# Triethylenetetramine-*N,N,N′,N″,N‴,N‴*-hexaacetic Acid (TTHA) and TTHA-Bis(butanamide) as Chelating Agents Relevant to Radiopharmaceutical Applications

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The *N,N′*-bis(butanamide) derivative of TTHA (TTHA = triethylenetetramine-*N,N,N′,N″,N‴,N‴*-hexaacetic acid), and its $Ga^{3+}$ and $In^{3+}$ complexes were synthesized and characterized. The crystal X-ray diffraction structure of $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$ was determined. The complex crystallizes in the monoclinic space group $P2_1/n$ with $a = 7.179(2)$ Å, $b = 20.334(3)$ Å, $c = 10.902(5)$ Å, $\beta = 101.90(2)°$, and $Z = 2$. Each gallium atom is bonded to six donor atoms ($N_2O_4$) in a slightly distorted octahedral geometry. The values of the protonation constants and the protonation sequence were determined by potentiometry and NMR. The stability constants of the $Al^{3+}$, $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ complexes of TTHA-$(BuA)_2$ and of the $Ga^{3+}$ complex of TTHA were determined by potentiometry. The structures, in solution, of the $Al^{3+}$, $Ga^{3+}$, and $In^{3+}$ complexes of TTHA-$(BuA)_2$ and TTHA were analyzed by $^1H$, $^{13}C$, $^{27}Al$, $^{71}Ga$, and $^{115}In$ NMR techniques. Derivatization of two terminal carboxylates by butanamide substituents leads to a significant decrease of the total ligand basicity (5.77 log units) and to a change of the solubility of the resulting complexes. The stability constant of the ML complexes of TTHA-$(BuA)_2$ with $Fe^{3+}$ exhibits the highest value of the series ($10^{23.92}$). The $In^{3+}$ complex is more stable than that of $Ga^{3+}$ and almost as stable as that of the $Fe^{3+}$. However, the decrease in indium and iron complex stability is less drastic going from TTHA to TTHA-$(BuA)_2$ (about 3 log units) than for $Al^{3+}$ or $Ga^{3+}$ (about 6 log units). pM values calculated under physiological conditions for DTPA, TTHA, and the bis(butanamide) derivatives have shown that while DTPA remains a ligand of choice to chelate $Fe^{3+}$ and $In^{3+}$ ions in vivo compared to transferrin as competitor ligand, TTHA, surprisingly, appears to be the best of these four ligands (pM = 22.71) to chelate $Ga^{3+}$.

## Introduction

Polyamino polycarboxylate ligands, both linear and cyclic, are currently used in medical applications to prepare metal compounds with radioisotopes for diagnostic imaging or radiotherapy or with lanthanides for magnetic resonance imaging (MRI).[1] These compounds combine favorable properties required in clinical use: high water solubility; low toxicity; high thermodynamic and kinetic stabilities. To expand their clinical applications, new chelates have been designed using functionalized polyamino polycarboxylate ligands. The functionalization can be used to increase the selectivity of the ligand and the stability of the metal chelates but also to optimize the hydrophilic/lipophilic balance or to covalently attach the ligand to a synthetic supramolecular support or to a protein. These last two possibilities are essential to increase the intracellular biodistribution of the compound and the diagnostic and radiotherapeutic targeting of disease sites with high specificity.[2]

To take advantage of the tumor cell's affinity for low-density lipoproteins (LDL) to improve the delivery of imaging agents to tumor sites,[3,4] we recently proposed a new method for labeling LDL with indium-111 using the *N,N″*-bis(stearamide) of diethylenetriaminepentaacetic acid (DTPA-$(SA)_2$) as a lipid chelating anchor (L) to stabilize the radionuclide on the LDL particles.[5] This acyclic ligand was selected in preference to macrocyclic derivatives because its rate of complexation is much higher than that of rigid cyclic ligands, which is an advantage to complex the $In^{3+}$ ion at the last step of the radiolabeling procedure. The flexibility of this DTPA derivative compared to the rigidity of macrocycles was also an advantage to enforce the insertion of the metal chelate into the lipid layer of LDL. In vitro and in

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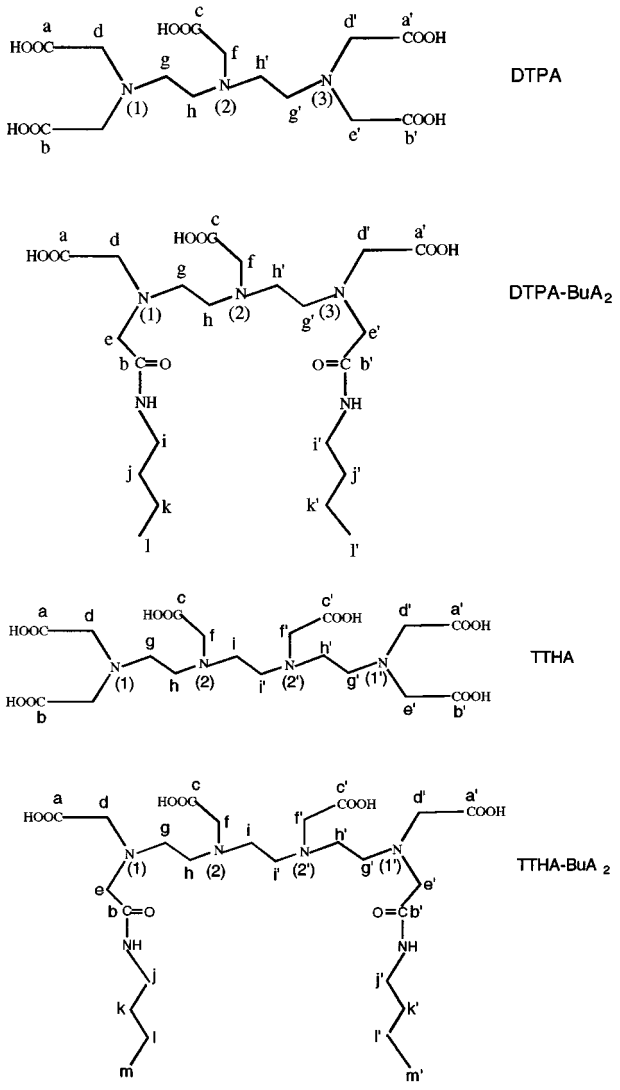


**Figure 1.** Structure and labeling of DTPA, DTPA-$(BuA)_2$, TTHA, and TTHA-$(BuA)_2$.

vivo studies have demonstrated that these In−L−LDL particles possess suitable properties to be evaluated as potential radiopharmaceuticals for tumor localization.[6]

The main physicochemical properties of the In(DTPA-$(SA)_2$) chelate are its neutral charge and insolubility in aqueous buffered solutions at pH 7.4, high lipophilicity and solubility in lipid phases (e.g. LDL), high stability constant, and the presence of an eight-coordinated $In^{3+}$ single structural isomer in aqueous solution. These last two properties were deduced by comparison with the results obtained from potentiometric and NMR solution studies for the DTPA-bis(butanamide) derivative (DTPA-$(BuA)_2$), which was used as a model for solubility reasons[7] (see Figure 1).

Decadentate bis(amide) derivatives of triethylenetetramine-*N*,*N*,*N*′,*N*″,*N*‴,*N*‴-hexaacetic acid (TTHA) may form negatively charged complexes with metal(III) ions. The aim of this work is to examine the influence of the coordination properties on the solubility, stability constants, and isomer populations in aqueous solution in order to further evaluate the potential of such chelates for metal labeling of lipoproteins or other radiopharmaceutical applications. TTHA was also studied in this work for comparison.

This report describes the following: (a) the synthesis and characterization of the TTHA-bis(butanamide) ligand and its $Ga^{3+}$ and $In^{3+}$ complexes; (b) the X-ray structure of $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$; (c) the protonation studies of TTHA-$(BuA)_2$ and TTHA; (d) the stability constants and structures in aqueous solution of $Al^{3+}$, $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ complexes of TTHA-$(BuA)_2$ and TTHA, using potentiometric and NMR techniques.

## Experimental Section

**Materials.** All chemicals were of analytical grade, used as received unless specifically noted, and obtained from the following sources: citric acid, butylamine, and metal salts from Aldrich, triethylenetetraminehexaacetic acid (TTHA) from Sigma Chemical Co., solvents from Prolabo. Sodiumsulfonate 4,4-dimethyl-4-sylapentane (DSS) from Bruker Spectrospin. Other materials are cited under specific sections.

**Instrumentation for Ligand Characterization.** C, H, and N analyses were performed by the Centre d'Analyze Interuniversitaire (ENSCT, Toulouse, France). Fast atom bombardment (FAB) mass spectra of samples dissolved in a glycerol/thioglycerol matrix were obtained on a Nermag R10-10H mass spectrometer at the Paul Sabatier University, Toulouse, France. Infrared spectra were recorded from KBr pellets on a Perkin-Elmer 883 spectrophotometer. $^1H$ and $^{13}C$ NMR spectra were recorded on Bruker AC 200 and AM 250 spectrometers. The residual proton signal from the solvent was used as internal standard for $^1H$ NMR.

**Synthetic Procedures and Characterization. Triethylenetetraminetetraacetic Acid Dianhydride, TTHAa.** It was prepared according to the reported method with some modifications.[8] To TTHA (1.33 g, 2.70 mmol) were added acetic anhydride (2 mL) and pyridine (1.3 mL). The mixture was stirred at 40 °C for 48 h and filtered. The cream precipitate was washed with acetic anhydride (100 mL) and diethyl oxide (100 mL) and dried under vacuum at 40 °C: yield 80%; mp 171−172 °C; IR ($\nu$/cm$^{-1}$) 2950, 2910 (CH alkyl), 1820, 1760 (CO anhydride), 720 (CH alkyl).

***N*,*N*′-Bis(butanamide) of TTHA, TTHA-$(BuA)_2$.** Butylamine (0.271 mL, 2.75 mmol) was added dropwise with stirring to a solution of triethylenetetraminehexaacetic acid dianhydride (0.627 g, 1.37 mmol) in dry dimethylformamide (100 mL, 40 °C). The colorless reaction mixture was stirred (40 °C, 1 h) and filtered. Chloroform (100 cm$^3$) was added to the filtrate, and after cooling at 4 °C for 2 h, the beige precipitate was filtered off, washed with diethyl oxide (100 mL), and dried at 40 °C overnight. This crude product was recrystallized from hot pyridine−ethanol (1/3 v/v; 120 mL) at 70 °C, giving a white powder after 1 day at room temperature, yield 60%. It was soluble in water (20 g·L$^{-1}$), dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) and insoluble in diethyl ether, chloroform, acetone, and hexane, mp 204−205 °C. Anal. Calcd for $C_{26}H_{48}N_6O_{10}\cdot 2H_2O$: C, 48.74; H, 8.18; N, 13.12. Found: C, 48.89; H, 7.70; N, 13.12. FAB MS: *m*/*e* of $(M + H)^+ = 604.3$, calc = 604.71. IR ($\nu$/cm$^{-1}$): 3342 (N−H amide), 3030, 2950, 2920, 2860 (C−H alkyl); 1730, 1710, (C═O acid); 1660, 1625 (C═O amide); 1560 (C−N amide); 720 (C−H alkyl). $^1H$ NMR [$\delta_H$ /ppm, 250 MHz; solvent and standard $D_2O$ at $\delta$ 4.78, pH = 3.1, 25 °C] (multiplicity; proton type, *J* (Hz) [carbon numbering, see Figure 1]): 0.87 (t; 6H, 7.3; 2 $CH_3$ [m]); 1.28 (m; 4H; 7.8; 2 × $CH_2$ [l]); 1.46 (q; 4H; 7.0; 2 $CH_2$ [k]); 3.22 (t; 4H; 6.9; 2 $CH_2$ [j]); 3.31 (s; 8H; 4 $CH_2$; [g and h]); 3.36 (s; 4H; 2 $CH_2$ [i]); 3.68 (s; 4H; 2 $CH_2$ [f]); 3.73 (s; 4H; 2 $CH_2$ [e]); 3.77 (s; 4H; 2 $CH_2$ [d]). $^{13}C$ NMR (62.9 MHz; solvent $D_2O$ and DSS as standard at $\delta$ = 0 ppm, pH = 3.1, 25 °C) ($\delta$/ppm (multiplicity; *J* (Hz) [carbon numbering]): 15.52 (q; 124; $CH_3$ [m]); 21.97 (t; 122 [l]); 32.95 (t; 124 [k]); 41.8 (t; 139 [j]); 53.5 (t; 143 [i]); 54.11 (t; 142 [h]); 54.4 (t; 143 [g]); 57.6 (t; 142 [e]); 59.6 (t; 142 [f]); 59.6 (t; 141 [d]); 171.03 (s [b]); 174.51 (s [c]); 175.04 (s [a]).

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**Table 1.** Crystallographic Data for $[Ga_2(OH_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$

| | |
|---|---|
| formula | $C_{18}H_{42}Ga_2N_4Na_2O_{22}$ |
| fw | 851.98 |
| temp | 20(2) °C |
| wavelength | 0.710 70 Å |
| cryst system | monoclinic |
| space group | $P2_1/n$ |
| unit cell dimens | $a = 7.179$ (2) Å, $\alpha = 90°$ |
| | $b = 20.334$ (3) Å, $\beta = 101.90(2)°$ |
| | $c = 10.902$ (5) Å, $\gamma = 90°$ |
| V | 1557.2(9) Å$^3$ |
| $Z$ | 2 |
| $D$(calcd) | 18.17 g/cm$^3$ |
| abs coeff | 18.57 cm$^{-1}$ |
| final $R$ indices [$I > 2\sigma(I)$] | $R_1 = 0.0232$, w$R_2{}^a = 0.0582$ |
| $R$ indices (all data) | $R_1 = 0.0260$, w$R_2{}^a = 0.0593$ |

[a] $wR_2 = [\{\sum w(F_o^2 - F_c^2)^2\}/\{\sum w(F_o^2)^2\}]^{1/2}$.

**Complexes. Preparation of Ga(TTHA-$(BuA)_2$), Which Hydrolyzed to Give $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$.** To an aqueous solution (8 mL) of TTHA-$(BuA)_2$ (0.2 g, 0.312 mmol) were added an aqueous mixture containing $GaCl_3$ (0.070 g, 0.312 mmol) and citric acid (0.066 g, 0.312 mmol). The pH of the solution was adjusted to 8.2 by adding aqueous NaOH (0.1 M). The mixture was stirred at 50 °C for 2 h. Evaporation of the solvent on a rotary evaporator afforded a white powder. The crude product was dissolved in a small volume (6 mL) of water/methanol/ethanol mixture (1:1:1 v:v:v) at 40 °C and filtered. Slow evaporation of the filtrate gave colorless crystals after 1 month. Yield: 25%. IR ($\nu$/cm$^{-1}$): 3320 (N−H amide), 2960, 2920, 2873 (CH alkyl); 1590, 1390 ($COO^-$); 1550, 720 (C−H alkyl); 303 (Ga−O). X-ray structural analysis revealed that the two amide bonds were hydrolyzed under these experimental conditions. The final compound isolated was a digallium(III) TTHA disodium salt complex, hexaaquodisodium dihydroxy($\mu$-triethylenetetraminehexaacetato)digallium-(III) dihydrate.

**Ga(TTHA-$(BuA)_2$)Na·2$H_2O$.** A solution of TTHA-$(BuA)_2$ (0.219 g, 0.362 mmol) was prepared in a mixture of NaOH (0.1 M)/ethanol (1:1 v:v, 10 mL). An aqueous mixture of $GaCl_3$ (1.3 mL, 0.362 mmol) and citric acid (0.076 g, 0.362 mmol) and a solution of NaOH (0.1 M) were alternatively added dropwise to the ligand solution. The pH of the mixture was kept constant at 6 during the synthesis. The mixture was stirred at 50 °C for 45 min, and evaporation of the solvent produced a yellow product. This crude compound was dissolved in a hot butanol−toluene mixture (3:1 v:v, 40 mL) and stirred at 50 °C for 30 min. After filtration to eliminate solid salts (sodium citrate), evaporation of the filtrate and vacuum-drying yielded the final pale yellow compound, which is soluble in water, pyridine, DMSO, and hot butanol and slightly soluble in methanol and DMF. Yield: 30%; mp = 210 °C. Anal. Calcd for $C_{26}H_{44}GaN_6O_{10}Na\cdot 2H_2O$: C, 42.81; H, 6.63; N, 11.52. Found: C, 42.65; H, 6.88; N, 11.45. FAB MS: $m/e$ of (M + H) = 693.0, calc = 693.39. IR ($\nu$/cm$^{-1}$): 3447 (N−H amide), 2964, 2873 (CH alkyl); 1653, 1385 ($COO^-$); 708 (C−H alkyl); 388 (In−N). $\delta_C$ (62.9 MHz; solvent $D_2O$ and DSS as standard at $\delta = 0$ ppm, pH = 6.0, 25 °C) ($\delta$/ppm [carbon numbering]): 15.57 [m,m′]; 22.00; 33.09; 41.34; 45.21 [l,l′] [k,k′] [j,j′]; 53.6; 54.00; 54.7 [h,h′] [i,i′] [g,g′]; 58.92; 60.00; 61.38 [d,d′] [e,e′] [f,f ′]; 176.02; 177.8; 181.52; 181.82; [a,a′] [b,b′] [c,c′].

**In(TTHA-$(BuA)_2$)Na·2$H_2O$.** A solution of TTHA-$(BuA)_2$ (0.3 g, 0.496 mmol) was prepared in a mixture of NaOH (0.1 M)/ethanol (1:1 v:v, 12 mL). An aqueous mixture (3 mL) of $InCl_3$ (0.110 g, 0.496 mmol) and citric acid (0.104 g, 0.496 mmol) and a solution of NaOH (0.1 M) were alternately added dropwise to the ligand solution. The synthesis was pursued as described above for Ga(TTHA-$(BuA)_2$). Yield: 30%; mp > 260 °C. Anal. Calcd for $C_{26}H_{44}InN_6O_{10}Na\cdot 2H_2O$: C, 40.32; H, 6.24; N, 10.85. Found: C, 40.31; H, 6.34; N, 10.82. FAB MS: $m/e$ of (M + H) = 738.0, calc = 738.47. IR ($\nu$/cm$^{-1}$): 3262 (N−H amide), 2960, 2920, 2873 (CH alkyl); 1627, 1381 ($COO^-$); 730 (C−H alkyl). $\delta_C$ (62.9 MHz; solvent $D_2O$ and DSS as standard at $\delta = 0$ ppm, pH = 7.0, 25 °C) ($\delta$/ppm [carbon numbering]): 14.47 [m,m′]; 20.93; 31.20; 31.40; 31.78; 40.56; 40.9; 41.80; 41.90 [l,l′] [k,k′] [j,j′]; 51.0; 54.00 [h,h′] [i,i′] [g,g′]; 56.3; 61.0; 62.9 [d,d′] [e,e′] [f,f ′]; 172.2; 172.3; 177.2; 178.2 [a,a′] [b,b′] [c,c′].

**Table 2.** Selected Bond Lengths (Å) and Angles (deg) for $[Ga_2(OH_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$[a]

| | | | |
|---|---|---|---|
| Ga−N(1) | 2.1168(14) | Ga−O(3) | 1.9745(13) |
| Ga−N(2) | 2.186(2) | Ga−O(5) | 1.9970(14) |
| Ga−O(2) | 1.976(2) | Ga−O(7) | 1.8420(13) |
| O(7)−Ga−O(3) | 97.95(6) | O(2)−Ga−N(1) | 82.67(6) |
| O(7)−Ga−O(2) | 95.88(6) | O(5)−Ga−N(1) | 88.61(6) |
| O(3)−Ga−O(2) | 96.40(6) | O(7)−Ga−N(2) | 98.61(6) |
| O(7)−Ga−O(5) | 93.03(6) | O(3)−Ga−N(2) | 160.65(5) |
| O(3)−Ga−O(5) | 88.28(6) | O(2)−Ga−N(2) | 91.79(6) |
| O(2)−Ga−O(5) | 169.26(5) | O(5)−Ga−N(2) | 80.93(6) |
| O(7)−Ga−N(1) | 177.64(6) | N(1)−Ga−N(2) | 83.32(6) |
| O(3)−Ga−N(1) | 80.39(6) | H(O(7))−O(7)−Ga | 113(2) |

[a] Symmetry operations: (a) $-x, -y + 1, -z$; (b) $-x + 1, -y + 1, -z + 1$; (c) $-x + 1, -y + 2, -z$; (d) $-x, -y + 1, -z + 1$; (e) $-x + {}^1/_2, y - {}^1/_2, -z + 1$; (f) $-x, -y, -z + 1$; (g) $-x + {}^1/_2, y + {}^1/_2, -z + 1$.

**X-ray Data Collection and Structure Determination of $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$.** Graphite−monochromated Mo K$\alpha$ radiation ($\lambda = 0.710\,73$ Å) was employed as X-ray source. Crystal X-ray diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer. Cell constants were determined by least-squares fitting of 25 accurately centered reflections. During data collection the intensities of three standard reflections were checked periodically for stability, and no loss of intensity was observed. The data set was corrected for Lorentz and polarization effects and absorption corrections (9 $\psi$-scans) were applied. Other data are given in Table 1. The structure was solved by direct methods. Program packages used for calculations, refinements, and plotting were, successively, MOLEN, SHELXS 86, SHELXL 93, and KPLOT.[9] Atomic scattering factors and anomalous dispersion terms were obtained from program packages or from ref 10. All hydrogen atoms were observed on Fourier difference maps and refined with fixed isotropic thermal parameters except those involved in hydrogen bonds which were refined with free isotropic thermal parameters. Selected interatomic distances and angles are listed in Table 2, and a plot of the molecule is shown in Figure 2 along with the labeling scheme. Complete tables of crystallographic data are included as Supporting Information.

**Potentiometric Measurements. Reagents and Solutions.** Metal ion solutions were prepared at about 0.025 M from the nitrate salts of the metals of analytical grade (from Aldrich) with demineralized water (obtained by a Millipore/Milli-Q system) and were standardized by titration with $Na_2H_2EDTA$ (disodium salt of ethylenedinitrilotetraacetic acid).[11] For $Ga^{3+}$ and $Al^{3+}$ back-titration with a standard solution of $ZnSO_4$ was made. The solutions of the trivalent metal ions were kept in an excess of nitric acid to prevent hydrolysis. Carbonate-free solutions of the titrant, $(CH_3)_4NOH$, were prepared as described before.[7] The solutions were standardized by titration with a solution of potassium hydrogen phthalate and discarded when the percentage of carbonate was about 0.5% of the total amount of base, verified by Gran's method.[12]

**Equipment for Potentiometric Measurements.** The equipment used was described before.[7] All the experiments were monitored by computer. The temperature was kept at 25.0 ± 0.1 °C; atmospheric $CO_2$ was excluded from the cell during the titration by passing purified $N_2$ across the top of the experimental solution in the reaction cell. The ionic strength of the solutions was kept at 0.10 M with $(CH_3)_4NNO_3$.

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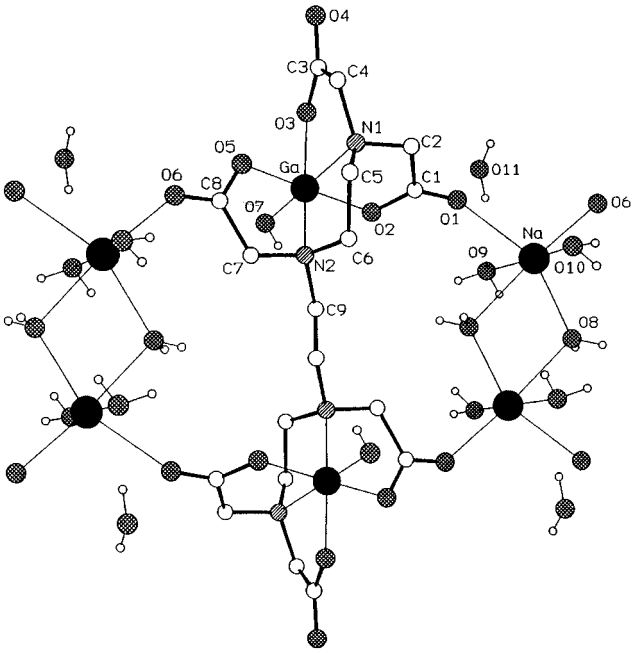


**Figure 2.** ORTEP drawing of the crystal structure of $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$, showing the atom numbering.

**Potentiometric Measurements.** The $[H^+]$ of the solutions was determined by the measurement of the emf of the cell, $E = E'^\circ + Q \log[H^+] + E_j$. $E'^\circ$ and $Q$ were obtained by previous calibration titrating a standard solution of known hydrogen-ion concentration at the same ionic strength, using the values of the acid range. The term p[H] is defined as $-\log [H^+]$. $E_j$, the liquid-junction potential, was found to be negligible in the experimental conditions used. The value of $K_W = ([H^+][OH^-])$ was determined from data obtained in the alkaline range of the calibration, considering $E'^\circ$ and $Q$ valid for the entire pH range, and was found to be equal to $10^{-13.80}$.

The potentiometric equilibrium measurements were made on 20.00 mL of ligand solutions $\cong 2.50 \times 10^{-3}$ M diluted to a final volume of 30.00 mL, 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 2:1, 1:1, and 1:1.5. The $E$ data were taken after additions of 0.0250 mL or 0.050 mL increments of standard 0.0978 M $(CH_3)_4NOH$ solution, and after stabilization in this direction, equilibrium was then approached from the other direction by adding 0.1000 M standard acid.

With $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ the degree of formation of the metal complexes with TTHA and TTHA-$(BuA)_2$, at the beginning of the titrations, was too high for the use of the direct potentiometric method, so it was necessary to perform ligand−ligand or metal−metal competition titrations to determine the constants. $K_2H_2EDTA$ was the second ligand for the $In^{3+}$ and $Fe^{3+}$ complexes of TTHA-$(BuA)_2$. Ratios of $C_L$:$C_{L'}$:$C_M$ of 1:1:1 and 1:1:2 were used (L is our ligand, for which the stability constant of the metal complex was to be determined, and L′ the reference ligand, $K_2H_2EDTA$, for which the stability constant of the complex of the same metal is known accurately). A competition reaction, which can be written in terms of equilibrium (1), occurs in good conditions when all the complexed species exist in solution at a concentration of at least 30% of the total metal ion.

$$(ML')^- + H_xL \rightleftharpoons (ML)^{(x-3)-} + (x-2)H^+ + (H_2L')^{2-} \quad (1)$$

$x = 6$ for L = TTHA $\quad x = 4$ for L = TTHA-$(BuA)_2$

Stability constants for the $Ga^{3+}$ complexes of TTHA and TTHA-$(BuA)_2$ were calculated by relying on the competition or displacement reaction 2.[13]

$$GaLOH^{(x-2)-} + 3OH^- \rightleftharpoons Ga(OH)_4^- + L^{x-} \quad (2)$$

$x = 6$ for L = TTHA $\quad x = 4$ for L = TTHA-$(BuA)_2$

At values of pH = 6 the constants corresponding to the formation of the species $GaL^{(x-3)-}$ and $GaLOH^{(x-2)-}$ were determined and used as constants in other parts of the titration curves (of low pH values) to obtain the constants of the other equilibrium reactions.

Stability constants of the complexes of $Fe^{3+}$ with TTHA were determined by a direct redox method. The potential of the titration of a solution containing $Fe^{2+}/Fe^{3+}$, with an excess of $Fe^{2+}$, with a solution of TTHA as titrant, at pH = 2, was followed by a Pt/reference pair of electrodes.[14] For the $In^{3+}$ complex of TTHA the same experimental method was used, following the competition reaction between $Fe^{3+}$ and $In^{3+}$ for the ligand.[14]

Although the equilibria of the competition reactions between two complexes, (1) and (2), were slow in being reached, automated titrations were still possible. It was necessary to wait for 10−15 min at each point of the titration in the pH range where the competition reaction took place. The same values of stability constants were obtained whether using the direct or the back-titration curves.

**Calculation of Equilibrium Constants.** Protonation constants

$$K^H_i = \frac{[H_iL]}{[H_{i-1}L][H]}$$

were calculated by fitting the potentiometric data obtained for the free ligand to the SUPERQUAD program.[15] The stability constants of the various species formed in the aqueous solution were obtained from the experimental data of the titrations of solutions with different ligand and metal ion ratios, using the same program.[15] The initial computations were obtained in the form of overall stability constants or values:

$$\beta_{M_mL_lH_h} = \frac{[M_mL_lH_h]}{[M]^m[L]^l[H]^h}$$

We found mononuclear species, ML, $MH_iL$ ($i$ = 1, 2, 3), ML-H (i.e. $\beta_{ML-H} = \beta_{MLOH}K_W$), and dinuclear species, $M_2L$, $M_2L$-H, and $M_2L$-$H_2$. Differences, in log units, between the values $\beta_{MLH}$ (or $\beta_{ML-H}$ or $M_2L$) and $\beta_{ML}$ or between the values $\beta_{MLH_i}$ and $\beta_{MLH_{i-1}}$ provide the stepwise reaction constants. The species introduced were limited to those which can be justified by established principles of coordination chemistry. Species distribution curves were plotted with the aid of the program SPE and SPEPLOT.[16] Titration curves for TTHA-$(BuA)_2$ and species distribution curves for TTHA are included as Supporting Information.

The errors quoted are the standard deviations ($\pm\sigma$) of the overall stability constants given directly by the program. In the case of the stepwise constants the standard deviations were determined by the normal propagation rules and do not represent the total experimental errors.

**Hydrolysis Species of the Trivalent Metal Ions.** The trivalent metal ions studied in the present work form several hydrolytic species in aqueous solution. Their constants are subject to some disagreements in the literature, but we used the values considered to be the most reliable and shown in Table 3.[7,17,18]

**NMR Measurements.** Proton and $^{13}C$ NMR spectra were recorded on Varian Unity 500 (499.843 and 125.697 MHz, respectively) and Varian XL-200 (200.053 and 50.300 MHz, respectively) spectrometers at the University of Coimbra. $^{27}Al$, $^{71}Ga$, and $^{115}In$ NMR spectra were obtained on the Varian Unity 500 spectrometer at 130.263, 152.426, and 109.545 MHz, respectively. Accurate measurement of the $^{27}Al$ NMR resonance line widths on this spectrometer was not possible due to the presence of a broad background $^{27}Al$ NMR signal from the

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**Table 3.** Hydrolysis Constants of the $Al^{3+}$, $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ Ions Used in This Work[7,17,18]

| quotient | $Al^{3+}$ | $Ga^{3+}$ | $Fe^{3+}$ | $In^{3+}$ |
|---|---|---|---|---|
| $MOH/M \times OH$ | 8.49 | 10.87 | 11.29 | 9.50 |
| $M(OH)_3/M \times (OH)^3$ | 24.76 | 30.33 | | 29.50 |
| $M(OH)_2/M \times (OH)^2$ | 16.84 | 20.95 | 21.74 | 18.20 |
| $M(OH)_4/M \times (OH)^4$ | 31.58 | 38.34 | | 33.80 |
| $M_2(OH)_2/M^2 \times (OH)^2$ | 20.04 | | 24.84 | 23.20 |
| $M_3(OH)_4/M^3 \times (OH)^4$ | 42.08 | | | |
| $M_4(OH)_4/M^4 \times (OH)^4$ | | | | 47.80 |

broadband probe used. Therefore, some $^{27}Al$ spectra were also obtained on a Varian VXR 400S NMR spectrometer (Delft Technical University) at the frequency of 104.215 MHz using either a nonspinning solid-state magic angle spinning (MAS) probe devoid of $^{27}Al$ NMR background signal or a broad-band liquids probe with subtraction of the broad $^{27}Al$ NMR background probe signal from the $^{27}Al$ signal of the $Al^{3+}$ chelate solutions studied. Comparison of both methods gave the same line width values. Proton chemical shifts were referenced to sodium 3-(trimethylsilyl)-[$^2H_4$]propionate and $^{13}C$ shifts to *tert*-butyl alcohol as internal reference (methyl signal at $\delta$ 31.2). Aqueous solutions (0.1 M) of the $Al^{3+}$, $Ga^{3+}$, and $In^{3+}$ nitrates were used as external references for the $^{27}Al$, $^{71}Ga$, and $^{115}In$ chemical shifts. Solutions of the complexes for the NMR measurements were made up in $D_2O$ (99.98% isotope purity, from Sigma Chemical Co.) in the 1:1 and 2:1 metal-to-ligand ratios by mixing equimolar solutions of the ligands $H_4L$ and hydrated nitrates of $Al^{3+}$, $Ga^{3+}$, and $In^{3+}$ followed by solution pH adjustment with dilute NaOD and DCl (from Sigma Chemical Co.). pH measurements were carried out on a Metrohm E520 pH-meter equipped with an Ingold 405-M3A glass electrode, and the values obtained were corrected for the deuterium isotope effect.[19] Proton and $^{13}C$ NMR spectra were assigned on the basis of data[7] from the literature and using the one-dimensional APT spectra and two-dimensional chemical shift correlated (COSY) spectra.

## Results and Discussion

**Ligand Synthesis.** The TTHA-bis(butanamide) ligand was prepared in two steps from TTHA. In the first step TTHA-bis(anhydride) (TTHAa) was prepared using the previously described procedure.[8] In the second step, the TTHA-bis(amide) derivative was obtained from the condensation reaction of TTHAa with 2 equiv of butylamine. The analytical and spectral data are completely consistent with the proposed formulation (Figure 1), and NMR data unambiguously confirm the symmetry of the ligand. TTHA-$(BuA)_2$ is soluble in water, DMF, and DMSO.

**Metal Complex Syntheses**. Gallium and indium complexes were prepared with the corresponding chloride salts. The gallium complex of TTHA-$(BuA)_2$ was tentatively synthesized in the presence of an equimolar quantity of citric acid in aqueous solution at pH 8.2, but this slightly basic pH was high enough to hydrolyze the amide bond and convert the bis(amide) derivative into the initial hexaacetic form. This reaction, probably templated by the gallium(III) ion, yielded $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$ as it was confirmed by the crystal X-ray structural analysis. This structure also revealed the binuclearity of the gallium chelate with TTHA. To avoid such hydrolysis in basic conditions, the pH of the reaction mixture was then kept constant at 7.0 without changing any other conditions in the synthetic procedure to prepare the gallium and indium complexes derived from TTHA-$(BuA)_2$. Ga(TTHA-$(BuA)_2$) and In(TTHA-$(BuA)_2$) complexes are soluble in water, pyridine, DMF, and hot butanol. Both complexes were characterized by IR, NMR, mass spectral methods, and elemental analysis. Attempts to get monocrystals of these last two complexes from different synthetic procedures in weakly acid, neutral, or weakly basic conditions were unsuccessful. The complexes show IR bands in the region 3300−3200 $cm^{-1}$ assigned to the N−H stretch of the coordinated secondary amide groups and around 1630−1620 and 1380−1370 $cm^{-1}$ for the $COO^-$ group vibrations. The bands characteristic of the nonionized carboxylic groups and of the amide CO groups are assumed to be masked by the very broad absorption band of $COO^-$ (130 $cm^{-1}$at half-height) centered at 1627 $cm^{-1}$. Upon coordination to the metal ion, the N−H frequencies undergo a bathochromic shift from 3342 to 3262 $cm^{-1}$ (≈80 $cm^{-1}$). The observation of broad bands at 3600−3200 $cm^{-1}$ suggests the presence of hydrogen bonding in these complexes. New bands appear below 600 $cm^{-1}$ in the IR spectrum of each coordinated ligand with bands at 300 and 247 $cm^{-1}$ which could be assigned to the M−N and M−O bonds, respectively.

**X-ray Structure of $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$.** An ORTEP drawing of the complex is given in Figure 2. There are two $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$ molecules in the monoclinic unit cell. Each gallium atom is bonded to one hydroxyl anion and to the completely deprotonated decadentate TTHA ligand through two of the four tertiary amino nitrogen donor atoms and three of the six deprotonated carboxylate oxygen atoms affording a 2-fold negative anion complex. Each $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$ molecule is centrosymmetric and contains one $[Ga_2(OH)_2(TTHA)]$ dianion, one $[Na_2(H_2O)_6]$ dication, and two molecules of water of hydration. Bonds between the two halves of the centrosymmetric molecule are brought about by two water O atoms bridging two sodium atoms and by carboxylate O atoms bridging the gallium and sodium atoms. These bridges describe a 20-membered crown-like ring around the center of symmetry (Figure 2).

Each gallium atom is bonded to six donor atoms ($N_2O_4$) in a slightly distorted octahedral coordination geometry (Table 2). One carboxylate O atom (Ga−O(3) = 1.975(1) Å) and one nitrogen atom (Ga−N(2) = 2.186(2) Å) occupy the axial sites while the other two carboxylate oxygen and nitrogen atoms occupy sites in the equatorial plane (Ga−N(1) = 2.117(1), Ga−O(2) = 1.976(2), Ga−O(5) = 1.997(1) Å). The fourth equatorial site is occupied by the hydroxyl oxygen atom giving the shortest gallium−oxygen bond length in this complex (Ga−O(7) = 1.842(1) Å). The bond lengths of Ga to the tertiary-amino nitrogen atoms and to the carboxylate oxygen atoms are consistent with those observed in (hydrogen ethylenediaminetetraacetato)aquogallate(III)[19] and in polydentate hydroxy-aromatic ligands.[20,21] Other bond lengths and angles of the TTHA backbone are comparable to those observed in metal complexes derived from this ligand.[22]

The trans angle N(2)−Ga−O(3) (160.65(5) Å) had the largest deviation from 180° (≈20°), probably caused by the steric constraints of the eight-membered chelate ring involved. The two trans angles, O(2)−Ga−O(5) (169.26(5)) and N(1)−Ga−O(7) (177.64(6)°), each involving two five-membered chelate rings, deviated less from 180°. The cis angles were in the 80−

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**Table 4.** Protonation (log $K_i^H$) Constants of the Ligands TTHA-$(BuA)_2$, TTHA, DTPA-$(BuA)_2$, and DTPA and Their Stability Constants (log $K_{M_mL_lH_h}$) with $Al^{3+}$, $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ ($T$ = 25.0 °C; $I$ = 0.10 M $(CH_3)_4NNO_3$)

| | ligands | | | |
|---|---|---|---|---|
| equil quotient | TTHA-$(BuA)_2$[a] | TTHA | DTPA-$(BuA)_2$[c] | DTPA[b] |
| [HL]/[L][H] | 9.82(4) | 10.63(5);[a] 10.62[b] | 9.36(1) | 10.71 |
| $[H_2L]/[HL][H]$ | 7.09(7) | 9.46(2);[a] 9.49[b] | 4.44(2) | 8.64 |
| $[H_3L]/[H_2L][H]$ | 4.23(9) | 6.11(3);[a] 6.10[b] | 3.31(2) | 4.28 |
| $[H_4L]/[H_3L][H]$ | 3.33(8) | 4.04(4);[a] 4.06[b] | <2 | 2.6 |
| $[H_5L]/[H_4L][H]$ | | 2.75(4);[a] 2.75[b] | <2 | 2.0 |
| $[H_6L]/[H_5L][H]$ | | 2.34(7);[a] 2.3[b] | | 1.6 |
| $[H_7L]/[H_6L][H]$ | | *a*; 1.80[b] | | 0.7 |
| $[H_3L]/[L][H]^3$ | 21.14 | 26.20;[a] 26.21[b] | 17.11 | 23.63 |
| $[H_4L]/[L][H]^4$ | 24.47 | 30.24;[a] 30.27[b] | ≅19 | 26.23 |
| [AlL]/[Al][L] | 14.59(2) | 20.23(2);[a] 21.0;[b] 18.74;[d] 19.7[e] | | 18.7; |
| [AlHL]/[AlL][H] | 4.40(2) | 5.97(1);[a] 5.85[b,e] | | 20.66[d] |
| [AlL]/[AlLOH][H] | 7.53(5) | | | 4.3 |
| $[Al_2L]/[AlL][Al]$ | 5.58(7) | 9.55(3);[a] 9.20;[b,e] 8.9[e] | | 6.6 |
| $[Al_2L]/[Al_2LOH][H]$ | 3.16(4) | 4.68(5);[a] - | | |
| $[Al_2L]/[Al_2L(OH)_2][H]^2$ | 6.92(6) | 9.87(6);[a] 11.7[b,e] | | |
| [GaL]/[Ga][L] | 21.57(2) | 27.75(7);[a] 28.21;[b] 23.60[d] | 18.18(5) | 24.3 |
| [GaHL]/[GaL][H] | 3.95(2) | 15.1[f] | 3.2(6) | 4.16 |
| $[GaH_2L]/[GaHL][H]$ | 2.87(3) | 4.8(1);[a] 5.30;[b] 4.52[f] | | |
| $[GaH_3L]/[GaH_2L][H]$ | | 3.9(1);[a] 3.96;[b] 3.54[f] | | |
| [GaL]/[GaLOH][H] | 7.18(4) | *a*; 2.57[b] | 4.57(3) | 7.51 |
| $[Ga_2L]/[GaL][Ga]$ | 6.6(1) | 9.43(4);[a] 9.64[b] | | |
| $[Ga_2L]/[Ga_2LOH][H]$ | 2.84(8) | 12.40(9);[a] 10.0[f] | | |
| $[Ga_2L]/[Ga_2L(OH)_2][H]^2$ | 6.6(1) | 3.25(9);[a] - | | |
| | | 7.46(7);[a] - | | |
| [FeL]/[Fe][L] | 23.92(5) | 27.66(4);[g] 26.8;[b,e] 29.4[h] | | 28.0 |
| [FeHL]/[FeL][H] | 4.97(4) | 7.49(2);[g] 7.55;[b] 7.60[e] | | 3.56 |
| $[FeH_2L]/[FeHL][H]$ | 1.86(6) | 7.51[h] | | |
| [FeL]/[FeLOH][H] | | 2.05(2);[g] 2.68;[b] 2.75[e] | | 4.12 |
| $[Fe_2L]/[FeL][Fe]$ | 7.02(2) | 2.60[h] | | |
| $[Fe_2L]/[Fe_2LOH][H]$ | 2.16(4) | -; 9.6[b,h] | | |
| $[Fe_2L]/[Fe_2L(OH)_2][H]^2$ | 4.38(4) | 12.13(2);[g] 13.7[b,e] | | |
| | | 2.11(3);[g] - | | |
| | | 5.91(5);[g] 6.4;[b] 7.0[e] | | |
| [InL]/[In][L] | 23.69(6) | 26.88(6);[g] 26.6[b] | 22.7(1) | 29.3 |
| [InHL]/[InL][H] | 4.68(2) | 7.30(3)[g] | 1.9(1) | |
| $[InH_2L]/[InHL][H]$ | (1.71(3)) | 2.33(4)[g] | | |
| [InL]/[InLOH][H] | | | 10.2(2) | 11.9 |
| $[In_2L]/[InL][In]$ | 5.66(9) | 9.0(1)[g] | | |
| $[In_2L]/[In_2LOH][H]$ | 2.38(5) | 4.2(1)[g] | | |
| $[In_2L]/[In_2L(OH)_2][H]^2$ | 7.33(7) | | | |

[a] This work. [b] $T$ = 25.0 °C; $I$ = 0.10 M, ref 23a. [c] Reference 7. [d] Reference 28. [e] Reference 29a. [f] Reference 29b. [g] Reference 14. Reference 29c.

89° range, the smallest value being observed for the N(1)−Ga−O(3) cis angle and the largest for the N(2)−Ga−O(7) cis angle.

Each $Na^+$ ion is coordinated by one carboxylate O atom (Na−O(1) = 2.252(2) Å) from one $[Ga_2(OH)_2(TTHA)]$ complex, one carboxylate O atom (Na−O(6) = 2.404(2) Å) from the other nearest $[Ga_2(OH)_2(TTHA)]$ complex, two bridging water O donor atoms (Na−O(8) = 2.397(2) Å), and two water O donor atoms (Na−O(10) = 2.344(2) Å).

In the solid state the ligand TTHA coordinates two $Ga^{3+}$ ions giving a dinuclear structure as also observed with copper,[22a] chromium,[22b] vanadium,[22c] and antimony,[22d] unlike metal ions with greater ionic radii which give a mononuclear structure, such as lanthanum,[22e] dysprosium,[22e] bismuth,[22f] and neodynium.[22g]

**Protonation Constants.** The protonation constants of TTHA-$(BuA)_2$ and TTHA are summarized in Table 4 together with those of DTPA-$(BuA)_2$[7] and DTPA[23a] for comparison. All the protonation constants of TTHA and also its stability constants with the trivalent metal ions shown in Table 4 were redetermined by us as the literature values were spread over a wide range, making any comparison with our ligand a difficult task. TTHA-$(BuA)_2$ ($H_4L$) has four titrated protons whose corresponding protonation constants can be determined by potentiometry. The titration curve presents two inflections at $a$ = 2 and $a$ = 3, $a$ being the number of equivalents of base added per equivalent of ligand.

The first protonation constant of TTHA-$(BuA)_2$ is a high value, and the second, a fairly high one, which correspond to the protonation of amine centers. The third and forth protonation constants are lower, of the same order of those corresponding to the protonation of carboxylate groups or amine centers at shorter distances. It is interesting to observe how different the values are of the second and third constant of TTHA, which are higher than those of TTHA-$(BuA)_2$ ($K_2^H$ differ by 2.37 and those of $K_3^H$ by 1.88, in log units). The information of the macroscopic constants obtained by potentiometry does not elucidate the sequence of the protonation, but it can be complemented by the identification of the microscopic

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protonation distribution among their various basic sites as a function of the pH, provided by a titration followed by proton NMR spectroscopy. The observed deshielding of the methylene protons of the ligand is correlated with the percentages of protonation of the amino or carboxylate groups, following the empirical procedure of Sudmeier and Reilley.[24] TTHA has been studied by this approach,[25,26] and an adequate fit of the NMR data could only be achieved with the shielding constants proposed by Letkeman and Westmore, $C_N = 0.75$ ppm, $C_{N'} = 0.25$ ppm, and $C_O = 0.20$ ppm.[27] The protonation fractions, $f_j$, for the nitrogen atoms ($f_1$ and $f_2$) and the carboxylate groups ($f_3$ and $f_4$), see labeling in Figure 1, were calculated for integer values of $n$ (=1, ..., 6, number of moles of acid added per mole of polyamino carboxylate).[27]

In this work we evaluated the protonation fractions for TTHA-$(BuA)_2$ for integer values of $n$ at pH values selected on the basis of the calculated log, using the same shielding constants as previously determined for TTHA.[27] Full least-squares treatment of the data was not carried out, as it gave large errors in the calculated protonated fractions. Instead, only a qualitative evaluation was undertaken in order to discuss the differences observed relative to TTHA. For $n = 1$ and 2 the values $f_3 = f_4 = 0$ were assumed.[27] The values of $f_2$ were calculated directly from the shift of protons i (Figure 1). Protons g and h gave estimates of $f_1$ and $f_2$, while d and f gave estimates of $f_1$ and $f_4$ and $f_2$ and $f_3$, respectively. The protons e of the amide derivatized acetates had anomalous pH-dependent shifts and were not used to obtain $f_1$ values. Assignments of the proton signals at different pH values were carried out on the basis of signal multiplicities, absence of signal crossovers over the whole pH range, and the comparison of experimental and calculated shifts using $f_j$ values obtained from assigned resonances.

When 2 equiv of acid is added to the fully unprotonated form of the ligands, which in both ligands bind exclusively to the nitrogens, the values for TTHA-$(BuA)_2$ ($n = 1$, $f_1 = 0.09$, $f_2 = 0.41$; $n = 2$, $f_1 = 0.38$, $f_2 = 0.62$) are quite different from those for TTHA ($n = 1$, $f_1 = 0.28$, $f_2 = 0.28$; $n = 2$, $f_1 = 0.52$, $f_2 = 0.40$).[27] In fact, for TTHA-$(BuA)_2$ there is a preference for the central backbone nitrogens N(2) relative to terminal ones N(1), while in TTHA there is a small preference for the terminal nitrogens. For $n > 2$ the fractional protonation values obtained are as follows: at $n = 3$ for TTHA-$(BuA)_2$ ($f_1 = 0.65$, $f_2 = 0.69$, $f_3 = 0.15$, $f_4 = 0$) versus TTHA ($f_1 = 1.00$, $f_2 = 0.52$, $f_3 = 0.17$, $f_4 = 0$);[27] at $n = 4$, for TTHA-$(BuA)_2$ ($f_1 = 0.69$, $f_2 = 0.69$, $f_3 = 0.46$, $f_4 = 0.16$) versus TTHA ($f_1 = 0.93$, $f_2 = 0.60$, $f_3 = 0.47$, $f_4 = 0.05$).[27] These values indicate that while the third and fourth equivalents of acid added to TTHA fully protonate the terminal nitrogens and bind the central acetate groups preferentially to terminal ones, in TTHA-$(BuA)_2$ the terminal nitrogens are not fully protonated but some preference is also shown for the central acetate groups. Thus, amide derivatization of two terminal carboxylates in TTHA makes the terminal nitrogens less basic and the remaining terminal carboxylate groups more basic, as shown before for DTPA-$(BuA)_2$.[7]

The overall basicity of TTHA-$(BuA)_2$ is 5.77 log units lower than that of TTHA; the formation of the two amide linkages in the TTHA structure led to a drop of 0.81 log units in $K_1$ and of 2.37 log units in $K_2$ relative to TTHA or of 0.89 and 1.55, respectively, relative to DTPA, which can be interpreted by considering the structures of both ligands at different protonation stages. In the case of TTHA, the protonated forms for $n = 2$ and 3, with the terminal nitrogen atoms preferentially protonated, are stabilized by internal hydrogen bonded rings between terminal carboxylates and nitrogen groups, leading to high values of the second and third protonation constants. In the case of TTHA-$(BuA)_2$, the fully unprotonated form is stabilized by internal hydrogen bonded rings between the hydrogens of side-chain amide groups and the terminal carboxylate oxygens, leading to the low values of the first three protonation constants of the ligand and to the lowered basicity of the terminal nitrogen atoms. A similar situation has been observed for the first two protonations of DTPA versus DTPA-$(BuA)_2$.[7]

Thus, the replacement of two carboxylate groups of TTHA by butanamide substituents leads to a significant drop in the overall basicity of the ligand which will have important implications on its metal complexing behavior.

**Metal Complexation Studies.** The stability constants of the complexes formed by TTHA-$(BuA)_2$ with $Al^{3+}$, $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ are also summarized in Table 4 together with corresponding values for the similar complexes of TTHA,[14,23a,b,28,29] DTPA[23a] and DTPA-$(BuA)_2$.[7] As can be observed, mono- (ML, MHL, $MH_2L$, and MLOH) and dinuclear ($M_2L$, $M_2LOH$, and $M_2L(OH)_2$) species were found for TTHA-$(BuA)_2$ according to the best model. The ML, and sometimes also the MLOH, constants were obtained by competition reactions with EDTA (for the $Fe^{3+}$ and $In^{3+}$ complexes) or with $OH^-$ (for the $Ga^{3+}$ complexes). The values for the $MH_iL$ species were obtained by direct titrations (1:1 ratio) considering constant the values for ML and MLOH first determined by the competition procedures. The lower values obtained for the $Al^{3+}$ complexes allowed their direct determination. The very strong tendency of $Ga^{3+}$ to undergo hydrolysis to $Ga(OH)_4^-$ was taken advantage of for the calculation of formation constants of our ligand with this metal ion.[30a,b] As the final constants will be affected by the uncertainties of the hydrolysis constants, the best literature values were used in the calculations.

The titration curves of the $M^{3+}$:TTHA-$(BuA)_2$ systems in 1:1 ratio show an inflection for values of $a = 4$. For $a = 3$ a slight increase of pH forming a new buffer region is perceived for $Fe^{3+}$ and $In^{3+}$, indicating the dissociation of the protonated species MHL. With the ratio 2:1 (prepared by increasing the amount of metal ion for the same amount of ligand), quite a different titration curve was obtained with a steep inflection at 6 equiv of base per equivalent of ligand, while the titration of $Cu^{2+}$, at the same ratio, maintained the equivalence point at $a = 4$. This may be explained by the formation of the neutral species $M_2L(OH)_2$, involving the trivalent metal ions, corresponding to the equilibrium

$$2M^{3+} + H_4L + 6OH^- \rightleftharpoons M_2L(OH)_2 + 4H_2O$$

although the formation of $M_2L^{2+}$ and $M_2L(OH)^-$ are also possible.

The alternative reaction

$$2M^{3+} + H_4L + 6OH^- \rightleftharpoons MHL + 3H_2O + M(OH)_3$$

gives rise to precipitation of the hydrolyzed trivalent metal ion.

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(30) (a) Harris, W. R.; Martell, A. E. *Inorg. Chem.* **1976**, *15*, 713. (b) Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1980**, *19*, 1646.

As no precipitation occurred in these pH regions, the first reaction seems to be the most pertinent, as also shown by the SUPERQUAD program.

TTHA-$(BuA)_2$ and TTHA with 10 donor atoms have a great tendency to form dinuclear complexes, $M_2L$. In such cases the octahedral coordination sphere of each metal ion is completed with one molecule of $H_2O$, forming $M_2L(OH_2)_2$. These molecules of water are directly coordinated and strongly bound to the metal ion, dissociating at very low pH to form $M_2L(OH)_2$. This species, mentioned previously for some of the complexes of TTHA, namely those of $Al^{3+}$ and $Fe^{3+}$,[23a,b] was observed in the present work by the crystal X-ray diffraction structure of the gallium complex of TTHA, which shows the hydroxyl oxygen atom directly bound to the metal ion having the shortest gallium−oxygen bond length of the complex.

The trend of stability constants for the ML complexes of TTHA-$(BuA)_2$ with the trivalent metal ions studied agrees with what is usually observed for the complexes of polyamino polycarboxylate ligands, the $Fe^{3+}$ complex exhibiting the highest value of the series.[18] The $In^{3+}$ complex is more stable than that of $Ga^{3+}$ and almost as stable as that of $Fe^{3+}$. However, the TTHA complexes of $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ present almost the same values for the stability constants, the $Ga^{3+}$ complexes being more stable than usual for this type of ligand.[18]

Data collected in Table 4 indicate that the formation of two amide bonds in TTHA decreases the thermodynamic stability constants (ML) by 5.64 for $Al^{3+}$ and by 6.18 for $Ga^{3+}$ complexes but only by 3.76 for the $Fe^{3+}$ and 3.19 for the $In^{3+}$ complexes (values in log units). A similar decrease was verified for the $M_2L$ species: 3.97 for the $Al^{3+}$, 5.8 for the $Ga^{3+}$, 5.11 for the $Fe^{3+}$, and 3.34 for the $In^{3+}$ complexes (also in log units). The formation of the bis(amide) derivative of TTHA leads to a decrease of the overall basicity of the ligand, of about 5.77 log units. This value parallels the decrease of the stability constants for the $Al^{3+}$ and $Ga^{3+}$ mononuclear complexes. A similar situation has been found for the chelates of DTPA vs DTPA-bis(amide) with trivalent lanthanides[31] and also with the $Ga^{3+}$ and the $In^{3+}$ complexes.[7] Neverthless, in the $Fe^{3+}$ and $In^{3+}$ complexes of TTHA vs TTHA-$(BuA)_2$ the decrease in stability is lower than expected.

The evaluation of the behavior of the trivalent metal ion complexes of TTHA-$(BuA)_2$ for medical applications cannot be made by the direct comparison of stability constant values. In the blood stream, other species are present which may compete with the metal (such as, $Ca^{2+}$, $Zn^{2+}$, or $H^+$) or with the ligand for the formation of the metal complex (other molecules and proteins, such as transferrin).[32] To avoid the transfer of the metal ion to the biomolecule, which can be fatal if toxic or radioactive ions are used, the complex formed with the specific ligand needs to be particularly stable, both thermodynamic and kinetically, and highly selective. pM values ($pM = -\log [M^{3+}]$), calculated from the stability constants of the complexes formed, taking into account the protonation constants of the ligand at physiological pH (pH = 7.4) when the total ligand concentration is twice of that of the total metal concentration,[33,34] are a more reliable choice for the evaluation of ligand effectiveness. Due 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. The larger the pM values, the more effective the ligand is.

**Table 5.** log $K_{ML}$ and PM Values for $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ Complexes of TTHA-$(BuA)_2$, TTHA, DTPA-$(BuA)_2$, DTPA, and Tranferrin

| | $Ga^{3+}$ | | $Fe^{3+}$ | | $In^{3+}$ | |
|---|---|---|---|---|---|---|
| ligands | log $K_{ML}$ | pM | log $K_{ML}$ | pM | log $K_{ML}$ | pM |
| TTHA-$(BuA)_2$ | 21.57 | 19.42 | 23.9 | 21.31 | 23.69 | 21.12 |
| TTHA | 27.75 | 22.71 | 27.66 | 22.97 | 26.88 | 22.09 |
| DTPA-$(BuA)_2$[b] | 18.18 | 19.09 | | | 22.7 | 20.74 |
| DTPA[b] | 24.3 | 19.97 | 28.0 | 26.7 | 29.0 | 24.72 |
| transferrin[c] | 20.3 | 20.4 | 20.7 | 20.7 | 19.2 | 18.9 |

[a] This work, values calculated for 100% excess of free ligand at physiological conditions, pH = 7.4; $C_M = 1.0 \times 10^{-5}$ M, $C_L = 2.0 \times 10^{-5}$ M. The calculations were made with the SPE program (ref 16). [b] Reference 7. [c] Reference 33.

In Table 5 we summarize the pM values determined for several ligands, and also transferrin complexes,[33] taken from the literature, among which are TTHA-$(BuA)_2$ and TTHA. Table 5 shows that despite the lower stability constants of the bis-(amide) derivatives compared with the parent ligands (TTHA or DTPA) this situation is partially compensated by the lower overall basicity of the former ligands. Thus, among the ligands mentioned in Table 5, DTPA is the best for biological applications using $Fe^{3+}$ and $In^{3+}$. However, TTHA-$(BuA)_2$ does not show significant differences from pM values from TTHA for these two metal ions, presenting higher values than those of transferrin. For the $Ga^{3+}$ complexes, TTHA seems to be the best ligand from this point of view. TTHA-$(BuA)_2$, DTPA-$(BuA)_2$, and even DTPA seem to have a similar behavior with pGa values lower than those of transferrin.

**NMR Studies.** The $Al^{3+}$, $Ga^{3+}$, and $In^{3+}$ complexes of TTHA and TTHA-$(BuA)_2$ were investigated in aqueous solution by $^{27}Al$, $^{71}Ga$, and $^{115}In$ NMR spectroscopy. The line widths of the metal resonances increased sharply in the above order, in agreement with the relative width factors for quadrupolar relaxation of those nuclei, which are 1.0:2.34:14.0.[35] The main information available from the spectra of aqueous solutions containing the $Ga^{3+}$ or $In^{3+}$ nitrates and the ligands TTHA or TTHA-$(BuA)_2$ is the existence (or not) in solution of the NMR-detectable high-symmetry species, namely the aqueous cations or their monomeric hydrolyzed forms, since the $^{71}Ga$ and $^{115}In$ NMR signals for the polyamino polycarboxylate complexes are usually too broad to be observed.[7,35,36] For the $Ga^{3+}$ complexes, $Ga^{3+}$/TTHA-$(BuA)_2$ solutions, either of 2:1 stoichiometry (0.10 M:0.05 M) at pH 6.5 or 1:1 at pH 7.2, produced only a very broad $^{71}Ga$ NMR signal, centered at $\delta = 134 \pm 5$ ppm with a line width $\Delta\omega_{1/2} = 35000 \pm 400$ Hz. Therefore, these solutions contain neither $Ga^{3+}(aq)$ nor $Ga(OH)_4^-$ species in significant amounts, as they would exhibit sharp and easily detectable $^{71}Ga$ signals (a 0.02 M gallium(III) nitrate solution at pH 2.0 gives a signal at 0 ppm and $\Delta\omega_{1/2} = 1680$ Hz, while at pH 10.0 the signal is at $\delta = 221.2$ ppm and $\Delta\omega_{1/2} = 136$ Hz). This is in agreement with the species distribution curves calculated from the thermodynamic data of Table 4, which in these conditions give a predominance of the dimer species $LGa_2(OH)_2^{2-}$ for the 2:1 ratio and of the $GaL^{3-}$ species for the 1:1 ratio (L = TTHA-$(BuA)_2$) (see Figure 3). For the $In^{3+}$ complexes, 1:1 (0.025 M:0.025 M) stoichiometric solutions of $In^{3+}$/L (L = TTHA or TTHA-$(BuA)_2$) at pH 7.2 did not give any sharp signals

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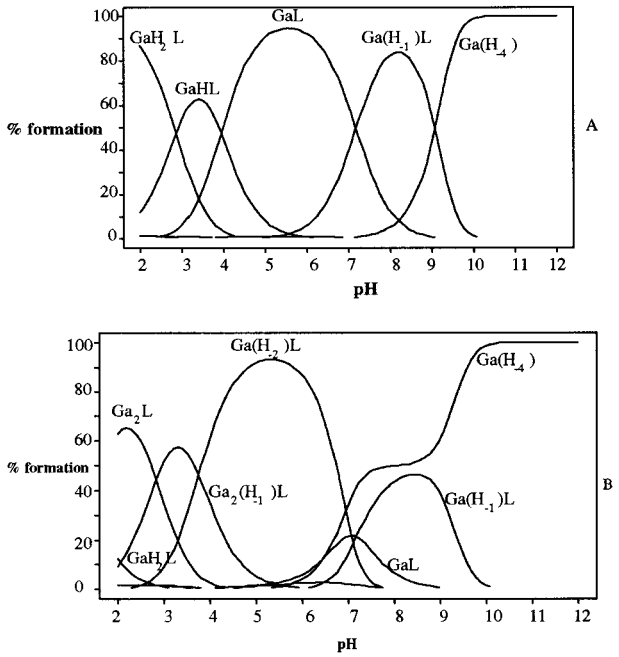


**Figure 3.** Species distribution curves calculated for the aqueous solution containing $Ga^{3+}$ and TTHA-(BuA)$_2$ (L) at a molar ratio of 1:1 (A) or 2:1 (B). Percent values represent concentrations relative to the total amount of gallium(III) at an initial value of $1.667 \times 10^{-3}$ M (A) or $1.667 \times 10^{-3}$ M (B).

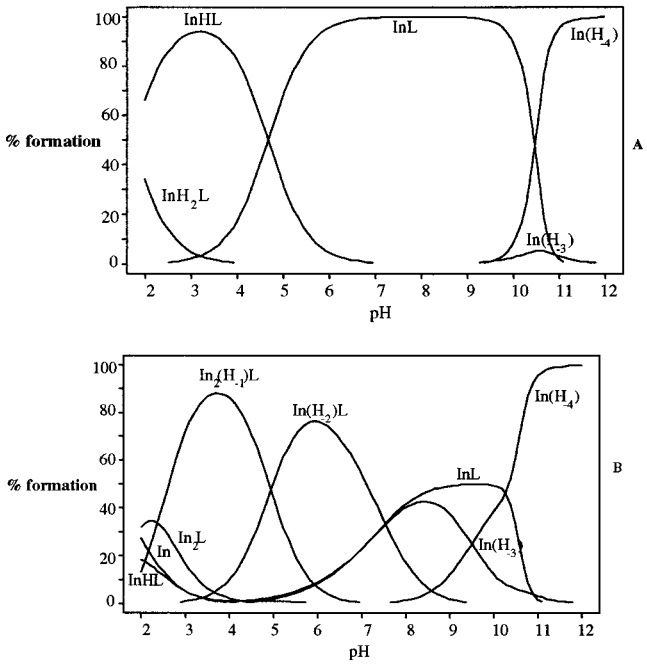


**Figure 4.** Species distribution curves calculated for the aqueous solution containing $In^{3+}$ and TTHA-(BuA)$_2$ (L) at a molar ratio of 1:1 (A) or 2:1 (B). Percent values represent concentrations relative to the total amount of indium(III) at an initial value of $1.667 \times 10^{-3}$ M (A) or $3.333 \times 10^{-3}$ M (B).

attributable to $In^{3+}$(aq) species (0.02 M indium(III) nitrate solution at pH 2.0 yields a $^{115}$In NMR signal with $\delta = 0$ ppm and $\Delta\omega_{1/2} = 18300$ Hz) but gave a very broad and badly defined resonance at a chemical shift very different from zero. As expected from the species distribution curves calculated from the thermodynamic data of Table 4, these solutions do not seem to contain the symmetric species $In^{3+}$(aq) or $In(OH)_4^-$ (see Figure 4).

$^{27}$Al NMR studies of Al(III) complexes of hydroxy carboxylate and polyamino polycarboxylate ligands[7,36,37] indicate that the chemical shift and line width of the metal nuclear resonance may yield very useful information on the coordination geometry and symmetry of the metal ion in the complex. Table 6 summarizes the $^{27}$Al resonance signals observed at pH 7.2 for aqueous solutions of $Al^{3+}$/L complexes (L = TTHA or TTHA-(BuA)$_2$) at the 1:1 and 2:1 stoichiometries and compares them with data from the literature for 1:1 $Al^{3+}$/L complexes (L = DTPA or DTPA-(BuA)$_2$).[7,37d] None of the solutions studied shows the sharp signals from the species $Al(H_2O)_6^{3+}$ at 0 ppm, or $Al(OH)_4^-$ at 80 ppm, indicating the absence of uncomplexed Al(III) species, in agreement with the species distribution curves obtained from potentiometric studies (see Figure 5) showing that $Al(OH)_4^-$ is absent from the solutions at pH $\leq$ 8. Taking into account the observed linear relationship of $^{27}$Al shifts of polyamino polycarboxylates with the ligand denticity and previous studies with $Al^{3+}$ complexes of DTPA and DTPA-(BuA)$_2$,[7,37d] the observed shift values are in agreement with penta- or hexacoordination of the ligands present in solution (Table 6). The signal line widths increase when going from the DTPA or TTHA chelates to their bis(amide) derivatives, due either to a reduced symmetry of the metal coordination or its slower tumbling, which affect the $^{27}$Al nuclear quadrupole relaxation. A solution of $Al^{3+}$/L (L =TTHA) (1:1, 0.2 M) shows a pH-dependent $^{27}$Al NMR resonance. At pH 6.8 a signal is observed at 31.0 ppm and 1420 Hz line width, from the pentacoordinate (via $N_2O_3$ donor atoms) unprotonated form $[AlL]^{3-}$, in good agreement with this work, while at pH 4.1 the shift decreases to 25.5 ppm, which was attributed to the formation of the protonated forms $[AlHL]^{2-}$ (pentacoordinate) and $[AlH_2L]^-$ (tetracoordinate).[37e] However, our distribution curves show that the predominant species at pH 4.1 is the dimeric form $Al_2L(OH)^-$. Thus the observed $^{27}$Al shift decrease with pH can be attributed to the formation of dinuclear species rather than to a ligand denticity decrease upon protonation.

**Table 6.** $^{27}$Al Resonance Signals Observed for Aqueous Solutions of $Al^{3+}$ Complexes with TTHA, TTHA-(BuA)$_2$, DTPA, and DTPA-(BuA)$_2$ (pH = 7.2)

| system[a] | $\delta$ (ppm) | $\Delta\omega_{1/2}$ (Hz) | ref |
|---|---|---|---|
| $Al^{3+}$/TTHA (1:1) | 35.7 ± 0.1 | 1450 ± 20 | *b* |
| $Al^{3+}$/TTHA (2:1) | 35.7 ± 0.1 | 1560 ± 20 | *b* |
| $Al^{3+}$/TTHA-(BuA)$_2$ (1:1) | 37.8 ± 0.1 | 3120 ± 20 | *b* |
| $Al^{3+}$/TTHA-(BuA)$_2$ (2:1) | 37.3 ± 0.1 | 2700 ± 20 | *b* |
| $Al^{3+}$/DTPA (1:1) | 37.2 ± 0.1 | 1835 ± 20 | *c* |
| $Al^{3+}$/DTPA-(BuA)$_2$ (1:1) | 40.3 ± 0.1 | 2600 ± 20 | *d* |

[a] The ligand concentration is 0.10 M. [b] This work. [c] Reference 37d. [d] Reference 7.

The $^{13}$C NMR spectra of the aqueous solutions at pH 7.0 of the 1:1 and 2:1 stoichiometric mixtures of the trivalent cations $Al^{3+}$, $Ga^{3+}$, or $In^{3+}$ and the ligand TTHA yielded useful information on the nature and structure of the complexes present through comparison of the observed shifts with those of the free ligand. This information is shown in Table 7 for the systems studied in this work and compared with data from the literature for 1:1 mixtures of $La^{3+}$, $Y^{3+}$, or $Lu^{3+}$ with TTHA.[38,39] Except for the $Al^{3+}$ and $La^{3+}$ systems, which give spectra

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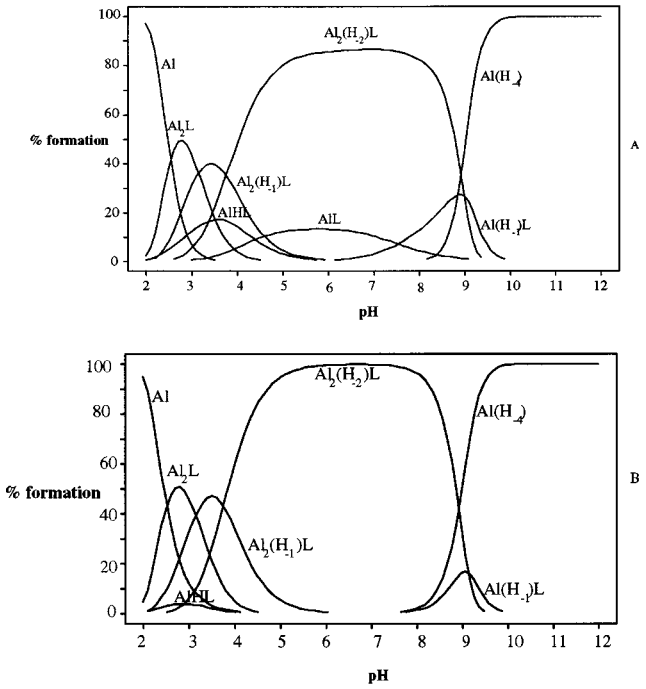


**Figure 5.** Species distribution curves calculated for the aqueous solution containing $Al^{3+}$ and TTHA-$(BuA)_2$ (L) at a molar ratio of 1:1 (A) or 2:1 (B). Percent values represent concentrations relative to the total amount of aluminum(III) at an initial value of $1.667 \times 10^{-3}$ M (A) or $3.333 \times 10^{-3}$ M (B).

corresponding to a single species in solution, in all the other cases many signals are observed corresponding to the presence of more than one species. The chemical shifts and intensities of the carboxylate signals directly give the number of free (in the 172−175 ppm range) and metal-bound (in the 176−182 ppm range) carboxylate groups in the complexes[40] (Table 8). The complexity of most of the $^{13}C$ spectra led us to complement this study with proton NMR spectra. The various AB patterns, in the 3.8−3.2 ppm shift region, corresponding to the bound acetate methylene protons in complexes with long metal−nitrogen and short metal−oxygen bond lifetimes,[41] were defined through the cross-peaks observed in the respective two-dimensional COSY spectra.[7] In the same proton shift range, singlets are also observed, corresponding to free acetate groups. Table 8 summarizes the minimum number and type of free and bound carboxylate groups, obtained from the proton and $^{13}C$ spectra of the TTHA complexes. The agreement beween both types of spectra is generally good, although the COSY spectra give more reliable information than the $^{13}C$ resonance assignments, due to possible $^{13}C$ signal overlaps.

We now correlate this NMR information with the number of complex species of TTHA present in solution as detected in each case by NMR, comparing with predictions from speciations calculated on the basis of the stability constants of Table 5, and propose possible structures. The validity of these structures is then discussed on the basis of previously proposed ones. For $Al^{3+}$ or $Ga^{3+}$, with maximum coordination number 6, possible structures for the mononuclear species ML include the following: C1 ($N_{(1)}N_{(2)}O_4$ donor atoms, three free carboxylates and three bound, one coordinated $H_2O$); C2 ($N_{(1)}N_{(2)}N_{(3)}O_3$ donor atoms, three free carboxylates and three bound); C3 ($N_{(1)}N_{(2)}N_{(3)}N_{(4)}O_2$ donor atoms, four free carboxylates and two bound). For the binuclear species $M_2L$, structure C4 (see Figure 2) has each cation with $N_{(1)}N_{(2)}N_{(3)}O_3(OH)$ donor atoms (all six carboxylates bound). In fact, structure C1 has been proposed for the complexes $(AlL)^{3-}$, $Al(HL)^{2-}$,[37e] and $Cu(HL)^{3-}$,[43] structure C3 for the complex $(PbL)^{4-}$,[26] and structure C4 for the complex $(Cu_2L)^{2-}$.[43] For the 2:1 solution of $Al^{3+}$/L, the simplicity of the $^{13}C$ NMR spectrum (three different types of bound carboxylates, Table 8) leads us to conclude that it exclusively contains the species $(Al_2L(OH)_2)^{2-}$, in agreement with the calculated speciation, with a structure C4. However, the observation of six bound carboxylate $CH_2$ cross-peaks in the COSY spectrum and the doubling of all the methylene $^{13}C$ resonances in the complex indicate two isomeric structures of the C4 type, with two different relative configurations of the two metal centers. One of these should correspond to the X-ray crystal structure determined in this work for the analogous binuclear $Ga^{3+}$ complex (Figure 2). In the 1:1 $Al^{3+}$/L solution, in which more than one species are present, five signals from bound carboxylates and two from free groups are observed. Their $^{13}C$ shifts and relative intensities indicate that the mononuclear complex $(AlL)^{3-}$ predominates, but the dinuclear complex $(Al_2L(OH)_2)^{2-}$ (structure C4) and the free ligand ($H_2L^{4-}$ ) are also present in significant amounts, again in agreement with the calculated speciation. The $(AlL)^{3-}$ species gives two different $^{13}C$ and proton signals from metal-bound carboxylates and two from free carboxylates, the later being of equal intensity, which points to structure C3 for this complex. For the $Ga^{3+}$/L solutions, with four bound and two free carboxylate signals, the 1:1 mixture shows a predominance of the species $(GaL)^{3-}$ and the presence of $(Ga_2L(OH)_2)^{2-}$. In addition, in the 2:1 solution those two complexes are again present with a predominance of the dinuclear species, in agreement with the calculated speciations. The carboxylate NMR signals observed indicate that $(Ga_2L(OH)_2)^{2-}$ has structure C4 in solution (with isomers). The $(GaL)^{3-}$ species gives three different $^{13}C$ and proton signals from metal-bound carboxylates and two from free carboxylates, but the free carboxylate signals have the same relative intensities, which points to a C1 or C2 structure for this complex.

In the case of the cations with coordination number 8−10 ($In^{3+}$, $La^{3+}$, $Y^{3+}$, and $Lu^{3+}$), the most probable mononuclear structures are C5 (nine-coordinate $N_4O_6$, one free carboxylate and five bound) and C6 (ten-coordinate $N_4O_6$, with all carboxylates bound), while in the binuclear structure C7 each cation is eight-coordinate $N_{(1)}N_{(2)}O_3(H_2O)_3$ (all carboxylates bound). The crystal structure of $(LaL)^{3-}$ was found to be of the type C6 (bicapped square antiprism), whereas that of $(DyHL)^{3-}$ is of the type C5, a monocapped square antiprism.[22e] It has been proposed that in aqueous solution $(ThL)^{2-}$ [25] and $(LaL)^{3-}$ [38,39] have rigid decadentate structures (C6).[25] Fluorescence studies of the $Eu^{3+}$/L system have shown that the $Eu_2L$ complex, predominating at low pH and/or at metal:ligand stoichiometries higher than 1, has a binuclear structure C7.[38] In our studies, the $In^{3+}$/L (1:1) solution gives at least four types of broad signals from bound carboxylates, which indicates a fluctional equilibrium among different structures differing in the number of bound and free carboxylates, e.g. C5 (one free carboxylate) and one with two free carboxylates. In the case of the lanthanide chelates, $(LaL)^{3-}$ generates three sharp signals from bound carboxylates, three methylene signals from acetate groups, and

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**Table 7.** $^{13}C$ Shifts ($\delta$, ppm) for the Complexes Present in Aqueous Solutions of $M^{3+}$ and the Ligand L = TTHA (p[H] 7.2, 298 K, 0.10 M Ligand Concentration)

| system | carboxylates (a,a′), (b,b′), (c,c′) | $CH_2$ acetates (d,d′), (e,e′), (f,f′) | $CH_2$ in (g,g′), (h,h′), (i,i′) |
|---|---|---|---|
| L[a] | 172.07; 172.07; 175.25 | 57.69; 57.69; 56.49 | 51.84; 51.57; 50.62 |
| L[b] | 176.94; 176.94; 177.48 | 59.22; 59.22; 58.04 | 52.87; 52.65; 52.24 |
| L[c] | 180.53; 180.53; 180.08 | 60.27; 60.27; 59.11 | 53.14; 53.14; 52.38 |
| AlL (1:1) | 176.16; 177.95; 177.77; 177.33; 174.85; 173.11 | 64.25; 61.95; 59.89; 58.31; 57.84; 56.70 | 56.24; 56.10; 54.4; 53.80; 53.10; 52.40; 52.20; 51.30; 49.50 |
| AlL (2:1) | 178.07; 178.02; 177.39 | 64.25; 61.95; 59.89; 59.76 | 56.24; 56.02; 54.46; 54.39 |
| GaL (1:1) | 179.39; 177.22; 177.10; 176.75; 175.09; 172.84 | 63.58; 61.03; 59.20; 58.80; 58.89; 57.77; 57.39 | 56.24; 56.02; 54.46; 54.39 |
| GaL (2:1) | 179.46; 177.37; 177.26; 176.90; 174.57; 172.95 | 63.76; 61.21; 59.10; 58.46; 58.14; 57.59 | 56.24; 55.59; 55.50; 54.40; 53.76; 53.04; 52.40; 52.22; 51.16; 49.51 |
| InL (1:1) | 179.25; 177.85; 177.51; 177.07 (broad) | 63.51; 62.16; 61.77; 60.8; 60.33; 59.31; 57.92; 55.80; 56.41 (broad) | 54.40; 52.30; 51.00 (broad) |
| LaL (1:1)[d] | 181.59; 181.30; 181.01 | 63.73; 62.8; 61.67 | 58.95; 57.22; 54.23 |
| YL (1:1)[d] | 181.58; 181.83; 180.99; 180.92; 180.61; 180.54; 180.43; 180.27; 177.46 | (many signals) | (many signals) |
| LuL (1:1)[d] | 182.17; 181.82; 181.48; 181.39; 181.25; 180.98; 180.78; 180.61; 179.89; 178.06 | many signals | many signals |

[a] p[H] 6.0. [b] p[H] 8.8. [c] p[H] 11.0. [d] Reference 38.

**Table 8.** Minimum Number of Free and Bound Carboxylate Groups of the Chemical Species Present in Solutions of M/L and ML′ (M = Al, Ga, In; L = TTHA, L′ = TTHA-$(BuA)_2$), As Obtained from Proton and $^{13}C$ NMR[a] and Comparison with Literature Information for ML (M = La, Y, Lu)

| | no. of bound carboxylates | | no. of free carboxylates | |
|---|---|---|---|---|
| system | $^1H$ | $^{13}C$ | $^1H$ | $^{13}C$ |
| AlL (1:1) | 5 | 5 | 2 | 2 |
| AlL (2:1) | 6 | 3 | 0 | 0 |
| GaL (1:1) | 3 | 4 | 2 | 2 |
| GaL (2:1) | 7 | 4 | 2 | 2 |
| InL (1:1) | 6 | 4 | 0 | 0 |
| LaL (1:1)[b] | 3 | 3 | 0 | 0 |
| YL (1:1)[b] | 6 | 9 | 0 | 0 |
| LuL (1:1)[b] | *c* | 10 | *c* | 0 |
| AlL′ (1:1) | 6 | 8 | 2 | 2(0) |
| AlL′ (2:1) | 10 | 5 | 0 | 3(0) |
| GaL′ (1:1) | 5 | 4 | 1 | 3(1) |
| GaL′ (2:1) | 9 | 3 | 0 | 3(1) |
| InL′ (1:1) | 5 | 2 | 2 | 2(2) |

[a] In the case of the L′ chelates, the number of carbonyl resonances assigned to free amides with $\delta$ < 173 ppm is shown in parentheses. [b] Reference 38. [c] Not studied.

three from the ethelenediamine bridges, indicating that all carboxylates and all amino groups of TTHA are coordinated to $La^{3+}$, in a well-defined decacoordinated structure,[38] such as a bicapped dodecahedron.[39] For the cases of $(YL)^{3-}$ and $(LuL)^{3-}$,[38,39] the large number (9−10) of proton and $^{13}C$ NMR signals observed (somewhat broadened at room temperature and with chemical shifts which do not correspond to permanently free carboxylates), indicates a rapid fluctional equilibrium between a symmetric form of the type C6, with all bound carboxylates, and an asymmetric form such as C5, with one unbound carboxylate group. This Ln(III)-unbound carboxylate is responsible for binding alkali metal ions in solution, which makes the $Dy(TTHA)^{3-}$ and $Tm(TTHA)^{3-}$ chelates useful $^{23}Na$ shift reagents for biomedical applications.[42]

The systems involving the ligand L′ = TTHA-$(BuA)_2$ were also studied by $^{13}C$ and proton (one-dimensional and COSY) spectra using the same methodology as described for the TTHA complexes. The $^{13}C$ shifts of free L′ are compared with those observed for its metal complexes (Table 9). In all cases, the methylene and methyl groups of the amide side chains give multiple signals, which may result from amide functions with free or bound carbonyl groups or from various isomers within the bound amide functions. The $^{13}C$ resonances from carboxylates and amide carbonyls show free and bound functions. Table 8 summarizes the minimum number of free and bound carboxylates, as well as the minimum number of signals from free amide functions, which have shifts close to the value observed for the free ligand. In the proton spectra, again all side-chain protons show multiple signals. In the $\delta$ = 3.8−3.2 ppm spectral region, the number of singlet resonances gave the number of $CH_2$ groups from free acetates, while the COSY spectra gave the number of AB systems ($CH_2$ groups from bound acetates) (Table 8). Thus, all the complex species present have free and bound carboxylate and amide groups, with structural isomers associated with the stereochemistry of the binding of the carbonyl groups to the metal, as found previously for the DTPA-$(BuA)_2$ complexes.[7] Due to the complexity of the systems, we used the species distribution functions obtained from the thermodynamic data of Table 4 to determine the species present in the solutions with 1:1 and 2:1 metal-to-ligand stoichiometries at pH 7.0 (see Figures 3−5). For the 2:1 $Al^{3+}$/L′ solution, the species $(Al_2L'(OH)_2)$ predominates (see Figure 5), with the dinuclear structure C4, as the number of free carboxylates and free amides is zero (Table 9). This structure shows various isomers from different binding stereochemistries of the two amide functions, one at each metal center. With the 1:1 $Al^{3+}$/L′ solution, the NMR information is compatible with the presence of free $H_2L'^{2-}$ and the complexes $(AlL')^-$ and $(Al_2L'(OH)_2)$, given by the calculated species distribution. The structure of $(AlL')^-$ (which cannot be obtained from the present data) leads to isomeric amide stereochemistries at the metal bond center. In the case of the 2:1 $Ga^{3+}$/L′ solution, $(Ga_2L'(OH)_2)$ (structure C3) predominates, while for the 1:1 solution the complexes $(GaL')^-$ and $(GaL'(OH))$ generate the variety of signals observed (Table 8). As for the 1:1 $In^{3+}$/L′ solution, there is a single species $(InL')^-$, with fluxional isomeric structures where one or two free carboxylates and/or amides are present.

## Conclusion

The replacement of two terminal carboxylates of TTHA by two amide groups to give TTHA-$(BuA)_2$ leads to a decrease of the ligand basicity and of the stability of the corresponding metal complexes of the trivalent cation ($Al^{3+}$, $Ga^{3+}$, $Fe^{3+}$, $In^{3+}$). The sum of the first four protonation constants is 24.47 for TTHA-

**Table 9.** $^{13}C$ Shifts ($\delta$, ppm) for the Complexes Present in Aqueous Solution of $M^{3+}$ and the Ligand L′ = TTHA-$(BuA)_2$ (p[H] 7.2, 298 K, 0.10 M ligand concentration)

| system | carboxylates and amide carbonyls (a,a′), (b,b′), (c,c′) | $CH_2$ acetates (d,d′), (e,e′), (f,f′) | $CH_2$ en (g,g′), (h,h′), (i,i′) | $CH_2$ alkyl groups (j,j′), (k,k′), (l,l), (m,m′) |
|---|---|---|---|---|
| L′ (pH = 3.5) | 175.04; 171.05; 174.52 | 59.00; 59.60; 57.60 | 54.11; 54.40; 53.50 | 41.80; 32.95; 21.97; 15.52 |
| AlL′ (1:1) | 180.26; 176.60; 178.28; 177.33; 176.98; 175.65; 175.42; 175.07; 174.40; 174.09 | 61.83; 61.21; 60.83; 60.09; 59.80; 57.57; 56.05 | 54.45; 54.26; 53.85; 53.27; 52.88; 52.41; 51.20 | 42.80; 31.98; 20.91; 14.50; 42.45; 31.09; 20.69; 14.20; 40.37 |
| AlL′ (2:1) | 180.22; 179.44; 179.29; 176.93; 175.78; 174.60; 174.40; 174.71 | 61.76; 61.27; 60.25; 59.86; 57.57; 56.18 | 54.35; 53.83; 52.92; 52.44; 51.20 | 42.80; 32.02; 20.90; 14.50; 42.56; 31.16; 20.65; 14.20; 40.49 |
| GaL′ (1:1) | 179.3; 178.97; 175.74; 175.58; 174.80; 173.49; 171.91 | 60.08; 57.38; 56.02 | 53.74; 52.79; 52.35 | 42.25; 31.94; 20.86; 14.41; 40.29; 31.08; 20.66; 14.20 |
| GaL′ (2:1) | 179.84; 179.04; 176.48; 173.60; 173.34; 172.85 | 63.75; 61.25; 61.00; 60.33; 60.07; 59.07; 57.43; 56.22 | 55.48; 54.48; 54.00; 53.01; 52.30; 50.92 | 42.80; 32.02; 20.16; 14.55; 42.57; 31.23; 20.78; 14.36; 40.48 |
| InL′ (1:1) | 178.20; 177.20; 172.80; 172.30 (br signals) | 61.0 (several br signals ranging from 60.29 to 56.53) | several br signals ranging from 54.00 to 51.00 | 41.90; 31.78; 20.93; 14.47; 41.80; 31.40; 40.90; 31.20; 40.56 |

$(BuA)_2$ and 30.24 for TTHA. These results are essentially comparable to other polyamino polycarboxylates, where, for example, the total basicity of DTPA-$(BuA)_2$ is lower than that of DTPA[7] (the sum of the first three protonation constants is 17.11 and 23.63, respectively). Thus, derivatization of two terminal carboxylates by butanamide substituents implies a significant decrease of the total ligand basicity, decreasing their negative charge and altering the solubility of the resulting complexes. However, the decrease in the indium complex stability is less drastic going from TTHA to TTHA-$(BuA)_2$ (26.88 to 23.69) than it was between DTPA and DTPA-$(BuA)_2$ (29.3 to 22.7). This can be explained by the presence of four carboxylate groups remaining in TTHA-$(BuA)_2$ (instead of three in DTPA-$(BuA)_2$) giving the metal the possibility to satisfy its greater affinity for carboxylate groups than for amide functions. But in the present work we expected a lesser decrease in the stability constant of the indium(III) complex of TTHA-$(BuA)_2$. The gain in stability (about 2.5 log units) was not very fruitful considering the greater number of structural isomers in solution. This does not compensate the greater solubility of the In(TTHA-$(BuA)_2$) complex compared to that of In(DTPA-$(BuA)_2$). For these reasons lipophilic DTPA-bis(amide) derivatives remain more appropriate for the metal labeling of lipoproteins or of other lipid vesicles as reported before.[6] To have a better prediction of the utility of the different ligands studied in this work, pM values under physiological conditions have been calculated. While DTPA remains a ligand of choice to chelate $Fe^{3+}$ and $In^{3+}$ ions in vivo compared to transferrin as competitor ligand, TTHA appears surprisingly as the best of these four ligands (pM = 22.71) to chelate $Ga^{3+}$. This ligand could be used in biological studies for in vitro and in vivo experimentations. From more basic considerations, the studies afford new thermodynamic data on M(III) complexes (M = Al, Ga, Fe, and In) with TTHA and TTHA-$(BuA)_2$. Strong differences appear between $Al^{3+}$ on one hand and $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ on the other hand. $Ga^{3+}$, $Fe^{3+}$, and $In^{3+}$ give mononuclear species in a 1:1 ratio and dinuclear species in the ratio 2:1 (M:L) at pH = 7, but for $Al^{3+}$, dinuclear species ($Al_2L(OH)_2$) are also formed in the 1:1 ratio, at the same pH.

In the solid state, in basic conditions and with a metal-to-ligand ratio of 1:1, the dinuclear species has been isolated with gallium and TTHA, showing the greater affinity of this metal for the hydroxyl anion than for the carboxylate anion, due to its acid character. When synthesis procedures were carried out under neutral conditions, the neutral mononuclear species, Ga−L and In−L, were obtained.

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**Supporting Information Available:** A figure depicting $[Ga_2(OH)_2(TTHA)][Na_2(H_2O)_6]\cdot 2H_2O$ in the cell, tables listing detailed crystallographic data, atomic positional and *U* parameters, anisotropic thermal parameters, isotropic thermal parameters for the hydrogen atoms, and bond length and angles, and figures showing titration curves for TTHA-$(BuA)_2$ and species distribution curves for TTHA (10 pages). Ordering information is given on any current masthead page.

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