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the complexes  $\text{LuL}^4$  and  $\text{NdL}^4$  clearly indicate the presence of at least 3 isomeric species. However, the intensities of the signals are different and it can be assumed that the chemical shifts of 2 isomers are very close and that the spectra obtained may represent the presence of the 4 possible wrapping isomers.

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## References

- 1 R. B. Lauffer, *Chem. Rev.*, 1987, 901.
- 2 M. F. Tweedle, in *Lanthanide Probes in Life, Chemical and Earth Sciences. Theory and Practice*, eds J. C. Bünzli and G. R. Choppin, Elsevier, Amsterdam, 1989, p. 127.

- 3 S. Aime, M. Botta, M. Fasano and E. Terreno, *Chem. Soc. Rev.*, 1998, 27, 19.
- 4 D. Parker and J. A. G. Williams, *J. Chem. Soc., Dalton Trans.*, 1996, 3613.
- 5 S. C. Quay, *U.S. Pat.*, 4, 687, 659, 1987.
- 6 H.-J. Weinmann, R. C. Brasch, W.-R. Press and G. E. Wesbey, *Am. J. Roentgenol.*, 1984, 142, 619.
- 7 H. P. Niendorf, A. Alhassan, V. R. Greens and W. Clauß, *Invest. Radiol.*, 1994, 29 (suppl. 2), S 179.
- 8 W. P. Cacheris, S. C. Quay and S. M. Rocklage, *Magn. Reson. Imag.*, 1990, 8, 467.
- 9 A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum, New York, 1974, vol. 1.
- 10 A. D. Sherry, W. P. Cacheris and K.-T. Kuan, *Magn. Reson. Med.*, 1988, 8, 180.
- 11 R. T. Dean, Y. Lin, R. W. Weber and D. H. White, *U.S. Pat.*, 4, 826, 673, 1989.
- 12 F. Jasanada and F. Nepven, *Tetrahedron Lett.*, 1992, 33, 5745.
- 13 S. W. A. Bligh, A. H. M. S. Chowdhury, M. McPartlin, I. J. Scowen and R. A. Bulman, *Polyhedron*, 1995, 14, 567.
- 14 C. F. G. C. Geraldes, A. M. Urbano, M. A. Hoefnagel and J. A. Peters, *Inorg. Chem.*, 1993, 32, 2426.
- 15 C. Paul-Roth and K. N. Raymond, *Inorg. Chem.*, 1995, 34, 1408.
- 16 Y.-M. Wang, T.-H. Cheng, G.-C. Liu and R.-S. Sheu, *J. Chem. Soc., Dalton Trans.*, 1997, 833.
- 17 S. Aime, F. Benetollo, G. Bombieri, S. Colla, M. Fasano and S. Paoletti, *Inorg. Chim. Acta*, 1997, 254, 63.
- 18 X. Zhao, R. Zhuo, Z. Lu and W. Lin, *Polyhedron*, 1997, 16, 2755.
- 19 Y.-M. Wang, S.-T. Liu, Y.-J. Wang and R.-S. Shen, *Polyhedron*, 1998, 17, 2021.
- 20 L. Ehnebo and B. F. Pedersen, *Acta. Chem. Scand.*, 1992, 46, 126.
- 21 Y.-M. Wang, Y.-J. Wang, R.-S. Sheu, G.-C. Lin, W.-C. Lin and J.-H. Liao, *Polyhedron*, 1999, 18, 1147.
- 22 J. F. Carvalho, S.-H. Kim and C. A. Chang, *Inorg. Chem.*, 1992, 31, 4065.
- 23 S. F. Franklin and K. N. Raymond, *Inorg. Chem.*, 1994, 33, 5794.
- 24 E. Bovens, M. A. Hoefnagel, E. Boers, H. Lammers, H. van Bekkum and J. A. Peters, *Inorg. Chem.*, 1996, 35, 7679.
- 25 H. Imura, G. R. Choppin, W. P. Cacheris, L. A. de Learie, T. J. Dunn and D. H. White, *Inorg. Chim. Acta*, 1997, 258, 227.
- 26 C. F. G. C. Geraldes, A. D. Sherry, W. P. Cacheris, K.-T. Kuan, R. D. Brown, R. H. Koenig and M. Spiller, *Magn. Reson. Med.*, 1988, 8, 191.
- 27 G. Schuhmann-Giampieri, H. Schmitt-Willich, T. Frenzel and H. J. Weinmann, *Invest. Radiol.*, 1991, 26, 963.
- 28 R. B. Lauffer, D. J. Parmelee, H. S. Ouellet, R. P. Dolan, H. Sajiki, D. M. Scott, P. J. Bernard, E. M. Buchanan, K. Y. Ong, Z. Tyeklar, K. S. Midelfort, T. J. McMurry and R. C. Walovitch, *Acad. Radiol.*, 1996, 3, S356.
- 29 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, 99, 2293.
- 30 S. Göhr-Rosenthal, H. Schmitt-Willich, W. Ebert and J. Conrad, *Invest. Radiol.*, 1993, 28, 789.
- 31 W. A. Volkert and T. J. Hoffman, *Chem. Rev.*, 1999, 99, 2269.
- 32 *Eur. Pat. Appl.*, EP 0, 331, 616, Schering AG, 1989.
- 33 *U.S. Pat.*, 5, 676, 926, Schering AG, 1987.
- 34 L. Burai, I. Fábrián, R. Király, E. Szilágyi and E. Brücher, *J. Chem. Soc., Dalton Trans.*, 1998, 243.
- 35 L. Zékány and I. Nagypál, in *Computational Methods for Determination of Formation Constants*, ed. D. J. Lege, Plenum, New York, 1985, p. 291.
- 36 J. L. Sudmeier and C. N. Reilley, *Anal. Chem.*, 1964, 36, 2699.
- 37 T. Moeller and L. C. Thompson, *J. Inorg. Nucl. Chem.*, 1962, 24, 499.
- 38 I. Grenthe and I. Tobiasson, *Acta. Chem. Scand.*, 1963, 17, 2101.
- 39 P.-K. Tse and J. E. Powell, *Inorg. Chem.*, 1985, 24, 2727.
- 40 J. J. Stezowski and J. L. Hoard, *Isr J. Chem.*, 1948, 24, 323.
- 41 B. G. Jenkins and R. B. Lauffer, *Inorg. Chem.*, 1988, 27, 4730.
- 42 S. Aime and M. Botta, *Inorg. Chim. Acta*, 1990, 177, 101.
- 43 É. Tóth, L. Burai, E. Brücher and A. E. Merbach, *J. Chem. Soc., Dalton Trans.*, 1997, 1587.
- 44 R. J. Day and C. N. Reilley, *Anal. Chem.*, 1964, 36, 1073.
- 45 G. R. Choppin, P. A. Baisden and S. A. Khan, *Inorg. Chem.*, 1979, 18, 1330.
- 46 J. A. Peters, *Inorg. Chem.*, 1988, 27, 4686.
- 47 W. A. Thomas, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1997, 30, 183.