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Equilibrium and Formation/Dissociation Kinetics of Some Ln^{III}PCTA Complexes

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The protonation constants (K_i^H) of 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid (PCTA) and stability constants of complexes formed between this pyridine-containing macrocycle and several different metal ions have been determined in 1.0 M KCl at 25 °C and compared to previous literature values. The first protonation constant was found to be 0.5–0.6 log units higher than the value reported previously, and a total of five protonation steps were detected (log $K_i^H = 11.36$, 7.35, 3.83, 2.12, and 1.29). The stability constants of complexes formed between PCTA and Mg^{2+}, Ca^{2+}, Cu^{2+}, and Zn^{2+} were also somewhat higher than those previously reported, but this difference could be largely attributed to the higher first protonation constant of the ligand. Stability constants of complexes formed between PCTA and the Ln^{3+} series of ions and Y^{3+} were determined by using an "out-of-cell" potentiometric method. These values ranged from log $K = 18.15$ for Ce(PCTA) to log $K = 20.63$ for Yb(PCTA), increasing along the Ln series in proportion to decreasing Ln^{3+} cation size. The rates of complex formation for Ce(PCTA), Eu(PCTA), Y(PCTA), and Yb(PCTA) were followed by conventional UV–vis spectroscopy in the pH range 3.5–4.4. First-order rate constants (saturation kinetics) obtained for different ligand-to-metal ion ratios were consistent with the rapid formation of a diprotonated intermediate, $Ln(H_2PCTA)^{2+}$. The stabilities of the intermediates as determined from the kinetic data were 2.81, 3.12, 2.97, and 2.69 log K units for Ce(H_2PCTA), Eu(H_2PCTA), Y(H_2PCTA), and Yb(H_2PCTA), respectively. Rearrangement of these intermediates to the fully chelated complexes was the rate-determining step, and the rate constant (k_r) for this process was found to be inversely proportional to the proton concentration. The formation rates (k_{OH}) increased with a decrease in the lanthanide ion size [9.68×10^7, 1.74×10^8, 1.13×10^8, and 1.11×10^9 M^{-1} s^{-1} for Ce(PCTA), Eu(PCTA), Y(PCTA), and Yb(PCTA), respectively]. These data indicate that the Ln(PCTA) complexes exhibit the fastest formation rates among all lanthanide macrocyclic ligand complexes studied to date. The acid-catalyzed dissociation rates (k_1) varied with the cation from 9.61×10^{-4}, 5.08×10^{-4}, 1.07×10^{-3}, and 2.80×10^{-4} M^{-1} s^{-1} for Ce(PCTA), Eu(PCTA), Y(PCTA), and Yb(PCTA), respectively.

Introduction

Recent biomedical applications of lanthanide complexes have catalyzed a growing interest in the synthesis and evaluation of new functionalized open-chain and macrocyclic ligands that form highly stable and kinetically inert complexes with various lanthanide ions (Ln^{3+}). The interest in paramagnetic and radioactive metal ions is largely driven by advances in magnetic resonance imaging (MRI) contrast agents (mostly Gd^{3+} complexes), nuclear medicine diagnostic agents [isotopes used in positron emission tomography (PET) and γ emitters such as $^{67}Ga^{3+}$, $^{111}In^{3+}$, or $^{169}Yb^{3+}$], and therapeutic radiopharmaceuticals (β emitters such as $^{90}Y^{3+}$, $^{177}Lu^{3+}$, $^{153}Sm^{3+}$, $^{166}Ho^{3+}$ or $^{149}Pm^{3+}$).[1] A total of seven Gd^{3+}

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complexes [four based upon diethylenetriaminepentaacetic acid (DTPA) and three based upon 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetate (DOTA)] have been used successfully as clinical MRI contrast agents for many years.[2,3] Macrocyclic ligands such as DOTA have some advantages over acyclic ligands such as DTPA for certain applications because the rigidity of the macrocyclic ring adds to the thermodynamic stability and kinetic inertness of the resulting complexes. The linear and macrocyclic-based ligands exhibit markedly different formation kinetics as well. Macrocyclic ligands tend to form metal ion complexes much more slowly,[3] a significant disadvantage when preparing therapeutic metal-based drugs involving short-lived radionuclides.

The development of novel $^{90}Y^{3+}$-labeled monoclonal antibodies and other therapeutic agents has stimulated interest in the discovery of new Y complexes that have more favorable formation kinetics and yet remain inert toward dissociation. $^{90}Y^{3+}$ is an attractive isotope for radio-immunotherapy because of its favorable emission energy and long half-life ($t_{1/2} = 64$ h). As the ionic radii, oxidation state, and coordination chemistry of Y^{3+} and the Ln^{3+} ions are similar, the same types of ligands can be used for these ions.[1] It is important to emphasize that the kinetic inertness of the complexes plays a crucial role in determining the amount of radioactive ion released in vivo. Even though the thermodynamic stabilities of DTPA-based ligands with $^{90}Y^{3+}$ are satisfactorily high, they are also more kinetically labile than required for some applications. Any $^{90}Y^{3+}$ or radiolanthanide ions released in vivo tend to accumulate in bone and can result in high radiation doses to bone marrow.[4] Thus, the general requirements for Ln^{3+} or Y^{3+} complexes for therapeutic applications include a satisfactory thermodynamic stability and kinetic inertness and rapid complex formation rates for those radioisotopes having short lifetimes.

A large number of 9−14-membered tri- or tetraazamacrocycles with three to four carboxylate-containing side arms have been examined. The formation rates of the Ln^{3+} complexes are typically slow, and on the basis of kinetic data (saturation kinetics), the existence of stable di- or monoprotonated complexes is often observed.[5−14] Protonated intermediates of $Ce(H_2DOTA)^+$ and $Eu(H_2DOTA)^+$ have been directly detected by UV−vis,[7−9] luminescence,[12] and NMR spectroscopy.[14] The synthesis of the pyridine-containing 12-membered tetraazatriacetate ligand 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid (H_3PCTA) has been described previously.[15−18] This ligand has some attractive features for use in biomedicine including the presence of an aromatic chromophore that acts as an antenna for Eu^{3+} and Tb^{3+} luminescence and a high water relaxivity ($q = 2$ complex) for the Gd^{3+} complex.[16,19−21] Complexes of $^{67}Ga^{3+}$, $^{68}Ga^{3+}$, or $^{111}In^{3+}$ ions with PCTA have been suggested as radiopharmaceuticals in radio-immunoscintigraphy and in PET[22] based on the calculated p*M* and low-osmolality values. These data compare favorably to the p*M* values for DOTA complexes at physiological pH.[23] The stabilities of PCTA complexes with Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Eu^{3+}, and Gd^{3+} have been reported,[16,19,23−25] but neither detailed thermodynamics nor formation/dissociation kinetics of the Ln(PCTA) complexes have been reported in detail.

The objective of the study was to determine the stability constants for the series of Ln(PCTA) complexes and compare those to the stability of the complexes formed with structurally similar macrocyclic ligands, NOTA (1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetate), DO3A (1,4,7,10-tetraazacyclododecane-1,4,7-triacetate), DOTA, TRITA (1,4,7,10-tetraazacyclotridecane-*N*,*N*′,*N*″*N*‴-tetracetate), and TETA (1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″*N*‴-tetracetate). The kinetics of Ln(PCTA) complex formation and acid-catalyzed dissociation were also evaluated and compared to other systems. The ligands involved in this work are presented in Chart 1.

Experimental Section

General Procedures. The ligand PCTA was prepared as previously described.[16]

Thermodynamic Stability Constants. Stock solutions of $MgCl_2$, $CaCl_2$, $ZnCl_2$, $CuCl_2$, YCl_3, and $LnCl_3$ were prepared from analytical-grade salts (Aldrich and Sigma, 99.9%). The concentrations of the stock solutions were determined by complexometric

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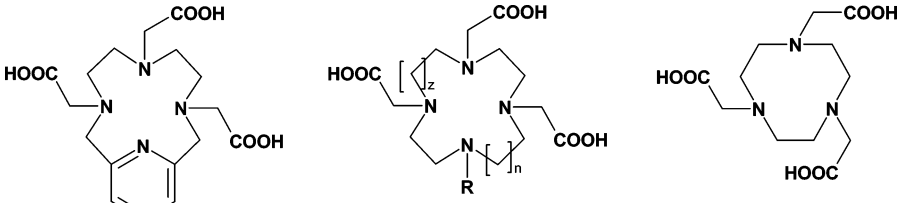
Chart 1. Structures of the Ligand Studied and Ligands Used for Comparison in This Work



n	z	R	
1	1	H	DO3A
1	1	CH_2—COOH	DOTA
2	1	CH_2—COOH	TRITA
2	2	CH_2—COOH	TETA

titration using a standardized Na_2H_2EDTA solution in the presence of eriochromeblack T ($MgCl_2$ and $ZnCl_2$), calconcarboxylic acid ($CaCl_2$), murexide ($CuCl_2$), or xylenol orange (YCl_3 and $LnCl_3$) as an indicator.[26] A stock solution of the ligand was prepared, and the ligand concentration was determined by pH potentiometry on the basis of the titration curves obtained in the absence and presence of excess $CaCl_2$. The protonation constants of the ligand were calculated from the data obtained by titrating 2 and 5 mM samples (374 data points) with a standardized KOH solution (0.2 M) in the absence of Ca^{2+} in the pH range of 1.7−12.2. The protonation constants of the ligand (log K_i^H) are defined as follows:

$$K_i^H = \frac{[H_iL]}{[H_{i-1}L][H^+]} \quad (1)$$

where $i = 1, 2, ..., 5$ and $[H_{i-1}L]$ and $[H^+]$ are the equilibrium concentrations of the ligand ($i = 1$), protonated forms of the ligand ($i = 2, ..., 5$), and hydrogen ions, respectively. The hydrogen ion concentrations were calculated from the measured pH values as described in the literature.[27] Briefly, a 0.01 M HCl solution (in 1.0 M KCl) was titrated with a standardized KOH solution, and the differences between the measured and calculated pH values were used to correct the measured pH values.[27] The ion product of water was also established in these experiments ($pK_w = 13.845$) and used for calculations. The ionic strength of all titrated solutions was maintained constant using 1.0 M KCl (Mallinckrodt). All equilibrium measurements (direct titrations) were carried out in 10.00-mL sample volumes with magnetic stirring under an Ar atmosphere at 25 °C using a 665 Metrohm Dosimat autoburet. The pH was measured with a Ross semi-microcombination electrode (Orion) combined with a Thermo Orion EA 940 ion analyzer. The electrode was calibrated by potassium phthalate (pH = 4.005) and sodium tetraborate (pH = 9.180) (Alfa Aesar). The titrant (KOH) was prepared using degassed, twice distilled water, and its concentration was standardized by titration with potassium phthalate. The solution was kept under an Ar atmosphere to prevent entry of CO_2.

The potentiometric measurements for systems that reach equilibrium rapidly (M^{2+} metal ions) were carried out by use of a computer-controlled automated titration system. Stability constants for doubly charged metal ions were determined from titration data preformed at 2:1, 1:1, and 1:2 metal-to-ligand ratios, and the number of points fitted varied between 176 and 321 data pairs. Owing to the slow formation reactions of Y(PCTA) and Ln(PCTA) complexes, an "out-of-cell" technique was applied for the stability determinations. Sixteen 1.5-mL samples containing a known amount of ligand (with known H^+ concentration) and Y^{3+} or Ln^{3+} were prepared, and the pH was adjusted to a range where complexation could be expected to take place based upon literature stability data for various Gd^{3+} and Eu^{3+} complexes.[16,19,25] The samples were sealed under a blanket of Ar and kept in an incubator at 45 °C for 1 week (this time period was established by preliminary spectrophotometric studies on duplicates of the most acidic and most basic samples kept together with the rest of the "out-of-cell" samples). After this time period, the samples were removed from the incubator and reequilibrated at room temperature (for 6−7 days). The samples were then opened, and the equilibrium pH was measured. The stability of the hydroxo complexes was determined in a separate titration after the complexes were fully formed. In these experiments, complex solutions with a 1:1 metal-to-ligand ratio were prepared at around pH = 6. At this pH, the full complexation takes only a few seconds. After pH stabilization, a titration was performed with standardized KOH. The equilibrium of the hydroxo complex formation was rapid, and the points were recorded with 1-min intervals. A total of 84−120 data points were recorded in the range of about pH = 6−12. The software *PSEQUAD* was used to process the titration data (calculation of the protonation and stability constants).[28] The reliability of the protonation and stability constants are characterized by the calculated standard deviation values shown in parentheses and the fitting of parameter values (ΔV, which is the difference between the experimental and calculated titration curves expressed in milliliters of the titrant).

Formation Kinetics. The formation rates of Ce(PCTA), Eu(PCTA), Y(PCTA), and Lu(PCTA) were studied at 25 °C and 1.0 M KCl ionic strength by direct spectrophotometry on a Cary 300Bio UV−vis spectrophotometer using thermostated cell holders and semi-micro quartz cells (Starna, optical path length 1 cm). Typically, the concentration of the ligand was 0.2 mM while the concentration of the metal ion was varied between 0.6 and 4 mM (the reaction is first-order even at comparable ligand and metal ion concentrations).

Over the range of pH = 3.5−4.4, complex formation is slow enough to follow by conventional spectrophotometry by following changes in the $\pi \rightarrow \pi^*$ absorption band of the ligand at $\lambda_{max} = 261$ nm ($\epsilon_{max} = 3.85 \times 10^3$ dm^3 mol^{-1} cm^{-1} at pH = 4.07). Upon coordination, this band undergoes a red shift ($\lambda_{max} = 269$ nm with $\epsilon_{max} = 4.91 \times 10^3$ dm^3 mol^{-1} cm^{-1} for the Ce^{3+} complex at pH = 4.07) as a result of metal ion coordination, and the kinetics of complex formation were followed by measuring changes in absorbance at 278 nm (where the absorbance of the metal ions is weak (Ce^{3+} and Eu^{3+}) or zero (Y^{3+} and Yb^{3+}). Formation of Ce-

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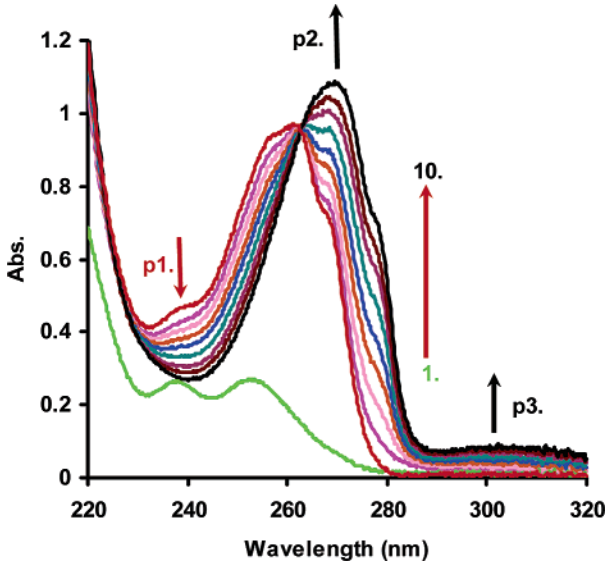

Figure 1. Absorbance changes in the UV region recorded for the formation of Ce(PCTA) after mixing equimolar amounts of Ce^{3+} and PCTA at pH = 4.07 [$C_{Ce(PCTA)}$ = 0.2 mM, 1.0 M KCl, 0.05 M DMP]. Each curve corresponds to (1) Ce^{3+} alone in a DMP buffer, (2) immediately after mixing Ce^{3+} with PCTA ("0" min), (3) after 1 min, (4) after 3 min, (5) after 6 min, (6) after 10 min, (7) after 19 min, (8) after 38 min, (9) after 70 min, and (10) at equilibrium.

(PCTA) could be studied in the UV range 275−320 nm, where the absorbances of the ligand and metal ion are negligible (Figure 1, peaks p2 and p3). The peak at λ_{max} = 269 nm is due to the absorbance of the pyridine unit in the complex, while complexed Ce^{3+} has λ_{max} = 304 nm (Figure 1, p3). Because the molar absorption coefficients (ϵ_{max}) for the $\pi \rightarrow \pi^*$ transitions are much higher than those for the 4f−5d transitions, complex formation was typically followed by changes in absorbance at 278 nm. An experiment in which the reaction was monitored at the absorption maximum (λ_{max} = 304 nm) in a more concentrated solution (C_{PCTA} = 1.0 mM) gave a formation rate identical with that of measurements at λ_{max} = 278 nm. All formation kinetic studies were carried out in the noncoordinating buffer *N*,*N*′-dimethylpiperazine (DMP; 0.05 M) to maintain a constant pH. The pH of each sample was remeasured after full complex formation (usually on the next day), and any samples with pH changes greater than 0.025 pH units were excluded from the calculations.

Equation 2 was used to calculate the first-order rate constants (k_{obs} for formation and k_d for dissociation), where A_0, A_e, and A_t are the absorbance values measured at the start of the reaction (t = 0), at equilibrium, and at time t, respectively. The kinetic curves had been acquired usually until equilibrium was reached, and to obtain the rate constants, the complete kinetic curves were fitted. However, the dissociaton reactions in $C \leq 0.5$ M of $HClO_4$ were followed only until 85−90% conversion. The data were fitted to eq 2 with the software *Scientist* (Micromath) using a standard least-squares procedure. The relative error for the fitting of the absorbance

$$A_t = A_e + (A_0 - A_e)e^{-k_{obs}t} \quad (2)$$

vs time curves in all cases was lower than 1%, and the calculated first-order rate constant k_{obs} and k_p values were reproduced within a 5% error as determined in five identical experiments.

Kinetics of Dissociation. Acid-catalyzed dissociation kinetics of Ce(PCTA), Eu(PCTA), Y(PCTA), and Lu(PCTA) were studied in 0.05−3.12 M $HClO_4$ [$NaClO_4$ was added to maintain the ionic strength of 3.12 M $(H^+ + Na^+)ClO_4^-$], and usually 17−24 data points were obtained at 25 °C. The $HClO_4$ stock solution (approximately 4.0 M) was standardized by acid−base titration against a standard NaOH solution in the presence of phenolphthalein as an indicator. The $NaClO_4$ solution was prepared by weighing out an appropriate quantity of $NaClO_4$. The reactions were followed in 0.25 mM complex solutions by direct spectrophotometry at 278 nm, and the complex concentrations were adjusted so that changes of ∼0.9 absorbance units were detected throughout the dissociation reaction.

Results and Discussion

Protonation and Stability Constant Determinations. The ligand PCTA has seven protonation sites, but only four protonation constants have been detected by using either ^1H NMR spectroscopy or pH potentiometry.[16,23−25] Delgado and co-workers originally ascribed the first protonation step to occur at the pyridine N,[23] yet Aime et al. later showed by ^1H NMR that the most basic site of the ligand is the N atom opposite to the pyridine ring.[16] The addition of a second proton results in a rearrangement of the ring protons such that the two protons shift to the two tertiary N atoms positioned trans to each other in the macrocyclic ring and cis to the pyridine N. In earlier studies, the protonation constants were determined using relatively low ligand concentrations (1.67 and 1 mM, respectively) in 0.1 M Me_4NNO_3 or KCl.[23−25] At such low concentrations, only a small portion of the added base is neutralized by the ligand protons in the range of pH = 11−12, and this results in a significant degree of uncertainty in the evaluation of the protonation constants in this range.[29] Here, we determined the protonation constants from titration data generated using two high ligand concentrations (2 and 5 times higher than those reported earlier[23−25]), and both titration curves were fitted simultaneously. The ion product of water, as determined by allowing this parameter to vary in the fitting procedure (pK_w = 13.85 ± 0.01) and as determined by a separate titration (pK_w = 13.845) in the range of pH = 10.92−12.21, was in close agreement. A fifth protonation constant was also included in the fitting procedure because the titration was initiated at a starting pH of 1.7. As the stability constants of the Ln(PCTA) and Y(PCTA) complexes were determined in acidic samples (the final equilibrium pH of the samples was in the range of pH = 1.75−2.50), the value of log K_5^H will impact this calculation. The protonation constants determined in this work are compared with previously reported literature values for PCTA and other related ligands in Table 1.

The stability constants of Mg^{2+}, Ca^{2+}, Zn^{2+}, and Cu^{2+} ions with $PCTA^{3-}$ were reported previously by Delgado et al.[23] However, because there was a significant difference between the total ligand basicity of the ligand determined here in comparison to the values reported by Delgado, the stabilities of these same complexes were redetermined in 1.0 M KCl. The stability constants of PCTA, NOTA, DO3A, and DOTA with Mg^{2+}, Ca^{2+}, Zn^{2+}, and Cu^{2+} are summarized in Table 2. The values obtained in this study are somewhat higher

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Table 1. Comparison of the Protonation Constants of **PCTA**, NOTA, DO3A, and DOTA (t = 25 °C)

ligand	I	log K_1	log K_2	log K_3	log K_4	log K_5	$\sum$log K_i
NOTA[a]	0.1 M KCl	11.96	5.65	3.17			20.78
	1.0 M $NaClO_4$[b]	10.77	6.03	3.16	1.96		21.92
DO3A[c]	0.1 M Et_4NCl	11.59	9.24	4.43	3.48		28.74
PCTA[d]	0.1 M Me_4NNO_3	10.90	7.11	3.88	2.27		24.16
	0.1 M KCl[e]	10.73	7.52	4.2	2.4		24.85
PCTA[f]	**1.0 M KCl**	**11.36 (0.01)**	**7.35 (0.01)**	**3.83 (0.01)**	**2.12 (0.01)**	**1.29 (0.03)**	**25.96**
DOTA[g]	0.1 M KNO_3	12.09	9.76	4.56	4.09		30.45
	0.1 M Me_4NCl[h]	12.60	9.70	4.50	4.14	2.32	33.26

[a] Reference 30. [b] Reference 31. [c] Reference 10. [d] Reference 23. [e] Reference 25. [f] $\Delta V = 1.87 \times 10^{-3}$ for 374 data pairs fitted. [g] Reference 32. [h] Reference 9.

Table 2. Stability Constants of the Complexes Formed with Some Double-Charged Cations Mg^{2+}, Ca^{2+} Cu^{2+}, and Zn^{2+} and Ligands NOTA, **PCTA**, and DOTA (I = 1.0 M KCl, 25 °C)

M^{2+}	eq quotient	NOTA[a]	PCTA[c]	**PCTA**[d]	DOTA[e]
Mg^{2+}	[ML]/[M][L]	9.69	11.82	**12.35 (0.01)**	11.92
	[MHL]/[ML][H]	4.6	3.70	**3.82 (0.02)**	4.09
Ca^{2+}	[ML]/[M][L]	8.92	12.38	**12.72 (0.01)**	17.23
	[MHL]/[ML][H]	5.06	3.66	**3.79 (0.05)**	3.54
Cu^{2+}	[ML]/[M][L]	21.63	17.79	**18.79 (0.04)**	22.25
	[MHL]/[ML][H]	2.77	4.03	**3.58 (0.04)**	3.78
	[ML]/[ML(OH)][H]		10.3	**11.28 (0.05)**	
Zn^{2+}	[ML]/[M][L]	18.3[b]	18.22	**20.48 (0.06)**	21.10[f]
	[MHL]/[ML][H]		3.64	**3.10 (0.06)**	4.18[f]
	[ML]/[ML(OH)][H]		9.4	**12.31 (0.08)**	

[a] Reference 31. [b] Reference 30. [c] Reference 23. [d] $\Delta V(Mg^{2+}) = 1.16 \times 10^{-2}$ mL, number of data pairs fitted = 176; $\Delta V(Ca^{2+}) = 1.25 \times 10^{-2}$ mL, number of data pairs fitted = 321; $\Delta V(Cu^{2+}) = 1.98 \times 10^{-2}$ mL, number of data pairs fitted = 293; $\Delta V(Zn^{2+}) = 1.21 \times 10^{-2}$ mL, number of data pairs fitted = 298. [e] Reference 33. [f] Reference 32.

than the values reported earlier largely because of differences in the ligand basicity ($\sum$log K_i^H) between the two studies. As noted by Delgado et al., the stability of $Zn(PCTA)^-$ is somewhat higher than that of $Cu(PCTA)^-$ (opposite to that predicted by the Irving−Williams order). In the absence of X-ray structures, it is difficult to explain the origin of these differences, but the higher stability of $Zn(PCTA)^-$ compared to $Cu(PCTA)^-$ suggests that PCTA could potentially be used for the selective complexation of Zn^{2+} over Cu^{2+}.

Fewer thermodynamic studies of the Ln(PCTA) complexes have appeared likely due to slow complexation kinetics that excludes the use of conventional pH potentiometry. The stability constant for Gd(PCTA) was, however, determined by Aime et al. by competitive binding between DTPA and PCTA for Gd^{3+}. The competition reaction was followed by relaxometry, and the stability constant was reported as log K_{GdL} = 21.0.[16] More recently, Siaugue and co-workers studied the luminescence properties of Eu^{3+} with azapyridinomacrocycles and reported stability constants for Eu(PCTA) also in the range of log K_{EuL} = 21.[19,25] Because of slow complex formation, we determined the stability constants of PCTA with Ce^{3+}, Nd^{3+}, Eu^{3+}, Y^{3+}, Ho^{3+}, and Yb^{3+} ions using an "out-of-cell" potentiometric method. Only mononuclear species (ML) were found for the Ln(PCTA) complexes, and for Ce^{3+}, Nd^{3+}, and Y^{3+}, the stability constants of the protonated MHL complexes could also be calculated from the "out-of-cell" data. The stability of the hydroxo complexes was determined in a separate titration after the complexes were fully formed. The stability constants for Ln(PCTA) and Y(PCTA) are listed in Table 3. These data differ somewhat from the published values (they are smaller than the reported literature values by about 0.6−0.8 log K units). This difference, however, is acceptable because of the relatively greater error associated with the competitive relaxometric method used by Aime et al.[16]

The stability constants of the Ln(PCTA) complexes increase from Ce^{3+} to Eu^{3+} and then remain relatively constant for the heavier lanthanides, with a slight increase toward the end of the series (Yb^{3+}). This trend, similar to that found for the Ln(NOTA) and $Ln(DOTA)^-$ complexes,[34−36] indicates that the size match between the lanthanide ion and the coordination cage determined by the four N atoms of the macrocyclic ring and the three carboxylate O atoms is more favorable for the heavier lanthanides (from Eu^{3+} through Lu^{3+}). The total basicity of PCTA ($\sum$log K_i^H) is about 3 orders of magnitude less than that of DO3A (Table 1) and 4 orders of magnitude higher than that of NOTA. On the basis of total basicity comparisons of NOTA, PCTA, and DO3A, one would predict a priori stability constants on the order of log $K \sim 17-18$ for Ln(PCTA) complexes. Interestingly, the experimentally determined values (Table 3) were ~2 log K units higher than that predicted on the basis of basicity alone. This difference may be attributed, in part, to preorganization of the macrocyclic cavity by the pyridine ring.

Kinetics of Complex Formation. The complex formation rates of Ln^{3+} ions with macrocyclic ligands are typically several orders of magnitude slower than the rates of complex formation with flexible multidentate ligands such as DTPA. PCTA has a convenient built-in chromophore due to a $\pi \rightarrow \pi^*$ transition of the pyridine moiety, the maximum of which exhibits a red shift upon complexation with a metal ion (Figure 1). The red shift was large enough to be used to monitor the complex formation. Owing to the high ϵ_{max} [for Ce(PCTA), $\epsilon_{max} = 4.91 \times 10^3$ dm^3 mol^{-1} cm^{-1} at λ_{max} = 269 nm and pH = 4.07], large changes in absorbance were

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Table 3. Stability Constants of the Ln^{3+} Complexes Formed with the Ligand **PCTA** (I = 1.0 M KCl, 25 °C)[a]

M^{3+}	eq quotient	NOTA[b]	DO3A[e]	**PCTA**	DOTA[b]
Ce^{3+}	[ML]/[M][L]	13.23	19.7	**18.15 (0.04)**	23.39
	[MHL]/[ML][H]		1.25	**2.89 (0.06)**	
	[ML]/[ML(OH)][H]			**11.30 (0.01)**	
Nd^{3+}	[ML]/[M][L]	13.17		**20.15 (0.04)**	22.99
	[MHL]/[ML][H]			**2.41 (0.08)**	
	[ML]/[ML(OH)][H]			**11.63 (0.02)**	
Eu^{3+}	[ML]/[M][L]	13.86		**20.26 (0.09)**[g]	23.45
	[ML]/[ML(OH)][H]			**11.31 (0.01)**	
Gd^{3+}	[ML]/[M][L]	14.27, 13.7[c]	21.1	**20.39 (0.04)**[h]	24.67, 25.3[f]
	[ML]/[ML(OH)][H]		2.06	**11.10 (0.03)**	2.8[f]
Ho^{3+}	[ML]/[M][L]	15.21		**20.24 (0.06)**	24.54
	[ML]/[ML(OH)][H]			**11.09 (0.01)**	
Y^{3+}	[ML]/[M][L]		21.1[f]	**20.28 (0.02)**	24.3[f]
	[MHL]/[ML][H]			**1.81 (0.15)**	
	[ML]/[ML(OH)][H]			**11.10 (0.01)**	
Yb^{3+}	[ML]/[M][L]	15.35, 15.95[d]	23.0[d]	**20.63 (0.06)**	25.0, 25.41[d]
	[ML]/[ML(OH)][H]			**10.26 (0.01)**	

[a] The literature values for Ln(NOTA), Ln(DO3A), and Ln(DOTA)$^-$ complexes are also listed for comparison. [b] Reference 34. [c] Reference 6. [d] Refers to log K_{LuL}. [e] Reference 35. [f] Reference 36. [g] The literature values of the stability constant for Eu(PCTA) are 20.9 and 21.1 from refs 19 and 25. [h] The literature value of the stability constant for Gd(PCTA) is 21.0 ± 0.5 from ref 16.

registered even in quite dilute solutions (0.2 mM). The kinetics of formation were studied for four metal ions, Ce^{3+}, Eu^{3+}, Y^{3+}, and Yb^{3+}, in the presence of 3−20-fold excess metal ion.

In the presence of excess Ln^{3+}, the rate of complex formation may be described as

$$d[LnL]_t/dt = k_{obs}[PCTA]_t \quad (3)$$

where $[PCTA]_t$ is the total concentration of the free ligand and k_{obs} is a pseudo-first-order rate constant. The formation reactions were investigated in the range of pH = 3.56−4.40 with seven to nine pH readings while varying the concentrations of Ce^{3+}, Eu^{3+}, or Y^{3+} and in the range of pH = 3.56−4.07 with five pH readings for formation of the Yb^{3+} complex. At higher pH values, complex formation was too fast to be followed by conventional spectrophotometry, and below pH 3, complex formation was not complete. A plot of pseudo-first-order rate constants vs concentrations of Ln^{3+} and/or Y^{3+} showed a saturation curve at all pH readings [Figure 2 for Eu(PCTA) and Y(PCTA) complexes and Figure S1 in the Supporting Information for Ce(PCTA) and Yb(PCTA) complexes]. This behavior can be described by rapid formation of an intermediate protonated complex that rearranges to the final product, Ln(PCTA), in a slow rate-determining step.[37] A similar mechanism has been reported for other Ln^{3+} macrocyclic polyaminopolycarboxylic acid ligands [Ln(NOTA), Ln(DO3A), Ln(DOTA)$^-$, Ln(TRITA)$^-$, and Ln(TETA)$^-$].[5−14]

The dependence of k_{obs} values on the metal ion concentration may be described by eq 4, where $K_{cond}^{Ln(H_2PCTA)^{2+}}$ is the

$$k_{obs} = \frac{k_r K_{cond}^{Ln(H_2PCTA)^{2+}}[Ln^{3+}]}{1 + K_{cond}^{Ln(H_2PCTA)^{2+}}[Ln^{3+}]} \quad (4)$$

conditional stability constant of the intermediate and k_r is the rate constant for deprotonation and rearrangement of the intermediate to the product. The diprotonated complex Ln(H$_2$PCTA)$^{2+}$ is identified as a common intermediate by pH potentiometry (Ce^{3+}, Eu^{3+}, and Y^{3+}) and UV−vis spectrophotometry (Yb^{3+}). When the reactants were mixed in a weakly buffered solution, a rapid pH drop was first observed

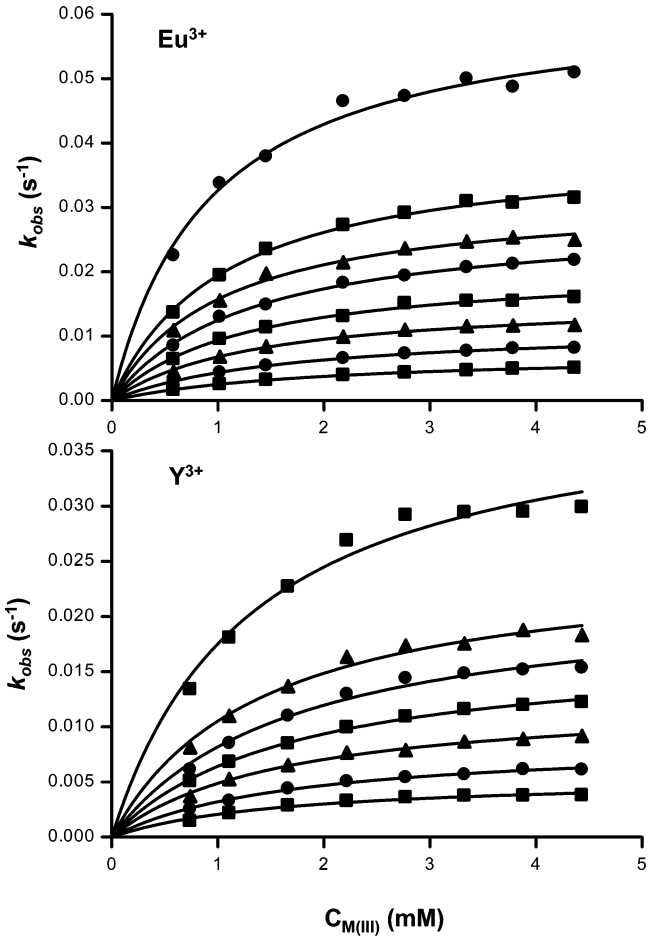

Figure 2. Pseudo-first-order rate constants, k_{obs}, as a function of the M^{3+} ion concentration at different pHs in the formation reaction of some M(PCTA) complexes [pH readings from top to bottom are 4.40, 4.21, 4.14, 4.07, 3.97, 3.86, 3.72, and 3.56 for Eu(PCTA) and 4.40, 4.21, 4.14, 4.07, 3.97, 3.86, and 3.72 for Y(PCTA)].

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followed by the slow decrease in the pH. The number of protons released in each of these steps could be determined in an independent titration from the amount of base needed to neutralize the same amount of a buffer solution. However, the number of the protons released in the first step depended upon the starting pH of the buffer used. For the metal ions examined here, the composition of the intermediate was determined at pH = 3.86 in weakly buffered solutions (0.01 M DMP). Upon mixing of solutions of the ligand in the form $H_{2.5}$PCTA and Ln^{3+} ($C_{PCTA} = 5 \times 10^{-4}$ M; $C_{Ln^{3+}} = 2.5 \times 10^{-3}$ M; $V = 10.00$ mL), the amount of protons released in the slow process was about 4 times more than that in the initial step. This suggests that the intermediate is the diprotonated complex $Ln(H_2PCTA)^{2+}$. Because of rapid formation of the Yb(PCTA) complex, pH changes upon complexation cannot be followed with an electrode. Therefore, a spectrophotometic method was used to ensure that the intermediate in this system is the diprotonated $Yb(H_2PCTA)^{2+}$ complex. The conditions for this experiment were similar to those listed above, but the pH changes upon mixing of the reactants were followed spectrophotometrically with the use of an acid−base indicator (methyl orange; range of pH = 3.1−4.4; $C_{MO} = 2 \times 10^{-5}$ M; $V = 1.20$ mL). The absorbance changes of the sample containing an indicator in weakly buffered solutions (0.01 M DMP) were calibrated by the addition of a known amount of acid (5 μL of 0.1084 M HCl). A kinetic curve was then recorded for the system $H_{2.5}$PCTA plus 5 equiv of Yb^{3+}. The changes in the absorbance can be expressed in the volume of added acid (0.1084 M HCl), and the approximate amount of protons released can thus be determined. Similarly to the other Ln^{3+} ions studied, the experiments strongly suggest the formation of a diprotonated intermediate, $Yb(H_2PCTA)$. Diprotonated intermediates have been identified in the complexation reactions of $Ln(DOTA)^-$, various LnDOTA derivatives, and $Ln(TRITA)^-$ and $Ln(TETA)^-$ complexes.[7−9,12−14,38] However, for Ln(NOTA) and for complexes of some DO3A derivatives, monoprotonated intermediates has been found.[5,10,11] It was found that the protonation degree of the intermediate depended on the relative values of protonation constants of the N atoms and on the differences between them. The first two protonation constants of some 9- and 10-membered macrocyclic ligands such as NOTA and DETA were found to differ with 6−8 orders of magnitude, which is possibly the reason for the presence of monoprotonated [Ln(HL)] intermediates.[5,6] In the case of PCTA, the differences between the first and second protonation constants were found to be ($\log K_1^H - \log K_2^H$) 3.99 log K units, which lies between the values obtained for DOTA and NOTA (2.33−2.90 and 4.74−6.31, respectively; Table 1).

The final form of the equations used for fittings is eq 5 (for additional equations, see the Supporting Information). The k_{obs} values at different pHs and Ln^{3+} ion concentrations were fitted to eq 6, and the rate constants k_r and the stability constants of the intermediates ($K_{therm}^{Ln(H_2PCTA)^{2+}}$) were calculated for each of the metal ions studied. Because of the relatively fast formation of the complexes, it was impossible to obtain stability constants of the intermediates by pH potentiometric titration (no reliable data can be obtained).

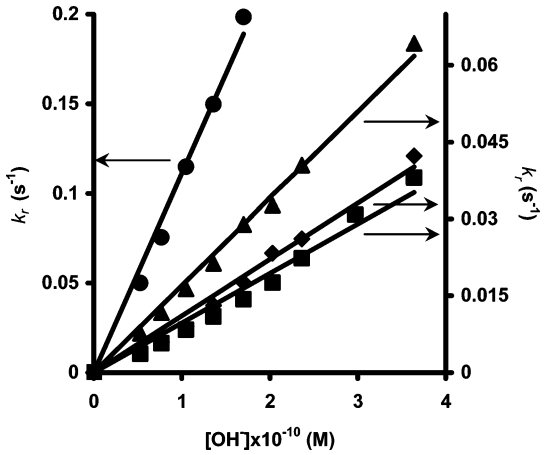

Figure 3. Formation rates of M(PCTA) complexes vs OH^- ion concentration for Ce (squares), Eu (triangles), Y (diamonds), and Yb (circles) (I = 1.0 M KCl; t = 25 °C).

$$k_{obs} = \frac{k_r \frac{K_{therm}^{Ln(H_2PCTA)^{2+}}}{\alpha_{H_2L}}[Ln^{3+}]}{1 + \frac{K_{therm}^{Ln(H_2PCTA)^{2+}-}}{\alpha_{H_2PCTA}}[Ln^{3+}]} \quad (5)$$

From kinetic data, the stability constants of the intermediate species were found to be reasonably pH-independent over the pH range examined (calculated stability constants at each pH reading are listed in Table S1 in the Supporting Information). The averages of the stability constants of the intermediates $Ce(H_2PCTA)^{2+}$, $Eu(H_2PCTA)^{2+}$, $Y(H_2PCTA)^{2+}$, and $Y(H_2PCTA)^{2+}$ were 2.81 ± 0.03, 3.12 ± 0.02, 2.97 ± 0.06, and 2.69 ± 0.06, respectively. The values obtained from the kinetic data are higher than those for the monoacetate complexes [$Ln(CH_3-COO)^{2+}$].[38] On the other hand, the stabilities of the La^{3+} and Y^{3+} complexes formed with dicarboxylic acid−glutarate are 3.02 and 3.25, respectively, which are close to the values obtained in our study, indicating that the number of acetate arms coordinated to the metal ions in the intermediates was likely greater than one and most likely two.[38] The stability constants calculated from the kinetic data of the intermediates are lower than those obtained from pH potentiometric titration for the $Ln(H_2DOTA)^+$ complexes (4.4, 4.3, and 4.2 for Ce, Eu, and Yb intermediates, respectively) and somewhat higher than those reported for a DO3A derivative ligand (2.4, 2.5, and 2.43 for the Ce, Eu, and Yb intermediates, respectively).[8,39]

The rate constants k_r are inversely proportional to the H^+ ion concentration as $k_{OH} = k_H/K_w$ ($[H^+][OH^-] = K_w$, where K_w is the ionic product of water; eq 6 and Figure 3). Similar relations were found for a large number of 9−14-membered tri- or tetraazamacrocycles with three or four carboxylate-

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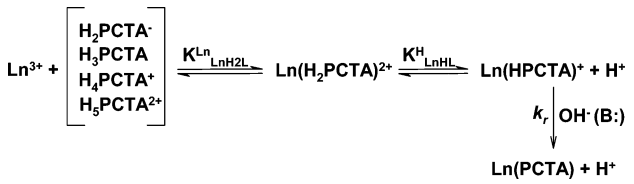
Table 4. Rate Constants, k_{OH}, Characterizing the Rearrangements of Ce(PCTA), Eu(PCTA), Y(PCTA), and Yb(PCTA) Complexes (I = 1.0 M KCl, 25 °C) Compared to the Literature Data for Complexes Formed with Macrocyclic Ligands

	k_{OH} (M^{-1} s^{-1})			
	Ce^{3+}	Eu^{3+}	Gd^{3+}	Yb^{3+}
NOTA[a]	6.3×10^7		7.1×10^7	5.5×10^7
DO3A[b]			2.1×10^7	
PCTA	$\mathbf{(9.68 \pm 0.34) \times 10^7}$	$\mathbf{(1.74 \pm 0.03) \times 10^8}$	$\mathbf{(1.13 \pm 0.03) \times 10^8}$ [c]	$\mathbf{(1.11 \pm 0.03) \times 10^9}$
DOTA[d]	3.5×10^6, 1.16×10^6,[e] 2.7×10^6 [f]	1.1×10^7, 7.2×10^6 [b]	5.9×10^6 [b]	4.1×10^7, 9.3×10^7 [f]
TRITA[g]	6.9×10^6		2.6×10^7	5.0×10^7
TETA[e]	5.58×10^6			

[a] Reference 5. [b] Reference 12. [c] Refers to Y(PCTA). [d] Reference 8. [e] Reference 40; for $Ce(TETA)^-$, the term with second-order dependence on the OH^- ion concentration was observed, too. [f] Reference 9. [g] Reference 13.

Scheme 1. Suggested Most Probable Formation Scheme of Ln(PCTA) Complexes



containing side arms.[5−14] Interestingly, among all acetate derivatives studied, $Ce(TETA)^-$ is an exception in that this complex shows a second-order dependence.[40] This behavior was explained by a hydroxide ion catalyzed rearrangement of the ligand TETA, a ligand that is less preorganized than DOTA. The k_{OH} values are presented and compared to different tri- and tetraazamacrocyclic ligands in Table 4.

$$k_r = k_H/[H^+] = k_{OH}[OH^-] \quad (6)$$

The tetraazamacrocycle PCTA can be regarded as a more preorganized triacetate derivative of DOTA or DO3A. Because of the similarities in their structures, the complex formation mechanism is expected to be similar to that of DOTA.[8,9] The linear dependence of the complex formation on the OH^- ion concentration can be interpreted as rapid deprotonation of the intermediate $Ln(H_2PCTA)^{2+}$ to $Ln(HPCTA)^+$ in an equilibrium step followed by release of the second proton in a rate-determining step. It is easy to prove that the concentration of the monoprotonated complex is inversely proportional to the H^+ ion (or linearly proportional to the OH^- ion) concentration. The validity of general base catalysis has been shown for DOTA and some DO3A derivatives,[9,39] and also in our case, the k_{obs} values depend linearly on the buffer concentration (basic form of the buffer, not shown). The evidence for the existence of general base catalysis supports the rate-controlling role of the deprotonation of the monoprotonated intermediate and the formation of the Ln(PCTA) complexes. Deprotonation is followed by a fast structural rearrangement in which the metal ion moves to the "coordination cage" defined by the ligand. The proposed reaction steps for the formation of Ln(PCTA) complexes are illustrated in Scheme 1. However, it is worth mentioning that the plot of k_r vs $[OH^-]$ for all studied metal ions can also be fitted with second-order dependence on the OH^- ion concentration, as it was found for the formation of $Ce(TETA)^-$.[40] In these cases, the k_{OH} rate constants deviate from the values presented in Table 4 by ~25%, but the errors in the k_{OH} and k_{2OH} values cannot be determined by the program, possibly because of the limited interval of pH used for the study.

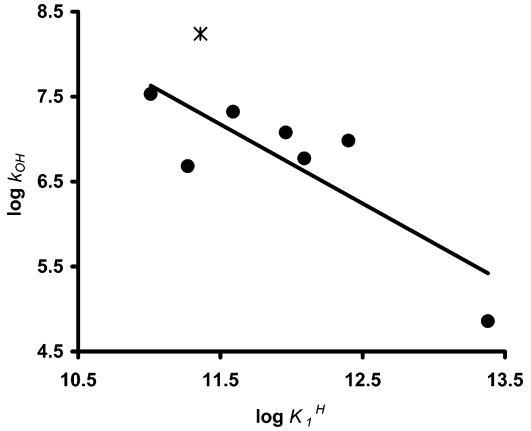
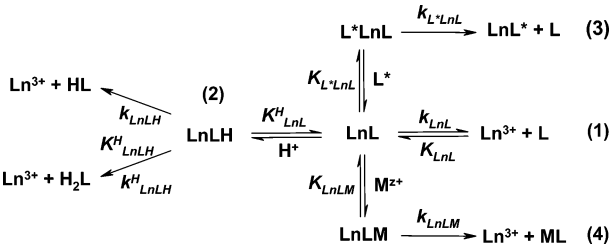


Figure 4. Correlation of log k_{OH} obtained for some 12-membered macrocyclic ligands with the first protonation constant (log K_1^H) of the ligand (k_{OH} value characterizing the formation rate of Eu(PCTA) marked with an asterisk).

The data of Table 4 indicate that the formation rates of the Ln(PCTA) complexes are at least 1 order of magnitude faster than they are for the corresponding $Ln(DOTA)^-$ complexes. Because of the presence of a pyridine moiety, PCTA seems to be more preorganized than other tetraazmacrocycles. Kumar et al. found a linear correlation between the rate of rearrangement of the intermediate and the first protonation constant of the ligand.[10] A similar correlation can be established for GdL complexes of several 12-membered tri- and tetraacetate (DOTA and DO3A derivatives) ligands based on the literature data (Figure 4; the values used for generation of the figure are listed in Table S3 in the Supporting Information). It is important to note, however, that the log K_1^H values of the ligands strongly depend on the ionic strength used in each experiment. The rate of formation of Eu(PCTA) (marked with an asterisk) as determined in this work is somewhat higher than expected based on the log K_1^H value, which can be attributed, in part, to the more preorganized structure of PCTA [the rates of formation for two neighboring Ln^{3+} ions differ only slightly, and Eu(PCTA) can be compared to Gd(PCTA)]. The X-ray structure of the parent macrocyclic amine pyclen indicates a preorganized, rigid structure,[20] but in the absence of X-ray

(40) Chang. C. A.; Liu, Y.-L.; Chen, C.-Y.; Chou, X.-M. *Inorg. Chem.* **2001**, *40*, 3448.

Scheme 2. Dissociation of LnL Complexes



crystallographic data for the PCTA ligand itself or some of the Ln(PCTA) complexes, it is difficult to ascribe the unusually fast formation kinetics exclusively to preorganization.

Kinetics of Dissociation. The kinetics of complex dissociation is an important parameter to consider if the Ln(PCTA) complexes are to be used in vivo. In the past 2 decades, the dissociation kinetics of several lanthanide(III) polyaminopolycarboxylate complexes formed with both open-chain and macrocyclic ligands have been studied by various methods.[3] On the basis of these data, the dissociation of the complexes in vivo can occur through one of the following pathways (Scheme 2): (1) spontaneous dissociation characterized by the rate constant k_{LnL}, (2) proton-assisted dissociation characterized with the protonation constants K^H_{LnHL} and $K^H_{LnH_2L}$ and rate constants k_{LnHL} and k^H_{LnHL}, (3) ligand-assisted dissociation characterized by the stability constant of mixed ligand complexes K_{LLnL*} and rate constant k_{LLnL*}, and (4) metal-ion-catalyzed dissociation characterized by the stability of dinuclear complexes K_{LnLM} and rate constant k_{LnLM}. The rate of the dissociation is directly proportional to the total concentration of the complex:

$$-d[LnL]_t/dt = k_d[LnL]_t \quad (7)$$

where k_d is a pseudo-first-order rate constant. Taking into account all possible dissociation pathways according to Scheme 2, the total concentration of the complex $[LnL]_t$ can be expressed as

$$[LnL]_t = [LnL] + [LnHL] + [LnH_2L] + [LnLM] + [LLnL^*] \quad (8)$$

In the systems of Ln^{3+} ion and octa- or nonadentate macrocyclic ligands, the probability of a ligand-exchange reaction is very low because formation of the ternary complex (LLnL*) is unlikely. The stabilities of the complexes of Ln^{3+} ions formed with endogenous ligands (e.g., citrate, glutamate) are several orders of magnitude less than they are for macrocyclic lanthanide(III) polyaminopolycarboxylate complexes.[38] On the other hand, the metal-ion-catalyzed dissociation has not been detected either for Ln(NOTA) and Ln(DO3A) or for $Ln(DOTA)^-$, possibly due to the absence of dinuclear LnLM complexes. However, this is an important dissociation route for Ln^{3+} complexes formed with DTPA derivatives and macrocyclic ligands derived from larger cyclic amines such as $Ln(TRITA)^-$ and $Ln(TETA)^-$.[3,13] In general, for 12-membered tetraazamacrocycles with three to four acetate pendant arms including PCTA, eq 8 can be simplified to

$$[LnL]_t = [LnL] + [LnHL] + [LnH_2L] \quad (9)$$

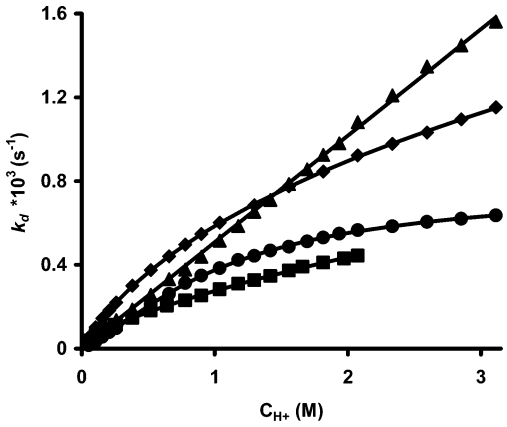


Figure 5. Dissociation rates (k_d) for CePCTA (squares), EuPCTA (triangles), YPCTA (diamonds), and YbPCTA (circles) as a function of the H^+ concentration.

The acid-catalyzed dissociation rates of Ln(PCTA) (Ce^{3+}, Eu^{3+}, and Yb^{3+}) and Y(PCTA) were studied in weak acid (0.05−3.12 M $HClO_4$), where the complexes are thermodynamically unstable and dissociate completely. The rates of dissociation can be expressed by eq 7, and the plot of the obtained k_d values vs H^+ concentration is shown in Figure 5.

In general, two types of curves were observed: (1) the rate of dissociation is linearly proportional to the acid concentration [Eu(PCTA) and Yb(PCTA) in the concentration range 0.05−1.0 mol dm^{-3}] and (2) saturation kinetics [Ce(PCTA), Y(PCTA), and Yb(PCTA) at high acid concentrations]. Similar results were reported for rates of dissociation of complexes formed with other macrocyclic ligands.[5−8,11,13,14,35,41] The saturation kinetics are in good agreement with the presence of an intermediate protonated complex, rapidly forming at the beginning of the reaction. The protonation likely occurs first on the acetate O atom(s), and then the proton is transferred to the N atom(s) of the ring. After the proton transfer, the metal ion moves out of the macrocyclic ring cavity ("coordination cage") and the dissociation takes place from this species where the metal ion is in an "out-of-cage" position. In slightly different scenarios, either the proton transfer or the structural rearrangement or both may occur slowly. During the rearrangements, a second protonation may occur on the ring N atom, and as a result of the electrostatic repulsion, Ln^{3+} leaves the "coordination cage".

For the linear correlation, the dissociation rate (k_d) can be expressed as follows:

$$k_d = k_0 + k_1[H^+] \quad (10)$$

where k_0 is a constant that describes dissociation independent

(41) Kang, S. I.; Ranganathan, R. S.; Emswiler, J. E.; Kumar, K.; Gougoutas, J. Z.; Malley, M. F.; Tweedle, M. F. *Inorg. Chem.* **1993**, *32*, 2912.

on the acid concentration (most likely the spontaneous dissociation) and k_1 a constant describing acid-catalyzed dissociation. Small, and in some cases even negative, values with large errors were obtained for k_0 during the fitting procedures (see the Supporting Information for details). This is not surprising because the spontaneous dissociation pathway is negligible in strongly acidic solutions, where the formation of a protonated species predominates. Therefore, during the fittings, the k_0 values have been set to 0. The rate of the spontaneous dissociation of the Ln(DOTA)$^-$ complexes was also reported to be extremely low and can be measured directly with high errors. However, when the spontaneous dissociation rate of the LnL complexes formed with 9−14-membered macrocyclic ligands is compared with the acid-catalyzed dissociation rate of these complexes, the rate of spontaneous dissociation can be predicted (at about 4−6 orders of magnitude slower than the acid-catalyzed dissociation). According to our assumption, the order of magnitude of the spontaneous dissociation of Ln(PCTA) complexes can be predicted and values of $10^{-8}-10^{-9}$ s^{-1} would be expected. The values are negligibly small, but it is worth noting that, at physiological conditions, spontaneous dissociation may have a larger contribution to the dissociation rate (eq 10) than the term $k_1[H^+]$ because of the low acid concentration at pH = 7.4.

The linear dependence of k_p on the H^+ ion concentration in the entire range was detected for Eu(PCTA), and it is in good agreement with the dissociation of the monoprotonated Eu(HPCTA)$^+$ complexes. For dissociation of Ce(PCTA), Y(PCTA), and Yb(PCTA), saturation curves were obtained. It can be interpreted by assuming an accumulation of diprotonated complexes with an increase of the H^+ ion concentration. For these complexes, dissociation will proceed with the formation of mono- and diprotonated [Ln(HPCTA)$^+$ and Ln(H$_2$PCTA)$^{2+}$] species. In these complexes, presumably the acetate group(s) are protonated in equilibrium processes. The proton transfer to the ring N atom(s) in the monoprotonated species can be the rate-determining step, especially at lower H^+ ion concentrations, where the concentrations of the protonated species are low. At higher proton concentration, the diprotonated species are predominant and they rearrange in the rate-determining step to an intermediate in which the metal ion is coordinated only by three acetate arms in the "out-of-cage" position. The dissociation of this intermediate will therefore be very fast. This dissociation mechanism is analogous to the one suggested by Tóth et al. for dissociation of Eu(DOTA)$^-$ and by other authors for dissociation of some DO3A derivatives [e.g., Kumar et al. for Gd(DO3A) and Gd(HP-DO3A) and Kang et al. for Gd(DO3MA)].[8,41] Taking into account all possible dissociation pathways, the rate of dissociation can be given as

$$-\frac{d[Ln(PCTA)]_t}{dt} = k_d[Ln(PCTA)]_t = k_0[Ln(PCTA)] + k_1[Ln(HPCTA)^+] + k_2[Ln(H_2PCTA)^{2+}] \quad (11)$$

The concentrations of the protonated complexes can be calculated with the stability constants of the protonated species according to

$$K_1 = \frac{[Ln(HPCTA)^+]}{[Ln(PCTA)][H^+]} \quad \text{and} \quad K_2 = \frac{[Ln(H_2PCTA)^{2+}]}{[Ln(HPCTA)^+][H^+]} \quad (12)$$

Finally, the pseudo-first-order rate constant k_d can be expressed with eq 9 as

$$k_d = \frac{k_0 + k_1K_1[H^+] + k_2K_1K_2[H^+]^2}{1 + K_1[H^+] + K_1K_2[H^+]^2} \quad (13)$$

As described earlier, k_0 was fixed to zero and the rate constants k_1 and k_2 (k_{LnLH} and k^H_{LnLH} from Scheme 2) and the protonation constants K_1 and K_2 (K^H_{LnL} and K^H_{LnLH} from Scheme 2) were fitted to eq 13.

It is possible to determine the K_1 value independently by pH potentiometry. For the complex Eu(DOTA)$^-$, Tóth and co-workers determined the protonation constant $K_1 = 14 \pm 1$.[8] Our attempts to determine the protonation constants of the complexes failed because no acceptable data could be obtained in titrations at about 5 mM complex solutions with a HCl solution. This is likely due to the fact that PCTA has lower protonation constants than DOTA and, because of the lower log K^H_i values of PCTA, lower log K^H_i values of the Ln(PCTA) complexes would be expected. The log K^H_1 for Eu(DOTA)$^-$ is already quite low (1.15),[8] and determining a protonation constant lower than 1.15 would be difficult. We did not consider the use of UV−vis spectrophotometry to determine the protonated complex stability because the protonation occurs presumably on the acetate arm, which is far away from the pyridine chromophore. The first-order constants (k_1 and k_2) obtained in the fittings of the k_d values to eq 13 for Ce(PCTA), Y(PCTA), and Yb(PCTA) must be converted to the second- and third-order rate constants for proper comparison with the literature data. The results of the fittings are listed and compared to some other macrocyclic LnL complexes in Table 5.

The uncertainty in the products k_1K_1 and $k_2K_1K_2$ cannot be determined, but the uncertainties in the first-order rate constants do provide some important insights. These values are $k_1 = (1.08 \pm 0.08) \times 10^{-4}$ s^{-1} and $k_2 = (1.79 \pm 0.25) \times 10^{-3}$ s^{-1} for Ce(PCTA), $k_1 = (6.24 \pm 0.58) \times 10^{-4}$ s^{-1} and $k_2 = (3.25 \pm 0.40) \times 10^{-3}$ s^{-1} for Y(PCTA), and $k_1 = (2.83 \pm 0.53) \times 10^{-4}$ s^{-1} and $k_2 = (7.98 \pm 0.24) \times 10^{-4}$ s^{-1} for Yb(PCTA).

When the k_d values for Ce(PCTA) and Y(PCTA) were fitted to eq 13, the rate constants k_1 and k_2 and the protonation constants K_1 and K_2 could be estimated with reasonable certainty (in all cases, the uncertainty was less than 10%). For dissociation of Yb(PCTA), a somewhat larger uncertainty was found when all of the data were fitted to eq 13. However, the correlation is linear in the 0.05−1.0 mol dm^{-3} H^+ ion concentration range, and fitting the data to eq 10 resulted in a value $k_1 = (3.87 \pm 0.04) \times 10^{-4}$ M^{-1} s^{-1}. This is somewhat larger than the value obtained from saturation kinetics

Table 5. Rates of Dissociation for Ce(PCTA), Eu(PCTA), Y(PCTA), and Yb(PCTA) Complexes (I = 1.0 M KCl, 25 °C)[a]

ligand	Ln^{3+}	Ce^{3+}	Gd^{3+}	Yb^{3+}
NOTA	k_0 (s^{-1})	2.5×10^{-5}	8.3×10^{-6}	2.7×10^{-6} [b]
	k_1 (M^{-1} s^{-1})	4.3×10^{-2}	2.3×10^{-2}	1.6×10^{-3} [b]
DO3A	k_0 (s^{-1})	1.8×10^{-3}	4.0×10^{-4}	
	k_1 (M^{-1} s^{-1})	1.12×10^{-1}	2.51×10^{-2} [c]	2.77×10^{-2} [c]
PCTA	$\boldsymbol{k_0}$ **(s^{-1})**	*d*	*d*	*d*
	$\boldsymbol{k_1}$ **(M^{-1} s^{-1})**		$\mathbf{(5.08 \pm 0.04) \times 10^{-4}}$ [f]	$\mathbf{(3.87 \pm 0.04) \times 10^{-4}}$ [h]
	$\boldsymbol{k_1K_1}$ **(M^{-1} s^{-1})**[e]	$\mathbf{9.61 \times 10^{-4}}$	$\mathbf{1.07 \times 10^{-3}}$ [g]	$\mathbf{2.80 \times 10^{-4}}$
	$\boldsymbol{k_2K_1K_2}$ **(M^{-2} s^{-1})**[e]	$\mathbf{2.07 \times 10^{-3}}$	$\mathbf{6.32 \times 10^{-4}}$ [g]	$\mathbf{8.85 \times 10^{-4}}$
	$\boldsymbol{K_1^H}$	$\mathbf{8.90 \pm 0.87}$	$\mathbf{1.72 \pm 0.17}$[g]	$\mathbf{0.99 \pm 0.16}$
	$\boldsymbol{K_2^H}$	$\mathbf{0.13 \pm 0.02}$	$\mathbf{0.11 \pm 0.03}$[g]	$\mathbf{1.12 \pm 0.10}$
DOTA	k_0 (s^{-1})		5×10^{-10} [j]	
	k_1 (M^{-1} s^{-1})	8×10^{-4} [i]	2×10^{-6},[j] 1.4×10^{-5} [k]	

[a] Data for some other complexes formed with macrocyclic ligands are also listed for comparison. [b] Refers to Er(NOTA). [c] Calculated from data in ref 35 for the dissociation of $Lu(H_2DO3A)^{2+}$. [d] Fixed to zero (see the text). [e] Calculated from determined k_1, k_2, K_1, and K_2 values (see the text). [f] Refers to Eu(PCTA). [g] Refers to Y(PCTA). [h] Fittings for the H^+ ion concentration range of 0.05−1.0 to eq 7. [i] The second-order dependence on the H^+ ion concentration with third-order rate constant 2.0×10^{-3} M^{-2} s^{-1} was also observed in ref 7. [j] Reference 9. [k] Calculated for $Eu(DOTA)^-$ from ref 8. k_2 also can be calculated $= 1.04 \times 10^{-3}$ M^{-2} s^{-1} with the use of $k_1 = 1 \times 10^{-6}$ s^{-1} and $k_2 = 6.2 \times 10^{-4}$ s^{-1} and protonation constants $K_1 = 14$ M^{-1} and $K_2 = 0.12$ M^{-1}.

($k_1 = 2.80 \times 10^{-4}$ M^{-1} s^{-1}). Mathematically, the rate constant k_1 is calculated from the data obtained in the range 0.05−0.5 M; at higher acid concentrations, the contribution of the term $k_1K_1[H^+]$ to k_d is very low (eq 13); therefore, the uncertainty in k_1 is high. Similar conclusions were drawn by Tóth et al. when dissociation of the $Eu(DOTA)^-$ complex was studied.[8] According to Tóth et al., the unity term can be neglected in the denominator when working at high H^+ ion concentrations so eq 13 simplifies to eq 14. Fitting k_d

$$k_d \approx \frac{k_2K_2[H^+]}{1 + K_2[H^+]} \qquad (14)$$

data collected in the range of 1.0−3.12 M H^+ ion concentrations to eq 14 gave $k_2 = (9.54 \pm 0.18) \times 10^{-4}$ s^{-1} and $K_2 = 0.67 \pm 0.03$ M^{-1}. These data indicate that the dissociation of $Yb(H_2PCTA)^{2+}$ at high acid concentration occurs mainly through the formation and dissociation of the diprotonated complex [from $Yb(HPCTA)^+$] as a result of the shift of the rate-controlling step. When these data are compared to the constants obtained by Kumar et al. for Gd(DO3A) ($k_1 = 4.0 \times 10^{-4}$ s^{-1}; $k_2 = 1.93 \times 10^{-2}$ s^{-1}; $K_2 = 1.3$ M^{-1}) and for Lu(DO3A) ($k_2 = 3.6 \times 10^{-3}$ s^{-1}; $K_2 = 7.7$ M^{-1}),[35] it can be concluded that the rate of direct dissociation of the protonated complex $Yb(HPCTA)^+$ is similar to the one found for Gd(DO3A) but the complex $Yb(HPCTA)^+$ appears to be more resistant to the acid-catalyzed dissociation. This can be attributed partially to the difference in the second protonation constants of the ligands (Table 1). The rate of proton-assisted dissociation of the complex Gd(DO3MA) was found to be very similar. In 0.1 M NaCl, the first two protonation constants are somewhat lower ($\log K_1^H = 10.82$; $\log K_2^H = 8.42$) than the value measured in 0.1 M Me_4NCl ($\log K_1^H = 13.38$; $\log K_2^H = 9.15$).[41] In those cases where a higher concentration of background electrolyte was used, this difference will be even higher and the values will probably be close to the values measured for the ligand PCTA (Table 1). The rate constants obtained for Gd(DO3MA) are $k_1 = 3.7 \times 10^{-5}$ s^{-1}, $k_2 = 5.2 \times 10^{-4}$ s^{-1}, and $K_2 = 5$ M^{-1}, characterizing the direct dissociation, the proton-catalyzed dissociation of the monoprotonated complex, and the protonation constant of the complex, respectively.[41] The rate of the proton-catalyzed dissociation of the monoprotonated complex (k_2) is very close to that found for Yb(PCTA). It appears that the protonation constants of the ligands are critical when the ligand is chosen for complexation and in vivo use. However, all of our attempts to find a correlation between the first (or a sum of the first and second) protonation constants of the ligands and the rate of the acid-catalyzed dissociation of the complexes failed. This is most likely due to the larger uncertainties in the kinetic data, differences in the ionic media used for the evaluation of both equilibrium (protonation constants) and kinetic data and differences in the dissociation mechanisms.

Conclusions

The thermodynamic stabilities of a few Ln(PCTA) complexes were determined and found to be in the range from 18.15 for Ce(PCTA) to 20.63 for Yb(PCTA). The trend of the stability constants is similar to that of the Ln(NOTA) and/or $Ln(DOTA)^-$ complexes involving a large increase at the beginning of the series, practically constant values for Ln^{3+} ions in the middle of the series, and a slight increase of the stability at the end for Yb(PCTA). The mechanism of complex formation is similar to that reported earlier for DOTA and DOTA derivatives, but formation of Ln(PCTA) proceeds at least 10 times faster than the corresponding Ln(DOTA) complexes. The formation rates increase with decreasing lanthanide size, and there is an order of magnitude difference between the k_{OH} values for Ce(PCTA) and Yb(PCTA). According to our studies, Ln(PCTA) complexes have the fastest formation rates among all 9−14-membered tri- or tetraazamacrocyclic ligands studied so far. The fast formation kinetics can be attributed, at least in part, to preorganization of the ligand by the pyridine ring.

Spontaneous dissociation of the Ln(PCTA) complexes was too slow to be evaluated here, but acid-catalyzed dissociation of Ln(PCTA) complexes occurred at about an order of magnitude faster than that of the corresponding $Ln(DOTA)^-$ complexes. The acid-catalyzed dissociation rates decrease with decreasing Ln^{3+} ion size. While Ln(PCTA) complexes dissociate somewhat faster than Ln(DOTA) complexes in

acid, these complexes are still kinetically quite inert at physiological pH values and appear to have sufficient kinetic inertness for in vivo applications.

The current study may have implications for the future design of complexing agents that have both favorably fast formation kinetics and favorable kinetic inertness toward dissociation. On the basis of the present thermodynamic and kinetic data, PCTA seems to be an attractive ligand for constructing radiopharmaceutical applications that require rapid formation kinetics.

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Supporting Information Available: Evaluation of the equations for formation data fittings, plot of the first-order rate constants, k_{obs}, as a function of the Ln^{3+} ion concentration at different pHs in the formation reactions of Ce(PCTA) and Yb(PCTA) complexes (Figure S1), table with conditional and thermodynamic stability constants obtained for several Ln(PCTA) complexes from formation kinetic data (Table S1), tables with the observed k_{OH} values for formation (Table S2) and k_p for dissociation of Ce(PCTA), Eu(PCTA), Y(PCTA), and Yb(PCTA) complexes (Table S4), and literature data used for the generation of Figure 4 (Table S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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