Inorg. Chem. **2002**, *41*, 685–692

Synthesis and Solution Studies of the Complexes of Trivalent Lanthanides with the Tetraazamacrocycle TETA-$(PO)_2$

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Received July 6, 2001

A new potentially multidentate hexaprotic ligand H_6[TETA-$(PO)_2$] has been prepared by reaction of ethylenediamine-*N*,*N*′-diacetic acid (EDDA), paraformaldehyde, and phosphinic acid; its coordination properties with three lanthanide ions (La^{3+}, Gd^{3+}, and Lu^{3+}) have been explored. The structures of the complexes were studied in aqueous solution by potentiometric pH titrations and by ^{31}P NMR spectroscopy. Four acidity constants were determined potentiometrically in the range 2.5 < pH < 14. The four measured pK_a values can be divided into two groups, and within each group the initial deprotonation was found to have little effect on the second. Variable temperature ^{31}P and $^{31}P\{^1H\}$ EXSY NMR spectra showed that, for [Lu(TETA-$(PO)_2$)]$^{3-}$, the two phosphorus atoms exist in different chemical environments and undergo an exchange process which is very fast on the NMR time scale at room temperature. This result is consistent with one of the phosphinate residues coordinating the metal ion and exchanging with a free analogue. In the case of [La(TETA-$(PO)_2$)]$^{3-}$, only one temperature invariant signal is observed in ^{31}P NMR spectra; it corresponds to both phosphinate residues remaining uncoordinated to La^{3+}. The stability of [Ln(TETA-$(PO)_2$)]$^{3-}$ has an order of $La^{3+} > Gd^{3+} > Lu^{3+}$. The coordination of one phosphinate residue to Lu^{3+} brings the metal ion closer to the plane of four nitrogens and farther from the four carboxylate arms, resulting in [Lu(TETA-$(PO)_2$)]$^{3-}$ having a lower stability than the corresponding La^{3+} and Gd^{3+} complexes. A pM–pH distribution diagram showed that introducing two phosphinate groups into TETA renders [Gd(TETA-$(PO)_2$)]$^{3-}$ more stable than [Gd(TETA)]$^-$. The selectivity factor of the ligand for Gd^{3+} vs Ca^{2+}, Zn^{2+}, and Cu^{2+} has been calculated, and the hydration number for [Dy(TETA-$(PO)_2$)]$^{3-}$ has been measured by ^{17}O NMR spectroscopy to be zero.

Introduction

Since the approval of [Gd(DTPA)(H_2O)]$^{2-}$ as a contrast agent for magnetic resonance imaging (MRI) in 1988, many other Gd(III) complexes have been investigated for potential use in diagnostic medicine.[1] Currently, this area is still of burgeoning research interest and is reviewed regularly.[1,2]

Since the number of suitable nuclei for use in MRI contrast agents is limited, new agents with improved properties will depend on the choice of the ligand used to complex the paramagnetic ion, usually Gd^{3+}. Although copious data have been accumulated over the past 2 decades, most of these concern polyaminopolycarboxylate ligands.[1] Phosphinate derivatives of macrocylic chelates have been explored by Parker and Sherry;[3−6] however, most of the ligands were designed by replacing the carboxylates in the polyaminopolycarboxylate ligands with phosphinates. Unfortunately, most of the Gd(III) complexes thus formed were found to have low hydration numbers ($q = 0$).[1] We have designed a

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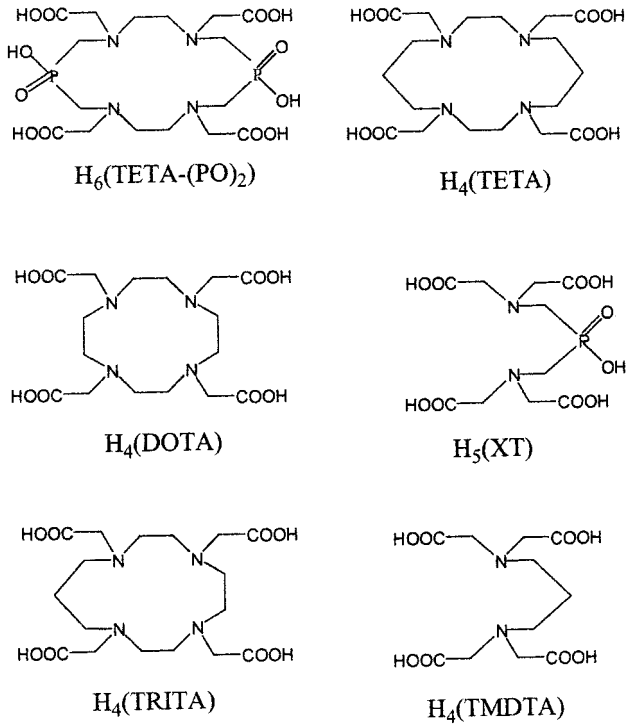
10.1021/ic010716a CCC: $22.00
Published on Web 01/31/2002

Chart 1



diphosphinate tetraazamacrocycle H_6[TETA-$(PO)_2$] (Chart 1) in which two of the methylenes in H_4[TETA] are replaced by phosphinates while the four carboxylate groups are maintained. We were interested to see how this replacement affected the stability and hydration number of the analogous Gd(III) complexes.

It is known that studying Gd(III) stability constants alone is insufficient to explain in vivo stability trends.[7,8] Cacheris et al.[8] proposed the use of a "selectivity" factor, log K_{sel}, by considering the relative affinity of a given ligand for Gd^{3+} as well as for biologically relevant cations such as Ca^{2+}, Zn^{2+}, and Cu^{2+}. These authors suggested that an increase in selectivity for Gd(III) over endogenous cations substantially contributes to the high LD_{50} (lethal dose for 50% of tests, indicating a lack of acute toxicity). These results have encouraged the study of Gd(III) complex stability in aqueous solution, although the thermodynamic selectivity index can be used only with those complexes which have sufficiently fast dissociation and substitution kinetics.[1]

In this study, we have measured the acidity constants of H_6[TETA-$(PO)_2$] and its aqueous complexation with La^{3+}, Gd^{3+}, and Lu^{3+} ions; the results are compared with the corresponding values for TETA and DOTA.[9] We have also determined the stability constants of the complexes of this ligand with Ca^{2+}, Zn^{2+}, and Cu^{2+} and calculated selectivity factors. To understand the structures and dynamic processes of the complexes in solution, variable temperature ^{1}H and ^{31}P NMR studies were undertaken. ^{17}O NMR was used to measure the hydration number of the $[Dy(TETA\text{-}(PO)_2)]^{3-}$ complex.

Experimental Section

Materials. Atomic absorption standard solutions of La^{3+} or Lu^{3+} were purchased from Sigma, while that of Gd^{3+} was from Aldrich. The NaOH (1 M) solution used as titer was from Fisher Scientific, and its concentration was established with potassium biphthalate from Anachemia Canada Inc. NaCl, for controlling the ionic strength, and HCl(aq) were also purchased from Fisher Scientific. Water used in the experiments was deionized (Barnstead D8902 and D8904 cartridges), distilled (Corning MP-1 Megapure still), and depleted in CO_2 by boiling under Ar for 30 min. D_2O, CD_3-OD, NaOD, and DCl used in the NMR studies were purchased from Cambridge Isotope Laboratories.

Instrumentation. Potentiometric measurements were carried out with an automatic titration system consisting of a Metrohm 713 pH meter equipped with a Metrohm 6.0233.100 electrode, a model 665 Metrohm Dosimat autoburet, water-jacketed titration vessels, and a Julabo UC circulating bath. Both the pH meter and autoburet were controlled by an IBM-compatible PC, and the titration was controlled by a locally written QBasic program. The electrode was calibrated before each titration by titrating a known amount of aqueous HCl with a known amount of NaOH. A plot of mV-(calculated) vs pH gave a working slope and intercept, so that pH could be read as -log[H^+] directly. The value of pK_w used at I = 0.16 was 13.76.[11] ^{1}H and ^{31}P NMR spectra were obtained on a Bruker AC-300 spectrometer, using a 5 mm QNP probe, at 300.13 and 121.49 MHz, respectively. ^{17}O NMR spectra were recorded at 54.24 MHz on a Bruker AC-400 spectrometer using a 5 mm BBI-Z probe.

Preparation of H_6[TETA-$(PO)_2$]. Ethylenediamine-*N*,*N*′-diacetic acid (EDDA, 3.4 g, 19.3 mmol) was suspended in 6 N HCl (50 mL), and the resulting mixture was heated to 105−110 °C under vigorous stirring. Paraformaldehyde (2.8 g, 93 mmol) was then added to give a clear solution. Phosphinic acid (2 mL of 50% aqueous solution, 19.3 mmol) was added in four equal portions over 15−20 min. The reaction mixture was heated to reflux for 3−4 h, during which time a white precipitate formed. The reaction mixture was allowed to cool to room temperature. The resulting white solid was separated by filtration, washed with 6 N HCl (5 mL) and acetone (5 mL), and dried under vacuum overnight. The yield was 0.65 g (∼13% based on EDDA). The crude product (>95% pure by NMR) was recrystallized by dissolving the sample in a minimal volume of 0.1 N NaOH solution, followed by titration with concentrated HCl to pH = 1.5. The white precipitate was collected by filtration and dried under vacuum overnight. Anal. Calcd (found) for $C_{16}H_{30}N_4O_{12}P_2\cdot 2HCl\cdot 2H_2O$: C, 29.97 (30.18); H, 5.66 (5.48); N, 8.74 (8.56). ^{1}H NMR (D_2O, KOD, δ ppm relative to TMS): 3.53 (s, 8H, NCH_2COOH); 3.44 (s, 8H, NCH_2CH_2N); 3.27 (d, 8H, NCH_2P, J_{H-P} = 12.9 Hz). ^{31}P NMR (δ ppm relative to external phosphoric acid): 41 ppm. ESMS (negative ion detection mode; *m*/*z* (relative intensity %, assignment)): 531.3 (8, $[M-H]^-$) ($M = C_{16}H_{30}N_4O_{12}P_2$), 265.1 (100, $[M-2H]^{2-}$), 132.0 (33, $[M-4H]^{4-}$).

Potentiometric pH Titration. The acidity constants for H_6-[TETA-$(PO)_2$] were determined by titrating 50 mL of aqueous 2.8 mM HCl (I = 0.16 M NaCl, T = 25 °C) in the presence of 0.3 mM H_6[TETA-$(PO)_2$] under N_2 with 2 mL of 0.11 M NaOH. The

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constants were calculated using the data within the range $2.5 \leq pH \leq 10.0$ on an IBM-compatible computer containing a Pentium II processor using a curve-fit procedure (a Newton−Gauss nonlinear-least-squares program). This corresponded to about 80% neutralization for the equilibrium $[H_5(TETA\text{-}(PO)_2)]^-/[H_4(TETA\text{-}(PO)_2)]^{2-}$ and about 97% neutralization for the equilibrium $[H(TETA\text{-}(PO)_2)]^{5-}/[TETA\text{-}(PO)_2]^{6-}$. The final results for the acidity constants were obtained from the average of 10 independent titrations.

The stability constants of La^{3+}, Gd^{3+}, Lu^{3+}, Ca^{2+}, Zn^{2+}, and Cu^{2+} with $H_6[TETA\text{-}(PO)_2]$ were determined under the same conditions as for the acidity constants. Because the metal ion concentration was very low (<0.3 mM), the ionic strength of the solution was controlled by NaCl ($I = 0.16$ M, 25 °C). The ligand to metal ion ratios used in these experiments were slightly greater than 1:1. The calculations were carried out by a previously described curve-fitting procedure.[12] The final results are the average of at least four independent titrations for each metal ion. Hydrolysis constants for all metal ions were taken from ref 13.

^{1}H, ^{31}P, and ^{17}O NMR Measurements. Variable temperature ^{1}H and ^{31}P NMR spectra for $H_6[TETA\text{-}(PO)_2]$, and the La− and Lu−TETA-$(PO)_2$ systems were obtained in D_2O or a mixture of CD_3OD (80%) and D_2O (20%), depending on the experiment. A stock solution of $[TETA\text{-}(PO)_2]^{6-}$ was prepared by dissolving (0.030 g, 0.0474 mmol) of $H_6[TETA\text{-}(PO)_2]$ in 3 mL of the appropriate solvent and adjusting the pD to 10.5 using 30% NaOD. A 1.0 mL aliquot of this stock solution was used to dissolve 0.0058 g (0.0156 mmol) of $LaCl_3 \cdot 7H_2O$ so that the ratio of La:L was 1:1 and the pD was subsquently adjusted to 9.0. Similarly, 0.006 g of $LuCl_3 \cdot 6H_2O$ (0.0156 mmol) was dissolved in 1.0 mL of the stock solution, and the pD was adjusted to 9.7. The lowest temperature reached in D_2O-CD_3OD was −35 °C (238 K), and the highest was 45 °C (318 K) in D_2O solution. At two temperatures (25 and 10 °C), the spectra of $Lu[TETA\text{-}(PO)_2]^{3-}$ were recorded in both solvents, and the results were almost identical. ^{1}H NMR spectra were referenced to a residual proton signal of the solvent, while ^{31}P NMR spectra were referenced to external 85% H_3PO_4. The variable temperature accessories were checked with the methyl alcohol calibration method (±1 °C).[14]

Two-dimensional $^{31}P\{^1H\}$ EXSY (exchange spectroscopy) spectra for $Lu[TETA\text{-}(PO)_2]^{3-}$ in D_2O were acquired in the phase sensitive mode, using the pulse sequence $[90° - t_1 - 90° - t_m - 90° - ACQ]$. In a typical EXSY experiment 128 FIDs were recorded in the F2 dimension, with each FID acquired in 128 scans over a 2.185 kHz spectral width. Line broadening was set at 1 Hz. At 298 K a mixing time of 100 ms was used, whereas mixing times of 10, 25, and 100 ms were employed at 278 K. A series of three one-dimensional spectra were extracted from each of the EXSY experiments at 278 K containing the 48 ppm diagonal peak and its off-diagonal correlation peak with the free ligand. Since the diagonal and off-diagonal resonances were found to have the same phase, one can be sure that these correlations arise as the result of chemical exchange and not from NOE interactions. The ^{17}O NMR experiments for Dy(III) were recorded at 298 K in D_2O, with a 90° pulse width of 14 μs, and an acquisition time of 0.943 ms was usually employed to give 4096 data points; 256 scans were collected per spectrum. ^{17}O NMR line widths were on the order of 60 Hz.

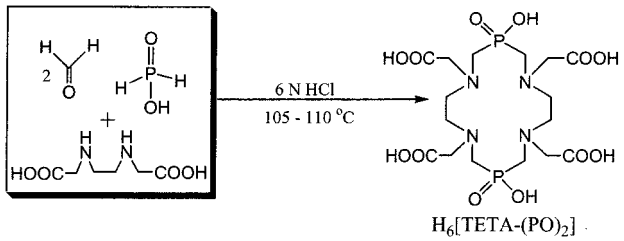
Table 1. Logarithms of the Acidity Constants for TETA-$(PO)_2$ Measured by Potentiometric pH Titration ($I = 0.16$ M NaCl, 25 °C) and the Corresponding Values for XT, [17] TETA [9] ($I = 0.10$ M KCl, 25 °C), and DOTA [9] ($I = 0.10$ M KCl, 25 °C)

compd	TETA-$(PO)_2$	TETA	XT	DOTA
pK^H_{H5L}	1.89 ± 0.05			
pK^H_{H4L}	4.08 ± 0.02	3.21	2.32 ± 0.18	3.95
pK^H_{H3L}	4.78 ± 0.04	4.15	2.84 ± 0.13	4.84
pK^H_{H2L}	7.76 ± 0.04	10.10	6.56 ± 0.06	9.69
pK^H_{HL}	8.45 ± 0.03	10.82	9.27 ± 0.05	11.14

Concentrations employed ranged for Dy(III) from 8.0 to 40.2 mM and for $[Dy(TETA\text{-}(PO)_2)]^{3-}$ from 7.8 to 39.2 mM.

Results and Discussion

Synthesis of TETA-$(PO)_2$. Phosphinic acid undergoes Mannich reactions with primary or secondary amines in the presence of excess paraformaldehyde under strongly acidic conditions.[15] Recently, Varga reported the synthesis of bis-(aminomethyl)phosphinic acids via a Mannich reaction.[16] In this report, a new macrocyclic chelator TETA-$(PO)_2$, has been prepared by reacting phosphinic acid with 1 equiv of EDDA in the presence of excess paraformaldehyde in 6 N HCl at 105−110 °C. TETA-$(PO)_2$ was isolated as its hydrochloride salt. For successful cyclization, high dilution is preferred. TETA-$(PO)_2$ has been characterized by elemental analysis, NMR (^{1}H, ^{13}C, and ^{31}P) and mass spectroscopic methods. The EI-MS and NMR data are completely consistent with the proposed structure.



$H_6[TETA\text{-}(PO)_2]$

Acidity Constants. The acidity constants for $H_6[TETA\text{-}(PO)_2]$ determined potentiometrically are summarized in Table 1 together with the values for XT,[17] TETA,[9] and DOTA[9] for comparison. From the structure of $H_6[TETA\text{-}(PO)_2]$ (Chart 1), one can deduce that 10 protons in total may be ionizable from the fully protonated $H_{10}[TETA\text{-}(PO)_2]^{4+}$ species, i.e., four from the carboxylate groups, four from the tertiary ammonium nitrogen atoms, and two from the phosphinate groups. On the basis of previous experience and the known acidity constants of $H_4[TETA]$,[9] it is not surprising to observe that the carboxylate and phosphinate groups exhibit pK_a values lower than 2. Although a value of $pK_a = 1.9 \pm 0.1$ has been determined, all six deprotonation steps will have only a small effect on the metal complex stability constants. NMR titrations proved that there are no further deprotonation reactions, beyond these reported in Table 1, up to pH 14.

The last two deprotonation steps ($n = 1, 2$ in eqs 1 and 2) most probably occur at trans-nitrogen atoms, and the other

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Table 2. Stability Constants of the La^{3+}, Gd^{3+}, and Lu^{3+} Complexes with TETA-$(PO)_2$ As Measured by Potentiometric pH Titration ($I = 0.16$ M NaCl, 25 °C) Together with the Values of Some Structurally Similar Ligands

ligand	stability const	La^{3+}	Gd^{3+}	Lu^{3+}	ref
TETA-$(PO)_2$	$\log K^{M}_{MH_2L}$	6.03 ± 0.03	7.11 ± 0.09	7.52 ± 0.16	this work
	$\log K^{M}_{MHL}$	9.89 ± 0.16	10.62 ± 0.09	10.82 ± 0.20	
	$\log K^{M}_{ML}$	13.82 ± 0.15	12.74 ± 0.14	11.71 ± 0.20	
TETA	$\log K^{M}_{MHL}$	7.37 ± 0.01	8.01 ± 0.05		9
	$\log K^{M}_{ML}$	11.60 ± 0.01	13.77 ± 0.02		
TRITA	$\log K^{M}_{MHL}$	11.2 ± 0.1	10.6 ± 0.2		9
	$\log K^{M}_{ML}$	17.02 ± 0.03	19.17 ± 0.06		
XT	$\log K^{M}_{MHL}$	7.9 ± 0.3	9.22 ± 0.16		17
	$\log K^{M}_{ML}$	13.0 ± 0.3	15.73 ± 0.25		
TMDTA	$\log K^{M}_{ML}$	8.7	11.0		9

two deprotonation steps ($n = 3$, 4 in eqs 1 and 2) may occur at two carboxylates or ammonium nitrogen atoms.[10]

$$[H_n(\text{TETA-}(PO)_2)]^{2-n} \rightleftharpoons [H_{n-1}(\text{TETA-}(PO)_2)]^{2-n-1} + H^+ \quad (1)$$

$$K^{H}_{HnL} = [H^+][[H_{n-1}(\text{TETA-}(PO)_2)]^{2-n-1}]/[[H_n(\text{TETA-}(PO)_2)]^{2-n}] \quad (2)$$

The four pK_a values > 2 in Table 1 for each ligand can be divided into two groups, those < 5 and those > 6. For H_6-[TETA-$(PO)_2$], the two pairs of acidity constants, K^{H}_{H4L}, K^{H}_{H3L} and K^{H}_{H2L}, K^{H}_{HL} differ internally by a factor of about $10^{0.7}$, which agrees with the corresponding value obtained for H_4[TETA], wherein the first two acidity constants differ by $10^{0.94}$ and the second two differ by $10^{0.72}$. The value of $10^{0.7}$ is also in quite close agreement with the purely statistical estimation of $10^{0.6}$, implying that the two protons in the pair are in similar chemical environments and that the two deprotonation steps in the pair have little effect on one another. In H_4[DOTA], however, the two higher pK_a values differ by $10^{1.45}$, which may be caused by strong five-membered ring hydrogen bonding of one proton among the four nitrogens. This does not seem plausible with the two six-membered hydrogen bonding rings in $H[TETA]^{3-}$ or H_6-[TETA-$(PO)_2$]. Introducing two phosphinate groups into TETA also makes the two groups of acidity constants closer in value. The difference (ΔpK_a) between the average of the two higher pK_a values and the average of the two lower ones is 3.68 for TETA-$(PO)_2$ (($\Delta pK_a = 7.76 + 8.45)/2 - (4.08 + 4.78)/2 = 3.68$), 2.71 for XT ($\Delta pK_a = 9.27 - 6.56 = 2.71$), whereas the corresponding value is 6.78 for TETA ($\Delta pK_a = (10.10 + 10.82)/2 - (3.21 + 4.15)/2 = 6.78$). It is known that this ΔpK_a value reflects the strength of the intramolecular hydrogen bond among the nitrogen atoms;[12,18] hence, the result again implies that the phosphinate groups have a large effect on intramolecular hydrogen bond formation. If one supposes that a phosphinate oxygen and a nitrogen form an intramolecular hydrogen bond, this would make the first two deprotonation steps from the nitrogen atom more difficult; i.e., higher pK_a values would be expected. However, it is hard to explain why the last two protons are released from nitrogen atoms more easily in TETA-$(PO)_2$ than in TETA.

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Stability Constants of TETA-$(PO)_2$ with La^{3+}, Gd^{3+}, and Lu^{3+}. Using potentiometric pH titrations, three stability constants in the range $2 \leq pH \leq 11$ could be determined for each of the three metal ions studied. The results are summarized in Table 2; according to the stability constants, species distribution diagrams were calculated and are shown in Figure 1. For comparison, the corresponding stability constants for TETA, TRITA, XT, and TMDTA from the literature[9,17] are also listed in Table 2.

As shown in Figure 1, almost all of each metal ion is coordinated to the ligand at pH > 6. Some differences between the metal ion complexes exist; $La(L)^{3-}$predominates, whereas, for Gd(III) and Lu(III), protonated species are more important. The diagrams in Figure 1 also show that hydrolysis of each metal ion does not play a role when pH < 10, though the corresponding constants have been included in the fit.

Comparing the stability constants listed in Table 2, it can be seen that for the protonated complexes of TETA-$(PO)_2$, the stabilities of analogous complexes are $La^{3+} < Gd^{3+} < Lu^{3+}$; this order agrees with the stabilities of these metal ions in complexes of TETA, TRITA, and many other ligands.[9] The fully deprotonated complexes ML^{3-}, however, display the opposite order, $La^{3+} > Gd^{3+} > Lu^{3+}$. As was discussed previously,[18] this must be related to the structures of the complexes (vide infra).

Structures of the Lanthanide Complexes. The ^{1}H and ^{31}P NMR spectra of TETA-$(PO)_2$ itself are simple; the single signal at about 41 ppm in the ^{31}P NMR spectrum shows that the two phosphorus atoms are equivalent. The ^{1}H NMR spectrum consists of four resonances at 2.87, 2.57, 2.53, and 2.47 ppm; the two resonances at 2.57 and 2.53 ppm exhibit a 10 Hz H−P coupling constant.

The ^{31}P NMR spectrum of $[Lu(\text{TETA-}(PO)_2)]^{3-}$ shows two signals at 48 and 34 ppm; when the ligand concentration was in excess, an additional resonance was observed at 41 ppm. This is different from the spectrum of $[La(\text{TETA-}(PO)_2)]^{3-}$, in which there is only one resonance at 34 ppm when [ligand]:[metal ion] = 1:1. This result shows that the two phosphorus atoms in $[Lu(\text{TETA-}(PO)_2)]^{3-}$ are inequivalent, whereas in $[La(\text{TETA-}(PO)_2)]^{3-}$, they are equivalent.

The variable temperature ^{31}P NMR spectra for $[Lu(\text{TETA-}(PO)_2)]^{3-}$ from −35 °C up to 45 °C are shown in Figure 2. As the temperature is increased, the two resonances at 48

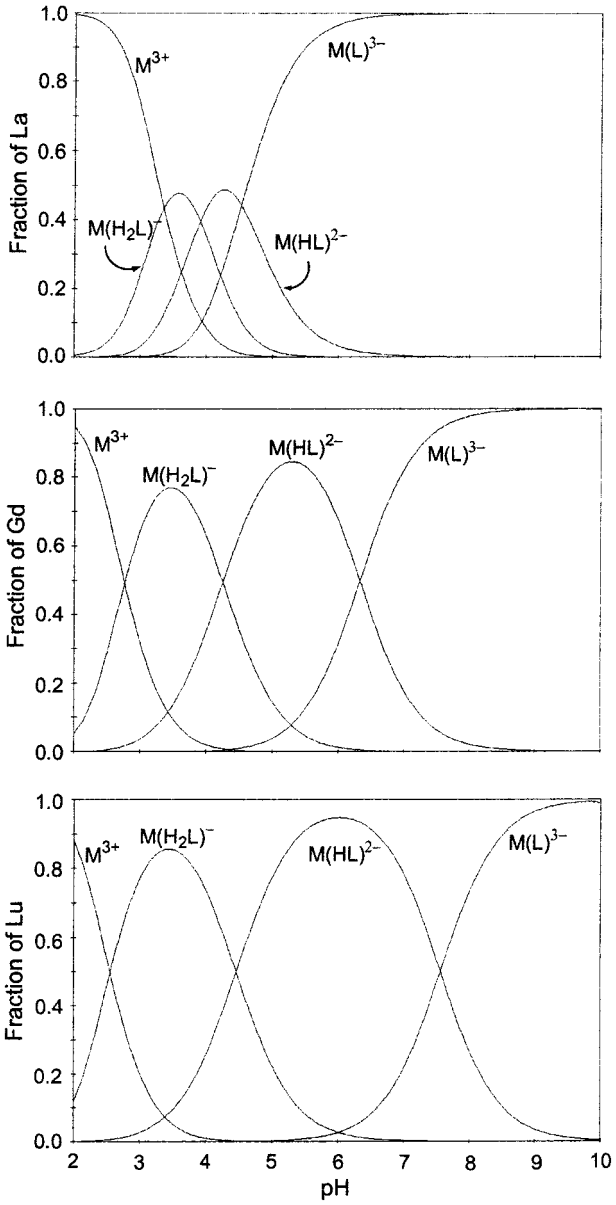

Figure 1. Species distribution diagram for the La^{III}–, Gd^{III}–, and Lu^{III}–TETA-$(PO)_2$ systems. The ligand concentration used in the calculation is 3.3×10^{-4} M, and the total metal ion concentration is 3×10^{-4} M.

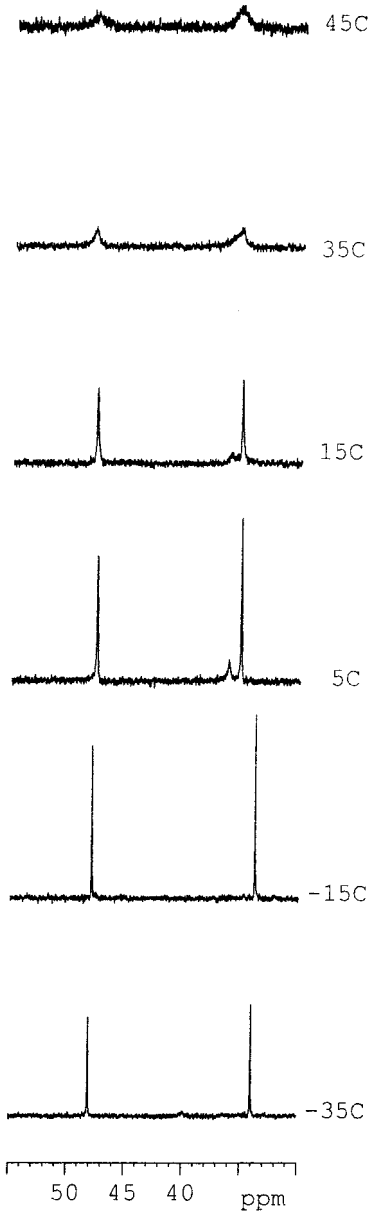

Figure 2. ^{31}P NMR spectra of $[Lu((TETA\text{-}(PO)_2)]^{3-}$ as a function of temperature from lowest to highest: spectra at $T \geq 5$ °C in D_2O; spectra at −15 and −35 °C in 1:9 D_2O:CD_3OD.

and 34 ppm broaden and begin to collapse into the baseline, indicating that a dynamic process is rendering equivalent the two phosphorus atoms in the macrocycle. This exchange process was further followed by 2D exchange spectroscopy (vide infra). For $[La(TETA\text{-}(PO)_2)]^{3-}$, signal broadening of the 34 ppm peak was not observable when the temperature was increased. The ^{31}P NMR results can be explained by one of the phosphinates coordinating to Lu(III) in $[Lu(TETA\text{-}(PO)_2)]^{3-}$, whereas in $[La(TETA\text{-}(PO)_2)]^{3-}$ both of the phosphinate groups are either coordinated or remain uncoordinated to the metal ion. The X-ray structure of a Lu^{3+} complex of $DOTA^{4-}$ showed that DOTA coordinates the metal ion as an octadentate ligand with four nitrogen and four oxygen donor atoms arranged in a square antiprismatic geometry (CSAP geometry) and a ninth axial coordination site occupied by a water molecule.[19] Crystals of the Lu(III), Eu(III), Gd(III), and Y(III) complexes of DOTA were isomorphous.[20] Many other complexes with similar ligands exhibit a comparable geometry, including a Tb(III) complex of TETA.[21] Unlike the related DOTA complexes, $[Tb(TETA)]^-$ did not have a water molecule in the inner coordination sphere.[21] As expected for square antiprismatic arrangements, the N_4 plane and the O_4 plane are parallel to each other and, interestingly, the metal ions typically do not occupy the center of the polyhedron but are shifted toward the O_4 plane.[21]

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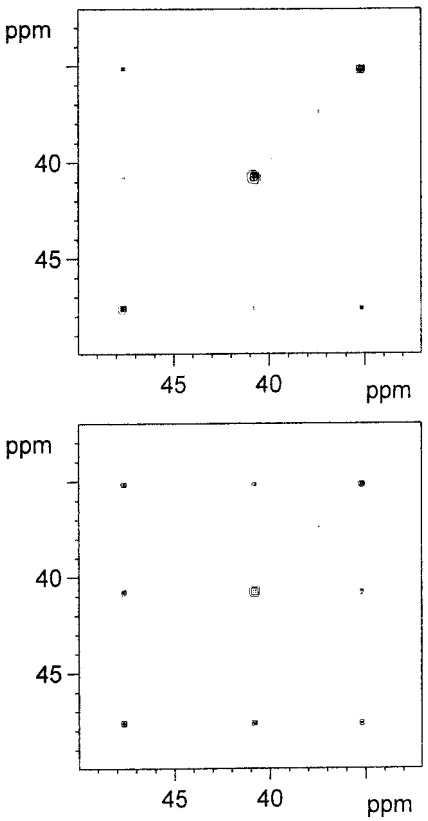

Figure 3. $^{31}P\{^1H\}$ EXSY NMR spectra for $[Lu(TETA\text{-}(PO)_2)]^{3-}$ recorded at 5 °C with mixing times of 100 (lower panel) and 25 ms (upper panel).

Considering these solid-state data, it is reasonable to suppose that the solution structure of the Ln(III) complex of TETA-$(PO)_2$ is similar to the solid-state structures of Ln^{III}−DOTA or −TETA complexes. This suggests that $Ln[TETA\text{-}(PO)_2]^{3-}$ might also be arranged in a CSAP geometry wherein the basal plane is occupied by four amine nitrogens and the capped plane is occupied by four carboxylate oxygens. In $Lu[TETA\text{-}(PO)_2]^{3-}$, it is possible that one of the phosphinate oxygens also coordinates Lu(III). Hence, two inequivalent phosphorus atoms would be observed in the ^{31}P NMR spectrum of $[Lu(TETA\text{-}(PO)_2)]^{3-}$. It is clear from the NMR data for the complex that the two phosphinates undergo exchange at a rate faster than the exchange between the coordinated and free ligand.

2D-EXSY NMR experiments conveniently map out exchanging sites and allow for the easy interpretation of exchange processes via intensity comparison. $^{31}P\{^1H\}$ EXSY NMR spectra for $[Lu(TETA\text{-}(PO)_2)]^{3-}$ recorded at 5 °C with mixing times of 100 ms and 25 ms are shown in Figure 3. The three resonances appearing along the diagonal at 48, 41, and 34 ppm correspond to uncoordinated phosphinate in the complex, free ligand phosphinate, and coordinated phosphinate in the complex, respectively, and these resonances exhibit chemical exchange. When the mixing time is 100 ms, the intensities of the cross-peaks between the free ligand and the complex are similar to the intensities of the cross-peaks between the two complex signals. When the mixing time is 25 ms, however, the intensity of the cross-peaks between the two complex signals is stronger. When the mixing time was further reduced to 10 ms (data not shown), the cross-peaks between the two complex signals remained strong but the cross-peaks between the complex and free ligand signals disappeared completely. When the temperature was increased to 298 K (mixing time = 100 ms; data not shown), the cross-peaks between the complex and the free ligand were evident, whereas the cross-peaks between complex signals were unobserved. These results conclusively prove that the intramolecular exchange (that between coordinated and uncoordinated phosphinate in $[Lu(TETA\text{-}(PO)_2)]^{3-}$) is more facile than the intermolecular coordinated/free ligand exchange. These results of the $^{31}P\{^1H\}$ EXSY NMR spectra of $[Lu(TETA\text{-}(PO)_2)]^{3-}$ support the proposed single bound phosphinate solution structure for the complex.

In $[La(TETA\text{-}(PO)_2)]^{3-}$, ^{31}P NMR spectra showed that when coordinated with La^{3+}, the only ligand signal had an upfield shift (from 41 ppm for the free ligand to 34 ppm for the La^{3+} complex). Coordination to a metal ion removes electron density from the phosphinate group, leading to a downfield shift of the NMR resonance. This analysis agrees with the result obtained previously for $[Ln(XT)]^{2-}$ complexes,[17] where coordination of the phosphinate group shifted the resonance of the ^{31}P NMR signal downfield (from 17 to 44 ppm). For the corresponding Co^{3+} complex, in which the phosphinate group was shown by an X-ray structure not to coordinate the metal ion, the ^{31}P NMR signal showed an upfield shift (from 17 to 12 ppm). By analogy therefore, it is reasonable to conclude that both of the phosphinate groups remain uncoordinated in $[La(TETA\text{-}(PO)_2)]^{3-}$.

Because increased denticity of the same ligand should increase the stability of its complexes,[22] according to the proposed structures for the La^{3+} and Lu^{3+} complexes, one would expect $[Lu(TETA\text{-}(PO)_2)]^{3-}$ to have a higher stability. It is clear that this is contrary to the results obtained potentiometrically (Table 2). Hence, there must be other factors that affect the stability. As was noted, according to the crystal structure of the Ln^{III}−DOTA or −TETA complexes,[19−21] the N_4 and the O_4 planes might be parallel to one another with the metal ion shifted toward the O_4 plane away from the center of the polyhedron. A 3D molecular model was constructed; it displayed significant strain in the approach of the phosphinate oxygen atom to the top of the O_4 plane. It is highly unlikely that both phosphinates could coordinate one metal ion. Furthermore, phosphinate group coordination might force the metal ion closer to the N_4 plane, weakening the strength of, or indeed rupturing, the metal carboxylate bonds. These bonding arguments could account for the lower stability of $[Lu(TETA\text{-}(PO)_2)]^{3-}$ compared with that of $[La(TETA\text{-}(PO)_2)]^{3-}$.

Comparing the Stability of $[Ln(TETA\text{-}(PO)_2)]^{3-}$ with Other Similar Complexes. In Table 2 are listed the stability constants of the complexes formed between La^{3+}, Gd^{3+}, or Lu^{3+} and several structurally similar ligands. Replacement of a five-membered chelate ring with a six-membered analogue reduced the lanthanide complex stability dramati-

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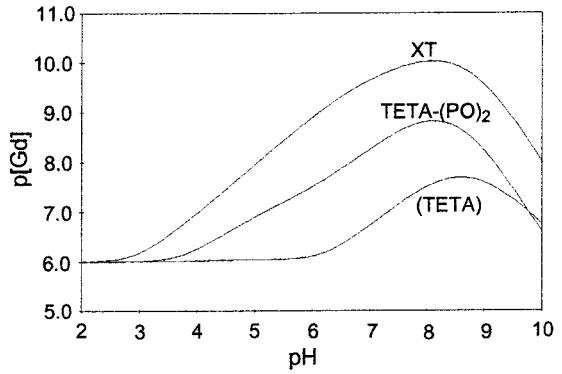

Figure 4. Plot of pM vs pH for the Gd^{3+}−TETA, −XT, and −TETA-$(PO)_2$ systems (L:Gd^{3+} = 1.1:1, pM = − log([M] + [M$(OH)_y$]), y = 1, 2, 3, 4; see text).

cally. Incorporating a phosphinate group in the ligand system seems to increase the stability of the resulting complexes. This is true when comparing the $[Ln(TMDTA)]^-$ and $[Ln(XT)]^{2-}$ systems, as well as the protonated complexes [Ln(HTETA)] and $[Ln(HTETA\text{-}(PO)_2)]^{2-}$; however, this latter trend no longer holds for $[Gd(TETA)]^-$ and $[Gd(TETA\text{-}(PO)_2)]^{3-}$. When a phosphinate group is present, the negative charge on the ligand is increased such that the stability of its complex with a positive metal ion is also increased, even when the phosphinate group does not coordinate. As discussed above, however, $[Gd(TETA\text{-}(PO)_2)]^{3-}$ does not follow this trend and this may be due to phosphinate group coordination with the resultant weakening or rupturing effect on the metal−carboxylate bonds. The stability constant of $[Gd(TETA\text{-}(PO)_2)]^{3-}$ is lower than that of $[Gd(TETA)]^-$, which may also be because the last two acidity constants of $[H_6(TETA\text{-}(PO)_2)]$ are smaller than those of $[H_4(TETA)]$.

To compare stabilities among ligands, a pM (pM = − log([M] + [M$(OH)_y$]), where y = 1, 2, 3, 4) vs pH distribution diagram was calculated; it is shown in Figure 4. Although the stability constant for $[Gd(TETA\text{-}(PO)_2)]^{3-}$ is smaller than the corresponding constant for $[Gd(TETA)]^-$, in the physiological pH range the Gd−(TETA-$(PO)_2$) system has higher pM values. This is because the protonated species $[Gd(HTETA\text{-}(PO)_2)]^{2-}$ is much more stable than its TETA analogue (Table 2). The pM values of the Gd−(TETA-$(PO)_2$) system, however, are substantially lower than for the Gd−(XT) system over the whole pH range, indicating that the macrocycle forms a less stable complex than its "open" analogue.

Selectivity of TETA-$(PO)_2$ for Gd^{3+} vs Physiologically Important Metal Ions: Ca^{2+}, Zn^{2+}, and Cu^{2+}. As mentioned in the Introduction, the relative affinity of potential ligands for Gd^{3+} and for biologically relevant cations such as Ca^{2+}, Zn^{2+}, and Cu^{2+} offers insight into the acute toxicity of the gadolinium complex.[8] A lower affinity of the ligand for Gd(III) means that the gadolinium complex could exhibit higher toxicity; hence, the stability constants of TETA-$(PO)_2$ with those three divalent metal ions have also been measured. These results are summarized in Table 3. The selectivity factor is defined in eq 3.[8]

Table 3. Stability Constants of the Ca^{2+}, Cu^{2+}, and Zn^{2+} Complexes with TETA-$(PO)_2$ As Measured by Potentiometric pH Titration (I = 0.16 M NaCl, 25 °C)

stability const	Ca^{2+}	Cu^{2+}	Zn^{2+}
log $K^M_{MH_2L}$	0.4 ± 0.4	8.90 ± 0.16	5.15 ± 0.15
log K^M_{MHL}	4.83 ± 0.03	12.18 ± 0.10	8.54 ± 0.14
log K^M_{ML}	6.24 ± 0.03	12.44 ± 0.10	8.63 ± 0.15

$$K_{sel} = (K_{GdL} + K_{GdH}[H]/K^H_{HL} + K_{GdH_2L}[H]^2/K^H_{H_2L}K^H_{HL} + ...) \times (\alpha_H^{-1} + \alpha_{CaL}^{-1} + \alpha_{CuL}^{-1} + \alpha_{ZnL}^{-1})^{-1} \quad (3)$$

The values of α are defined in eq 4−7.

$$\alpha_H^{-1} = 1 + K^H_{HL}[H] + K^H_{H_2L}K^H_{HL}[H]^2 + ... + K^H_{HnL}...K^H_{H_2L}K^H_{HL}[H]^n \quad (4)$$

$$\alpha_{CaL}^{-1} = K_{CaL}[Ca^{2+}] + K_{CaHL}[Ca^{2+}][H]/K^H_{HL} + K_{CaH_2L}[Ca^{2+}][H]^2/K^H_{H_2L}K^H_{HL} + ... \quad (5)$$

$$\alpha_{CuL}^{-1} = K_{CuL}[Cu^{2+}] + K_{CuHL}[Cu^{2+}][H]/K^H_{HL} + K_{CuH_2L}[Cu^{2+}][H]^2/K^H_{H_2L}K^H_{HL} + ... \quad (6)$$

$$\alpha_{ZnL}^{-1} = K_{ZnL}[Zn^{2+}] + K_{ZnHL}[Zn^{2+}][H]/K^H_{HL} + K_{ZnH_2L}[Zn^{2+}][H]^2/K^H_{H_2L}K^H_{HL} + ... \quad (7)$$

Because protonated species are important in the physiological pH region, they must be included in calculating the selectivity factor. If the in vivo concentrations of the components used were $[Ca^{2+}]$ = 2.5 mM, $[Zn^{2+}]$ = 50 μM, and $[Cu^{2+}]$ = 1 μM,[8] the selectivity factor log K_{sel} calculated according to the equations above for Gd−TETA-$(PO)_2$ is 5.47. Compared to values for other MRI agent systems (Gd−DTPA, 7.04; Gd−DTPA−BMA, 9.04;[8] Gd−DOTA, 8.3 [23]) it is clear that the selectivity is less than that in the commercial systems.

Hydration Number of $[Dy(TETA\text{-}(PO)_2)]^{3-}$. Figure 5 shows a plot of the dysprosium-induced ^{17}O NMR shift (Dy.I.S.) of D_2O versus concentration for Dy^{3+}(aq) and $[Dy(TETA\text{-}(PO)_2)]^{3-}$. For Dy^{3+}(aq), the slope of the straight line is −373 ppm/M (r^2 = 0.9997). This value is somewhat higher than, but in the same range as, values measured by other workers in H_2O (−357 to −360 ppm/M).[24−26] If the hydration number of Dy(III) is taken to be 8,[27] then a slope of −373/8 = −46.5 would be indicative of one bound water per Dy^{3+} ion. It is clear, however, that the slope for $[Dy(TETA\text{-}(PO)_2)]^{3-}$ is zero (0.25 ppm/M) in the error limit, indicating that no water is directly bound to Dy(III). As was mentioned initially, most lanthanide complexes with phos-

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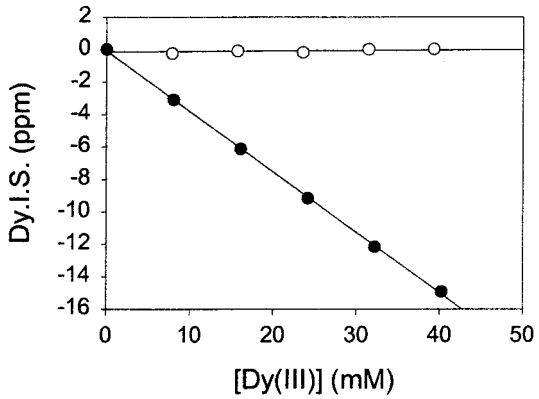

Figure 5. Plot of Dy-induced ^{17}O NMR chemical shift (Dy.I.S.) of $D_2{}^{17}O$ vs [Dy(III)] (●) or [Dy(TETA-$(PO)_2)^{3-}$] (○) at 25 °C, pH 9.

phonate-containing ligands have low hydration numbers q (e.g. DOTP, $q = 0.4 \pm 0.5$;[28] DOTMP, $q = 0.01$;[29] DoTBzP, $q = 0$ [29]).

Summary

Four acidity constants were determined for the TETA-$(PO)_2$ system in aqueous solution in the range 2.5 < pH < 14. The corresponding four protons are most probably associated with two trans-nitrogen atoms and two carboxylates, and the four pK_a values measured can be divided into two groups. In each group the initial deprotonation has little effect on the second. Variable temperature ^{31}P NMR and $^{31}P\{^1H\}$ EXSY NMR spectra suggest that for [Lu(TETA-$(PO)_2)]^{3-}$ one of the phosphinates is coordinated to the metal ion and undergoes exchange with the free phosphinate, whereas, in [La(TETA-$(PO)_2)]^{3-}$, both phosphinates remain uncoordinated. The coordination of a phosphinate to Lu^{3+} may explain the observed lower stability of this complex when compared to the corresponding La^{3+} and Gd^{3+} complexes in aqueous solution. Introducing two phosphinate groups into TETA makes [Gd(TETA-$(PO)_2)]^{3-}$ more stable than $[Gd(TETA)]^-$ but less stable than $[Gd(XT)]^{2-}$. The selectivity factor of the ligand for Gd^{3+} vs Ca^{2+}, Zn^{2+}, Cu^{2+} is lower than the corresponding values for DTPA, DTPA−BMA, and DOTA. The hydration number for [Dy(TETA-$(PO)_2)]^{3-}$ is zero as measured by ^{17}O NMR, and this result agrees with many other ligands containing phosphinates.

Acknowledgment. The authors acknowledge Dr. Nick Burlinson for assistance with the EXSY experiments. This work was supported by the Natural Sciences and Engineering Research Council of Canada and by Du Pont Pharmaceuticals Inc. T.S. acknowledges an NSERC postgraduate scholarship.

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