DALTON
FULL PAPER

Phosphate ester hydrolysis by yttrium(III) complexes with Bis-Tris propane and Tris ligands†

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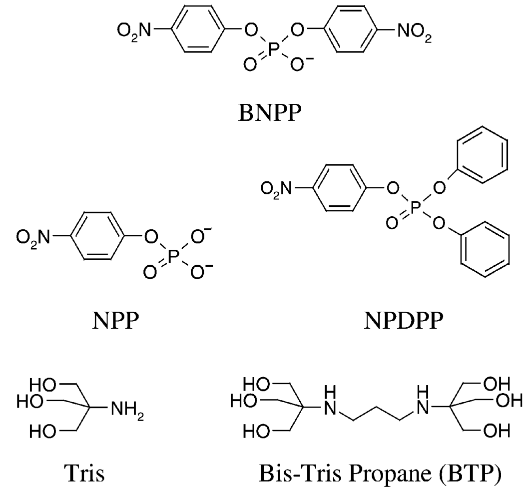
Unusual second-order, in metal, kinetics were observed for the hydrolysis of bis(4-nitrophenyl) phosphate (BNPP) and 4-nitrophenyl diphenylphosphate (NPDPP) catalyzed by Y(III) in the presence of Bis-Tris propane (BTP) and Tris ligands at 25 °C in weakly basic aqueous solutions. Potentiometric and ^{1}H-NMR titrations of BTP in the presence of Y(III) indicate formation of the mononuclear $Y(BTP)^{3+}$ complex and dinuclear hydroxo complexes $Y_2(BTP)(OH)_n{}^{6-n}$ where $n = 2, 4, 5$ or 6. Titrations of Tris in the presence of Y(III) were limited by the low stability of the system and were fitted to a tentative model involving formation of $Y(Tris)^{3+}$ and a single dinuclear complex $Y_2(Tris)_2(OH)_5{}^+$. Comparison of concentration and pH-dependences of the reaction rates with the species distribution diagrams show that the catalytic hydrolysis of BNPP involves a simultaneous interaction of the substrate with $Y_2(BTP)(OH)_4{}^{2+}$ and $Y_2(BTP)(OH)_5{}^+$ species in the Y(III)/BTP system and interaction with two $Y_2(Tris)_2(OH)_5{}^+$ species in the Y(III)/Tris system. The half-life for the hydrolysis of BNPP is only *ca.* 5 min at 25 °C and pH 8.5 in the presence of 4 mM Y(III) and 20 mM BTP. A rate enhancement by Y(III) in the hydrolysis of triester NPDDP is much smaller than that for diester BNPP. The hydrolysis of mono 4-nitrophenyl phosphate, the intermediate in BNPP hydrolysis, is zero-order in Y(III) indicating Michaelis–Menten type "saturation" kinetics.

Introduction

Metal complex catalysis in the hydrolysis of phosphoric and carboxylic acid esters is an area of active research.[1] The hydrolysis of phosphodiesters, which are extremely resistant to nucleophilic attack even with an activated 4-nitrophenolate leaving group,[2] is especially important because 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 research. Much attention has also been paid to the catalytic hydrolysis of more reactive phosphate triesters in connection with detoxification problems.[3]

Promising results were reported using aquo ions and complexes of lanthanides as the catalysts.[1d,4–18] There is much convincing evidence that the cleavage of phosphate esters occurs *via* the concerted action of a metal cation and a nucleophile (OH^- or RO^-) within metal hydroxide or alkoxide complexes.[1] The use of appropriate ligands, which could stabilize reactive hydroxo lanthanide species and, in favorable cases, pre-organize them for an optimum interaction with the substrate, is one of the key factors in the development of active catalysts. The stabilization is required because of the low solubilities of lanthanide hydroxides in combination with the relatively high pK_a values of lanthanide(3+) aquo ions,[19] which require basic conditions in order to generate active hydroxo complexes. Besides numerous sophisticated macrocyclic ligands, including calixarenes,[1i] a family of simple amino alcohols, derivatives of tris(hydroxymethyl)aminomethane (Tris) (Scheme 1), have attracted attention recently. Tris was successfully employed for the stabilization of the extremely acidic Zr(IV) catalyst,[20] Bis-Tris was found to form esterolytically active complexes with lanthanum(III).[10] We observed high hydrolytic activity in a system containing lanthanide(III) and Bis-Tris Propane (BTP, Scheme 1) in weakly basic solutions.[21] In this paper we report the use of BTP and Tris for the stabilization of hydroxo complexes of Y(III).



Scheme 1

Cation Y^{3+} (ionic radius r_i = 104 pm in six-coordination) is similar in size to Ho^{3+} (r_i = 104.1 pm in six-coordination) and by its acidity (pK_a = 8.61 at ionic strength I = 0.3 M)[19] to Gd^{3+} (pK_a = 8.62 at I = 0.3 M),[19] but the solubility product for $Y(OH)_3$ 6.3 × 10^{-24} M^4 is considerably larger than for both lanthanides (*ca.* 1.5 × 10^{-26} M^4) and therefore it is less susceptible to precipitation in basic solutions. Another advantage of Y(III) is the generally higher stability of its solutions, which do not undergo "ageing" in contrast to lanthanides.[22] Polymeric

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† Electronic supplementary information (ESI) available: Tables of original titration data for five different combinations of total BTP and yttrium(III) perchlorate concentrations and for three different combinations of total Tris and yttrium(III) perchlorate concentrations; Tables of stability constants; Figs. illustrating fitting of the titration data to the model given in Scheme 2. See http://www.rsc.org/suppdata/dt/b1/b103163p/

Y(III) hydroxo complexes of general structure $Y[Y(OH)_2]_n^{(3+n)+}$ or $[Y(OH)_2]_n^{n+}$, stabilized by 1.25 equivalents of anthranilic acid, were found to catalyze the hydrolysis of 4-nitrophenyl methylphosphonate.[22] The reaction kinetics were of a Michaelis–Menten type and the catalytic rate constant was *ca.* 10^6 times larger than the rate constant for the uncatalyzed hydrolysis. Comparison of catalytic activities of different metal cations in the hydrolysis of 4-nitrophenyl methylphosphonate shows that Y(III) is 100 times more active than La(III) and only 10 times less active than highly electrophilic cations like Th^{4+}, Ce^{4+} and Zr^{4+}.[23] To our knowledge the catalytic activity of Y(III) in phosphate ester hydrolysis has not been studied.

We employed bis(4-nitrophenyl) phosphate (BNPP) and 4-nitrophenyl phosphate (NPP), which is the intermediate product of BNPP hydrolysis (Scheme 1), as principal substrates for this study. The hydrolysis of more reactive 4-nitrophenyl diphenylphosphate (NPDPP), which is typically used as a model substrate for testing potential catalysts for the hydrolysis of toxic phosphates, was studied in less detail. These substrates are often used for studies of the phosphoesterase activity of metal complex catalysts and this allows one to make comparisons with other relevant systems.

Experimental

Materials

Bis(4-nitrophenyl) phosphate (Aldrich) was recrystallized from ethanol–water. 4-Nitrophenyl diphenylphosphate was prepared as described in the literature.[24] 4-Nitrophenyl phosphate di(Tris) salt, Bis-Tris propane {1,3-bis[tris(hydroxymethyl)-methylamino]propane} and Trizma base [tris(hydroxymethyl)-aminomethane] were all available from Sigma and used as supplied. $NaClO_4$ and reagent grade yttrium(III) nitrate or perchlorate were used as supplied from Aldrich. The concentration of metal ions in stock solutions was determined by adding excess of ethylenediaminetetraacetic acid and then back titrating with $ZnCl_2$ using Eriochrome Black T as indicator.[25] Distilled and deionized water (Barnstead nanopure system) was used.

Potentiometry

Potentiometric titrations were performed following the general recommendations given in ref. 26. All titrations were performed in a 75 ml thermostatted 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 non-linear least-squares regression in the Origin 3.5 program to the equation:

$$V + (V_0 + V)[H']f/C_B - (V_0 + V)K_w/(C_B[H']f) - V_e = 0 \quad (1)$$

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.[27] A value for pK_w of 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. 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 $-\log[H^+] = pH - \log f$.[27] 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.[28] Titrations of BTP and its mixtures with yttrium perchlorate were performed in the concentration range 5–20 mM for each component; 2 equivalents of $HClO_4$ per 1 mole of BTP were always added to the titrating mixture before the start of the titration. Titrations of Tris and its mixtures with yttrium perchlorate were performed in the concentration range 10–50 mM of Tris and 5–10 mM of Y(III); 1 equivalent of $HClO_4$ per 1 mole of Tris was always added to the titrating mixture before the start of the titration. Species distribution diagrams were calculated by using Species version 0.8 (Academic Software, Sourby Old Farm, Timble, Otley, Yorks, 1999).

Kinetics

Kinetic measurements were performed using a Hewlett-Packard 8453 diode array spectrophotometer equipped with a Peltier thermostatted cell compartment. Reaction solutions were prepared by combining appropriate amounts of metal and ligand stock solutions to the desired volume and pH was adjusted by adding small volumes of strong acid. We found that more reproducible results were obtained when the pH was adjusted by adding acid to mixtures of Y(III) and BTP which were more basic than required. Additions of concentrated base often induced local precipitation of the metal hydroxide. Reactions were initiated by adding an aliquot of the substrate solution. Special care was taken to avoid CO_2 absorption during reaction runs. Solution pH was measured after each run and all kinetic runs in which pH variation was larger than 0.05 were excluded. In order to have sufficiently high buffer capacity for the solution and stability in respect of metal hydroxide precipitation over a wide pH range, the reaction kinetics were studied with excess of BTP or Tris over the yttrium salt. It is worth noting that BTP has a wide pH buffer range from 6.5 to 9.5 due to the presence of two dissociating groups with pK_a values of *ca.* 7 and 9 (see Results section).

The course of all hydrolysis reactions was monitored spectrophotometrically by the appearance of 4-nitrophenolate anion at 400 nm. Stock solutions of BNPP and NPP were freshly prepared in water. Stock solutions of NPDPP were prepared in dioxane and the final reaction mixture contained 2 vol% of dioxane. Kinetic measurements used 40 μM substrate and varied concentrations of metal salts (typically from 0.5 to 5 mM) 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

^1H-NMR spectra were recorded on a Varian Gemini 300 NMR spectrometer. The instrumental pH values measured in D_2O were corrected to the respective pD values using the equation pD = pH + 0.4.[29]

Results and discussion

Complexation equilibria in yttrium(III)–BTP and yttrium(III)–Tris systems

Attempts to isolate complexes of Y(III) with BTP and Tris were unsuccessful and the information regarding the stoichiometry and stability of these complexes in solution was obtained by potentiometric and ^1H-NMR titrations. Fig. 1(a) shows pH titration curves for 10 mM BTP and for 10 mM 1 : 1 BTP to yttrium(III) perchlorate mixtures in the presence of 2 equivalents of $HClO_4$ [Table S1 in the ESI gives the numerical titration results for all BTP and Y(III) concentrations employed]. Fitting

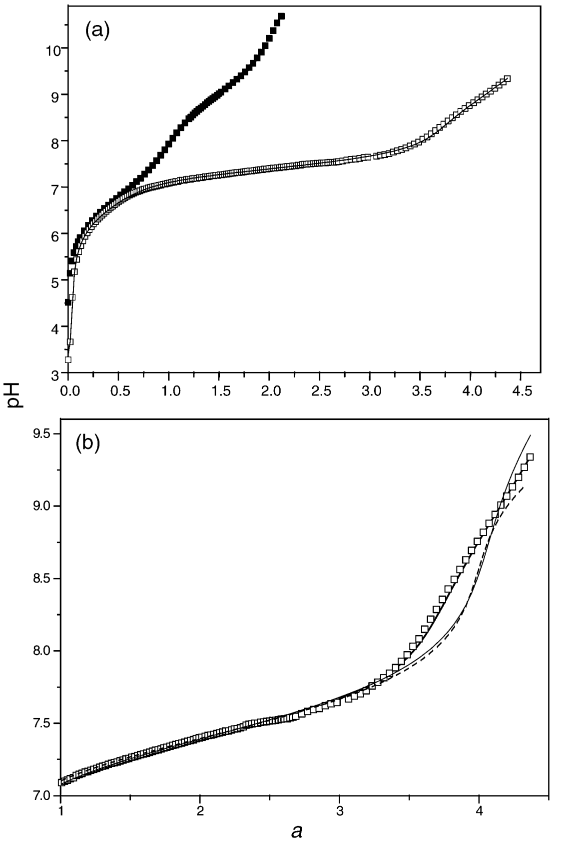


Fig. 1 (a) Titration curves of 10 mM BTP (solid squares) and a 10 mM 1 : 1 BTP to yttrium perchlorate mixture (open squares) in 0.1 M $NaClO_4$. Two equivalents of $HClO_4$ were added at the beginning of each titration; *a* is the number of added NaOH equivalents. (b) The magnified part of the titration curve for BTP/Y(III) at pH above 7. The thick solid line is the fitting curve for the model shown in Scheme 2 (see text), the thin solid line is the fitting curve for a model involving monomeric complexes $Y(BTP)(OH)_n^{3-n}$ and the dashed line is the fitting curve for a model involving dimeric complexes with two BTP molecules $Y_2(BTP)_2(OH)_n^{6-n}$.

of the titration curve for BTP gives pK_{a1} = 6.83 ± 0.02 for $(BTP)H_2^{2+}$ and pK_{a2} = 9.06 ± 0.02 for $(BTP)H^+$, in good agreement with published values (pK_{a1} = 6.75 and pK_{a2} = 9.10).[30] The titration curve in the presence of 1 equivalent of metal salt shows consumption of more than 2.5 equivalents of NaOH per 1 equivalent of metal besides 2 equivalents of NaOH consumed for the BTP titration (the range of titration was limited by precipitation at pH above 9.7). These results indicate formation of metal hydroxo complexes or deprotonation of the hydroxymethyl groups of BTP affording respective alkoxo complexes. Such ambiguity is common for titrations of metal complexes with alcohol ligands.[1a,12,31–33] It was not resolved for relevant aminoalcohol lanthanide(III) complexes,[12] but for Zn(II) complexes with macrocyclic ligands bearing a pendant alcohol the formation of alkoxo complexes was proven by crystal structures of isolated complexes.[31,33] Deprotonation of the Zn(II) bound alcohol group induced a considerable (*ca.* 0.3 ppm) upfield shift of the signals for the methylene protons of the CH_2OH group,[31] and in looking for the possibility of discriminating between hydroxo and alkoxo complexes in solution we turned our attention to this observation expecting to observe a qualitatively similar effect in the case of Y(III). To this end, the ^1H NMR pD titration of BTP in the presence of Y(III) was performed.

Fig. 2 shows chemical shifts corresponding to the singlet of the methylene protons of CH_2OH groups *vs.* pD in D_2O in the absence (open circles) and in the presence (solid squares) of $Y(NO_3)_3$. Chemical shifts of methylene protons of the propylene chain in positions α and β to the nitrogen atom of the free ligand showed similar pD-profiles, but underwent very small changes in the presence of metal. Analysis of the titration curves obtained in the absence of yttrium salt gave mutually agreed values of dissociation constants equalling pK_{a1} = 7.55 ± 0.03 for $(BTP)D_2^{2+}$ and pK_{a2} = 9.81 ± 0.03 for $(BTP)D^+$. Larger pK_a values in D_2O solvent as compared to H_2O agree with known H/D isotope effects on acid dissociation constants.[34] Points for solutions containing Y(III) deviate from the titration curve of the free ligand in the same pH range where the deviation is observed in the potentiometric pH titration curve [*cf.* Fig. 1(a)]. No attempts were made to fit these results because this would require too many adjustable parameters, but qualitatively a considerable downfield instead of upfield shift of the signal for the methylene protons α to the OH group agrees with a complex structure in which no ligand deprotonation occurs. Much smaller shifts in the signals of the methylene protons of the propylene chain, as compared to the signals of methylenes of CH_2OH groups in the presence of $Y(NO_3)_3$, are indicative of predominant metal binding to the alcohol groups of BTP.

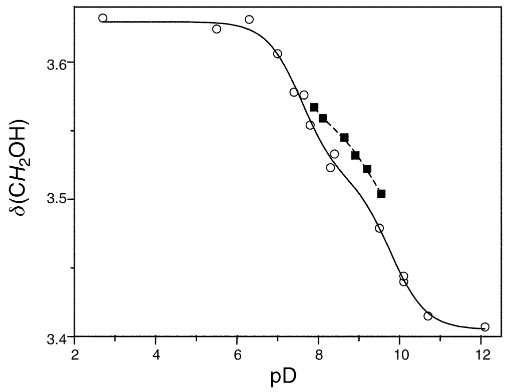


Fig. 2 ^1H-NMR pD titration of 10 mM BTP in D_2O. Open circles and solid squares show measurements in the absence and in the presence of 10 mM $Y(NO_3)_3$ respectively.

The simplest reaction scheme which follows from the potentiometric titration results would involve formation of a simple 1 : 1 $Y(BTP)^{3+}$ complex and a series of mixed mononuclear hydroxo complexes of general composition $Y(BTP)(OH)_n^{3-n}$ with *n* ranging from 1 to 3 [stability constants of binary Y(III) hydroxide complexes $Y(OH)^{2+}$, $Y_2(OH)_2^{4+}$ and $Y_3(OH)_5^{4+}$ were taken from the literature[19a,b] and used as fixed parameters in all fittings]. However, fitting of the results to such a scheme, illustrated by the thin solid line in Fig. 1(b), was unsatisfactory. Better fitting [Figs. 1(a),(b)] was obtained for a more complex scheme involving formation of dinuclear hydroxo complexes $Y_2(BTP)(OH)_n^{6-n}$ as presented by the set of equilibria shown in Scheme 2 [Fig. S1(a–e) in the ESI illustrates the quality of the fits for all titrations]. Another scheme involving two ligand molecules in the dimeric complexes of composition $Y_2(BTP)_2(OH)_n^{6-n}$ was also tested but gave a less satisfactory fitting as shown by the dashed line in Fig. 1(b). 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 yttrium(III)–BTP mixtures at different total concentrations, which agreed reasonably well with each other (stability constants obtained from all titrations are given in Table S2, ESI).

Titrations of Tris–yttrium(III) mixtures were possible only in the presence of a considerable excess of the ligand over metal. Fig. 3 shows titration curves for free Tris and for its 2 : 1 mixture with Y(III). Fitting of the titration curve for Tris gives pK_a = 8.01 ± 0.02 for $(Tris)H^+$ in good agreement with the published value (pK_a = 8.09).[19c] Titration of 10 mM Tris in the presence of 5 mM Y(III) was terminated below pH 8 because of metal hydroxide precipitation, however, it clearly shows the

$$\mathrm{BTP + H^+ \rightleftharpoons BTPH^+}$$
$$\mathrm{BTP + 2H^+ \rightleftharpoons BTPH_2^{2+}}$$
$$\mathrm{Y^{3+} + BTP \rightleftharpoons Y(BTP)^{3+}}$$
$$\mathrm{2Y^{3+} + BTP + 2OH^- \rightleftharpoons Y_2(BTP)(OH)_2^{4+}}$$
$$\mathrm{2Y^{3+} + BTP + 4OH^- \rightleftharpoons Y_2(BTP)(OH)_4^{2+}}$$
$$\mathrm{2Y^{3+} + BTP + 5OH^- \rightleftharpoons Y_2(BTP)(OH)_5^{+}}$$
$$\mathrm{2Y^{3+} + BTP + 6OH^- \rightleftharpoons Y_2(BTP)(OH)_6}$$

$$\mathrm{Tris + H^+ \rightleftharpoons TrisH^+}$$
$$\mathrm{Y^{3+} + Tris \rightleftharpoons Y(Tris)^{3+}}$$
$$\mathrm{2Y^{3+} + 2Tris + 5OH^- \rightleftharpoons Y_2(Tris)_2(OH)_5^{+}}$$

Scheme 2

Table 1 Stability constants for the formation of yttrium(III) complexes with BTP and Tris and for ligand protonation (25 °C, 0.1 M $NaClO_4$)

Equilibrium	log*K*[a]
[BTP–H]/[BTP][H]	9.06 ± 0.02[b]
$[BTP–2H]/[BTP][H]^2$	15.87 ± 0.02[b]
[BTP–Y]/[BTP][Y]	3.55 ± 0.26
$[BTP–2Y–2OH]/[BTP][Y]^2[OH]^2$	18.66 ± 0.24
$[BTP–2Y–4OH]/[BTP][Y]^2[OH]^4$	31.87 ± 0.46
$[BTP–2Y–5OH]/[BTP][Y]^2[OH]^5$	36.55 ± 0.22
$[BTP–2Y–6OH]/[BTP][Y]^2[OH]^6$	40.66 ± 0.61
[Tris–H]/[Tris][H]	8.01 ± 0.01[b]
[Tris–Y]/[Tris][Y]	2.23 ± 0.14
$[2Tris–2Y–5OH]/[Tris]^2[Y]^2[OH]^5$	40.04 ± 0.32

[a] Errors are the standard errors of the mean values of the equilibrium constants calculated by averaging the values determined in five titrations of Y(III)–BTP mixtures and three titrations of Y(III)–Tris mixtures with variable total metal and ligand concentrations. [b] Protonation constants for free ligands.

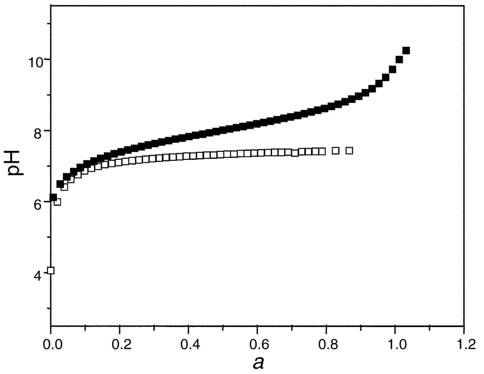
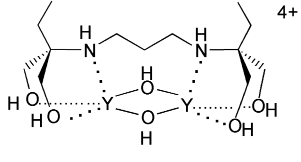


Fig. 3 Titration curves of 10 mM Tris (solid squares) and 10 mM Tris in the presence of 5 mM yttrium perchlorate (open squares) in 0.1 M $NaClO_4$; 1 equivalent of $HClO_4$ per equivalent of Tris was added at the beginning of each titration; *a* is the number of added NaOH equivalents per equivalent of the ligand.

complexation of Tris with Y(III) already at a pH below the pK_a of the ligand. The same models as above were tested to fit the titration plot. The best fit was observed for a model involving formation of a mononuclear $Y(Tris)^{3+}$ complex and a single dimeric complex, but with two ligand molecules: $Y_2(Tris)_2(OH)_5^-$ (Scheme 2). Since titrations were possible only over a narrow pH-range we consider this model rather tentative, although it satisfactorily fits titrations at different total concentrations of components [see ESI, Table S3, Fig. S2(a–c)]. Logarithms of the equilibrium constants for each step are collected in Table 1.

The stability constant for the $Y(Tris)^{3+}$ complex (Table 1) is close to that reported for $Eu(Tris)^{3+}$ (log*K* = 2.44).[35] BTP forms a substantially more stable complex with Y(III) indicating possible coordination of both halves of its molecule which are chemically similar to Tris.

Yttrium(III) has a strong tendency to form polymeric hydroxide complexes in water.[18,19] Dinuclear dihydroxo $Y_2(OH)_2^{4+}$ and trinuclear pentahydroxo $Y_3(OH)_5^{4+}$ species were reported among the products of Y(III) hydrolysis.[19] The potentiometric titration of yttrium(III) in the presence of 1.25 equivalents of anthranilic acid indicated formation of polymeric species like $[Y(OH)_2]_n^{n+}$ containing two equivalents of OH^- per Y(III).[22] Polynuclear species are also typical for chemically similar lanthanide(III) hydroxo complexes. The $La_2(OH)_2^{4+}$ unit is formed in a dinuclear macrocyclic lanthanum complex.[18] The potentiometric titration of $LaCl_3$ with alkali revealed formation of $La_2(OH)_5^+$ species,[8] relevant to $Y_2(BTP)(OH)_5^+$ and $Y_2(Tris)_2(OH)_5^+$ complexes in Scheme 2. Apparently, complexation with BTP or Tris stabilizes the species of lower nuclearity preventing formation of polymeric complexes and eventually the metal hydroxide precipitation. Based on the reported structure of the lanthanum(III) complex with neutral Bis-Tris ligand,[10] which has a common structural fragment with Tris and BTP, a structure of $Y_2(BTP)(OH)_n^{6-n}$ complexes may by proposed in a form exemplified by the structure **1** for $Y_2(BTP)(OH)_2^{4+}$ with two bridging OH anions. Similarly, a hypothetical structure **2** may be proposed for $Y_2(Tris)_2(OH)_5^-$.



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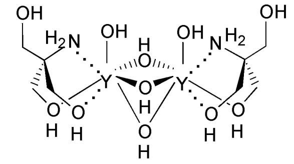


2

Kinetics of BNPP hydrolysis

The absorbance *A vs.* time *t* profiles monitored at the absorption maximum of 4-nitrophenolate anions followed a double-exponential eqn. (2) derived for the two-step kinetics of BNPP hydrolysis *via* an NPP intermediate:

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

where ε_{NP} and $[BNPP]_0$ are the molar absorptivity of 4-nitrophenolate and initial substrate concentration respectively, and observed first-order rate constants k_1 and k_2 refer to BNPP and NPP hydrolyses respectively. As discussed below the observed first-order rate constants for the BNPP hydrolysis refer to k_1 values in eqn. (2). Similar values of k_2 were found both from the kinetics of BNPP hydrolysis by using eqn. (2) and from independently studied kinetics of NPP hydrolysis indicating that NPP is the true reaction intermediate.

In the Y(III)–BTP system the reaction rate grows rapidly on increasing the pH above 7 and tends to level off at pH 9. The reaction order in metal was studied at pH 8.5, Fig. 4. Observed first-order rate constants (k_{obs}) of BNPP hydrolysis show a quadratic dependence on the total metal concentration:

$$k_{obs} = k[Y(III)]_T^2 \quad (3)$$

The species distribution diagram calculated for the conditions of Fig. 4 indicates that the predominating species are two hydroxo complexes $Y_2(BTP)(OH)_4^{2+}$ and $Y_2(BTP)(OH)_5^+$, which contribute *ca.* 79% and 19% to the total Y(III) concentration respectively in the whole concentration range of Y(III).

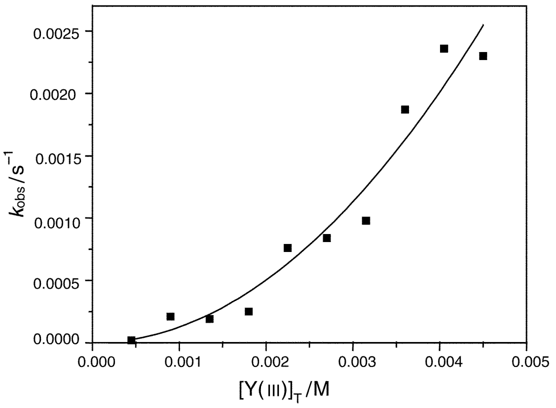


Fig. 4 Observed first-order rate constants for the hydrolysis of BNPP *vs.* Y(III) concentration in the presence of 20 mM BTP at pH 8.5 and 25 °C. The solid line is the fit to eqn. (3).

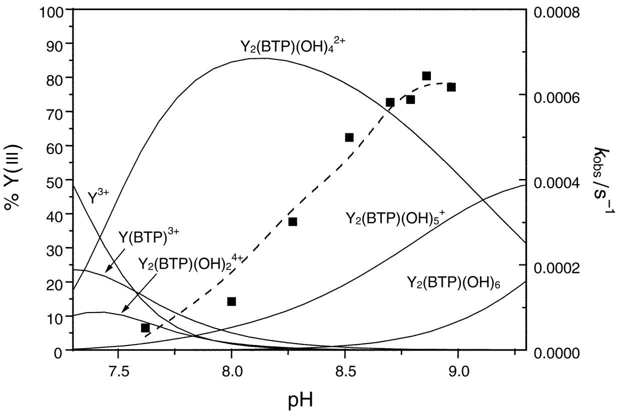


Fig. 5 Observed first-order rate constants for the BNPP hydrolysis at 25 °C (solid squares) and the species distribution diagram for hydroxo-complexes (Scheme 2) for 1.8 mM yttrium(III) and 20 mM BTP as a function of pH. The dashed line is the theoretical k_{obs} *vs.* pH-profile calculated in accordance with eqn. (4).

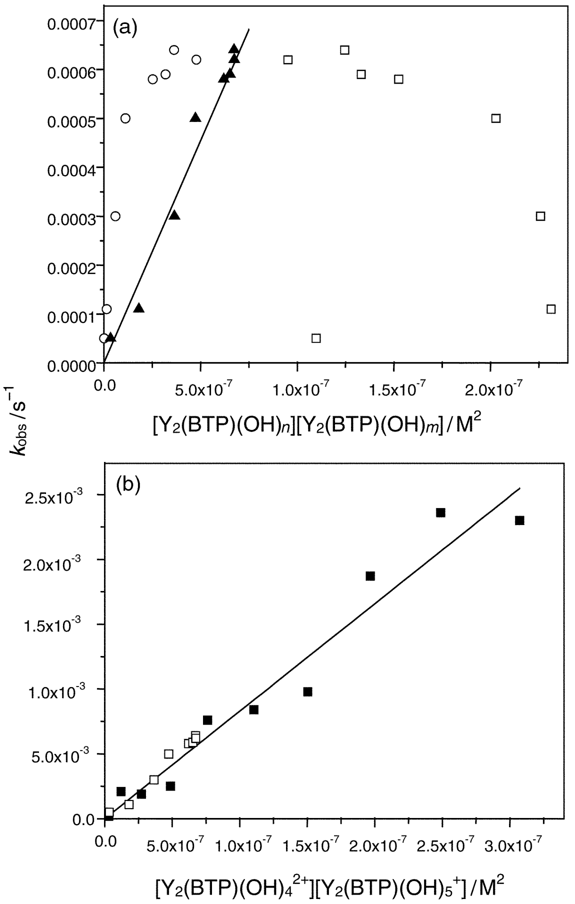


Fig. 6 (a) Observed first-order rate constants for BNPP hydrolysis (data from Fig. 5) *vs.* $[Y_2(BTP)(OH)_4^{2+}]^2$ (open squares), $[Y_2(BTP)(OH)_5^+]^2$ (open circles), and $[Y_2(BTP)(OH)_4^{2+}][Y_2(BTP)(OH)_5^+]$ (solid triangles). (b) Observed first-order rate constants for BNPP hydrolysis (data from Fig. 5 shown by open and data from Fig. 4 by solid squares) *vs.* $[Y_2(BTP)(OH)_4^{2+}][Y_2(BTP)(OH)_5^+]$.

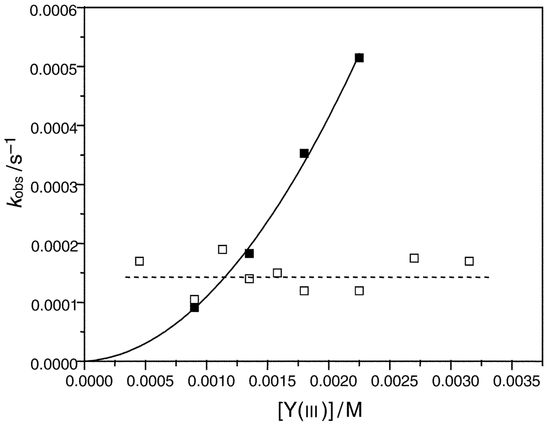


Fig. 7 Observed first-order rate constants for the BNPP (solid squares) and NPP (open squares) hydrolyses *vs.* Y(III) concentration in the presence of 20 mM Tris at pH 8.5 and 25 °C. Solid line is the fitting curve in accordance with eqn. (5).

Therefore, the second-order kinetics in total Y(III) cannot be related to increased dimerization of Y(III) at higher concentrations, but indicates participation of two dimeric complexes in the reaction with one BNPP ion. It is worth noting in this connection that even third-order in metal kinetics were reported for the reaction of BNPP with dimeric lanthanum(III) macrocyclic complexes.[18] Since the distribution of species remains practically unchanged on variation of Y(III) concentration under the conditions of Fig. 4, one cannot determine from these results whether the reaction involves two $Y_2(BTP)(OH)_4^{2+}$ complexes or two $Y_2(BTP)(OH)_5^+$ complexes or their combination. More information comes from the study of pH-dependence of the reaction rate, which provides a wide variation in the concentrations of both hydroxo complexes.

In order to analyze pH-profiles of k_{obs}, the species distribution diagram was calculated for the conditions of kinetic experiments in accordance with the equilibrium constants given in Table 1, and experimental values of k_{obs} were plotted on this diagram as shown in Fig. 5. Inspection of Fig. 5 reveals that the dominating species in the range of pH employed are again $Y_2(BTP)(OH)_4^{2+}$ and $Y_2(BTP)(OH)_5^+$. Taking into account the fact that the reaction is second-order in metal, the values of k_{obs} were plotted against the square of the concentrations of these species $\{[Y_2(BTP)(OH)_4^{2+}]^2$ or $[Y_2(BTP)(OH)_5^+]^2\}$ as well as against the product $[Y_2(BTP)(OH)_4^{2+}][Y_2(BTP)(OH)_5^+]$, Fig. 6(a). Evidently, k_{obs} correlates only with the last function. Fig. 6(b) shows the results from both the pH-dependence at fixed Y(III) concentration (open squares) and the concentration dependence at fixed pH (solid squares) in accordance with eqn. (4):

$$k_{obs} = k_3[Y_2(BTP)(OH)_4^{2+}][Y_2(BTP)(OH)_5^+] \quad (4)$$

from which $k_3 = 830 \pm 40$ M^{-2} s^{-1} can be calculated.

In the Y(III)–Tris system the kinetics of BNPP hydrolysis are also higher than first-order in metal, Fig. 7 (solid squares), although with some contribution from the first-order reaction. There is a single hydroxo complex in this case and the results in Fig. 7 show a good fit to eqn. (5):

$$k_{obs} = k_2[Y_2(Tris)_2(OH)_5^+] + k_3[Y_2(Tris)_2(OH)_5^+]^2 \quad (5)$$

with $k_2 = 0.012 \pm 0.007\ M^{-1}\ s^{-1}$ and $k_3 = 97 \pm 15\ M^{-2}\ s^{-1}$. The pH-profile of the observed rate constant is superimposed on the species distribution diagram in Fig. 8 (solid squares). The dashed line in Fig. 8 shows the fit to eqn. (5) with $k_2 = 0.12 \pm 0.05\ M^{-1}\ s^{-1}$ and $k_3 = 110 \pm 35\ M^{-2}\ s^{-1}$. The values of k_3 from both pH and concentration dependences agree within experimental error, but k_2 values are difficult to compare due to a low precision in k_2.

Kinetics of NPP and NPDPP hydrolysis

In both Y(III)–BTP and Y(III)–Tris systems the hydrolysis of NPP is zero-order in metal, Figs. 7 and 9 (open squares). It proceeds faster than the BNPP hydrolysis at low, but slower at higher Y(III) concentrations. Most probably the observed zero-order reflects the "saturation" Michaelis–Menten type kinetics with a large stability constant for the NPP–Y(III) intermediate complex. Kinetics of this type were also observed for NPP hydrolysis by lanthanide(III)–BTP hydroxo complexes.[21b] The observation of saturation kinetics with NPP, but no saturation with BNPP agrees with the known better ligating properties of the former substrate, which is a dianion capable, in principle, of metal chelation in contrast to monoanionic BNNP.[1a,36–38] Profiles of k_{obs} *vs.* pH for NPP hydrolysis initially show an increase in k_{obs} on increase in pH, like in the case of BNPP, but at pH > 7 k_{obs} becomes independent of pH, Figs. 8 and 10 (open squares).

Kinetics of the hydrolysis of NPDPP were only studied with the more stable Y(III)–BTP system. It has much in common with the kinetics of BNPP hydrolysis: kinetic order in Y(III) is higher than 1 (Fig. 9, solid squares) and the pH-profile of k_{obs} shows a large increase on going to higher pH values (Fig. 10, solid squares). Testing the correlation of k_{obs} with concentrations of different hydroxo species in the same manner as was done for BNPP above we found that results of both the concentration dependence and pH-dependence of k_{obs} at pH > 8 show the best fit to an equation similar to the eqn. (5), but involving now BTP instead of Tris pentahydroxo complexes:

$$k_{obs} = k_2[Y_2(BTP)(OH)_5^+] + k_3[Y_2(BTP)(OH)_5^+]^2 \quad (6)$$

with $k_2 = 0.28 \pm 0.09\ M^{-1}\ s^{-1}$ and $k_3 = 295 \pm 50\ M^{-2}\ s^{-1}$. The dashed line in Fig. 10 is the theoretical profile calculated in accordance with eqn. (6). It deviates significantly from the experimental points below pH 7. Apparently, other species, *e.g.* the dihydroxo complex, contribute to the reaction rate in this pH range.

Comparison with related systems and general discussion

The second-order with respect to metal kinetics of BNPP hydrolysis by Y(III) represents a new feature in lanthanide-catalyzed phosphodiester hydrolysis. Previously reported rate equations involved a "saturation" kinetics of a Michaelis–Menten type with aquo-ions and some macrocyclic complexes of lanthanides(III),[14,15,39,40] simple first-order kinetics with lanthanide(III) cryptates[9] and BTP–lanthanide(III) complexes,[21] and third-order kinetics with a dinuclear lanthanum(III) macrocycle.[18] Such diversity in rate laws makes it difficult to compare the activities of different catalysts in terms of the rate constants, which have different dimensions. Comparison in terms of k_{obs} shows that the phosphodiesterolytic activity of Y(III) in the presence of BTP is substantially higher than for the majority of reported lanthanide(III)-based systems. At [Y(III)] = 4.5 mM the k_{obs} for BNPP hydrolysis reaches the value of $0.0025\ s^{-1}$ at 25 °C and pH 8.5 (Fig. 4), which corresponds to a reaction half-life of only 4.6 min. Activities of lanthanide(III)–BTP systems are *ca.* one order of magnitude lower even at higher pH.[21] The most active among lanthanide(III) aquo-ions Er^{3+} has $k_{obs} = 0.00053\ s^{-1}$ at "saturation" and 50 °C.[15] Reported rate constants for BNPP cleavage by lanthanide(III) macrocyclic complexes[9,13,16] reach the level of $0.001\ s^{-1}$ only at elevated temperatures.

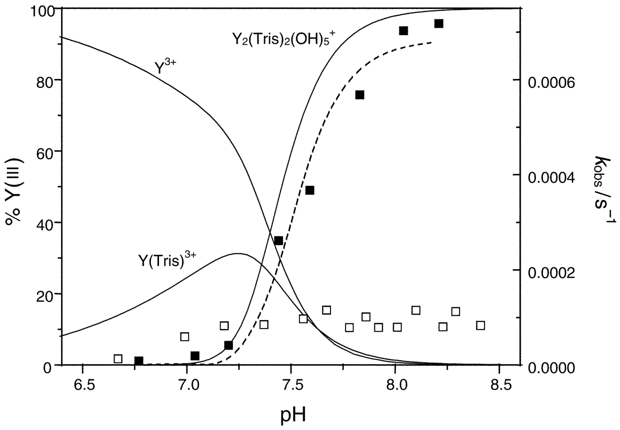


Fig. 8 Observed first-order rate constants for the BNPP (solid squares) and NPP (open squares) hydrolyses at 25 °C and the species distribution diagram for hydroxo-complexes (Scheme 2) for 2 mM yttrium(III) and 20 mM Tris. The dashed line is the theoretical k_{obs} *vs.* pH-profile calculated in accordance with eqn. (5).

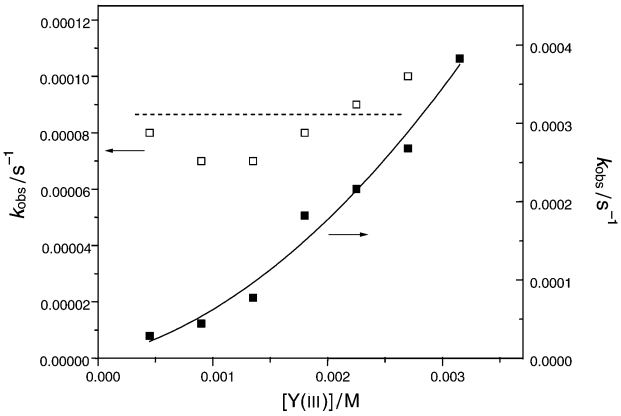


Fig. 9 Observed first-order rate constants for the hydrolysis of NPDPP (solid squares) and NPP (open squares) *vs.* Y(III) concentration in the presence of 20 mM BTP at pH 8.5 and 25 °C.

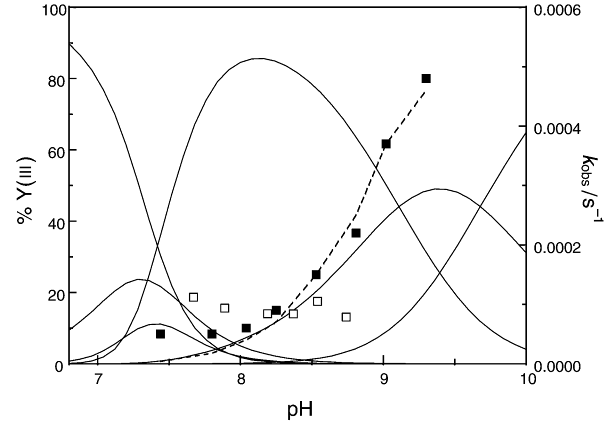
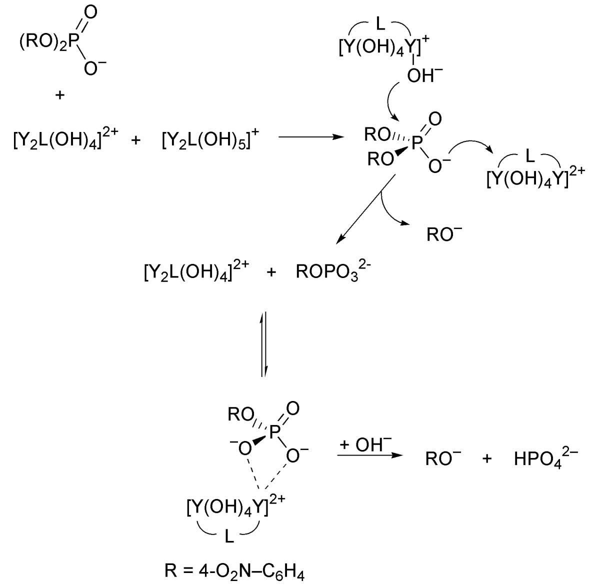


Fig. 10 Observed first-order rate constants for the hydrolysis of NPDPP (solid squares) and NPP (open squares) and species distribution diagrams for hydroxo-complexes (Scheme 2) for 1.8 mM yttrium(III) and 20 mM BTP as a function of pH. The dashed line is the theoretical k_{obs} *vs.* pH-profile calculated in accordance with eqn. (6).

The observed rate constants for the hydrolysis of triester NPDPP are of the same order of magnitude as those for BNPP (*cf.* Figs. 4, 5, 9 and 10). However, NPDPP is much more reactive than BNPP: reported first-order rate constants for spontaneous hydrolysis of NPDPP at 25 °C are 9.7×10^{-6} and 4.2×10^{-5} s^{-1} at pH 8 and 9 respectively,[41] while the rate constant for uncatalyzed BNPP hydrolysis at 25 °C is 1.1×10^{-11} s^{-1}.[42] Therefore, the rate enhancement factor at the highest Y(III) concentrations employed (3–5 mM) is *ca.* 20 for NPDPP, but 2×10^8 for BNPP.

The "stoichiometric" reaction mechanism for the cleavage of BNPP in the Y(III)–BTP system, which follows from the study of concentration and pH-dependences of the reaction rate, involves a third-order reaction of BNPP with $Y_2(BTP)(OH)_4^{2+}$ and $Y_2(BTP)(OH)_5^{+}$ complexes leading to formation of NPP, Scheme 3. In general terms the mechanism of phosphate ester hydrolysis by metal hydroxo complexes involves a combination of a nucleophilic attack by coordinated OH^- on phosphorus(V) and an electrophilic assistance by the metal cation directed either to phosphoryl oxygens or to the leaving group.[1f] Usually both the nucleophile and electrophile are the components of the same metal hydroxo complex, however when OH^- is the bridging ligand its nucleophilicity may be very low.[18,31,43] Certainly, some of the hydroxo ligands in $Y_2(BTP)(OH)_n^{6-n}$ complexes are bridging and probably with $n = 2$ and 4 they all are bridging and with fairly low reactivity. Therefore, it is logical to assume that the tetrahydroxo complex $Y_2(BTP)(OH)_4^{2+}$ provides only an electrophilic assistance to the substrate while the pentahydroxo complex $Y_2(BTP)(OH)_5^{+}$, with more basic hydroxo anions, acts as a nucleophile towards phosphorus(V), Scheme 3.



Scheme 3

In fact, some degree of association between anionic BNPP and cationic Y(III) hydroxo complexes can be anticipated. However, reported stability constants for association of BNPP with Co(III)[42] or Zn(II)[1a] complexes are of the order of 1 M^{-1} and one can hardly expect any indication for BNPP association with Y(III) from kinetic data obtained in a mM range of total metal concentrations. In contrast to BNPP, the dianion of NPP does form stable metal complexes, *e.g.* with $Zn([12]aneN_3)^+$ $\log K = 3.1$ for NPP, but $\log K < 0.5$ for BNPP.[1a] We believe that this increased affinity of NPP as a ligand explains the observed zero-order in metal kinetics for this substrate. Another important difference between BNPP and NPP is that the hydrolysis of phosphate monoesters proceeds *via* a monomolecular mechanism without direct nucleophilic attack.[44,45a] Therefore, the expected mechanism for metal catalysis in phosphomonoester hydrolysis is the leaving group stabilization rather than the nucleophile or phosphoryl activation.[44] However, it has been shown that the hydrolysis of metal-bound monoesters involves the nucleophilic attack by the coordinated hydroxide and the principal role of the metal ion is the mutual approximation of the substrate and nucleophile.[45b,46] An increase in k_{obs} for NPP hydrolysis on increase in pH below 7.5 (Fig. 8, open squares) indicates the participation of coordinated hydroxide. The independence of the rate of NPP hydrolysis of pH in more basic solutions (Figs. 8 and 10) is not surprising taking into account the "saturation" kinetics for this substrate: apparently NPP remains completely bound to a single Y(III) hydroxocomplex, *e.g.* $Y_2(BTP)(OH)_4^{2+}$, Scheme 3. Evidently, the present results are not sufficient for a discussion of the mechanism of hydrolysis of bound NPP. By analogy with mechanisms proposed for other metal hydroxo complexes[1f,45b,46] it should involve the intramolecular attack of hydroxide on the phosphoryl group of bound NPP.

With Tris as a ligand only one hydroxo complex $Y_2(Tris)_2(OH)_5^{+}$ exists in solution and the combination of reacting species shown in Scheme 3 is impossible. A point worthy of note, k_3 for the third-order reaction of BNPP with Tris complexes is *ca.* 10 times smaller than k_3 for the reaction with BTP complexes (110 and 830 M^{-2} s^{-1} respectively, see above). The hydrolysis of NPDPP involves a significant contribution of a simple second-order reaction of the substrate with the pentahydroxo complex, eqn. (6). For triesters the reactivity of metal hydroxo complexes correlates well with their basicity[47] and this explains the participation of only more basic pentahydroxo complexes. The third-order path for NPDPP also involves two higher hydroxo complexes $Y_2(BTP)(OH)_5^{+}$, eqn. (6), but due to the kinetic lability of Y(III) complexes this reaction path is indistinguishable from a path involving a combination of $Y_2(BTP)(OH)_4^{2+}$ and $Y_2(BTP)(OH)_6$.

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