Synthesis and NMR Studies of Three Pyridine-Containing Triaza Macrocyclic Triacetate Ligands and Their Complexes with Lanthanide Ions

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The synthesis of three triazamacrocycles containing the pyridine moiety and three acetate pendant arms (PCTA) is reported. The three systems differ due to the number of carbon atoms in the macrocyclic ring forming ligands PCTA-[12], -[13], and -[14], endowed with different coordination capabilities toward lanthanide(III) ions. Microscopic protonation sequences for the three ligands have been investigated by ^{1}H NMR spectroscopy. Complexes of PCTA-[12], -[13], and -[14] with La(III), Gd(III), and Lu(III) have been prepared. Relaxometric measurements on the aqueous solutions of the paramagnetic Gd(III) complexes in the presence of competitive ligands gave the following stability constants: log $K_f = 20.8$ for Gd-PCTA-[12], log $K_f = 19.3$ for GdPCTA-[13], and log $K_f = 12.5$ for GdPCTA-[14]. The measurement of water relaxation rates indicated a tendency to decrease the degree of hydration upon increasing the ring size. The VT ^{1}H and ^{13}C-NMR spectra of the diamagnetic La(III) and Lu(III) complexes exhibit a large variability of the solution structures dictated by the matching of the size of the lanthanide ion and the macrocyclic cavity. This results in noticeable differences in their stereochemical nonrigidity, hydration state, and thermodynamic stability. To some extent the changes observed in continuing from the 12−14-membered ring macrocyclic complexes parallels the behaviors shown by the octacoordinated lanthanide(III) complexes with DOTA and TETA. GdPCTA-[12] and -[13] feature promising properties in view of their possible use as contrast agents for magnetic resonance imaging.

Introduction

Recent years have witnessed a growing interest toward lanthanide(III) complexes mainly because of their involvement as diagnostic agents in magnetic resonance imaging (MRI).[1] In dealing with these applications, Gd(III) is the ion of choice for its high effective magnetic moment and for the long electronic relaxation time associated with the f^7 configuration. A good ability to enhance the water proton nuclear magnetic relaxation rates represents obviously a basic prerequisite for any paramagnetic complex to be considered as a potential contrast agent (CA) in MRI.[2] As far as toxicity is concerned, the chemist is asked to provide complexes characterized by a very high thermodynamic, and possibly kinetic, stability. Prototypes of complexes endowed with these characteristics are GdDTPA[1] and GdDOTA,[1] both routinely utilized in clinical practice.

A route to markedly improve the relaxation enhancement capability of Gd(III) complexes lies in the possibility of lengthening their molecular reorientational time (τ_R).[1c,3] This target may be pursued through the formation of macromolecular complexes either by the covalent[4] or noncovalent[5] linking of the complex to a slowly moving substrate. However, it has been found that the expected relaxation enhancement could be partly "quenched" by the occurrence of a long exchange lifetime (τ_M) of the coordinated water molecule.[5b] Relatively long τ_M values were reported for the GdDTPA and GdDOTA complexes[6] (about 0.2 μs at 25 °C), both displaying one coordinated water molecule (hydration number $q = 1$). The long τ_M value appears to be related to the dissociative exchange mechanism of the water molecule from the coordination site to the bulk.[6,7] It seems therefore reasonable that a shortening of τ_M may be pursued with complexes with $q = 2$. Furthermore, such complexes should possess an intrinsically higher relaxivity than those complexes of similar size with $q = 1$.

Herein we report on the synthesis of three pyridine-containing triaza macrocycle triacetate ligands (PCTA) which act as heptacoordinating chelators, thus allowing a higher hydration of the lanthanide(III) ions in their complexes (Chart 1).

Furthermore, the introduction of the pyridine in the macrocycle is expected to increase the stereochemical rigidity of the resulting complexes, a property often associated with an increase in their thermodynamic stability. The pyridine moiety may also provide a suitable site for a successive functionalization which facilitates the process of molecular recognition toward specific targets.

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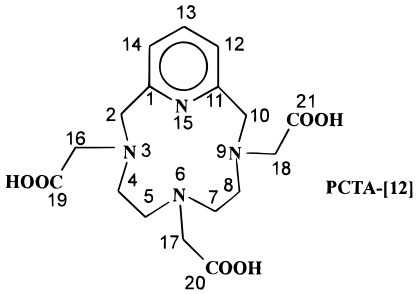
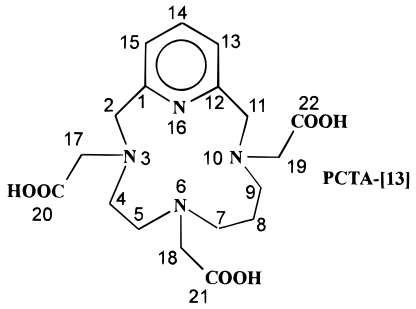
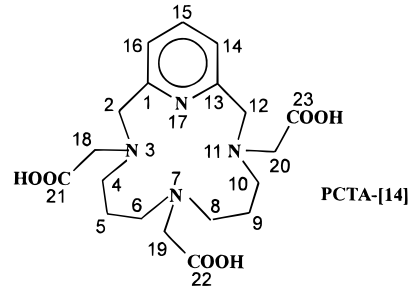
Chart 1







Recently Sherry et al.[8,9] have investigated some polyaza macrocyclic acetate ligands containing pyridine and their complexes with Gd(III) for possible use as MRI contrast agents. These studies included relaxometric measurements and animal tissue biodistribution using radioactive ^{153}Sm and ^{159}Gd complexes on the 12-membered PCTA system also considered in this work. A preliminary communication,[10] dealing with the NMR properties of Eu, Yb, and Gd complexes with the 13-membered PCTA ligand, has also appeared.

Experimental Section

FT-IR spectra were measured with a Perkin-Elmer 1720 X spectrophotometer with a ZnS cell. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on Bruker AC 200 (200 MHz and 50.2 MHz, respectively) and JEOL EX-400 (400 and 100.4 MHz) spectrometers. Mass spectra were recorded with a VG 7070 EQ spectrometer (at 70 eV; *m*-nitrobenzyl alcohol or glycerol as matrix in the FAB$^+$ ionization, isobutane in CI technique). Elemental analyses were performed with a Perkin-Elmer 240 apparatus. Melting points were determined with a Buchi 520 apparatus.

1,4,7-Tritosyl-1,4,7-triazaheptane and 1,4,8-tritosyl-1,4,8-triazaoctane were prepared with a reported procedure[11] in 54.5 and 63.0% yields after crystallization. 2,6-Bis(chloromethyl)pyridine was purchased from Fluka. 3,7,11,17-Tetrazabicyclo[11.3.1]heptadeca-1(17),13,15-triene (**2a**) was synthesized in 81% yield following the procedure described by Costa and Delgado.[14]

Synthesis of the Ligands. 3,6,9-Tritosyl-3,6,9,15-tetraazabicyclo-[9.3.1]pentadeca-1(15),11,13-triene (1c). A solution of 2,6-bis-(chloromethyl)pyridine (2.07 g, 11.8 mmol) in anhydrous acetonitrile (15 mL) was added dropwise, over a period of 1 h, to a suspension of 1,4,7-tritosyl-1,4,7-triazaheptane (6.9 g, 12.2 mmol) and Na_2CO_3 (5.8 g, 41.9 mmol) in anhydrous refluxing acetonitrile under a nitrogen atmosphere. The reaction was refluxed overnight. The solvent was evaporated and the residue extracted with aqueous sodium hydroxide−methylene chloride; the organic phase was dried over sodium sulfate and concentrated to give a solid which was recrystallized from acetone to give pure compound **1c** (7.7 g, 94%). Mp: 215−217 °C. ^{1}H-NMR ($CDCl_3$): δ 2.42 (s, 3H), 2.44 (s, 6H), 2.75 (bs, 4H), 3.33 (t, J = 8.0 Hz, 4H), 4.29 (s, 4H), 7.00−8.00 (m, 15H). ^{13}C-NMR ($CDCl_3$): δ 21.39, 21.43, 47.28, 50.21, 54.84, 124.12, 126.99, 127.10, 129.70, 129.84, 135.17, 135.86, 138.89, 143.37, 143.68, 155.19. FT-IR: 2929, 2856, 1595, 1456, 1343, 1157 cm^{-1}. Anal. Calcd for $C_{32}H_{36}N_4O_6S_3$: C, 57.46; H, 5.42; N, 8.38. Found: C, 57.67; H, 5.53; N, 8.42.

3,6,9,15-Tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene, Hydrobromide (2c·3HBr). Compound **1c** (3.2 g, 4.85 mmol) was refluxed for 12 h with a 48% aqueous hydrobromic acid solution (89.4 g, 530 mmol), glacial acetic acid (26.2 g, 436 mmol), and phenol (2.76 g, 29.4 mmol). The solution was then diluted with water and extracted with methylene chloride (3 × 60 mL). The aqueous phase was evaporated under reduced pressure, and the same quantity of reactants was added to the residue; the resulting solution was refluxed for a further 12 h. After a partitioning between water and methylene chloride, the aqueous phase was concentrated (3 mL) and diethyl ether (50 mL) was added. The white-yellow crystals obtained after filtration were washed with diethyl ether and dried under vacuum to give compound **2c**·3HBr (1.73 g, 79.5%). Mp: 249−253 °C (lit.[14] mp 250−4 °C). ^{1}H-NMR (D_2O): δ 3.32 (m, 8H), 4.52 (s, 4H), 7.42 (d, J = 7.5 Hz, 2H), 7.88 (t, J = 7.5 Hz, 1H). Anal. Calcd for $C_{11}H_{18}$·3HBr·H_2O: C, 28.29; H, 4.96; N, 12.00. Found: C, 28.30; H, 4.96; N, 11.98.

3,6,9,15-Tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene (2c). A solution of potassium hydroxide (5 g, 89.1 mmol) in water (5 mL) was added under stirring to a flask containing compound **2c**·3HBr (1.66 g, 3.70 mmol) precooled to 0 °C. After 15 min the solution was extracted with methylene chloride (6 × 10 mL), and the organic layers were dried over sodium sulfate and evaporated to give an oily residue which later solidified in the white, waxy compound **2c** (0.64 g, 84%). ^{1}H-NMR ($CDCl_3$): δ 2.23 (m, 4H), 2.67 (m, 4H), 3.93 (s, 4H), 6.98 (d, J = 7.6 Hz, 2H), 7.49 (t, J = 7.6 Hz, 1H). ^{13}C-NMR ($CDCl_3$): δ 48.84, 49.10, 53.69, 119.7, 136.3, 159.6. Mass spectrum: found EI *m/e* 206 (M$^+$), CI *m/e* 207 (MH$^+$); calcd for $C_{11}H_{18}N_4$ 206.

3,6,9,15-Tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic Acid, Hydrochloride (4c). A solution (3 mL) of potassium chloroacetate, obtained from chloroacetic acid (0.792 g, 8.38 mmol) and potassium hydroxide (0.47 g, 8.38 mmol), was added dropwise to a solution of compound **2c** (0.509 g, 2.47 mmol) in H_2O (5 mL), maintained at 80 °C. The reaction mixture was heated for 20 h, maintaining a pH = 10 (persistent pink color of phenophthalein previously added) with potassium hydroxide. The solution was then acidified with 2 N hydrochloric acid, the solvent was evaporated, and the residue was purified by column chromatography on Sephadex G-10, eluting with water. Compound **4c** was obtained as a powder, after evaporation of the solvent (0.78 g, 64%). ^{1}H-NMR (D_2O): δ 2.80 (bs, 4H), 3.42 (bs, 4H), 3.52 (s, 2H), 4.17 (s, 4H), 4.72 (s, 4H), 7.37 (d, J = 7.5 Hz, 2H), 7.90 (t, J = 7.5, 1H). ^{13}C-NMR (D_2O): δ 53.46, 56.88, 57.17, 58.90, 62.09, 125.22, 143.48, 152.05, 171.45, 177.01. Mass spectrum FAB-MS: 381 (M + H)$^+$, 403 (M + Na)$^+$, 419 (M + K)$^+$, calcd for $C_{17}H_{24}N_4O_6$ = 380. Anal. Calcd for $C_{17}H_{28}Cl_4N_4O_6$: C, 38.79; H, 5.32; N, 10.65. Found: C, 38.69; H, 5.50; N, 10.48. UV (H_2O, pH = 1): λ_{max} = 262.0 nm.

3,6,10,16-Tetrazabicyclo[10.3.1]hexadeca-1(16),12,14-triene, Hydrobromide (2b·4HBr). The hydrolysis was performed as described for **2c**. Starting from **1b** (1.93 g, 2.83 mmol), compound **2b**·4HBr

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(1.15 g) was obtained in 73% yield. Mp: 122−123 °C (lit.[14] mp 122−124 °C). ^{1}H-NMR (D_2O): δ 2.11 (m, 2H), 3.11 (m, 4H), 3.40 (m, 4H), 4.34 (s, 2H), 4.40 (s, 2H), 7.32 (d, J = 7.7 Hz, 1H), 7.37 (d, J = 7.7 Hz, 1H), 7.79 (t, J = 7.7 Hz, 1H). ^{13}C-NMR (D_2O): δ 22.76, 43.41, 44.33, 44.84, 45.82, 51.45, 51.96, 127.14, 127.44, 142.47, 152.58, 152.78.

3,6,10,16-Tetrazabicyclo[10.3.1]hexadeca-1(16),12,14-triene (2b). The conditions of this step were the same as described for **2c**: from **2b**·4HBr (1.06 g, 1.9 mmol), compound **2b** was obtained in 98.5% yield (0.410 g). ^{1}H-NMR ($CDCl_3$): δ 1.66 (q, J = 6 Hz, 2H), 2.56 (t, J = 6 Hz, 2H), 2.66 (t, J = 6 Hz, 2H), 2.68 (m, 4H), 3.88 (s, 2H), 3.90 (s, 2H), 7.02 (d, J = 7.7 Hz, 2H), 7.56 (t, J = 7.7 Hz, 1H). FT-IR: 3365, 1595, 1577, 1456, 1363, 1161, 1001, 747 cm^{-1}. Mass spectrum: found CI *m/e* 221 (MH^+); calcd for $C_{12}H_{20}N_4$ 220.

3,6,10,16-Tetrazabicyclo[10.3.1]hexadeca-1(16),12,14-triene-3,6,-10-triacetic Acid Methyl Ester (3b). Methyl bromoacetate (0.066 g, 0.43 mmol) in anhydrous tetrahydrofuran (3 mL) was added dropwise, under nitrogen atmosphere, to a stirring mixture of compound **2b** in anhydrous tetrahydrofuran (3 mL) and silver carbonate (0.068 g, 0.248 mmol). After 20 min the reaction mixture was allowed to cool to room temperature and was stirred overnight. The reaction mixture was filtered, and the filtrate was evaporated; $Na_2S·9H_2O$ was added in slight excess to the residue dissolved in 2 M HCl (10 mL). The reaction mixture was stirred for 15 min and filtered. The filtrate was concentrated to dryness and the crude solid partitioned between NH_3 8% (5 mL) and methylene chloride (3 × 5 mL). The organic layer was dried over sodium sulfate and the solvent evaporated to give compound **3b** (0.055 g, 91.3%) as an oil, pure enough for the following step. ^{1}H-NMR ($CDCl_3$): δ 1.71 (m, 2H), 2.5−2.8 (m, 8H), 3.22 (s, 2H), 3.49 (s, 2H), 3.53 (s, 2H), 3.67 (s, 3H), 3.75 (s, 3H), 3.77 (s, 3H), 3.97 (s, 2H), 3.98 (s, 2H), 7.17 (d, J = 7.7 Hz, 1H), 7.19 (d, J = 7.7 Hz, 1H), 7.63 (t, J = 7.7 Hz, 1H). ^{13}C-NMR ($CDCl_3$): δ 23.42, 47.95, 50.55, 50.88, 51.28, 51.51, 56.07, 56.90, 58.99, 122.43, 136.73, 157.04, 157.19, 171.58, 171.74. FT-IR: 1733, 1591, 1436, 1196, 1175, 768 cm^{-1}. Mass spectrum: found EI *m/e* 436 (M^+); calcd for $C_{21}H_{32}N_4O_6$ 436.

3,6,10,16-Tetrazabicyclo[10.3.1]hexadeca-1(16),12,14-triene-3,6,-10-triacetic Acid, Tripotassium Salt (4b). A methanolic solution of potassium hydroxide (0.0105 g, 0.187 mmol in 2 mL of methanol) was added to a solution of compound **3b** (0.027 g, 0.062 mmol) in methanol (8 mL). After 30 min at 0 °C under stirring, the solution was allowed to warm to room temperature and was maintained at this temperature for a further 30 min. The solvent was then evaporated, and compound **4b** was obtained as a pale, yellow powder, in quantitative yield. ^{1}H-NMR (D_2O): δ 1.21 (m, 2H), 2.26 (m, 4H), 2.34 (s, 4H), 2.77 (s, 2H), 3.04 (s, 2H), 3.08 (s, 2H), 3.64 (bs, 4H), 7.19 (d, J = 7.5 Hz, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.63 (t, J = 7.5 Hz, 1H). ^{13}C-NMR (D_2O): δ 23.32, 51.21, 53.70, 54.45, 60.57, 61.15, 61.59, 62.08, 62.56, 126.25, 140.81, 159.23, 159.45, 179.50, 181.13, 181.23. Mass spectrum FAB-MS: 395 $(M + H)^+$, 417 $(M + Na)^+$, 433 $(M + K)^+$, calcd for $C_{17}H_{24}N_4O_6$ 394.

3,7,11,17-Tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-triene-3,7,11-triacetic Acid Methyl Ester (3a). This compound was prepared, in a 95.8% yield (oil), by the same procedure described for **2b**. ^{1}H-NMR ($CDCl_3$): δ 1.48 (m, 4H), 2.51 (m, 4H), 2.65 (m, 4H), 3.12 (s, 2H), 3.42 (s, 4H), 3.62 (s, 3H), 3.77 (s, 6H), 3.92 (s, 4H), 7.26 (d, J = 7.7 Hz, 2H), 7.63 (t, J = 7.7 Hz, 1H). ^{13}C-NMR ($CDCl_3$): δ 25.32, 49.49, 51.01, 51.34, 54.77, 55.78, 60.84, 123.08, 137.21, 157.77, 171.53, 171.72. Mass spectrum: found EI *m/e* 450 (M^+); calcd for $C_{22}H_{34}N_4O_6$ 450.

3,7,11,17-Tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-triene-3,7,11-triacetic Acid, Tripotassium Salt (4a). Compound **4a** was synthesized, in quantitative yield, by the procedure described for **4b**. ^{1}H-NMR (D_2O): δ 1.18 (m, 4H), 2.22 (t, J = 7.3 Hz, 4H), 2.34 (t, J = 7.3 Hz, 4H), 2.82 (s, 2H), 3.11 (s, 4H), 3.77 (s, 4H), 7.29 (d, J = 7 Hz, 2H), 7.65 (t, J = 7 Hz, 1H). ^{13}C-NMR (D_2O): δ 25.56, 53.15, 54.86, 61.27, 62.51, 62.62, 126.42, 140.64, 159.81, 181.32, 181.41. Mass spectrum FAB-MS: 407 $(M + H)^+$, 429 $(M + Na)^+$, 445 $(M + K)^+$; calcd for $C_{17}H_{24}N_4O_6$ 406.

Complexation Procedure. The potassium salt of the appropriate ligand (0.2 mmol) was dissolved in H_2O (5 mL). The pH of the solution was adjusted to 6.5 with HCl (1 N). A solution of lanthanide trichloride (0.2 mmol) in H_2O was added, and the pH was adjusted to 7 with 1 N NaOH. At room temperature the complex formation is instantaneous. The solution was then evaporated under reduced pressure and the residue dried at 70 °C overnight.

NMR Measurements. Solutions of the ligand (0.02 M) for the acidic/basic titrations were made up in D_2O (99.8%), and the apparent pD was adjusted with DCl or NaOD. Apparent pD was measured with a glass combination electrode standardized with H_2O on the basis of pH buffers. The pD values were converted in pH values using the equation: pH = pD − 0.4.[15] Solutions of the La and Lu complexes (0.1 M) were prepared in D_2O, and the pD was adjusted to 7 with NaOD. ^{1}H- and ^{13}C-NMR spectra were obtained at 9.4 T on a JEOL EX-400 spectrometer and the chemical shifts referenced to *tert*-butyl alcohol (1%) used as an internal chemical shift standard (δ_H = 1.29 ppm; δ_C = 31.3 ppm). The variable-temperature accessory was calibrated with methanol.[16] Water proton longitudinal relaxation measurements (20 MHz; 25 °C) were carried out on five solutions (0.4−5 mM) of the Gd complexes with a Stelar Spinmaster spectrometer (Stelar, Mede (PV), Italy) operating at 20 MHz, by means of the standard inversion−recovery technique. A typical 90° pulse width was 3.5 μs, and the reproducibility of T_1 data was ±0.5%.

Results

Syntheses of PCTA-[12], -[13], and -[14] Ligands. The polyaza macrocyclic ligands were obtained as depicted in Scheme 1. The 12-membered PCTA ligand (**4c** or PCTA-[12]; vide infra) was previously known.[17] However, the synthetic route herein proposed is new and provides higher yields.

The synthesis of the polyaza macrocyclic ring is the crucial step in the preparation of these compounds, and the associated difficulties are strictly dependent on the size of the macrocycle. 3,7,11,17-Tetraazabicyclo[11.3.1]heptadeca-1(17), 13,15-triene (**2a**) was prepared by a template synthesis from 2,6-pyridinedicarbaldehyde and 1,5,9-triazanonane in the presence of copper(II) nitrate followed by reduction of the diimine with sodium borohydride as described by Costa and Delgado.[14] Attempts were made to apply the same cyclization method to the synthesis of 3,7,10,16-tetraazabicyclo[10.3.1]hexadeca-1(16),12,14-triene (**2b**) and 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15)11,13-triene (**2c**), but even varying the nature of the metal ion [Cu(II), Ag(I), Co(II), Ni(II)] no trace of the desired products was detected.[18] Compounds **1b** and **1c** (R = Ts) were firstly obtained in moderate yields by reacting 2,6-bis(chloromethyl)-pyridine[19] with disodium salt of 1,4,8-tritosyl-1,4,8-triazaoctane or 1,4,7-tritosyl-1,4,7-triazaheptane, respectively, in anhydrous dimethylformamide according to Richman and Atkins.[19] The low yields of this preparation prompted us to explore other routes to improve the cyclization step. We found that by employing anhydrous acetonitrile as solvent and potassium carbonate as base in heterogeneous conditions, the yields of the cyclization can be raised to 90% for **1b** and to 75% for **1c**. This synthetic improvement allows us to carry out multigram preparations (up to 30 g of these intermediates). Hydrolysis of the tritosylates **1b** and **1c**, with hydrobromic acid followed by alkalinization as previously reported,[19] gave **2b** and **2c** pure enough for the following step. As depicted in Scheme 1, the method adopted for introducing the acetate moieties on compounds **2** depends on the size of the macrocycle.

While compounds **2a** and **2b** could be alkylated in high yields with ethyl bromoacetate in the presence of Ag_2CO_3 as halide scavenger, in the case of **2c**, a mixture of products was obtained

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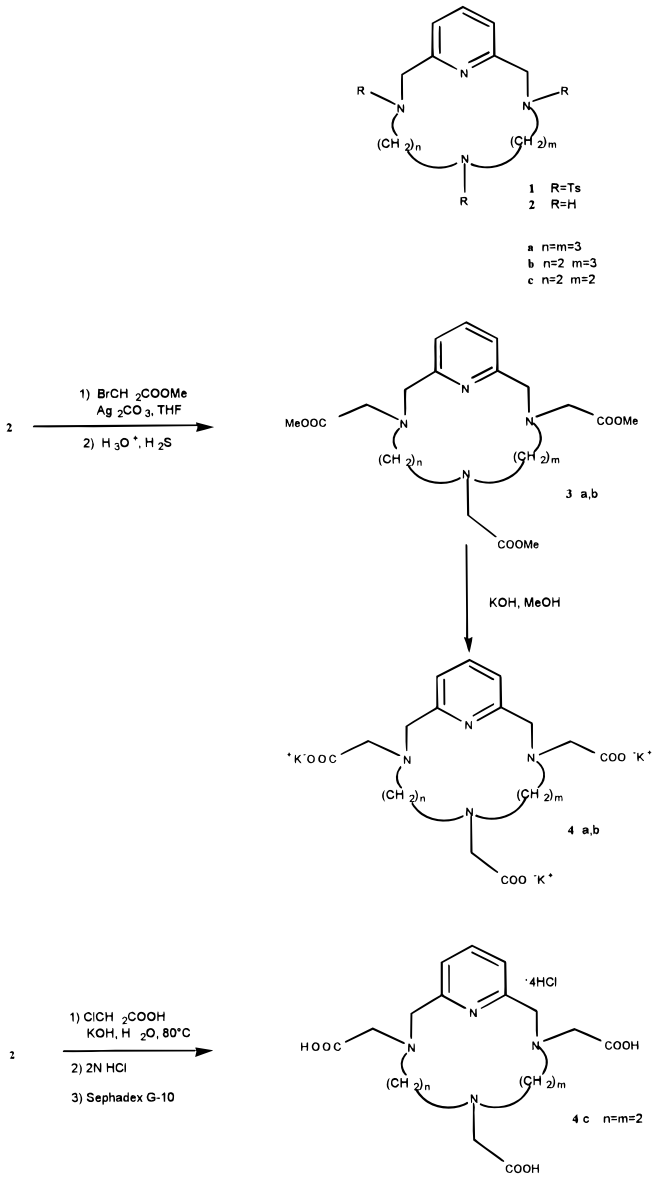
Scheme 1



by using the same method. Compounds **3a** and **3b** were subjected to hydrolysis with stoichiometric amounts of potassium hydroxide affording the potassium salts **4a** and **4b** which were used for metal complexes preparation without further purification.

Attempts to alkylate compound **2c** were carried out by reaction with glyoxylic acid and sodium cyanoborohydride under various conditions of temperature and solvent. Although this alkylating method proved to be successful in other macrocyclic systems,[20] it did not provide appreciable results in this case. Finally, the alkylation of **2c** was obtained by reaction with chloroacetic acid in the presence of potassium hydroxide at 80 °C and maintaining the pH of the solution at a value of about 10. The control of the pH of the reaction mixture plays an important role for the success of the alkylation: in fact, by taking into consideration the pK_a values (vide infra), a strong alkaline medium is necessary to deprotonate the nitrogen atom opposing the pyridine. In the following paragraphs, the obtained ligands will be indicated as PCTA-[12], PCTA-[13], and PCTA-[14] for **4c**, **4b**, and **4a**, respectively.

(20) Giovenzana, G. B.; Jommi, G.; Pagliarin, R.; Sisti, M.; Aime, S.; Botta, M.; Geninatti Crich, S. *Recl. Trav. Chim. Pays-Bas* **1996**, *115*, 94.

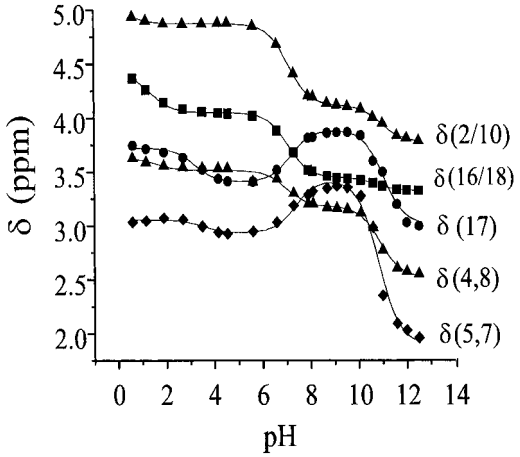


Figure 1. pH dependence of the ^{1}H-NMR resonances of ligand PCTA-[12] recorded at 400 MHz and 25 °C.

Microscopic Protonation Sequence. It is well established that, with poly(amino carboxylate) ligands, it is possible to assess the order of protonation by means of ^{1}H-NMR spectroscopy.[21] This is due to the fact that protonation of a basic site results in a deshielding of the adjacent nonlabile protons of the ligand.

The plot of proton chemical shifts of PCTA-[12], as a function of pH, shows that three protonation steps take place in the pH range 3−12 (Figure 1).

The first H^+ addition involves the nitrogen opposing the pyridine (pK_a ≅ 10.9), as clearly illustrated by the large downfield shift shown by methylenic resonances 5/7 and 17. Since methylenic resonances 4/8 and 2/10 display an analogous, although smaller, downfield shift, it is likely that some positive charge is located on N3 and N9. This may be the result of the formation of an intramolecular hydrogen bond resulting from the protonation on N6. Next, as the pH is further decreased (in the range 8.5−5), there is a downfield shift of resonances 2/10, 16/18, and 4/8 coupled to an upfield shift of signals 5/7 and 17. This behavior is strongly consistent with the occurrence of a second protonation step (pK_a ≅ 7.1) along with a shift of the former proton to afford a bis-protonated species on N3 and N9. Such a proton shift thus allows a better charge separation, although it is reasonable to think that a partial positive charge may still remain on N6 through its involvement in the reversible hydrogen bond formation with the protonated N3 and N9 sites. At the acidic limit (pH = 5−3) the protonation occurs on the carboxylate group of the acetate arm bound to N6 (pK_a ≅ 3.3).

In the larger PCTA-[14] ligand the protonation pattern follows the guidelines shown by PCTA-[12] with some interesting differences. On the L^{3-} ligand the first protonation step occurs at the N7 site as found in PCTA-[12] with a similar pK_a value of ∼10.9. The addition of the second proton takes place at a slightly higher pH (pK_a ≅ 7.9) than in PCTA-[12] and clearly occurs at N3 or N11, as shown by the large shift of 2/12, 4/10, and 18/20 resonances. However the CH_2 resonances adjacent to N7 do not suffer the upfield shift observed for PCTA-[12] on passing from pH 9 to pH 8. This would probably mean that the larger cycle of PCTA-[14] minimizes the repulsion between positive charges on N3/N11 and N7, thus limiting the deprotonation of this site. The third protonation again deals with a carboxylate groups, but now it appears to affect all three acetate arms to a similar extent (pK_a ≅ 4.8).

In the less symmetrical PCTA-[13] the formation of HL^{2-} species at the basic limit is accompanied, as expected, by a large

(21) Sudmeier, J. L.; Reilly, C. N. *Anal. Chem.* **1964**, *36*, 1698.

shift of resonances 18, 5, and 7, but it occurs at lower pH values (approximate pK_a of 10.1).

As shown above for PCTA-[12] and PCTA-[14], this protonation step centered on N6 affects to some extent resonances 2 and 11, which correspond to methylenic protons six bonds away from N6. However, resonance 11 is more shifted than resonance 2 in the pH range from 12 to 9, thus indicating that there is a preference in the hydrogen bond formation in the HL^{2-} species through N6 and N10 rather than N3; i.e. it appears more likely that a hydrogen bond is formed across the propylene rather than the ethylene moiety. Resonances 9 and 4 are shown to vary their chemical shift over a large pH range from 12 to 7, thus affected by both the first and the second protonation step (pK_a $\cong$ 7.7). From the behavior of resonances 18 and also 7 and 5, whose chemical shifts decrease significantly in the pH range 10 to 7, we draw the conclusion that upon the formation of the H_2L^- species the deprotonation at N6 occurs to a similar extent as in PCTA-[12]. From pH 5 the protonation of the acetate arm on N6 (pK_a $\cong$ 3.7) is clearly detected by the downfield shift of resonance 18.

It is interesting to compare the results obtained on PCTA-[12] and PCTA-[14] with those obtained on the parent macrocycles reported by Delgado et al.[14] In py[12]aneN4 the first protonation takes place mainly at the nitrogen atom facing the pyridine nitrogen as in PCTA-[12] and -[14], whereas the second equivalent of acid protonates the pyridine nitrogen. We have no evidence for a protonation at this site in PCTA-[12], -[13], and -[14] as the pyridine resonances show only a minor change in their chemical shifts along the whole range of investigated pH's.

Preparation of the Ln Complexes. The complexation of lanthanide(III) ions to the potassium salts of the ligands PCTA-[12], PCTA-[13], and PCTA-[14] has been carried out by adding stoichiometric amounts of the lanthanide(III) chlorides to the aqueous solutions of the ligands at neutral pH and at ambient temperature. In the case of La and Lu derivatives the formation of the complexes has been followed by high-resolution ^{1}H-NMR spectroscopy, by observing the disappearance of the signals of the free ligand.

Gd(III) Complexes. The long electronic relaxation time associated with Gd(III) ion results in severe induced line broadening of the resonances which prevents the detection of high-resolution NMR spectra of the complexes of this ion. However, some information may be gained by measuring and analyzing the relaxation rate of solvent water protons of their solutions. In fact, in the presence of a fast exchange between coordinated and bulk water, the paramagnetism of the metal ion is transferred to the bulk solvent.[22] This property is currently exploited in the application of the Gd(III) complexes as contrast agents for MRI. The ability to enhance water proton relaxation rates is usually expressed in relaxivity units, i.e. the increase of the water proton relaxation rate in a 1 mM solution of the paramagnetic complex. The measured relaxivity (R_{1p}) receives contributions from the magnetic interactions of the water molecules with the paramagnetic center both in the inner and the outer coordination spheres of the complex.

For low molecular weight poly(amino carboxylate) complexes such as GdDOTA and GdDTPA that contain a single coordinated water molecule ($q = 1$), the contribution from the outer sphere hydration accounts for about 50% of the observed relaxivity when measured at 20 MHz. On this basis, it may be estimated that one coordinated water molecule gives a contribution of ca. 2.5 s^{-1} mM^{-1} units to the overall relaxivity value at

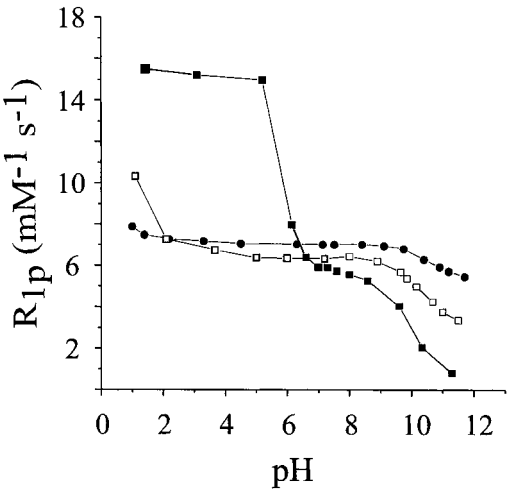


Figure 2. pH dependence of the longitudinal relaxation rate R_1 for 1 mM solutions of GdPCTA-[12] (●), GdPCTA-[13] (□), and GdPCTA-[14] (■), measured at 20 MHz, 25 °C.

20 MHz and 25 °C. Under the same experimental conditions, the measured relaxivities for Gd(III) complexes of PCTA-[12], PCTA-[13], and PCTA-[14] at pH = 7 are 6.9, 6.3, and 5.9 mM^{-1} s^{-1}, respectively. Thus, on the basis of the above considerations, these results indicate that a decrease in the overall hydration of the paramagnetic ion accompanies the increase in the size of the macrocycle. Actually, in the presence of isostructural complexes the small increase of MW on going from the 12- to 14-membered macrocyclic rings is expected to induce a certain lengthening of the reorientational correlation time of the complex with a concomitant relaxivity enhancement. Some structural differences among the three complexes may then be responsible for the observed behavior. The dependence of R_{1p} of the three complexes upon the pH of the solution is reported in Figure 2. The relaxivity of GdPCTA-[12] is almost unchanged from pH 1.5 to 10, and then it slowly decreases at higher pH values. This was already noted by Sherry et al.[8] and tentatively ascribed to the competition of OH^- for the coordination to Gd(III) ion. GdPCTA-[13] displays a constant R_{1p} value over a slightly smaller pH range (2−9.5), whereas GdPCTA-[14] shows a very limited range (between pH 6.5 and 8.0) where this parameter remains almost constant. As the pH is decreased below 6.5 there is an abrupt increase of relaxivity to reach values typical of free Gd(III) ion, whereas at pH >8 the relaxivity steadily decreases with the concomitant formation of a white insoluble precipitate of Gd hydroxide. Thus, GdPCTA-[14] shows a much lower stability than GdPCTA-[12] and GdPCTA-[13] as it forms only in a restricted pH range around neutrality. These differences prompted us to get an estimation of the thermodynamic stability constants of the three complexes at pH 7. This determination was carried out by measuring the relaxation rates of the aqueous solutions of the three complexes in the presence of variable amounts of suitable ligands L which are able to form complexes of analogous stability but with different relaxivity. In this case, the observed relaxation rate of the solutions (R_{1obs}) is given by the sum of three contributions:

$$R_{1obs} = R_{1p(Gd-PCTA)}[GdPCTA] + R_{1p(GdL)}[GdL] + R_{1W} \quad (1)$$

where R_{1W} is the relaxation rate of pure water at 25 °C.

At the neutrality, when L = DTPA, the relative concentrations of the various species depend on the following equilibrium:

$$GdPCTA + H_2L^{3-} \leftrightarrow GdL^{2-} + H_2PCTA^- \quad (2)$$

(22) Banci, L.; Bertini, I.; Luchinat, C. *Nuclear and Electron Relaxation*; VCH: Weinheim, Germany, 1991.

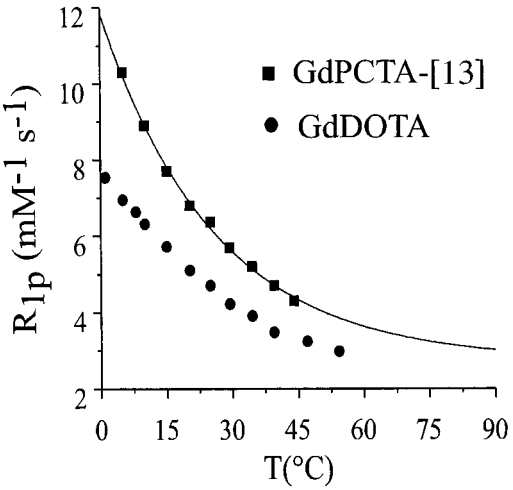


Figure 3. Temperature dependence of the longitudinal relaxation rate for 1 mM solutions of GdPCTA-[13] (■) and GdDOTA (●), measured at 20 MHz, pH = 7.

Whereas the competition with BA-DTPA (bis-amide-DTPA) affords a reaction equilibrium analogous to that of DTPA, in the case of L = CDTA ((±)-*trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetracetic acid), being different the degree of protonation of the ligands, eq 2 must be replaced by

$$\mathrm{GdPCTA} + \mathrm{HL}^{3-} + \mathrm{H}^{+} \leftrightarrow \mathrm{GdL}^{-} + \mathrm{H_2PCTA}^{-} \qquad (3)$$

By working out eqs 2 and 3, the ratio between the K_f values of the two Gd(III) complexes involved in each equilibrium can be evaluated:

$$K = K_{f[\mathrm{GdL}]}/K_{f[\mathrm{GdPCTA}]} \qquad (4)$$

The ligands DTPA (R_{1p} = 4.7,[1c] log K_f = 22.5[23]), CDTA ((±)-*trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetracetic acid) (R_{1p} = 8.3,[24] log K_f = 19.5[23]) and BA-DTPA (bis-amide-DTPA) (R_{1p} = 4.0,[24] log K_f = 13.0[24]) resulted particularly suitable for the determination of K_f values of PCTA-[12], PCTA-[13], and PCTA-[14], respectively. The evaluation of the observed R_{1obs} data on the basis of eqs 1−3 yielded log K_f values of 21.0 ± 0.5,[25] 19.3 ± 0.6, and 12.5 ± 0.5 for PCTA-[12], PCTA-[13], and PCTA-[14], respectively.

GdPCTA-[12] displays then a K_f value similar to that found for GdDO3A (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid), a related complex with an analogous heptadentate ligand based on DOTA structure. The only slightly lower stability of GdPCTA-[13] suggests a close analogy in the structure of the two complexes. On the other hand GdPCTA-[14] is much less stable, in agreement with the pH dependence of the relaxivity.

At pH = 7, all three Gd(III) complexes show an exponential decrease of the observed relaxivity upon increasing the temperature in the range 0−60 °C. This behavior is typical of the fast exchange condition, which occurs when the residence lifetime of the coordinated water molecule is much shorter than its relaxation time T_{1M}. In Figure 3 are reported, for comparison, the data for GdDOTA and GdPCTA-[13]. We may note that for the latter the fast exchange condition is met even in the lower temperature range whereas in the case of GdDOTA significant deviations from the exponential behavior are observed. This finding supports the hypothesis that a fast water exchange rate may occur in neutral complexes provided that the hydration number $q > 1$. This is in agreement with the observations reported in ref 8 for some Gd complexes endowed with negative, neutral, or positive charge and $q > 1$.

Solution Structures of Ln(III)−PCTA[12], −PCTA-[13], and −PCTA-[14]. La(III) Complexes. Some years ago it was reported that the ^{139}La NMR chemical shift represents a reliable parameter to assess the number and the type of the donor atoms in the coordination sphere provided by poly(amino carboxylate) ligands.[26] Actually, it was found that each coordinated amine nitrogen and carboxylic oxygen gives a downfield shift (measured at 70 °C, with respect to the aquaion) of ca. 50 and 30 ppm, respectively.[26] For the three La(III) complexes considered in this work, the observation of a very similar ^{139}La chemical shift (290 ± 5 ppm, at 70 °C) is then an indication of the occurrence of an analogous set of donor atoms around the lanthanide(III) ion. By assuming that the contributions from amine and carboxylate groups are unchanged in respect to those reported for other poly(amino carboxylate) complexes, it turns out that the contribution from the pyridine nitrogen corresponds to ca. 50 ppm.

The ^{1}H-NMR resonances of LaPCTA-[12] appear rather broad in the spectrum at room temperature; thus their assignment was carried out on the basis of the spectrum recorded at 50 °C where rather narrow resonances are observed. Whereas the resonances of the pyridine (7.4, 8.1 ppm) and ethylenic (2.3, 2.9 ppm) protons can be easily assigned, the assignment of methylenic protons 2,2′/10,10′ and 16,16′/18,18′ is more problematic. By comparison with the spectrum of the free ligand, we attribute the AX spin system (4.5 and 3.9 ppm, J = 16 Hz) to protons 2,2′/10,10′ and the AB spin system centered at 3.6 ppm (J = 16 Hz) to the acetic (16,16′/18,18′) protons. The singlet resonance at 3.4 ppm is assigned to the methylenic proton pair 17/17′. The observation of a singlet for this pair of acetic hydrogens is an indication of the occurrence, at high temperature, of a fast intramolecular rearrangement which makes equivalent these two protons on the NMR time scale. The ^{13}C NMR spectrum at 50 °C (Figure 4) shows signals corresponding to two carboxylates at 182.0 and 181.5 ppm, three pyridine carbons at 159.4 (C1/11), 142.3 (C13), 123.8 (C12/14) ppm, two CH_2 acetate at 64.4 (C 16/18) and 64.2 (C17) ppm, a methylenic at 63.9 ppm (C 2/10), and two ethylenic carbons at 57.0 and 56.0 ppm. In both ^{13}C- and ^{1}H-NMR spectra an extensive broadening of the resonances takes places as the temperature is decreased below room temperature. At 0 °C several resonances are clearly detected in the ^{13}C spectrum (Figure 4). The observed behavior does not appear consistent with the simple "freezing out" of the interconvertion between two isomeric species as found in the related DOTA complexes. Rather, the observation of a large dispersion of ^{13}C resonances is taken as an indication of the formation of polymeric species in solution. It is likely that this tendency to polymerize has to be associated with an untight wrapping of the heptadentate ligand around the large lanthanum(III) ion. Inspection into the X-ray structures of Ln(III) poly(amino carboxylate) complexes reveals that it is not uncommon for complexes with heptadentate ligands to find polymeric structures at the solid state.[27]

The overall VT behavior of the ^{1}H- and ^{13}C-NMR spectra of LaPCTA-[13] complex resembles those ones found for LaPCTA-[12], further complicated by the intrinsic asymmetry of the N4-ane-13 ring. The proton assignment has been carried out at 50

(23) Martell, A. E.; Smith, R. *Critical Stability Constants*; Plenum Press: New York, 1977; Vol. 3.
(24) Unpublished results.
(25) A value of log K_f = 20.8 has been measured by potentiometric titration. L. Calabi (Bracco Spa, Milano, personal communication).
(26) Geraldes, C. F. G. C.; Sherry, A. D. *J. Magn. Reson.* **1986**, *66*, 274.
(27) Kang, S. I.; Ranganathan, R. S.; Emswiller, J. E.; Kumar, K.; Gougoutas, J. Z.; Malley, M. F.; Tweedle, M. F. *Inorg. Chem.* **1993**, *32*, 2912.

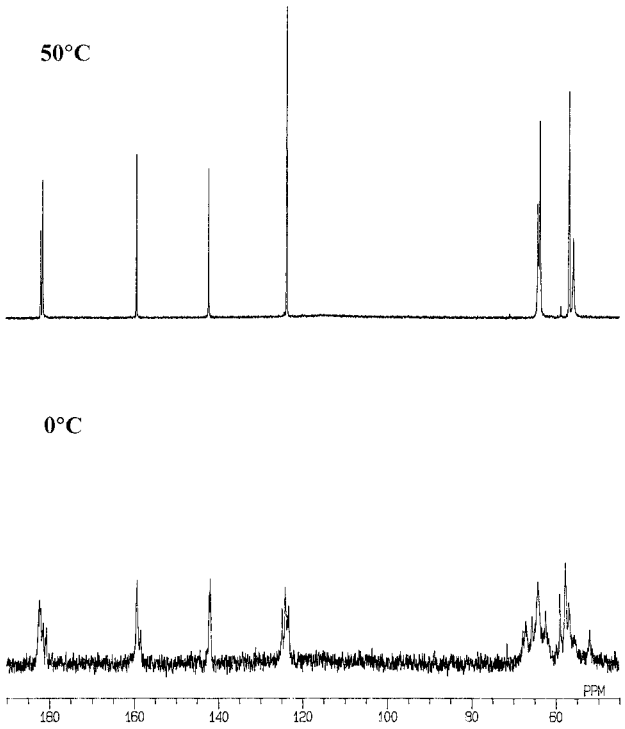


Figure 4. ^{13}C-NMR spectra of LaPCTA-[12] recorded at 100.4 MHz, in D_2O (pD = 7) at 50 and 0 °C. *tert*-Butyl alcohol was added as an internal reference (δ = 31.1 ppm).

°C on the basis of a homonuclear 2D-COSY experiment: the signals detected at 8.0 (J = 8 Hz), 7.5 (J = 8 Hz), and 7.5 ppm correspond to protons 14 and 15/13, respectively. The doublets at 4.4 (J = 15 Hz) and 3.7 ppm (J = 15 Hz) are assigned to protons 11,11′ and those at 4.4 (J = 15 Hz) and 4.0 ppm (J = 15 Hz) to protons 2,2′. In the region between 3.6 and 3.2 ppm there are the absorptions of protons 18/18′, 17/17′, and 19/19′, whereas the two AB systems centered at 3.0 and 2.9 ppm (J = 13 Hz) correspond to protons 4/4′ and 5/5′. The propylenic protons fall between 2.7 and 1.6 ppm. As well as in the case of the related PCTA-[12] complex, a reversal in the assignment of the proton pairs 2/11 with 17/19 is possible. On the other hand, in this case the methylenic protons of the central acetate arm (18,18′) are no longer equivalent, even in the presence of a fast inversion of the ring.[28] The ^{13}C NMR spectrum (at 50 °C) consists of 18 signals assigned, on the basis of a 2D HETCOR experiment: 182.2 (C21), 181.8 and 181.7 (C20/22), 159.5 and 159.4 (C1/12), 142.6 (C14), 124.9 and 124.5 (C15/C13), 65.8 (C19), 63.9 (C18), 63.8 and 63.6 (C2/11), 62.1 (C17), 61.9 and 61.4 (C9/7), 58.1 and 54.5 (C4/5), and 23.4 ppm (C8).

The broadening of ^{13}C and ^{1}H resonances noticed in the low-temperature spectra of LaPCTA-[12] and LaPCTA-[13] is less pronounced in the case of the LaPCTA-[14]. Thus, the enlargement of the macrocycle both increases the overall mobility in the system and prevents the formation of oligomeric adducts. As in the case of LaPCTA-[12] the presence of a plane of symmetry reduces the number of resonances, whereas some differences have been found in the chemical shift of 2/12 proton resonances (two doublets at 4.5 and 3.7 ppm, J = 13 Hz). In the aromatic region a triplet and a doublet are present at 8.0 (J = 8 Hz) and at 7.5 ppm (J = 8 Hz) as usual, and the acetate proton signals appear as two doublets at 3.7 (J = 16.5 Hz) and 3.3 ppm (J = 16.5 Hz) and a singlet at 3.3 ppm. The protons 5,5′,9,9′ fall at 1.9 and 1.7 ppm whereas the other methylenic proton resonances are in the region between 1.1 and 2.8 ppm. The ^{13}C spectrum shows 10 resonances: 182.0 (C21/23), 181.2 (C22), 158.9 (C1/13), 142.7 (C15), 124.5 (C16/14), 65.7 (C19), 64.9 (C2/12), 61.9 (C18/20), 57.0 (C4/10 and C6/8), and 24.3 ppm (C5/9).

On the body of these observations we concluded that all three ligands are able to coordinate La(III) ion by means of the four nitrogens of the macrocycle and the three oxygens of the acetate arms. The resulting complexes display an overall stereochemical nonrigidity that is higher for the larger cycle.

Lu(III) Complexes. LuPCTA-[12] appears highly fluxional over the investigated temperature range (0−90 °C). At 50 °C, the pyridine protons fall at 8.1 ppm (J = 8 Hz) and 7.6 ppm (J = 8 Hz); protons 2/2′ and 10/10′ absorb at 4.6 and 4.3 ppm (J = 17 Hz) and 16/16′ and 18/18′ at 3.82 and 3.7 ppm (J = 16 Hz), respectively; the methylenic protons of the central acetate arm (17/17′) fall at 3.6 ppm, whereas protons 4/4′, 8/8′ and 5/5′, 7/7′ afford three complex signals at 3.0, 2.7, and 2.7 ppm.

The ^{13}C spectrum consists of two carboxylate resonances at 181.9 and 181.4 ppm, three pyridine carbons at 160.0, 143.9, and 123.2 ppm, two methylenic acetate carbons at 66.2 and 63.5 ppm, and three macrocyclic methylenic resonances at 64.3, 60.1, and 59.0 ppm. No evidence was gained in either ^{1}H- and ^{13}C-NMR spectra of broadening of the resonances at the lowest attainable temperature (0 °C).

The ^{1}H-NMR spectrum of LuPCTA-[13] is rather complex and displays some significant differences from the corresponding spectrum of La(III) complex. For instance, 2/2′ or 11/11′ here give rise to an AB spin system centered at 4.4 ppm (J = 16 Hz) whereas in the La(III) complex they appear as two AX doublets separated by ∼0.8 ppm. Conversely, the other resonances assigned to 2/2′ or 11/11′ pairs are slightly more separated in this complex as they appear as two doublets at 4.3 and 3.8 ppm (J = 17 Hz). Other assigned signals are three AX doublets at 3.9, 3.7 (J = 17 Hz), at 3.9, 3.3 (J = 16 Hz), and at 3.6, 3.3 ppm (J = 17 Hz) relative to the acetic protons, and two multiplets at 1.8 and 1.6 ppm (H 8,8′). The remaining macrocyclic methylenic protons fall between 2.4 and 3.1 ppm. Upon the temperature being decreased, there is an overall broadening of the resonances which is very dramatic for some of them. The VT ^{13}C-NMR spectra resulted more informative. The ^{13}C-NMR spectrum of LuPCTA-[13] at 85 °C consists of 17 sharp resonances as expected for 17 structurally different carbons in the complex: at 181.9 and 181.8 (C20/22), 181.2 (C21), 160.5 and 159.1 (C1/12), 143.7 (C14), 124.7 and 124.0 (C13/15), 66.4, 64.5, 64.4, 63.7, 63.3, 60.2, 59.4, and 57.1 (acetic and macrocyclic methylenic carbons), and 23.5 ppm (C8). As shown in Figure 5 (methylenic region only), as the temperature is decreased most of the resonances markedly broaden then they sharpen again at the lowest attainable temperature. This process is accompanied by marked changes in the chemical shifts. The observed behavior is clearly indicative of an exchange process involving a largely dominant species and other one(s) at very low concentration. The actual maximum broadening of any resonance is then dependent upon the chemical shift separation between the signals of the exchanging species. Thus at 0 °C the exchange has been frozen out although we can detect only the resonances of the largely dominant isomer.

Lu(III)PCTA-[14] appears more fluxional than Lu(III)PCTA-[13] as some broadening of the ^{1}H-NMR resonances is detected only at temperatures lower than ambient. The ^{1}H-NMR spectrum (Figure 8c) consists of two pyridine absorptions at 8.1 (J = 8 Hz) and 7.6 (J = 8 Hz), an AB spin system assigned to 2/2′, 12/12′ protons centred at 4.3 ppm (J = 16 Hz), an AX spin system assigned to 18/18′, 20/20′ protons at 3.9 and 3.5

(28) This point has been checked by measuring the ^{1}H spectrum at higher magnetic field strength (600 MHz).

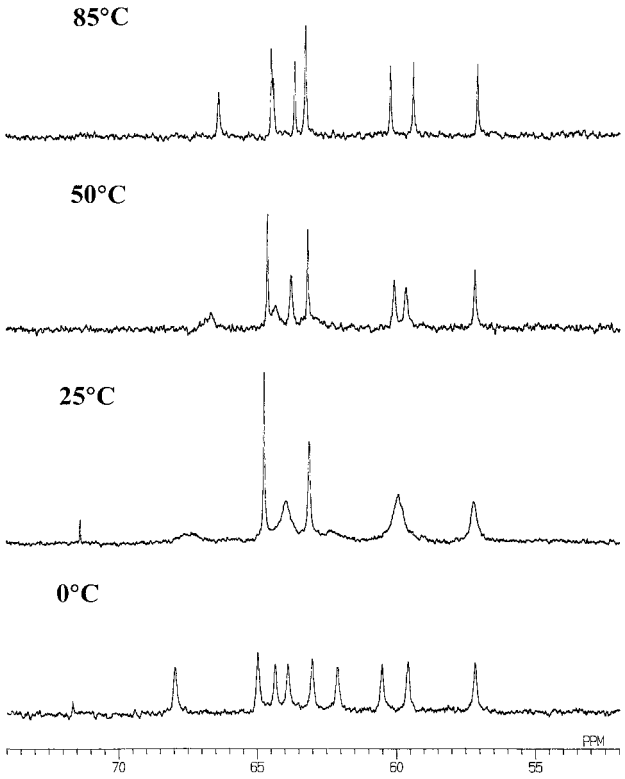


Figure 5. Variable temperature ^{13}C-NMR spectra of LuPCTA-[13] (methylenic region only) in D_2O (pD = 7).

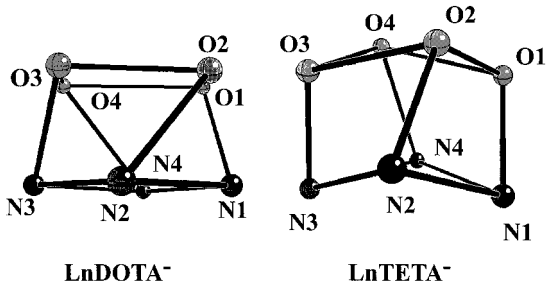
ppm (J = 17 Hz), a singlet at 3.4 corresponding to the two equivalent CH_2 proton of the central acetate arm, and a complex set of absorptions due to the propylenic moieties between 3.0 and 2.6 (4/4′, 10/10′ and 6/6′, 8/8′) and 2.15 and 1.85 (5/5′, 9/9′) ppm. The high-temperature ^{13}C NMR spectrum (T = 50 °C) shows 11 signals at 181.5 (C22), 181.1 (C21/23), 158.8 (C15), 143.5 (C1/13), 124.4 (C14/16), 65.5 (C2/12), 64.9 (18/20), 64.6 (C19), 60.8 and 58.6 (C4/10, C6/8), and 24.0 ppm (C5/9). The observed pattern is fully consistent with the occurrence of a fast intramolecular rearrangement involving both the acetate arms and the flexible part of the macrocycle. Upon the temperature being decreased, some broadening of the resonances occurs, and at 0 °C, this is particularly evident for methylenic resonances at 65.5, 64.9, and 60.8 ppm, thus confirming that an incipient freezing of the exchange process is taking place.

Discussion

In order to get more insight into the relationships between the ligand properties and the solution structures and dynamics of Ln(III)−PCTA-[12], -[13], and -[14] complexes, it is useful to recall some observations reported on related octacoordinated macrocyclic complexes. To this purpose we think that DOTA may represent a good reference system for PCTA-[12] and TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) for PCTA-[14].

In the case of the Ln(III)DOTA[29] and Ln(III)TETA[30] complexes, two distinct coordination geometries were found, namely a square antiprismatic coordination for the former and a dodecahedral one for the latter (Chart 2). The two coordination schemes bring about two important differences: (i) Relaxivity measurements unambiguously indicate that q = 1 in the case of GdDOTA whereas q = 0 in the case of GdTETA. These findings have been confirmed by luminescence studies of EuDOTA[31] and EuTETA[31] and from the X-ray structure determinations of EuDOTA[32] and TbTETA.[30] (ii) The thermodynamic stability is several orders of magnitude higher in DOTA than in TETA complexes.

Chart 2



In principle the interconversion between the two structural arrangements appears possible. On going from TETA to DOTA this transformation may be envisaged to occur through the flattening of the dihedral angles in N4 and O4 planes coupled to the inversion of ring conformation and/or inversion of the helicity of the acetate arms. Now, we should ask ourselves what remains valid of these considerations on passing from octacoordinating ligands such as DOTA and TETA to the heptacoordinating ligands. GdDO3A appears to have a high thermodynamic stability (log K_f = 21.1[33]), and the luminescence studies[34] suggest, for the hydration number, a value close to 2. Thus, an antiprismatic DOTA-like structure may be anticipated for such a complex. On the other hand, this structure was actually found in the case of the related GdDO3MA (DO3MA = (1*R*,4*R*,7*R*)-α,α′,α″-trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) complex containing three methyl groups on the acetate arms.

The stabilities of GdPCTA-[12] and PCTA-[13] are not too different from that of DO3A complex, thus suggesting the occurrence of a DOTA like structure for these species as well. The asymmetry of the macrocycle is expected to cause an increased rigidity of PCTA-[13] complexes with respect to PCTA-[12] as clearly shown in the VT ^{13}C-NMR spectra of LuPCTA-[13]. This agrees with a preliminary report on YbPCTA-[13][10] which showed the occurrence of two isomeric species (in the relative concentration of 1:10) in the low-temperature limiting spectrum. Thus, it seems reasonable to suggest that DOTA-like coordination modes largely characterize the limiting structures involved in the exchange process of LnPCTA-[12] and LnPCTA-[13] complexes. It follows that one of the coordination positions in the O4 plane of DOTA left vacant by the lack of an acetate arm in DO3A, DO3MA, and PCTA-[12] and PCTA-[13] complexes is occupied by a water molecule to keep an overall antiprismatic geometry with one capping water around the lanthanide ion.

Now, on going from GdPCTA-[13] to the larger, GdPCTA-[14] complex, we noted both an unexpected decrease of relaxivity and a dramatic decrease of the stability of the complex. Both effects may be taken as an indication that there has been a change in the coordination geometry which parallels to some extent that discussed above for DOTA/TETA transformation.

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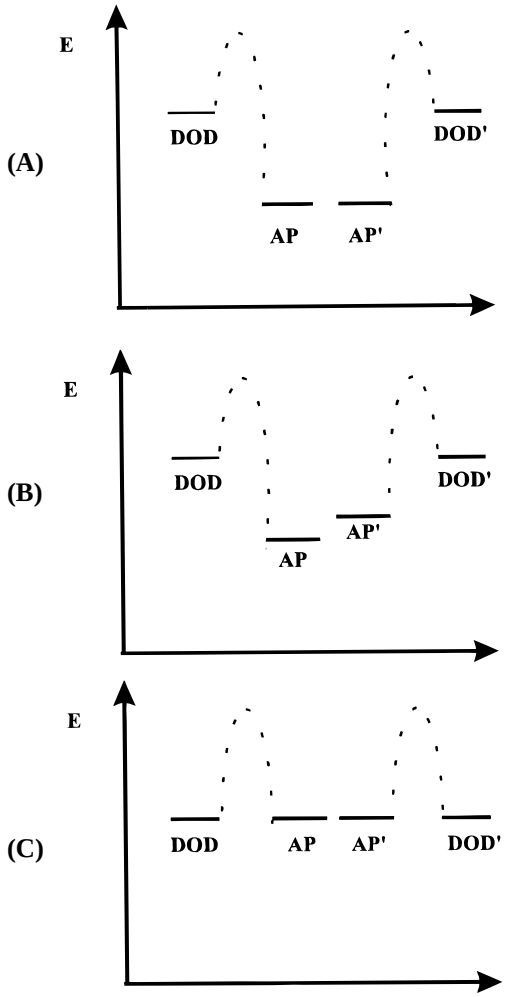
Chart 3



Said in other words we think that the larger macrocycle enables the PCTA-[14] ligand to wrap around the lanthanide ion in such a way to result in a reduced inner sphere hydration than PCTA-[12] and PCTA-[13]. On the basis of these considerations centered on the shift of geometry between two limiting coordination polyhedra represented by the square antiprism, DOTA-like structure, and the dodecahedral, TETA-like geometry, the size of the lanthanide ion has to be considered as an important determinant of the resulting structure, in addition to the dimension of the macrocyclic ring. It follows then that LaPCTA-[12] and LuPCTA-[14] may be considered the extremes in such an idealized framework. LaPCTA-[12] should own a DOTA-like structure with the highest hydration sphere and be more susceptible to forming polymers in solution through the fitting of an acetate arm of a neighbouring complex into the "large hole" provided by the exchanging water molecules. On the other hand, the smaller Lu(III) ion sinks into the bottom and allows the PCTA-[14] ligand to wrap around it in a TETA-like fashion.

One may get further insight into the coordination frame of these PCTA−Ln complexes by considering the qualitative energy diagrams for the exchange between the structures displaying antiprismatic (AP) and dodecahedral (DOD) geometries as reported in Chart 3, where AP′ and DOD′ are the enantiomeric forms of AP and DOD in the case of the symmetrical PCTA-[12] and PCTA-[14] complexes, but they are structural isomers in the case of PCTA-[13] complexes.

The transformation between AP and AP′ occurs through the inversion of the ethylenic and propylenic moieties as well as through the motion of the acetate arms, i.e. it corresponds to the racemization process observed in DOTA complexes. In the presence of AP ⇔ DOD (and AP′ ⇔ DOD′) interchanges, which is based on concerted upward and downward motions of four donor atoms (two N and two O), the overall asymmetry of the complexes does not cause a reduction of the number of ^{1}H and ^{13}C resonances.

The prototype complex whose exchange process may be represented by diagram A is LaPCTA-[12]. The large size of the metal ion coupled to the small macrocyclic dimension suggest that a fast exchange occurs between two DOTA-like structures. On going from PCTA-[12] to PCTA-[13], the substitution of an ethylenic with a propylenic moiety introduces a large asymmetry in the cycle that causes a net energy difference between AP and AP′ structures. In the case of LuPCTA-[13] it has been possible to "freeze out" this motion and we found that one AP isomer is largely dominant, as expected on the basis of diagram B. A further enlargement of the macrocycle causes a stabilization in energy of DOD forms which is minimized in the case of the smallest Lu(III) ion (diagram C).

In summary, we believe that the solution structures along the lanthanide series of PCTA-[12], -[13], and -[14] complexes exhibit a large variability dictated by the matching of the sizes of the lanthanide ion and the macrocyclic cavity. The overall result is a large variation in the stereochemical nonrigidity, inner coordination hydration sphere, and thermodynamic stability. This is consistent with the observation made by Sherry et al.[8] of significantly different *q* values measured for related Tb(III) and Eu(III) complexes of several pyridine-containing macrocyclic ligands.

As far as the applications in MRI are concerned, from the relaxometric investigations on Gd(III) complexes we have established that the lifetime of the coordinated water molecules is significantly shorter than in GdDOTA. Thus we expect to observe large relaxation enhancements upon binding of these Gd−PCTA-[12], -[13], and -[14] complexes to macromolecular systems, as the resulting relaxivity should not be "quenched" by exceedingly long exchange rates between the inner coordination sphere and bulk water. To reach this goal we are currently working on the preparation of GdPCTA-[12] complexes, bearing suitable functionalities on the pyridine moiety, that are able to target human serum albumin.

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Supporting Information Available: Figures of the pH dependence of the ^{1}H-NMR resonances of the ligands PCTA-[14] (Figure S1) and PCTA-[13] (Figure S2) and figures of the ^{1}H-NMR spectra of the PCTA-[12], -[13], -[14] complexes with La(III) (Figure S3) and with Lu(III) (Figure S4), respectively (5 pages). Ordering information is given on any current masthead page.

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