Phosphodiester Hydrolysis by Lanthanide Complexes of Bis-Tris Propane

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Potentiometric titrations of the mixtures of lanthanide(III) perchlorates and bis-Tris propane (BTP) reveal formation of dinuclear hydroxo complexes $M_2(BTP)_2(OH)_n^{6-n}$, where M = La(III), Pr(III), Nd(III), Eu(III), Gd(III), and Dy(III) and n = 2, 4, 5, or 6, in the pH range 7−9. ESI-MS data confirm the presence of dinuclear species. Kinetics of the hydrolysis of bis(4-nitrophenyl) phosphate (BNPP), mono-4-nitrophenyl phosphate (NPP), and 4-nitrophenyl acetate (NPA) in the lanthanide(III)−BTP systems has been studied at 25 °C in the pH range 7−9. The second-order rate constants for the hydrolysis of BNPP by individual lanthanide hydroxo complexes have been estimated by using the multiple regression on observed rate constants obtained at variable pH. For a given metal, the rate constants increase with increasing in the number n of coordinated hydroxide ions. In a series of complexes with a given n, the second-order rate constants decrease in the order La > Pr > Nd > Eu > Gd > Dy. Hydrolysis of NPP follows Michaelis−Menten-type "saturation" kinetics. This difference in kinetic behavior can be attributed to stronger binding of NPP dianion than BNPP monoanion to the lanthanide(III) species. Activities of lanthanide complexes in the hydrolysis of NPA, which is 10^6 times more reactive than BNPP in alkaline or aqueous hydrolysis, are similar to those in BNPP hydrolysis indicating unique capability of lanthanide(III) cations to stabilize the transition state of phosphate diester hydrolysis. Results of this study are analyzed together with literature data for other metal cations in terms of the Brønsted correlation and transition state−catalyst complexation strength.

Introduction

Metal complex catalysis in the hydrolysis of phosphoric and carboxylic acid esters continues to be an area of very active research.[1] Particularly challenging is the development of efficient catalysts for the cleavage of phosphodiesters, which are extremely resistant to hydrolysis even with activated 4-nitrophenolate leaving group.[2] Phosphodiester bonds form the backbone of DNA and RNA macromolecules and sufficiently active catalysts of their hydrolysis can find important applications as artificial nucleases in biochemical and medicinal researches.

Among the literature catalytic systems reported for the phosphodiester hydrolysis, the most active are complexes of highly charged metal cations: lanthanides(III),[1d,3−17] Ce(IV),[1d,18,19] Th(IV),[20] and Zr(IV).[21−23] Within this group of catalysts,

lanthanides(III) have the advantage of being less sensitive to the metal hydrolysis in neutral solutions, which leads eventually to metal hydroxide precipitation and loss of activity. Free aquo ions of lanthanides(III) are relatively weak catalysts. With typically employed substrate bis(4-nitrophenyl) phosphate (BNPP) (Figure 1) and metal ion concentrations in mM range, the half-life for the hydrolysis approaches 10−20 min at 50 °C.[14] Kinetics of BNPP hydrolysis is of the Michaelis−Menten type

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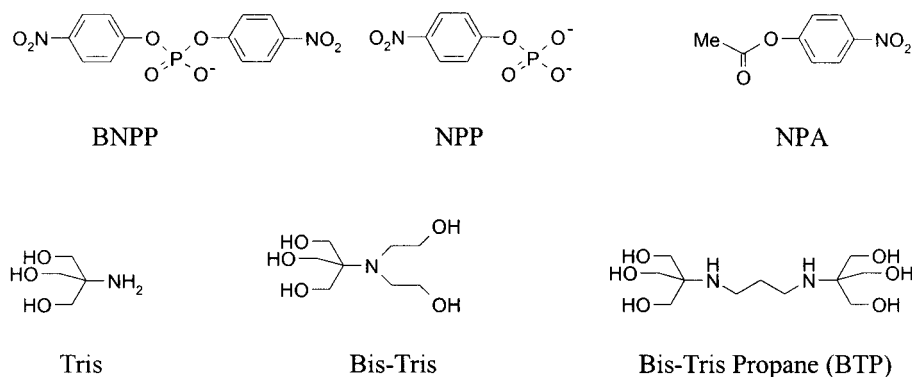(23) Stulz, E.; Leumann, C. *Chem. Commun.* **1999**, 239−240.

Figure 1. Structures of substrates used in this work and ligands derivatives of tris(hydroxymethyl)aminomethane (Tris).

with K_M values in the range 2−3 mM for all cations.[14] This imposes a further restriction on the observed rate constant, which cannot be larger than the catalytic rate constant k_C (8.6×10^{-4} s^{-1} at 50 °C for the most active Er^{3+})[14] even at elevated metal concentrations. There is much convincing evidence that the cleavage of phosphodiesters occurs via concerted action of a metal cation and a nucleophile, OH^- or RO^-.[1] Therefore, low activities of lanthanide aquo cations probably result from low concentration of OH^- or active metal hydroxo complexes in neutral solutions (since lanthanides are kinetically labile ions, a reaction mechanism involving simultaneous participation of metal cation and OH^- and a mechanism involving second-order reaction with metal hydroxo complex are kinetically indistinguishable). Indeed, much higher activities were reported for the systems involving hydroxide or alkoxide lanthanide(III) complexes.[7,9,11,12,17] Another important aspect is the high efficiency of the substrate activation by several (up to three) lanthanide(III) cations.[1f,g,24] Noteworthy, a recently reported remarkably active phosphodiesterolytic system based on a dinuclear dihydroxo lanthanum(III) macrocyclic complex [17] combines both of the above-mentioned features: metal−hydroxide ion cooperation and polynuclearity.

Despite considerable progress, the development of catalytic systems for phosphodiester hydrolysis that would be stable in water at pH close to neutrality and sufficiently active at room temperature still needs more effort. An obvious approach is testing different ligands, which could stabilize reactive hydroxo lanthanide species and in favorable cases pre-organize them for an optimum interaction with the substrate. The stabilization is required because of low solubilities of lanthanide hydroxides in combination with relatively high pK_a values of lanthanide(3+) aquo ions. For example, in the case of Eu^{3+}, the solubility product for $Eu(OH)_3$ equals 2.5×10^{-26} M^4 and pK_a of Eu_{aq}^{3+} is 8.6. [25] This means that in 1 mM solution the precipitation of $Eu(OH)_3$ will occur at pH ≥ 6.5 when the degree of formation of $Eu(OH)^{2+}$ is still less than 1%. Besides numerous sophisticated macrocyclic ligands, including calixarenes,[1i] a family of simple amino alcohols, derivatives of tris(hydroxymethyl)-aminomethane (Tris) (Figure 1) has attracted attention recently. Tris was successfully employed for stabilization of very active Zr(IV) catalyst,[22] bis-Tris was found to form hydrolytically active complexes with lanthanum(III).[9] We observed high hydrolytic activity in the system consisted of a lanthanide(III) and bis-Tris propane (BTP) in weakly basic solutions.[26]

The latter system differs from other lanthanide-based systems in several aspects. First, the reaction kinetics is first-order in metal without any "saturation" observed with several other lanthanide-based systems.[13,14,27] Second, the reaction rate vs pH profiles are very steep, indicating deprotonation of at least two coordinated water molecules (or ligand OH groups) in catalytically active complexes. The results of a more detailed study of the lanthanide(III)−BTP system, presented in this paper, show that in fact the active species are various dinuclear BTP−hydroxo complexes, some of which are related to previously proposed hypothetical active dinuclear hydroxo species.[7,9] Third, in the series of lanthanide(III) cations, the largest activity was observed for La(III) followed by Nd(III), Pr(III), and Eu(III). This trend is opposite to that observed for lanthanide aquo cations.[14,16] Larger activities for smaller cations are usually interpreted as being due to their larger charge densities and consequently stronger electrophilic substrate activation. We show in this paper that the basicity of metal-bound hydroxide ion is equally important, and when reactivities of individual mononuclear hydroxo complexes of different lanthanides are compared, they show rather small variation for different metals.

Besides the typically employed substrates BNPP and 4-nitrophenyl phosphate (NPP) (Figure 1), which is the intermediate product of BNPP hydrolysis, we also used in this study much more reactive 4-nitrophenyl acetate (NPA) (Figure 1). Mechanisms of nucleophilic substitution in esters of carboxylic acids and phosphates are generally similar, but carboxylates and phosphotriesters are much more reactive than phosphodiesters.[28] In accordance with this, hydrolytic activity of metal complex catalysts toward BNPP was found to be much lower than that toward NPA or 4-nitrophenyl phosphotriester derivatives for complexes of $Zn(II)^{1a,29-33}$ and Cu(II).[34] Surprisingly, with hydroxo complexes of lanthanides, we found similar activities for BNPP and NPA, which differ 10^6 times in rate constants of alkaline hydrolysis. An additional aspect of this work is an

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Table 1. Stability Constants for the Formation of Lanthanide(3+) Complexes with BTP (25 °C, 0.1 M NaClO$_4$)

		log $K,^a$ M =					
equilibrium		La^{3+}	Pr^{3+}	Nd^{3+}	Eu^{3+}	Gd^{3+}	Dy^{3+}
[BTP − H]/[BTP][H]	9.06^b						
[BTP − 2H]/[BTP][H]^2	15.87^b						
[BTP − M]/[BTP][M]		2.30					
[2BTP − 2M − 2OH]/[BTP]^2[M]^2[OH]^2		18.11	20.80	21.71	23.62	23.44	23.86
[2BTP − 2M − 4OH]/[BTP]^2[M]^2[OH]^4		28.96	31.66	32.77	34.95	34.90	38.12
[2BTP − 2M − 5OH]/[BTP]^2[M]^2[OH]^5		33.62	36.14	37.20	39.29	33.39	
[2BTP − 2M − 6OH]/[BTP]^2[M]^2[OH]^6						42.73	48.73
[M − OH]/[M][OH]^c		4.67	5.18	5.30	5.42	5.38	5.63

^{a} Error within ±(0.02−0.08). ^{b} Protonation constants for BTP. ^{c} Formation constants for monohydroxo complexes at 25 °C and ionic strength 0.3 M.[25a]

attempt to analyze our and other available kinetic data for esterolytic and phosphoesterolityc activities of metal−hydroxo complexes in terms of Brønsted correlations. Such correlations clearly show important differences in the activation modes of these two types of substrates.

Experimental Section

Materials. Bis(4-nitrophenyl) phosphate (Aldrich) was recrystallized from ethanol−water. 4-Nitrophenyl acetate (Aldrich) was recrystallized from acetic acid−ethyl acetate. *p*-Nitrophenyl phosphate di(Tris) salt, bis-Tris propane (1,3-bis[tris(hydroxymethyl)methylamino]propane), and Trizma base (tris[hydroxymethyl]aminomethane) from Sigma, NaClO$_4$, and reagent-grade lanthanide(III) perchlorates as 40 wt % aqueous solutions from Aldrich were used as supplied. The concentration of metal ions in stock solutions was determined by adding excess ethylenediaminetetraacetic acid and then back-titrating with ZnCl$_2$ with Eriochrome Black T as indicator.[35] Distilled and deionized water (Barnstead Nanopure system) was used.

Potentiometry. Potentiometric titrations were performed following the general recommendations given in ref 36. All titrations were performed in a 75-mL thermostated cell kept under nitrogen at 25 °C. The initial volume of titrating solution was 50 mL. The ionic strength was kept constant with 0.1 M NaClO$_4$. Measurements of pH were taken on an Orion Model 710-A research digital pH meter as carbonate-free NaOH solution was added to the system in small increments. Careful removal of carbonate and exclusion of CO$_2$ absorption during the titration are extremely important for obtaining reproducible titration curves. The electrode was calibrated from pH readings of 45 separate titration points of a titration of dilute (ca. 0.01 M) standardized HClO$_4$ with NaOH with the same background electrolyte. The results were fitted by using nonlinear least-squares regression in Origin 3.5 program to the equation

$$V + (V_0 + V)[H']f/C_B − (V_0 + V)K_W/(C_B[H']f) − V_e = 0$$

where V is the volume of titrant of concentration C_B added, V_0 is the initial volume of sample, K_W is the ionic product of water, V_e is the equivalence volume, [H'] is the uncorrected concentration of H$^+$ taken directly from pH measurements ([H'] = 10$^{−pH}$) and f is the correction factor, which is a function of activity coefficients and the junction potential.[37] The pK_w = 13.79 ± 0.04 was found under our conditions as one of the fitting parameters, and f was determined before each titration as the other one. The value −log f (typically in the range 0.1−0.2) was used to correct the pH meter scale, which gave after correction the values of −log [H$^+$] = pH − log f.[37] Thus, the electrode was calibrated in terms of proton concentration. The program Hyperquad 2000 Version 2.1 NT was used to calculate all equilibrium constants. Titrations of BTP and its mixtures with lanthanide salts were performed in the concentration range 5−20 mM of each component; 2 equiv of

HClO$_4$ per 1 mol of BTP was always added to the titrating mixture before the start of titration.

Kinetics. Kinetic measurements were performed by using a Hewlett-Packard 8453 diode-array spectrophotometer equipped with a Peltier thermostated cell compartment. Reaction solutions were prepared by combining appropriate amounts of metal and ligand stock solutions to the desired volume, and the pH was adjusted by adding small volumes of strong acid or base solutions as necessary. Reactions were initiated by adding an aliquot of the substrate solution. Special care was taken to avoid CO$_2$ absorption during reaction runs. The solution pH was measured after each run, and all kinetic runs in which pH variation was larger than 0.05 were excluded. To have sufficiently high buffer capacity of the solution and stability in respect of metal hydroxide precipitation in wide pH interval, the reaction kinetics was studied with excess of BTP over the lanthanide salt. It is worth noting that since BTP has two pK_a values around 7 and 9 (see Table 1) it has a wide buffer range between pH 6.5 and 9.5.

The course of BNPP, NPP, and NPA cleavage was monitored spectrophotometrically by appearance of 4-nitrophenolate anion at 400 nm. Stock solutions of BNPP and NPP were freshly prepared in water. Stock solutions of NPA were prepared in dry acetonitrile. Kinetic measurements used 40 μM substrate and varied concentrations of metal salts and BTP (used in appropriate pH intervals) at 25 °C. The observed first-order rate constants (k_{obs}) were calculated by the integral method or, for slow reactions, from initial rates.

NMR Spectroscopy. ^{1}H NMR spectra were recorded on a Varian Gemini 200 NMR spectrometer. The instrumental pH values measured in D$_2$O were corrected to the respective pD values with equation pD = pH + 0.4.[38]

Electrospray Ionization Mass Spectrometry. ESI-MS experiments were performed on VG Platform II quadrupole mass spectrometer with electrospray ionization source. Samples were run by direct infusion from an Harvard Apparatus Model 22 syringe pump at a rate of 20 μL/min. Data were collected and analyzed on VG Masslynx software running on a Pentium PC.

Results and Discussion

Complexation Equilibria in Lanthanide(III)−BTP System. Figure 2 shows pH titration curves for 10 mM BTP and for 10 mM BTP to lanthanide(III) perchlorate 1:1 mixtures in the presence of 2 equiv of HClO$_4$ (Table S1 in the Supporting Information gives the numerical titration results for all metals). Fitting of the titration curve for BTP gives pK_{a1} = 6.83 ± 0.02 for BTPH$_2^{2+}$ and pK_{a2} = 9.06 ± 0.02 for BTPH$^+$ in good agreement with published values (pK_{a1} = 6.75 and pK_{a2} = 9.10).[39] Titration curves in the presence of 1 equiv of metal salt show consumption of more than 2 equiv of NaOH per 1 equiv of metal. In the case of La(III), precipitation occurs at pH above 9, but titration with excess BTP (2:1 BTP to metal)

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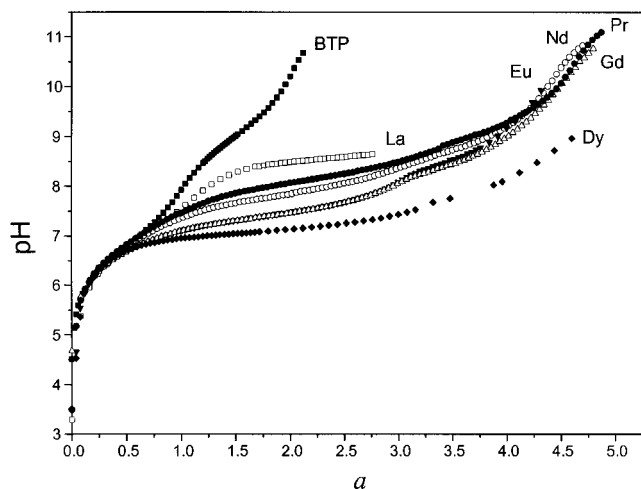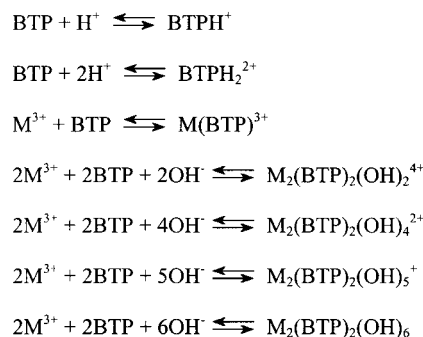
Figure 2. Titration curves of 10 mM BTP and 10 mM 1:1 BTP/lanthanide perchlorate mixtures in 0.1 M NaClO$_4$. Two equivalents of HClO$_4$ was added at the beginning of each titration. a is the number of added NaOH equivalents.

allowed us to reach pH 10 and observe consumption of ca. 2.5 equiv of NaOH per 1 equiv of La(III) as with other metals. These results indicate formation of metal hydroxo complexes or deprotonation of hydroxymethyl groups of BTP affording respective alkoxo complexes. In previous studies on lanthanide(III)−amino alcohol and polyol systems, this ambiguity was not resolved.[11,13] In some cases, the product analysis shows formation of phosphorylated alcohol ligand in the reaction of BNPP cleavage, which indicates probable alcohol deprotonation.[12] In our case, the product analysis showed the formation of only inorganic phosphate in the reaction with BNPP.[26] Therefore, on this criterion more probable is the existence of hydroxo complexes. To get more insight on this problem, ^{1}H NMR pH titration of BTP in the presence of La(ClO$_4$)$_3$ was performed. We determined on the basis of observation that deprotonation of the Zn(II)-bound alcohol group in a Zn(II) macrocyclic complex with pendent alcohol group induced considerable (ca. 0.3 ppm) upfield shift of the signals of methylene protons in a −CH$_2$OH group,[29] and we expected to have qualitatively similar effects in case of La(III) also. Figure 3 shows chemical shifts vs pD profiles in D$_2$O corresponding to the singlet of methylene protons in the α-position with respect to the OH group (Figure 3a) and central signals of multiplets of methylene protons of the propylene chain in α- and β-positions to nitrogen atom of BTP (Figure 3b,c, respectively) in the absence and in the presence of La(ClO$_4$)$_3$. Titration curves obtained in the absence of lanthanum salt were fitted to eq 1

$$\delta_{obs} = (\delta_{BTP} + \delta_{BTPH}[D^+]/K_{a2} + \delta_{BTPH2}[D^+]^2/K_{a1}K_{a2})/$$
$$(1 + [D^+]/K_{a2} + [D^+]^2/K_{a1}K_{a2}) \quad (1)$$

where δ_{BTP}, δ_{BTPH}, and δ_{BTPH2} are chemical shifts of a given proton in neutral, mono- and diprotonated BTP respectively and K_{a1}, K_{a2} are the first and second acid dissociation constants of protonated forms of BTP. The fitting of experimental profiles for chemical shifts of different BTP protons gave mutually agreed values of dissociation constants. The average values are pK_{a1} = 7.55 ± 0.03 for BTPD$_2^{2+}$ and pK_{a2} = 9.81 ± 0.03 for BTPD$^+$. Larger pK_a values in D$_2$O solvent as compared to H$_2$O agree with known H/D isotope effects on acid dissociation constants.[40] Points for solutions containing lanthanum perchlorate deviate from the titration curves of free ligand in the same

Scheme 1

$$BTP + H^+ \rightleftharpoons BTPH^+$$

$$BTP + 2H^+ \rightleftharpoons BTPH_2^{2+}$$

$$M^{3+} + BTP \rightleftharpoons M(BTP)^{3+}$$

$$2M^{3+} + 2BTP + 2OH^- \rightleftharpoons M_2(BTP)_2(OH)_2^{4+}$$

$$2M^{3+} + 2BTP + 4OH^- \rightleftharpoons M_2(BTP)_2(OH)_4^{2+}$$

$$2M^{3+} + 2BTP + 5OH^- \rightleftharpoons M_2(BTP)_2(OH)_5^+$$

$$2M^{3+} + 2BTP + 6OH^- \rightleftharpoons M_2(BTP)_2(OH)_6$$

pH range where the deviation is observed in the potentiometric pH titration curve (cf. Figure 2). Considerable downfield instead of upfield shifts of the signal of methylene protons in α-position to OH group agrees with a complex structure in which OH groups are bound to the metal ion without deprotonation. Much smaller also downfield shifts of signals of protons of propylene chain indicate that nitrogen atoms are also involved in the ligand binding.

A simple reaction scheme, which follows from the potentiometric titration results would involve formation of a series of mixed mononuclear hydroxo complexes of general composition M(BTP)(OH)$_n^{3-n}$ with n ranging from 1 to 3. However, fitting of the results to such a scheme was statistically unsatisfactory. Satisfactory fitting was obtained for a more complex scheme involving formation of dinuclear hydroxo complexes presented by the set of equilibria shown in Scheme 1 (Figure 1Sa,b in the Supporting Information illustrates the fitting quality). Logarithms of the equilibrium constants for each step are collected in Table 1. They represent the mean values of the constants obtained by titrations of lanthanide(III)−BTP mixtures at different total concentrations, which reasonably agreed with each other. Surprisingly, only for lanthanum the simple 1:1 metal to BTP complex contributes sufficiently to allow us the determination of its stability constant. For all other cations the stability of hydroxo complexes is so high that at pH close to 8 and above, where M(BTP)$^{3+}$ complexes should contribute significantly to the total metal concentration (assuming similar to La(III) stability constants for 1:1 complexes of other metals), the first of hydroxo complexes M$_2$(BTP)$_2$(OH)$_2^{4+}$ already becomes the dominating species (see also distribution curves in Figure 5). The stability constant of La(BTP)$^{3+}$ complex (Table 1) is close to that reported for Eu(Tris)$^{3+}$ (log K = 2.44).[41] This agrees with idea that BTP uses for coordination with lanthanides only half of its molecule which is chemically similar to Tris (see, however, below). Much higher stability (log K = 4.7) was reported for La(bis-Tris)$^{3+}$ complex,[9] but this ligand provides two additional hydroxyethyl groups for the binding to metal.

Lanthanides have the very strong tendency to form polymeric complexes in water.[17] Formation of dinuclear dihydroxo species M$_2$(OH)$_2^{4+}$ is known for all lanthanides(III) used in this work.[25] The La$_2$(OH)$_2^{4+}$ unit is formed also in a dinuclear macrocyclic lanthanum complex.[17] The potentiometric titration of LaCl$_3$ with alkali revealed formation of La$_2$(OH)$_5^+$ species,[7] relevant to M$_2$(BTP)$_2$(OH)$_5^+$ complexes in Scheme 1. Complexation with BTP stabilizes such species preventing formation of complexes

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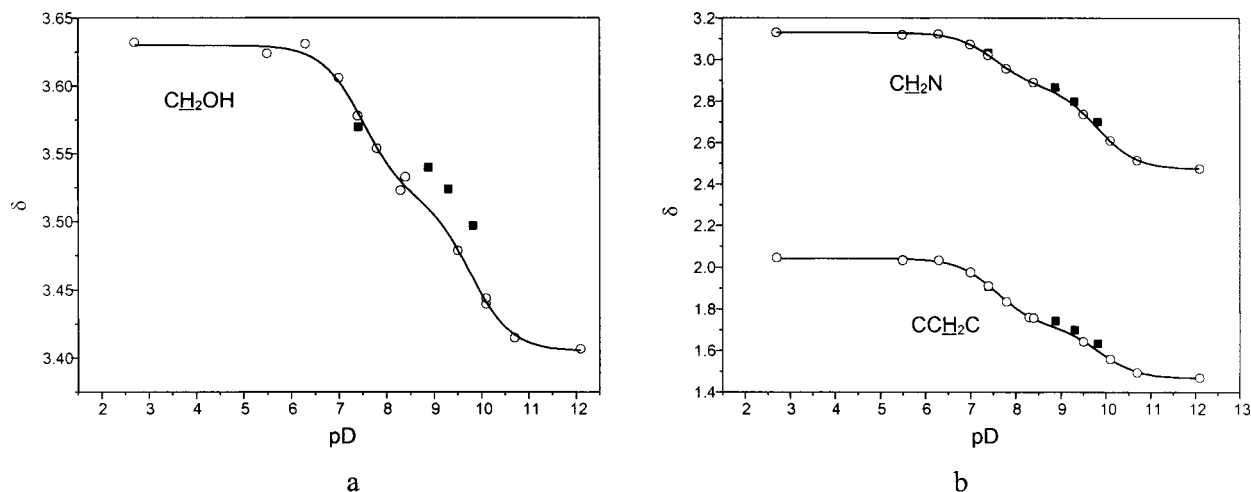
Figure 3. ^{1}H NMR pH titration of 20 mM BTP in D$_2$O. Open circles and solid squares show measurements in the absence and in the presence of 5 mM La(ClO$_4$)$_3$ respectively. Solid lines are the fitting curves to eq 1.
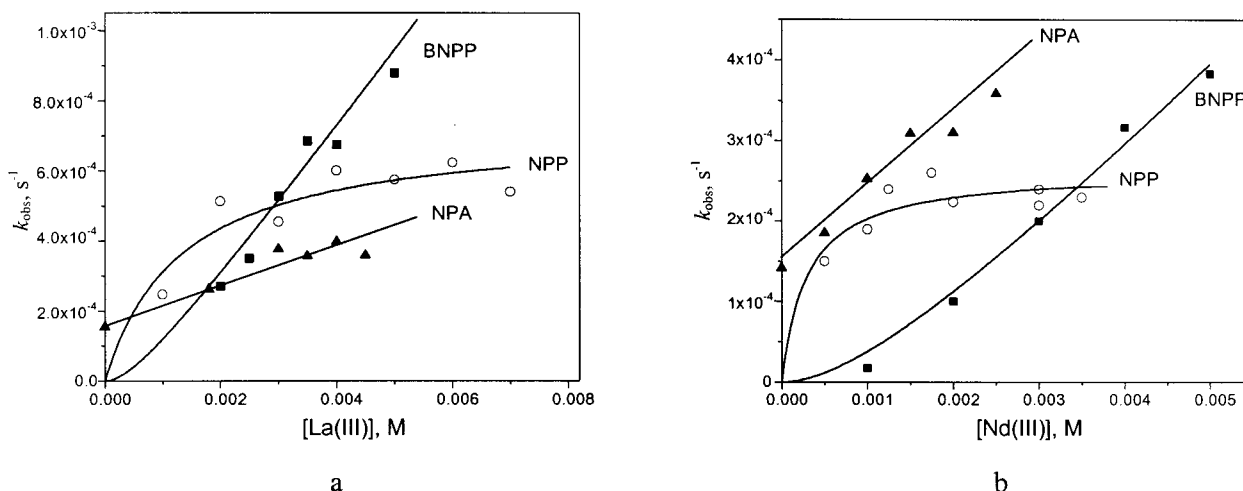

Figure 4. Observed first-order rate constants for the hydrolysis of BNPP, NPP, and NPA vs metal concentrartion: (a) lanthanum in the presence of 20 mM BTP, pH 9.0; (b) neodimium in the presence of 20 mM BTP, pH 8.5. Data for BNPP at metal concentrations above 2 mM were fitted to eq 3 with the rate constants given in Table 3; at lower concentrations, a deviation to the second-order kinetics was observed; see the text. Data for NPP were fitted to eq 4. Data for NPA showed a simple linear dependence.

of higher nuclearity and eventually the metal hydroxide precipitation. A hypothetical structure **1**, which takes into

1
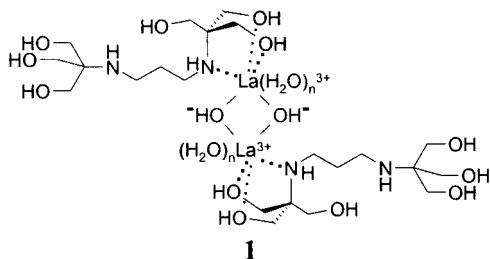
account discussed above mode of BTP binding, can be proposed for the M$_2$(BTP)$_2$(OH)$_2{}^{4+}$ complexes. One should expect similar behavior also with Tris as a ligand, but titrations of Tris–lanthanide mixtures always lead to precipitation of metal hydroxide at pH above 8. Therefore, at least some additional binding of the second half of BTP molecule to metal cation probably occurs and explains difference between Tris and BTP. Deprotonation of bound to metal water molecules in **1** leads to formation of higher hydroxo complexes shown in Scheme 1.

To get some additional information on types of species presenting in BTP–lanthanum mixtures in basic solutions, the

electrospray ionization mass spectra of 1 mM solution of lanthanum nitrate and 20 mM BTP at pH 9.2 were recorded. In several instances, ESI-MS has been applied successfully to the characterization of metal complexes.[42] This technique produces the gas-phase ions directly from solution with little or no fragmentation thus providing valuable qualitative information on the nature of charged species existing in solution. Of course, additional processes may occur in the gas phase leading to observation of so-called "false positives", i.e., species, which are formed only under the gas-phase conditions and do not exist in solution.[43]

Mass spectra of pure ligand solution at pH 9.2 consisted of a series of peaks corresponding to protonated BTP oligomers (BTP)$_n$H$^+$ where $n = 1-4$ and a sodium cationized peak at $m/z = 305.1$ (Table 2, entries 1–5). Certainly, only BTPH$^+$ cation really exists in solution while the sodium adduct and oligomers are formed in the gas phase. In the presence of lanthanum nitrate, eight additional peaks identified as shown in Table 2 (entries 6–13) were observed. In addition, two

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(43) Cunniff, J. B.; Vouros, P. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 437.
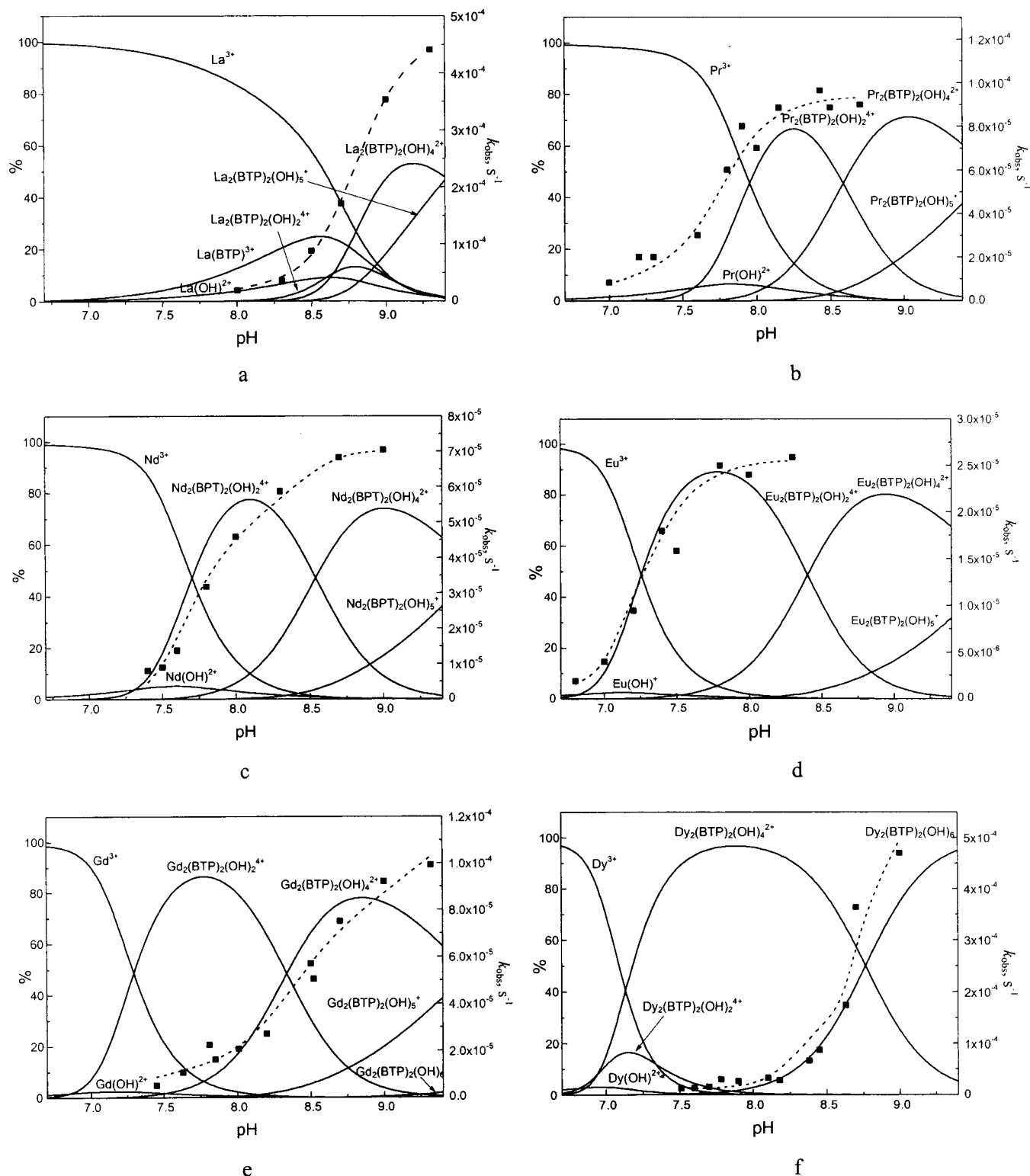
Figure 5. Observed rate constants for the BNPP hydrolysis at 25 °C (solid squares) and species distribution diagrams for hydroxo complexes (Scheme 1) for 2mM lanthanides(III) and 20 mM BTP. Dash lines are the theoretical k_{obs} vs pH profiles calculated in accordance with eq 3 and rate constants for the individual hydroxo complexes given in Table 2.

unidentified peaks at $m/z = 365.1$ and 409.2 were observed, which probably belong to sample impurities. Peaks at $m/z = 351.1$, 419.1, and 482.0 by their isotopic structure belong to dicationic species. Three peaks (Table 2, entries 7, 8, and 11) correspond to dimeric complexes and others to monomeric species, all of which contain single or doubly deprotonated BTP ligand. This may speak in favor of ligand deprotonation rather than formation of hydroxide complexes in solution, but it is

highly probable that the gas-phase conditions favor the dehydration of complexes converting a $M(OH^-)(ROH)$ fragment into $M(RO^-) + H_2O$. Indeed, in contrast to aqueous phase, the gas-phase basicity of OH^- is much higher than that of RO^-; i.e., the proton-transfer reaction $OH^-_{gas} + EtOH_{gas} \rightarrow EtO^-_{gas} + H_2O_{gas}$ is very exothermic with the energy change of -56 kJ/mol.[44] We believe therefore that the more probable route to these species is the gas-phase dehydration of hydroxide complexes.

Table 2. Calculated and Observed m/z Values for BTP + La(NO$_3$)$_3$ System at pH 9.2

	species	formula	calcd m/z	obsd m/z
1	BTP H$^+$	C$_{11}$H$_{27}$N$_2$O$_6$	283.192	283.2
2	BTP Na$^+$	C$_{11}$H$_{26}$N$_2$O$_6$Na	305.174	305.1
3	BTP$_2$ H$^+$	C$_{22}$H$_{53}$N$_2$O$_6$	565.376	565.4
4	BTP$_3$ H$^+$	C$_{33}$H$_{79}$N$_2$O$_6$	847.560	847.5
5	BTP$_4$ H$^+$	C$_{44}$H$_{105}$N$_2$O$_6$	1129.744	1129.7
6	La(BTP H$_{-1}$)(BTP)$^{2+}$	C$_{22}$H$_{51}$N$_4$O$_{12}$La	351.133	351.1
7	La$_2$(BTP H$_{-2}$)$_2$$^{2+}$	C$_{22}$H$_{48}$N$_4$O$_{12}$La$_2$	419.074	419.1
8	La$_2$(BTP H$_{-1}$)$_2$(NO$_3$)$_2$$^{2+}$	C$_{22}$H$_{50}$N$_6$O$_{18}$La$_2$	482.070	482.0
9	La(BTP H$_{-2}$)(BTP)$^+$	C$_{22}$H$_{50}$N$_4$O$_{12}$La	701.258	701.2
10	La(BTP H$_{-1}$)(BTP)(NO$_3$)$^+$	C$_{22}$H$_{51}$N$_5$O$_{15}$La	764.254	764.3
11	La$_2$(BTP H$_{-2}$)$_2$(NO$_3$)$^+$	C$_{22}$H$_{48}$N$_5$O$_{15}$La$_2$	900.156	900.1
12	La(BTP H$_{-2}$)(BTP)$_2$$^+$	C$_{33}$H$_{76}$N$_6$O$_{18}$La	983.442	983.4
13	La(BTP H$_{-1}$)(BTP)$_2$(NO$_3$)$^+$	C$_{33}$H$_{77}$N$_7$O$_{21}$La	1046.438	1046.4

The most abundant species in the solution used for ESI-MS measurements is La$_2$(OH)$_4$(BTP)$_2$$^{2+}$ (see the species distribution diagram in Figure 5a) which after dehydration affords La$_2$(BTP H$_{-2}$)$_2$$^{2+}$ (Table 2, entry 7). Also is detected an adduct of this cation with nitrate (Table 2, entry 11). Another dimeric species La$_2$(BTP H$_{-1}$)$_2$(NO$_3$)$_2$$^{2+}$ (Table 2, entry 8) is probably formed by dehydration and subsequent nitrate capture of the dihydroxo complex La$_2$(OH)$_2$(BTP)$_2$$^{4+}$. The amount of mononuclear lanthanum complexes La(BTP)$^{3+}$ and La(OH)$^+$ in solution is still significant at the conditions employed. Therefore, monomeric gas-phase ions may be produced by exchange and dehydration reactions between these monomeric solution species. On the other hand, with high excess of BTP over La(III) the dinuclear gas phase ions may undergo cleavage to monomers. The mutual electrostatic repulsion of highly charged La^{3+} ions inside the dinuclear complexes should be much stronger in the gas phase than in water and the relative stability of dinuclear complexes should be lower. Thus, cations La(BTP H$_{-2}$) (BTP)$^+$ and La(BTP H$_{-1}$)(BTP)(NO$_3$)$^+$ may be formed by the cleavage of La$_2$(BTP H$_{-2}$)$_2$$^{2+}$ and La$_2$(BTP H$_{-1}$)$_2$ (NO$_3$)$_2$$^{2+}$, respectively, by BTP and formation of other monomeric cations is readily explicable by subsequent capturing or loss of BTP and nitrate. In general, ESI-MS of lanthanum−BTP system shows a complicated pattern, but it supports the presence of dinuclear complexes with metal:ligand ratio 2:2 proposed on the basis of potentiometric titration.

Kinetics of BNPP Hydrolysis. It has been shown previously that the products of BNPP hydrolysis in the BTP−lanthanide system are 1 equiv of inorganic phosphate and two equivalents of 4-nitrophenol.[26] Depending on reaction conditions, absorbance A vs time t profiles monitored at the absorption maximum of 4-nitrophenolate anions followed simple first-order kinetics, when hydrolysis of NPP intermediate was fast, or more complex two-exponential eq 2 (ϵ_{NP} and [BNPP]$_0$ are the molar absorptivity of p-nitrophenolate and initial substrate concentration respectively), when the observed first-order rate constants k_1 and k_2 of BNPP and NPP hydrolysis are of similar magnitude.

$$A = \epsilon_{NP}[\text{BNPP}]_0\{2 + (k_1 e^{-k_2 t} + (k_1 - 2k_2)e^{-k_1 t})/(k_2 - k_1)\} \quad (2)$$

Discussed below observed first-order rate constants for the BNPP hydrolysis refer to k_1 values in the eq 2.

The reaction rate grows rapidly on increase in pH above 7 and tends to level off at pH 9. The reaction order in metal was studied in the interval of pH 8.5−9. Observed first-order rate

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constants (k_{obs}) of BNPP hydrolysis for all lanthanides employed are linear functions of metal concentration in the range above 2 mM, but with La(III) and Nd(III) at lower metal concentrations they show approximately quadratic dependence on total metal concentration (see Figure 4a,b). Reaction is strongly inhibited by NaClO$_4$: increase in total (buffer plus added salt) perchlorate concentration from 0.01 to 0.1 M leads to ca. 10-fold decrease in the reaction rate. Additions of NaCl or NaNO$_3$ caused a much smaller effect, indicating possible specific ion-pairing of perchlorate anion with positively charged lanthanide reactive species. This effect is responsible for a strong inhibition of the reaction rate by increased BTP concentrations at constant pH adjusted with HClO$_4$ without background NaClO$_4$ that was erroneously interpreted previously[26] as being due to formation of higher inactive complexes.

To analyze pH profiles of k_{obs}, the species distribution diagrams were calculated for the conditions of kinetic experiment in accordance with equilibrium constants given in Table 1, and experimental values of k_{obs} were plotted on these diagrams as shown in Figure 5. Inspection of Figure 5 reveals that the reactivity always increases on going from aquo ions to hydroxo complexes, as observed in other related systems. [7,9,11,12,17,22,24,27,29−33] For a given metal, the observed rate constant does not correlate with concentration of a single species, but there are some species that give the major contribution to the observed reactivity. On going to heavier and more electrophilic cations from La(III) to Dy(III), progressively higher dinuclear hydroxo complexes provide major contributions to the reactivity. Thus, if for Pr(III) the k_{obs} vs pH profile goes closely to the distribution curve for M$_2$(BTP)$_2$(OH)$_2$$^{4+}$ (Figure 5b), for Dy(III) this profile is shifted to the distribution curve for M$_2$(BTP)$_2$(OH)$_6$ (Figure 5f). There is also a contribution, most clearly seen in Figure 5a,b for La(III) and Pr(III), of monohydroxo mononuclear complexes, which probably are the sole reactive species in studies with lanthanide(III) aquo ions in neutral solutions. Analysis of the species distribution curves calculated at different metal concentrations shows also that at pH values between 8.5 and 9 the dinuclear complexes constitute more than 90% of total metal concentration in the range above 2 mM for all metals in agreement with observed first-order kinetics in these conditions. At the same time, for La(III), Pr(III), and Nd(III) there is a considerable contribution of mononuclear species below 1 mM concentration, that also agrees with the change of the reaction order to two at low concentrations of these metals (see above).

Second-order rate constants for BNPP hydrolysis by individual species were estimated by using the multiple regression on pH-dependence of k_{obs} in accordance with eq 3:

$$k_{obs} = k_1[\text{M(OH)}^{2+}] + k_2[\text{M}_2(\text{BTP})_2(\text{OH})_2^{4+}] + \\ k_3[\text{M}_2(\text{BTP})_2(\text{OH})_4^{2+}] + k_4[\text{M}_2(\text{BTP})_2(\text{OH})_5^{+}] + \\ k_5[\text{M}_2(\text{BTP})_2(\text{OH})_6] \quad (3)$$

Concentrations of hydroxo complexes were calculated from known total metal and BTP concentrations at each pH value and used as independent variables in eq 3. The resulting rate constants k_1-k_5 are given in Table 3. Missing rate constants for some species result from insignificant contributions of such species in the observed rate constant under conditions employed. In some cases, e.g., for k_1 values for Eu(III) and Dy(III), the standard deviations of mean values calculated from the multiple regression were very large, but exclusion of the respective term by setting $k_1 = 0$ led to a considerable decrease in fitting quality. Therefore, we included in Table 3 these and some other rate

Table 3. Rate Constants for the BNPP Hydrolysis by Different Lanthanide(3+) Complexes with BTP at 25 °C

	La(III)	Pr(III)	Nd(III)	Eu(III)	Gd(III)	Dy(III)
k_1, M^{-1} s^{-1}	0.26 ± 0.07	0.26 ± 0.09		0.038 [a]		0.81 [a]
k_2, M^{-1} s^{-1}	0.140 ± 0.075	$(3.7 \pm 1.2) \times 10^{-2}$	$(2.5 \pm 0.2) \times 10^{-2}$	$(1.1 \pm 0.1) \times 10^{-2}$	$(5.6 \pm 3.0) \times 10^{-3}$	
k_3, M^{-1} s^{-1}	0.195 ± 0.030	$(5.4 \pm 1.8) \times 10^{-2}$	$(4.4 \pm 0.4) \times 10^{-2}$	$(1.7 \pm 0.2) \times 10^{-2}$	$(3.5 \pm 0.3) \times 10^{-2}$	2.5×10^{-3} [a]
k_4, M^{-1} s^{-1}	0.275 ± 0.040		3.4×10^{-3} [a]		$(8.5 \pm 1.1) \times 10^{-2}$	
k_5, M^{-1} s^{-1}						0.33 ± 0.03

[a] Relative error in this constant is ca. 100%, but its exclusion leads to a substational decrease in the fitting quality.

Table 4. Parameters for the Rate Eq 4 for the NPP Hydrolysis by Lanthanide(3+) Complexes with BTP at pH 9.0 and 25 °C[a]

	La(III)	Pr(III)	Nd(III)	Eu(III)	Gd(III)	Dy(III)	Dy(III)[b]	Dy(III)[c]
k_C, s^{-1}	7.3×10^{-4}	2.5×10^{-4}	2.9×10^{-4}	1.5×10^{-4}	1.5×10^{-4}	1.5×10^{-3}	2.6×10^{-4}	1.6×10^{-4}
K_S, M^{-1}	750	>5000	1690	1760	3500	220	530	830

[a] Relative errors in k_C and K_S are within 20–30%. [b] pH 8.5. [c] pH 8.0.

constants with similarly large standard deviations considering them as approximate estimates.

Comparisons of k_1-k_5 values for any given metal show that among dinuclear complexes the reactivity increases with increase in number of bound OH$^-$, but the most reactive species are mononuclear monohydroxo complexes. The actual contribution of these complexes to k_{obs} is small, however, because the fraction of these species is very small; see Figure 5. Increased reactivity of dinuclear complexes possessing larger numbers of bound OH$^-$ anions parallels the situation with dinuclear Zn(II)[29,30] and La(III)[17] hydroxo complexes with macrocyclic ligands: for both metals M$_2$(OH)$_2$ species are more reactive than M$_2$(OH) complexes. Apparently, OH$^-$ anions in the higher complexes are more basic and may function as stronger nucleophiles toward phosphorus(V). When complexes of the same composition but with different metals are compared, their reactivities decrease on going from La(III) to Dy(III) (see trends in k_2, k_3, and k_4 values in Table 3, which refer to complexes M$_2$(BTP)$_2$(OH)$_2^{4+}$, M$_2$(BTP)$_2$(OH)$_4^{2+}$, and M$_2$(BTP)$_2$(OH)$_5^+$, respectively). In this series, the size of lanthanide(III) cations decreases leading to an increase in the charge density and electrophilicity of heavier cations. This should lead to a decrease in basicity of bound hydroxide anions. Thus, complexes with more electrophilic cations and less basic OH$^-$ anions possess lower reactivity. Therefore, within this series of metals in dinuclear complexes, the basicity of bound OH$^-$ appears to be more important than the electrophilicity of lanthanide cation, although the effect is rather moderate.

While reactivities of M$_2$(BTP)$_2$(OH)$_2^{4+}$ and M$_2$(BTP)$_2$-(OH)$_4^{2+}$ complexes decrease monotonically on going from La(III) to Dy(III), the observed first-order rate constants at a given metal concentration and at pH about 9 follow a different order: La > Pr > Nd > Eu < Gd < Dy. This happens because at this pH heavier lanthanides generate larger fractions of higher and more reactive hydroxo complexes. Thus, more electrophilic metal cations deactivate bound OH$^-$ by reducing too strongly its basicity, but this deactivating effect is compensated by ability of such cations to form higher hydroxo complexes in which bound OH$^-$ anions are more basic.

Kinetics of NPP and NPA Hydrolysis. Kinetics of the hydrolysis of these substrates were studied in less details mainly for comparative purposes. Hydrolysis of both substrates followed simple first-order kinetics in the presence of a high excess of metal. Rate constants found for NPP agreed well with k_2 values found from the fitting of the kinetic curves for BNPP hydrolysis to eq 2 under similar conditions. Generally, the reactivities of lanthanide BTP hydroxo complexes toward NPP are similar to those toward BNPP, but in contrast to BNPP, dependences of k_{obs} on metal concentration in this case clearly show a
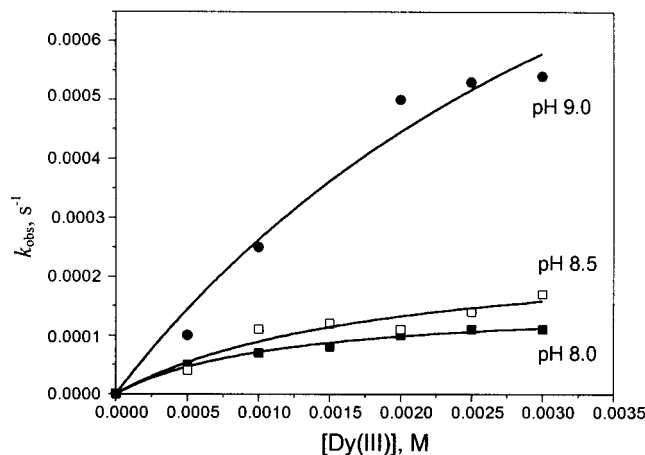

Figure 6. Observed first-order rate constants of NPP hydrolysis vs concentration of dysprosium(III) in the presence of 20 mM BTP at different pH values.

"saturation" behavior exemplified in Figure 4a,b for La(III) and Nd(III). Results for all metals were fitted to the Michaelis–Menten-type eq 4

$$k_{obs} = k_C K_S[M]/(1 + K_S[M]) \qquad (4)$$

where K_S is the formation constant of an intermediate substrate–lanthanide complex, k_C is the first-order rate constant for the hydrolysis of NPP inside this complex (catalytic constant), and [M] is the total metal concentration. The values of k_C and K_S for different lanthanides at pH 9 are given in Table 4. Both parameters are purely apparent constants, which refer to the total metal concentration. No attempt was made to separate contributions of individual species, but a qualitative discussion given below points out some essential features of the reaction mechanism.

Both the catalytic and the intermediate complex formation constants do not show regular trends within the lanthanide series. This is not unexpected because for different metals different species participate in the reaction at a given pH. Indeed, parameters of the eq 4 determined at different pH values are significantly different. In Figure 6, the plots of k_{obs} vs metal concentration for Dy(III) at different pH values are shown and kinetic parameters found from the fitting of these results to the eq 4 are given in Table 4. The intermediate formation constant decreases and the catalytic constant increases on going to lower pH values where more highly charged species predominate (cf. Figure 5f). Both tendencies are expectable, as a matter of fact, because NPP dianion should interact stronger with species bearing higher positive charge and the reactivity should be
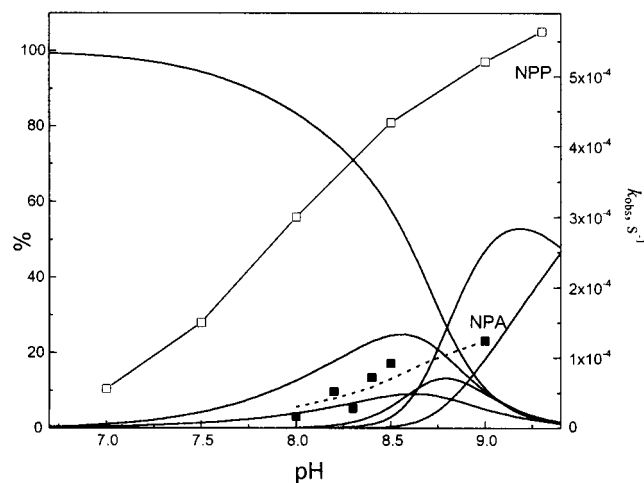
Figure 7. Observed first-order rate constants for the hydrolysis of NPP (open squares) and NPA (solid squares) in the presence of 2 mM lanthanum(III) and 20 mM BTP as function of pH. Solid lines are the species distribution curves (see Figure 5a). Dashed line is the theoretical profile calculated in accordance with eq 3 with the rate constants for the individual species given in the text.

higher for the species with larger number of bound hydroxide ions by analogy with BNPP hydrolysis. Profiles of k_{obs} vs pH for NPP hydrolysis show an increase in k_{obs} on increase in pH, like in the case of BNPP, but are much more flat. As an illustration, the plot of k_{obs} vs pH for La(III) is shown in Figure 7 together with the species distribution curves. This and other similar plots for other lanthanides cannot be interpreted in terms of eq 3, which implies the first-order kinetics in respect of the metal complexes. One can say, however, that complexes with larger number of bound hydroxide anions are more reactive toward NPP like in the case of BNPP.

The observation of saturation kinetics with NPP, but first-order kinetics with BNPP with the same reactive species reflects better ligating properties of the former substrate, which is a dianion capable, in principle, to metal chelation in contrast to monoanionic BNNP.[1a] Low affinity of phosphate diesters to metal cations, as well as increased affinity of phosphate monoesters is well documented.[1a,44−47] For example, stability constants for binding of BNPP and $(PhO)_2PO_2^-$ to Cu(ter-pyridine)$^{2+}$ equal 15.8 M^{-1} and 316 M^{-1}, respectively.[47] However, these values were determined in 75% EtOH solvent, which favors ionic association. For Zn([12]aneN$_3$)$^{2+}$ in water log K < 0.5 with BNPP, but log K = 3.1 with NPP.[1a] Under our conditions, strictly first-order kinetics in metal with BNPP as the substrate is observed at concentrations of lanthanides below 10 mM which means that the substrate-metal binding constants should be about or below 10 M^{-1}. Also, no "saturation" in metal concentration up to 4 mM was observed for BNPP hydrolysis in the presence of lanthanide(III) cryptates.[8] Nevertheless, in several systems "saturation" kinetics of BNPP cleavage with K_S values in the range 10^2−10^3 M^{-1} similar to those observed in our system with NPP as the substrate were reported.[13,14,27,48] The reasons for the unusually high affinity of phosphate diesters to metal centers in these systems are not clear. It should be taken into account, however, that the K_S value calculated from eq 4 or its modifications from kinetic data may be or may be not equal to the true equilibrium binding constant

depending on values of rate constants of subsequent transformations of the bound substrate.[49] Also, a self-aggregation of lanthanide species on increase in total metal concentration with formation of nonreactive polynuclear complexes may lead to the apparent "saturation" behavior.

Observed first-order rate constants of the hydrolysis of a carboxylic acid ester NPA were linear functions of lanthanide(III) concentration, as illustrated in Figure 4a,b for La(III) and Nd(III). This substrate is 10^6 times more reactive than BNPP in the alkaline hydrolysis: second-order rate constants of the alkaline hydrolysis (k_{OH}) at 25 °C are 9.5 M^{-1} s^{-1} for NPA [50] and 5.8×10^{-6} M^{-1} s^{-1} for BNPP. [51] Due to the fast alkaline hydrolysis of NPA, the rate of spontaneous reaction at pH about 9 is rather high for this substrate in contrast to BNPP and NPP for which the spontaneous hydrolysis does not contribute at all to the observed rate in the presence of lanthanides, Figure 4a,b. Surprisingly, the rate of lanthanide-catalyzed hydrolysis of NPA is similar to that of BNPP and NPP. Only reaction with La(III) was studied in detail. Figure 7 shows the dependence of the observed first-order rate constant of NPA hydrolysis in the presence of 2 mM La(III) and 20 mM BTP corrected for the rate of spontaneous reaction. The profile is similar to that observed for BNPP, Figure 5a, and fitting of these results to the eq 3 shows that in the given pH interval major contributions are from La(OH)$^{2+}$ and La$_2$(BTP)$_2$(OH)$_4^{2+}$ with the second-order rate constants k_1 = 0.37 M^{-1} s^{-1} and k_3 = 0.092 M^{-1} s^{-1}. From these results, we see that OH$^-$ coordinated to La(III) has higher reactivity in BNPP hydrolysis than free OH$^-$ (k(LaOH)/k(OH) = 4.4 × 10^4), but with NPA as the substrate coordination to La(III) only deactivates OH$^-$ (k(LaOH)/k(OH) = 0.039). This comparison clearly shows that the catalytic effect of lanthanides in the hydrolysis of a carboxylic acid ester results only from decrease of pK_a of coordinated water molecules, which makes hydroxide ions available at low pH although with reduced intrinsic reactivity, but catalysis of the phosphate ester hydrolysis involves certain activation of the substrate by metal cations.

Analysis of the Reaction Mechanism in Terms of Brønsted Correlation and Transition-State Stabilization. The role of metal ions in the mechanism of hydrolysis of phosphate esters may be discussed in terms of three effects, which take place in the first coordination sphere of the metal:[1f] (1) Lewis acid activation of the substrate via coordination of phosphoryl oxygens to the metal, (2) nucleophile (OH$^-$ or RO$^-$) activation via coordination to the metal, which brings nucleophile in the close proximity to the phosphoryl group, (3) leaving group activation, which involves decrease in pK_a of the leaving alcohol. The first and third effects require possibly high Lewis acidity of the metal cation, which agrees with the fact that generally most active are complexes of highly charged metal cations (see the Introduction). However, the second effect requires the bound nucleophile to conserve its reactivity, which for "hard" reaction sites, like phosphoryl and carbonyl groups, generally correlates with the nucleophile Brønsted basicity. Therefore, too strong decrease in pK_a of the conjugated acid of bound nucleophile may deactivate it considerably and a certain optimum in metal Lewis acidity should exist for the optimum catalytic activity. The basicity of bound nucleophile was considered as an important factor in the reactivity of hydroxo complexes,[1c,27] and

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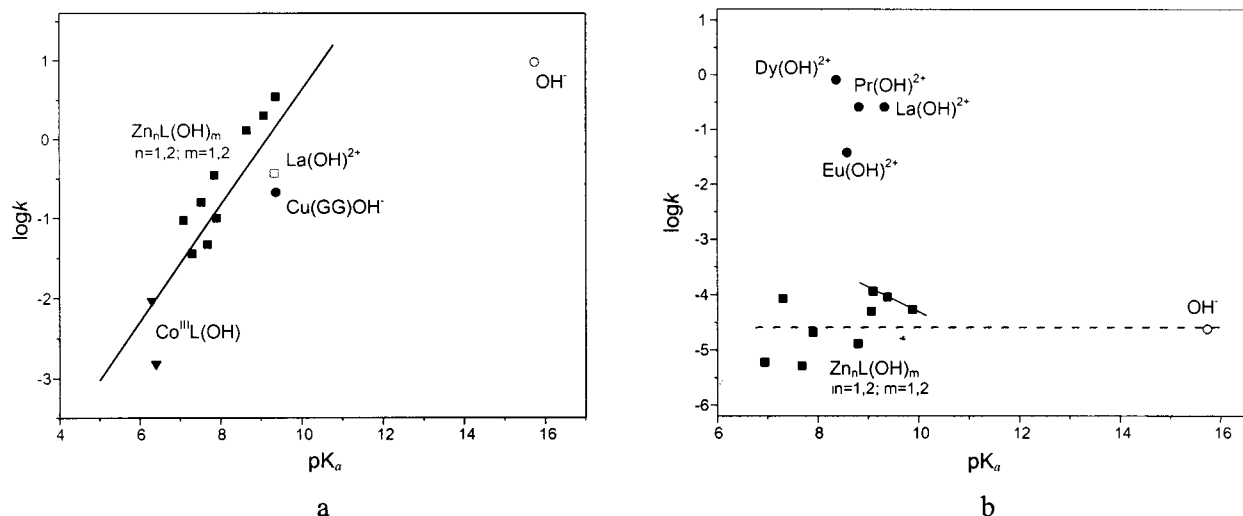
a

b

Figure 8. Second-order rate constants for the cleavage of NPA (a) and BNPP (b) by metal hydroxo complexes vs pK_a of their conjugated acids. Data for NPA are from refs 54 (Co(III) complexes), 1a and 30 (mononuclear and dinuclear Zn(II) complexes with macrocyclic ligands), 58 (Cu(GlyGly)OH$^-$), this work (La(OH)$^{2+}$), and 50 (OH$^-$), all at 25 °C. Data for BNPP are from refs 30, 31, 33, and 59 (Zn(II) complexes and OH$^-$ at 35 °C) and this work (Ln(OH)$^{2+}$ at 25 °C).

our present results also demonstrate higher reactivity of lanthanide complexes with more basic hydroxide. On the other hand, it was demonstrated that within a series of Co(III) complexes with different amine ligands the reactivity in BNPP hydrolysis varies depending on the ligand structure by several orders of magnitude while pK_a of coordinated water remains constant.[52] Thus, basicity of bound hydroxide cannot be the sole factor, but it also cannot be ignored.

Commonly, the search of an interrelation between basicity and reactivity is based on analysis of the Brønsted correlation between logarithms of the rate constants and pK_a values of the conjugated acids of nucleophiles. Such correlations for Zn(II)-catalyzed NPA and BNPP hydrolysis were discussed in qualitative terms.[1a] Here, we present a quantitative treatment on an extended database including lanthanides(III) (see Table 2S in the Supporting Information). The Brønsted correlation with the slope close to unity was demonstrated for the cleavage of NPA by organic nucleophiles of different types.[50] The points of this general correlation are rather scattered, but when nucleophiles of only one type are considered the correlation becomes good. In particular, for oxygen nucleophiles RO$^-$ the Brønsted correlation is given by eq 5.[53]

$$\log k(\text{RO}^-) = 0.75\text{p}K_a(\text{ROH}) - 7.28 \qquad (5)$$

Rate constants for NPA cleavage by complexes [Co([15]-aneN$_5$)(OH)]$^{2+}$ and [Co(NH$_3$)$_5$OH]$^{2+}$ were found to be qualitatively in line with the general correlation for organic nucleophiles.[54] Figure 8a shows results for the cleavage of NPA by 13 hydroxo complexes of Co(III), Zn(II), Cu(II) and La(III), which follow eq 6.

$$\log k(\text{M} - \text{OH}^-) = 0.73\text{p}K_a(\text{M} - \text{OH}_2) - 6.68 \qquad (6)$$

Parameters of eq 6 are very close to those of (5). Also, in both cases the point for free OH$^-$ strongly deviates negatively

from the line for RO$^-$ or M $-$ OH$^-$ nucleophiles. The point for La(OH)$^{2+}$ fits very well the dependence for hydroxo complexes of other metals. We see, therefore, that in respect of NPA hydrolysis La(III) behaves like other cations, which just reduce the pK_a of bound water molecules making OH$^-$ a nucleophile similar to organic O-nucleophiles without any substrate activation by the metal cation.

Results for BNPP are shown in Figure 8b. Results for three dinuclear Zn(II) complexes with macrocyclic ligands of similar structures show a negative slope [30] (these points are connected by a solid line in Figure 8b), but considering all available data for Zn(II) complexes one can see that the rate constants for hydroxo complexes of Zn(II) are similar to that of free OH$^-$ indicating the Brønsted slope close to zero. This means that the loss in reactivity of OH$^-$ upon binding to the metal is compensated in this case by the Lewis acid substrate activation. Results for lanthanides tend to form another horizontal line, but at the level ca. 10^4 higher (note that the rate constants for lanthanides are at 25 °C but those for zinc complexes are at 35 °C). It seems, therefore, that the lanthanide cations produce an additional activation effect, as compared to zinc, but variations in the Lewis acidity of these cations are relatively insignificant.

A discussion of the nature of activation effect of lanthanides(III) in terms of above-described contributions (1)−(3) is rather difficult because it requires the detailed knowledge of the mechanisms of elementary steps of the catalytic reaction. A less mechanistically demanding approach, widely used in the interpretation of enzyme and model reactions, [55] is based on estimation of the stability constants of catalyst-transition state complexes. Simplified structures **2** and **3** (R = 4-nitrophenyl) of the transition states for the alkaline hydrolysis of NPA and BNPP are shown in Chart 1. Known rate constants for the alkaline (see above) and neutral ($k_0 = 5.5 \times 10^{-7}$ s^{-1} for NPA[50] and 1.1×10^{-11} s^{-1} for BNPP[52a] at 25 °C) hydrolysis of these esters allow one to estimate pK_a values of the conjugated acids of the transition states **2** and **3** (pK^*_a) in accordance with eq 7:[56]
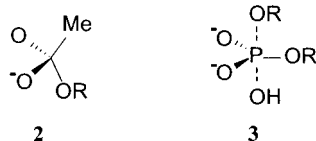
$$\text{p}K^*_a = \log(k_0/k_{\text{OH}}) + 14 \qquad (7)$$

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Chart 1

2　　　　　　　　**3**

$pK_a(\mathbf{2H}) = 6.8$　　　　$pK_a(\mathbf{3H^-}) = 8.3$

$\log K(La\mathbf{2}^{2+}) = 3.26$　　$\log K(La\mathbf{3}^+) = 9.32$　　$\log K(LaOH^{2+}) = 4.67$

$\log K(ZnL\mathbf{2}^+) = 4.50$　　$\log K(ZnL\mathbf{3}) = 5.58$　　$\log K(ZnLOH) = 5.36$

The resulting pK^*_a values given in Chart 1 show that the transition states for these reactions have similar basicities. Following the same approach as for derivation of the eq 7,[56] one can easily obtain the expression for stability constant of metal (M)−transition state (T) complex (K^*_{MT}), which refers to the equilibrium

$$M + T \rightleftharpoons MT$$
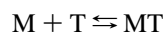
in the form:

$$\log K^*_{MT} = \log(k(M - OH^-)/k_{OH}) + 14 - pK_a(M - OH_2) \tag{8}$$

Using the k_1 value, equivalent to $k(M - OH^-)$ in eq 8, for La(III) from Table 2 (BNPP) and $k_1 = 0.37\ M^{-1}s^{-1}$ (NPA, see above) together with given above k_{OH} values for these substrates one obtains the stability constants of La^{3+}−transition state complexes with **2** and **3** given in Chart 1. Chart 1 also shows the association constants for **2** and **3** with a dinuclear Zn(II) complex $[Zn_2L1(OH)]^{3+}$ (L1 is an azocrown ether; see the Supporting Information for its structure), which corresponds

to $[Zn_2L1(OH)_2]^{2+}$, a complex possessing the highest activity in BNPP hydrolysis among all reported Zn(II) complexes (rate constants for the hydrolysis of BNPP and NPA by $[Zn_2L1-(OH)_2]^{2+}$ equal 1.15×10^{-4} and $1.3\ M^{-1}\ s^{-1}$ at 35 and 25 °C, respectively, pK_a values of the conjugated acid are 9.1 and 8.64 at 35 and 25 °C, respectively).[30] In terms of eq 8 for this system $[Zn_2L1(OH)_2]^{2+} = M - OH$ and $[Zn_2L1(OH)]^{3+} = M - OH_2$. For comparison, Chart 1 gives also the association constants of La^{3+} and $[Zn_2L1(OH)]^{3+}$ with OH^- at 25 °C. Binding of the transition state **2** to the zinc complex is stronger than to La^{3+} in accordance with higher affinity of the former to OH^-. As expected, for both metals affinity to less basic **2** is lower than to OH^-. With dianionic transition state **3** for both metals stability constants become larger than with the monoanion **2**, but for Zn(II) the increase in K^*_{MT} is only 10 times and for La(III) 10^6 times. A possible reason for such a difference is that **3** in contrast to **2** can act as a bidentate ligand, but with a very small chelate ring size, which is favored for metals of larger size.[57]

Supporting Information Available: Table 1S contains original titration data for 10 mM BTP and 10 mM lanthanide(III) perchlorate for all lanthanides studied; Figure 2Sa,b illustrates fitting of the titration data to the model given in Scheme 1 and a set of stability constants given in Table 1; Table 2S contains pK_a values of 26 hydroxo complexes of Zn(II), Cu(II), Co(III), and lanthanides(III) and the second-order rate constants of the cleavage of BNPP and NPA by these complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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