Phosphate diester hydrolysis within a highly reactive dinuclear cobalt(III) complex. Ligand effect on reactivity, transition state and dissociation

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The hydrolysis of methyl aryl phosphate diesters coordinated to a dinuclear Co(III) complex ($[Co_2(tame)_2(OH)_2\{O_2P(OAr)(OMe)\}]^{3+}$; tame = 1,1,1-tris(aminomethyl)ethane; **2**) has been studied in aqueous solution at 25 °C. Hydrolysis of the phosphate diester is base catalysed and occurs 30 to 60 fold faster than in analogous complexes where tame is replaced by 1,4,7-triazacyclononane (tacn) (**1**). The second order rate constants for base catalysed hydrolysis of **2** are highly sensitive to the basicity of the aryloxy leaving group with $\beta_{lg} = -1.29 \pm 0.03$. This leaving group dependence is similar to that of **1** ($\beta_{lg} = -1.38 \pm 0.01$), showing that the ligand affects reactivity without greatly altering the transition state at phosphorus. The slight decrease in β_{lg} is consistent with previous rationalisations of this high sensitivity. Dimethyl phosphate coordinated to both types of complex (**3**, tame; **4**, tacn) only dissociates from the complex, with no hydrolysis. Base catalysed dissociation is slower with tame (**3** 20 fold slower than **4**) but the pH independent reaction is faster (**3** 10 fold faster than **4**). These data suggest that the reactivity and turnover properties of these dinuclear complexes may be tuned rationally and independently.

Introduction

Many enzymes that hydrolyse phosphate ester bonds are activated by two or more metal ions.[1] There are many plausible interactions through which the metal ions might contribute to catalysing these reactions, and over the years there has been considerable interest in understanding the important modes of interaction.[2] Metal ion complexes also form the basis of the most effective artificial systems which have been created in efforts to reproduce enzyme-like reactivity;[3] these will be most productively developed through dissecting and quantifying the effect of these interactions. Dinuclear complexes, which can use several of these interactions in combination, are the most effective systems reported so far and are likely to provide the best route to useful artificial catalysts for phosphate diester hydrolysis.[4] Phosphate diesters are kinetically extremely stable to hydrolysis under physiological conditions,[5] and accelerating this rate to a viable level is still a major challenge. Since the ligands surrounding the metal ions are the only means to control modes of action and activity, it is important to understand how activity and ligand structure are related to enable development.

The mechanism and reactivity towards phosphate diester hydrolysis of complex **1** has been reported,[6] followed more recently by an extensive analysis and rationalisation of the transition state structure at phosphorus.[7] These complexes are extremely reactive in promoting the hydrolysis of the coordinated phosphate diester, accelerating base catalysed phosphate diester hydrolysis by 3×10^{10} fold for **1**, X = 4-NO_2. They also show a very high sensitivity to the pK_a of the leaving group, which has been rationalised in terms of a highly strained transition state/intermediate which involves several fused 4-membered rings (Fig. 1b).

Systematic work by Chin's research team established the importance of the effect of ligand structure on the reactivity of mononuclear Co(III) complexes in promoting hydrolysis of phosphate diesters,[8] and Komiyama *et al.* have more recently analysed the effect of ligand structure on Co(III) promoted 3′5′-cAMP hydrolysis.[9] These data can be rationalised by considering the increase in strain at the metal ion centre as it becomes part of a 4-membered ring including the reacting phosphate diester (as shown schematically in Fig. 1a). The angle φ becomes more acute as the transition state (or high energy intermediate) is formed, and this is facilitated if the ligand can accommodate an obtuse complementary bite angle (θ in Fig. 1). Thus, large increases in rate accelerations can be achieved through the rational design of the ligands which bind the metal ion.

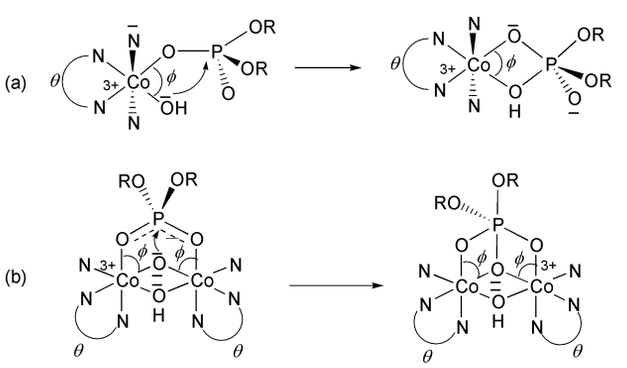

Fig. 1 Schematic representation showing how the angle φ at the Co(III) centre is reduced on forming the transition state/high energy intermediate in the course of promoting phosphate diester hydrolysis (a) for a mononuclear complex; (b) for the dinuclear complexes investigated in this work. This small angle can be better accommodated if the ligand allows the complementary angle θ to expand.

This suggests that the reactivity of the dinuclear complex should be especially sensitive to ligand variation around the Co(III) as both ions will be simultaneously benefiting from this effect, *i.e.* changing to a ligand which allows θ to increase more easily as φ decreases in the course of the reaction should have a bigger impact here than in the mononuclear case. In this

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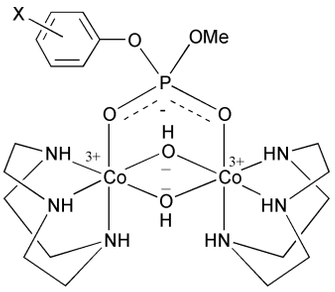

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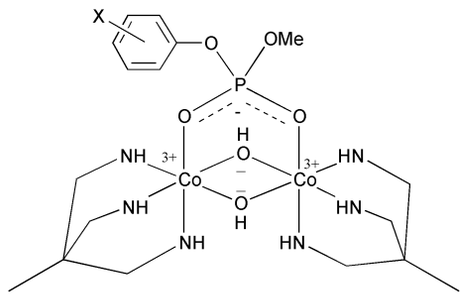
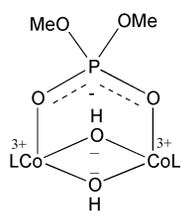


2a, X = $4NO_2$
2b, X = $3NO_2$
2c, X = 3Cl
2d, X = 4Cl
2e, X = H



3, L = tame
4, L = tacn

report we describe the effect on the reactivity of this dinuclear core of changing the ligand bound to Co(III) from 1,4,7-triazacyclononane (tacn) to 1,1,1-tris(aminomethyl)ethane (tame).

Tame was selected as an alternative ligand so that only one stereoisomeric species is formed; like tacn, it can only form facially coordinated complexes with Co(III) which are symmetrical. However, the chelate rings are now 6-membered, so within these complexes the complementary ring to the 4-membered rings involving the metal ion, nucleophilic oxide and the phosphate will always be 6-membered. In the analogous tacn complexes, the complementary chelate ring is always 5-membered. We have measured the rate of base catalyzed hydrolysis of a range of methyl aryl phosphates in the complexes **2a**–**e** and the rate of dissociation of dimethyl phosphate from the complexes **3** and **4**.

Experimental

Instruments

^{1}H NMR (250 MHz), ^{13}C NMR (100 MHz) and ^{31}P NMR (100 or 160 MHz) spectra were obtained with a Bruker AC 250 or AMX 400 MHz spectrometer. Chemical shifts are reported in ppm with 3-(trimethylsilyl)propane-1-sulfonic acid (0 ppm) and 85% phosphoric acid (0 ppm) used as external references. Kinetic studies were carried out by monitoring changes in the UV–vis spectra of dilute solutions in the thermostatted cell compartment of a Cary 1 Bio spectrometer. Product identification by HPLC was performed on a Hewlett-Packard 1100 Liquid Chromatograph using a 4.6 mm × 250 mm Luna ODS C-18 reversed-phase column.

Materials

$[(1,1,1\text{-Tris(aminomethyl)ethane})_2Co_2(OH)_3](NO_3)_3$ (**5**):[10] TameCoCl$_3$[11] was dissolved in water and the pH adjusted to 7 with NaOH. The orange solution was concentrated *in vacuo* and recrystallised from 0.1 M $NaNO_3$ solution to yield brownish-red plates of the triply hydroxide bridged dinuclear complex. This complex was used to prepare stock solutions for kinetic analysis.

δ_H (D_2O): 0.63 (6H, s, CH_3), 2.13 (12H, s, CH_2N). δ_H (DMSO-d_6): −0.59 (3H, μOH), 0.63 (6H, s, CH_3), 2.08 (12H, s, CH_2N), 4.72 (12H, br s, NH). δ_C (D_2O): 21.3 (CH_3), 41.7 (CCH_3), 46.2 (CH_2N).

CAUTION: Perchlorate salts of metal ion complexes are potentially explosive and should be handled with care. Although we have experienced no problems with related complexes, we have handled the complexes described here primarily as dilute aqueous solutions.

General procedure for preparation of stock solutions of **2a**–**e**, **3**, **4** and **6**. The complexes were formed following the same general procedure for making the perchlorate salt of the dinuclear complex with 1,4,7-triazacyclononane as the terminal ligands. $[(1,1,1\text{-Tris(aminomethyl)ethane})_2Co_2(OH)_3](NO_3)_3$ (**5**, 25 mg, 42 μmol) is mixed with two mole equivalents of phosphate diester and 0.5 cm^3 perchloric acid (0.1 M in D_2O). Monitoring the reaction by ^{31}P NMR showed the presence of only bridging diester and free diester. In all cases, the complexed phosphate diesters showed the characteristic ~13 ppm shift downfield of the free diester.[12] This stock solution was used directly for kinetic measurements. **6** was prepared using methyl phosphate monoester to identify the products of hydrolysis. **4** could also be isolated as previously described.[7]

δ_P (D_2O, 0.01 M $HClO_4$): Complex **2a**: 10.5 (free diester: −2.5). Complex **2b**: 10.6 (free diester: −2.4). Complex **2c**: 10.52 (free diester: −2.5). Complex **2d**: 10.7 (free diester: −2.3). Complex **2e**: 10.9 (free diester: −2.2). Complex **3**: 16.8 (free diester: 3.5). Complex **4**: 17.8 (free diester: 3.5). Complex **6**: 17.3 (free monoester: 5.5).

Product analysis

Stock solutions prepared as described above were adjusted to pH 3 and eluted through Sephadex® QAE cationic resin with 0.001 M HCl to remove excess diester, and frozen as it was eluted off the column. Lyophilising these solutions and dissolving the resultant solids in pH 3 solution showed that only complexed phosphate diester was present. Subsequent ^{31}P NMR reveals that under acidic conditions (pH 3) only dissociation of diester from **2a**–**e** is observed. To determine the fate of the phosphate diesters at high pH, the solutions which eluted off the Sephadex column were quenched directly with pH 11 CAPS buffer. **2a**–**d** react to give **6** (17.63 ppm; confirmed by addition of a complex prepared separately using methyl phosphate) and free methyl phosphate (5.54 ppm) in a ratio of 1.7 : 1. **2e** both hydrolysed and released a small proportion of phosphate diester at high pH; the percentage hydrolysis is 90 ± 5, as measured by integrating the ^{31}P NMR signals. The appearance of phenol and free diester were confirmed by HPLC analysis. Both **3** and **4** only release dimethyl phosphate at both high and low pH.

Kinetic methods

Hydrolysis of the complexes (0.05–0.1 mM) under pseudo first order conditions (50 mM aqueous buffer, I = 0.1 M ($NaClO_4$)) at 25 °C was followed by monitoring changes in absorbance with time and accurately obeyed first order kinetics for at least 3 half lives. Wavelengths used: **2a**, 340 or 400 nm; **2b**, 345 nm; **2c**, 263 nm; **2d**, 250 or 295 nm; **2e**, 261 nm; **3**, 295 nm; **4**, 296 nm. The free diesters are stable under these conditions, and show no observable hydrolysis over the timescales used. If the phos-

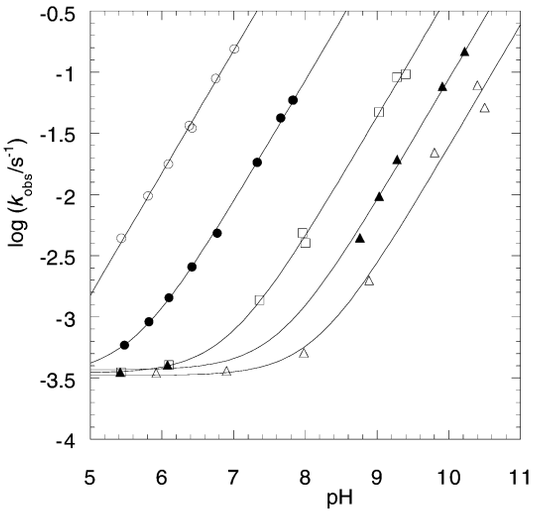


Fig. 2 The pH–rate profile for hydrolysis and dissociation of **2a** (open circles), **2b** (closed circles), **2c** (open squares), **2d** (closed triangles) and **2e** (open triangles) at 25 °C, 50 mM buffer, I = 0.1 M ($NaClO_4$). Solid lines are from eqn. (1) given in the text and rate constants given in Table 1.

phates are not bound to the complex *via* the acidic treatment, then no hydrolysis is observed. We obtained the same observed rate constants when the excess diester was separated from the reaction mixture as described in the product analysis, and when **4** was isolated as previously described. Similarly, we obtain exactly the same kinetic data whether we use the isolated complexes of **1** as substrates, or the acidic solution used in their synthesis.

Buffers used were: MES (2-[*N*-morpholino]ethanesulfonic acid), HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[ethanesulfonic acid]), EPPS (*N*-[2-hydroxyethyl]piperazine-*N*′-[propane-2-sulfonic acid]), CHES (2-[*N*-cyclohexylamino]ethanesulfonic acid), CAPS (3-[*N*-cyclohexylamino]propane-1-sulfonic acid).

The pK_a of the leaving group phenols were determined by potentiometric (I = 0.1 M ($NaClO_4$), titrant 0.1 M NaOH) or UV–vis spectroscopic (50 mM buffer, I = 0.1 M ($NaClO_4$)) titration under our experimental conditions and gave values of 6.95 for 4-NO_2-phenol, 7.95 for 3-NO_2-phenol, 9.00 for 3-Cl-phenol, 9.43 for 4-Cl-phenol and 9.90 for phenol. These values are used in this paper where our data are reported.

Results and discussion

Mixing phosphate diesters and complex **5** at neutral or basic pH leads to no observable hydrolysis of the phosphate. Under these conditions, the free diesters are very stable and hydroxide effectively blocks the coordination sites of the metal ions under these conditions. ^{31}P NMR shows that hydrolysis is only observed after a bridging phosphate complex is formed, which is achieved under acidic conditions. This reflects the behaviour of complexes **1**.[7] The changes in UV–Vis absorbance of fully aqueous solutions of **2a**–**e** across a range of pHs (0.05–0.1 mM substrate, 50 mM aqueous buffer, I = 0.1 M ($NaClO_4$) at 25 °C) accurately followed a first order rate equation for at least 3 half lives to give observed pseudo first order rate constants. These rate constants are plotted against pH in Fig. 2.

In all cases except for **2a**, which only showed specific base catalysis, a hydroxide catalysed and a pH independent region are apparent, so the data were fit to eqn. (1) (for **2a**, k_0 is omitted). The rate constants derived from this analysis are collected in Table 1.

$$k_{obs} = k_0 + k_1[HO^-] \quad (1)$$

Product analysis shows that the diesters only dissociate from the complex at pH 3, where k_0 is the only significant term. Hence, the pH independent region defined by k_0 represents pH independent dissociation of the diester from the complexes. At pH 11 all the reactions are specific base catalysed with negligible contribution from k_0. Only hydrolysis is observed for **2a**–**d** and accounts for 90% of the reaction of **2e**. Hence, k_1 represents specific base catalysed hydrolysis of the complexed phosphate diesters (0.9 k_1 in the case of **2e**).

Table 1 Rate constants for reactions of **2a**–**e**, **3** and **4** obtained by non-linear least squares fit of eqn. (1) to the pH–rate profiles as described in the text. All rate constants were measured in aqueous solution at 25 °C and I = 0.1 M ($NaClO_4$)

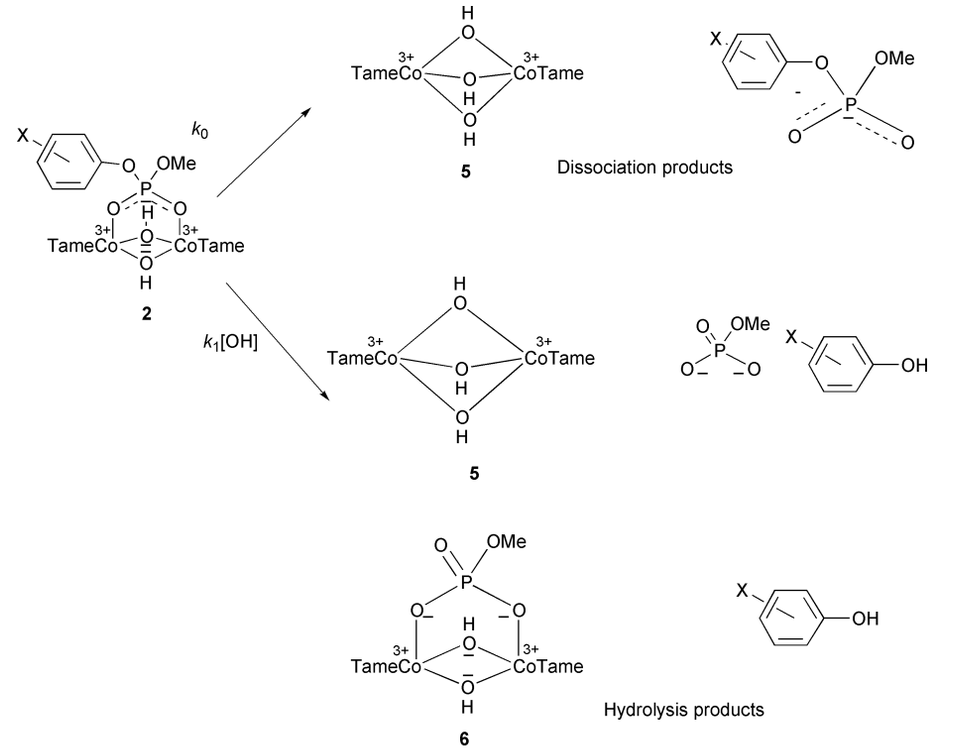
	k_1/M^{-1} s^{-1}	k_0/s^{-1}
2a	$1.50 \pm 0.04 \times 10^6$	–[a]
2b	$8.5 \pm 0.2 \times 10^4$	$3.3 \pm 0.3 \times 10^{-4}$
2c	$4.3 \pm 0.2 \times 10^3$	$3.5 \pm 0.3 \times 10^{-4}$
2d	$8.8 \pm 0.5 \times 10^2$	$3.7 \pm 0.3 \times 10^{-4}$
2e	$2.4 \pm 0.3 \times 10^2$	$3.3 \pm 0.6 \times 10^{-4}$
3	2.8 ± 0.3	$1.5 \pm 0.1 \times 10^{-4}$
4	$4.3 \pm 0.3 \times 10$[b]	$1.4 \pm 0.1 \times 10^{-5}$

[a] Not observed [b] Previously[7] reported as $4.0 \pm 0.4 \times 10^1$; this work includes a wider pH range so that the full data set can be fitted to eqn. (1).

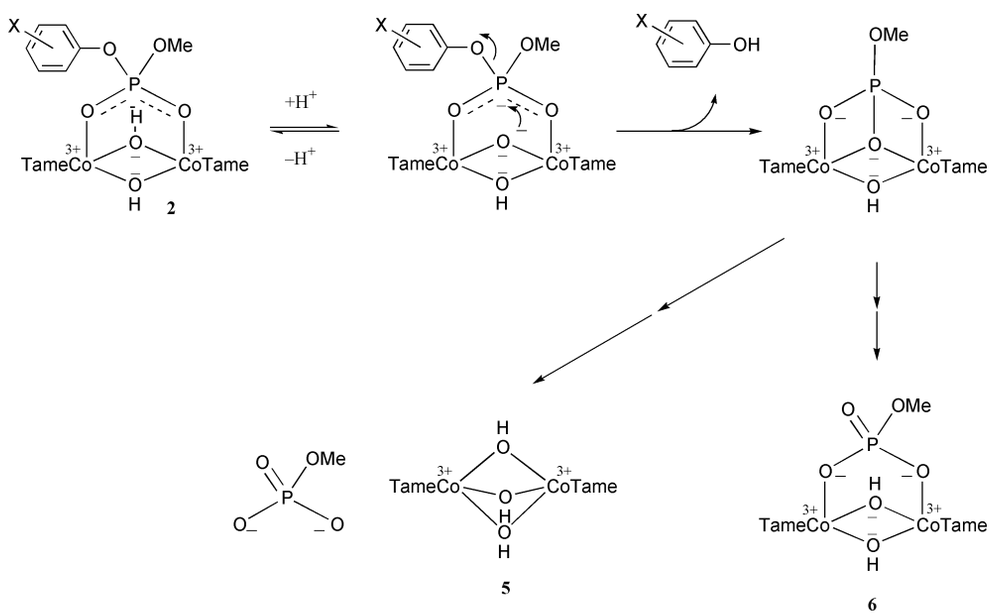
These observations are summarised in Scheme 1. The base catalysed hydrolysis reaction is rationalised by the same mechanism established for complex **1**,[7] and this is shown in Scheme 2. The hydrolysis of **2** closely resembles hydrolysis of **1**, except that **2** both releases methyl phosphate and rearranges to the bridging monoester complex **6** in a ratio of 1.7 : 1 (at pH 11). We explain this observation as partitioning of the intermediate formed after the bridging oxide has attacked the phosphate and expelled the leaving group. In the hydrolysis of complexes **1**, only the bridging product analogous to **6** was observed, and so the analogous intermediate apparently does not partition in the same way. However, we note that similar product analysis with these complexes has not been carried out at this high pH. Although we have not explored this observation in detail, we note that the partitioning process appears to be pH dependent because when **2a** is quenched at pH 7, only hydrolysis to **6** is observed, with no release of methyl phosphate.

Comparing the second order rate constant for hydroxide catalysed hydrolysis of **2a** with **1**, we find that the catalytic core of these dinuclear complexes is 30 fold more reactive when the Co(III) ions are coordinated with tame instead of tacn. At pH 7 and 25 °C, the half life for methyl 4-nitrophenyl phosphate coordinated in complex **1** is 2.5 minutes. Under identical conditions, coordination to the tame complex reduces the half life to 5 seconds (k_{obs} = 0.15 s^{-1}). The second order rate constant for hydroxide catalysed hydrolysis of methyl 4-nitrophenyl phosphate at 25 °C is 1.5×10^{-6} M^{-1} s^{-1},[7] so the background rate at pH 7 through this mechanism is 1.5×10^{-13} s^{-1} (half life ~150 000 years) and complex **2a** has a rate acceleration over the background reaction of some 12 orders of magnitude. Hence, as we had anticipated, the reactivity of this core can be significantly enhanced by the use of a more flexible ligand. For the least reactive diester studied here (methyl phenyl phosphate in **2e**) this rate differential approaches 2 orders of magnitude. We also note that base catalysed dissociation of methyl aryl diesters from **2** is much less significant than in **1**. This provides a ≤10% contribution to the base catalysed reaction of **2e** (≥90% hydrolysis); in contrast, methyl phenyl phosphate coordinated to **1** undergoes 98% dissociation and only 2% hydrolysis. This feature is discussed further below.

Although we show here that a successful rational development in reactivity is possible on the basis described in the introduction, it is unlikely that this approach will form the basis for a quantitative analysis. To do this, structural data from a mimic for the transition state/high energy intermediate would be



Scheme 1



Scheme 2

needed. This would allow the angle θ (Fig. 1) to be measured which might be expected to contribute to a quantitative insight into the reactivity differential; unfortunately, a suitable mimic for the dinuclear complex is not available (*i.e.* a complex which resembles the intermediate in Fig. 1b). In attempts to rationalise the relative activity of mononuclear Co(III) complexes, chelated carbonate complexes have been used for this role. Carbonate chelation gives an acute O–Co–O angle, and those ligands which compensate for this with a larger θ angle generally form more effective complexes for promoting phosphate ester hydrolysis. However, when analysing the effect of carboxylate chelation to mononuclear Co(III) complexes, Chin *et al.* point out that the complementary ring angle cannot be used as a quantitative guide to reactivity in promoting phosphate diester hydrolysis.[13] For example, reactivity in promoting BNPP [bis(4-nitrophenyl) phosphate] hydrolysis follows the series trpn [*N*,*N*-bis(3-aminopropyl)propane-1,3-diamine] > cyclen (1,4,7,10-tetraazacyclododecane) > tren [*N*,*N*-bis(2-aminoethyl)ethane-1,2-diamine], whereas in structures of these complexes containing 4-membered carbonate chelates, the complementary angles are 98°, 103° and 88° respectively. Similarly, Buckingham and Clark[14] have analysed the decarboxylation of Co(III) carbonate chelates, which is also sensitive to ligand structure, and conclude that although there is a qualitative decrease in rate for complexes where the complementary angle is large, the ligands also significantly affect a pre-rate limiting tautomerism, and so a simple structure–reactivity correlation is not possible.

The absolute value of the acceleration is surprisingly small; similar changes in mononuclear complexes lead to a similar or greater impact on reactivity. It may be that the status of the pentacoordinated phosphorus species has a bearing on this observation *i.e.* whether this is an intermediate or a transition state, and investigations into this are ongoing.

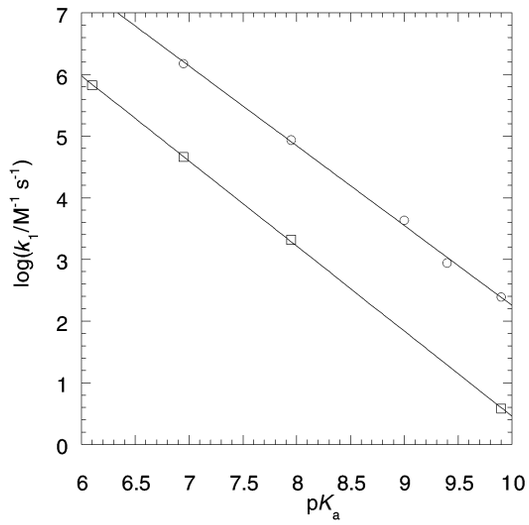


Fig. 3 The dependence of the second order rate constant for hydroxide catalysed hydrolysis of **2a**–**e** on the pK_a of the leaving phenol (circles). The line has slope −1.29 ± 0.03, intercept 15.8 ± 0.3 (r = 0.9991). The lower line (slope −1.38 ± 0.01, intercept 14.26 ± 0.08, r = 0.99995) shows the analogous fit for the tacn complex (squares).[7]

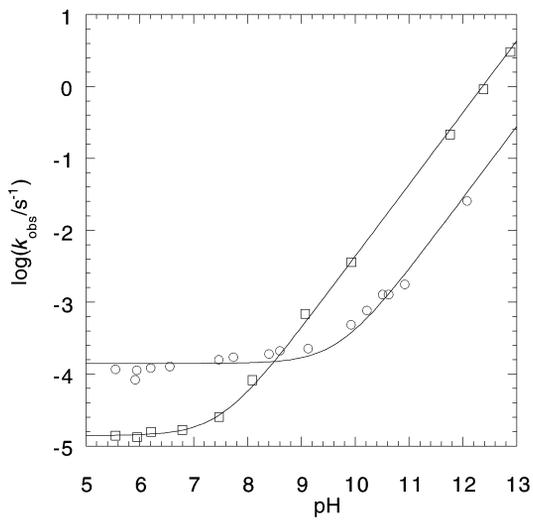


Fig. 4 The pH–rate profile for the dissociation of dimethyl phosphate from **3** (circles) and **4** (squares) at 25 °C, 50 mM buffer, I = 0.1 M ($NaClO_4$). Solid lines are from eqn. (1) given in the text and rate constants given in Table 1.

Another consequence of enhancing the reactivity of this complex in this fashion may be to alter the nature of the transition state at phosphorus. To probe the transition state of the hydrolysis reaction at the phosphate, we can compare the second order rate constants for hydroxide catalysed hydrolysis of **2a**–**e** against the pK_a of the leaving group phenols (Fig. 3).

The slope of this Brønsted plot reveals a β_{lg} = −1.29 ± 0.03 (intercept 15.8 ± 0.3; r = 0.9991). This high sensitivity to the leaving group implies that bond cleavage to the phenolate is well advanced in the transition state, but to estimate the degree of bond cleavage, we need to consider the changes in partial charges involved in the reaction.[15] The effect of coordinating both phosphoryl oxygens to Co is equivalent to a single protonation,[16] so the partial charge on the leaving group oxygen will be similar to that on a phenyl oxygen in neutral phenyl phosphate (+0.83). This changes to −1.0 in the product, so the overall change in charge at the aryl oxygen is −1.83 over the course of the reaction. Comparing this to the value at the transition state revealed by the β_{lg} shows that we have 0.70 bond cleavage in the transition state (=1.29/1.83). For comparison, we have also plotted the analogous data for complexes **1** in Fig. 3. These complexes show slightly higher sensitivity to leaving group pK_a (β_{lg} = −1.38 ± 0.01; intercept 14.26 ± 0.08; r = 0.99995),[7] and so slightly more bond cleavage in the transition state is evident. The same analysis as described above has been applied to this data, revealing 0.75 bond cleavage to the leaving group. This shows that the *difference* in reactivity between the tacn and tame complexes is rather insensitive to the leaving group (this difference only doubles on changing 4-nitrophenol to phenol as a leaving group). Both complexes are very sensitive to the leaving group of the diester, showing that this is a feature of the reactive core.

In a previous explanation for this high degree of bond cleavage to the leaving group, the main factor was ascribed to the strain which is expected to develop in the transition state.[7] As several fused 4-membered rings form, we expect both Hammond and anti-Hammond movement of the transition state relative to the intermolecular attack of hydroxide. Overall, this manifests itself in greater bond cleavage to the leaving group. In changing to a more flexible ligand, the energy of the transition states/intermediates should be lowered. This should reduce both forms of transition state movement, and consequently we expect reduced cleavage to the leaving group. Our observations reported here are in keeping with this explanation.

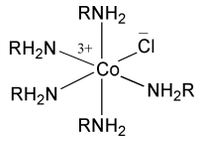
We also examined the dissociation of the phosphate diesters from **3** and **4** to determine the effect of the ligand change on phosphate diester dissociation. For such complexes to be viable catalytically, exchange needs to be rapid. Co(III) is relatively substitutionally inert, and so despite the favourable rate accelerations that have been reported for Co(III) based complexes, they are unlikely to be useful as true catalysts which can undergo turnover. As Co(III) undergoes a dissociative exchange process, ligand flexibility will also be expected to enhance this as it will help facilitate formation of a pentacoordinated intermediate.

As expected, complexes **3** and **4** only release dimethyl phosphate with no observable hydrolysis, and this reaction was measured over a range of pHs (Fig. 4). Both complexes show the same pH dependence as the aryl methyl diesters, and were fit to eqn. (1) (rate constants gathered in Table 1).

The pH independent reaction of **3** is accelerated relative to **4** by a factor of 10; similarly, complexes **2b**–**e** all have the same pH independent rate constant for dissociation (3.5×10^{-4} s^{-1}), which is also 10 fold faster than the analogous tacn complexes **1** (3×10^{-5} s^{-1}, X = 3-NO_2 or H). This is in keeping with the observation that increases in activity of mononuclear complexes is paralleled by increasing rates of substitution at the metal ion.[8,13] This is readily rationalised by noting that the ligand features which encourage high reactivity (as discussed above) will also facilitate the formation of a 5-coordinate intermediate as part of the dissociation pathway.

However, the hydroxide catalysed reaction of **3** is about 20 fold slower than for **4**. We had expected a similar rate increase, as the base catalysed reaction should also be ligand dependent. This observation accounts for the minimal contribution of dissociation of the diesters from the complexes **2** under basic conditions, where only **2e** showed a minor contribution from dissociation to the overall rate. The rate constant derived from 10% dissociation contributing to the base catalysed reaction of **2e** (24 ± 12 M^{-1} s^{-1}) corresponds to ~5 fold decrease in rate compared to **1**, X = H (133 M^{-1} s^{-1}). Hence, although specific base catalysed hydrolysis has been accelerated ≥30 fold in each case, the corresponding base catalysed dissociation has been inhibited and no longer makes a significant contribution to the reaction at higher pHs.

It is well known that dissociation from Co(III) complexes is very sensitive to the nature of the ligand;[17] under basic conditions, where an $E1_{cb}$ mechanism is followed, this needs to be



8, R = H
9, R = Me

rationalised by considering the pK_a of the ligands as well as the ability of the ligand to accommodate 5-coordinate Co(III). More acidic amines lead to a higher concentration of the conjugate base, which is the active species for the expulsion of the leaving group, and hence a faster overall rate of reaction. Comparing the rates of specific base catalysed dissociation of Cl from **8** and **9**, it is observed that for **9**, where the Co(III) is coordinated with 1° amines, loss of chloride is ~1000 fold more rapid than for **8**, which is coordinated with ammonia. Analysis by Lay[18] attributes this difference to poorer solvation of the coordinated 1° amines, which hence have a lower pK_a. A similar analysis accounts for our observation, with ligand flexibility partially compensating for the presumed pK_a difference of the metal coordinated NHs in tacn (2° amines) and tame (1° amines). If the base catalysed dissociation shows a similar ligand effect to the spontaneous dissociation, which is 10 fold faster for complexes **2**, then a comparable reactivity difference due to different substitution levels at the amine ligands as seen for **8** and **9** (where ligand flexibility is not a factor) reasonably accounts for the difference we observe between **3** and **4**.

This suggests that reactivity and dissociation can be controlled rationally and independently. Clearly ligand flexibility can be used to enhance the reactivity, but it may also be possible to make these complexes usefully labile by incorporating more acidic coordinating amines. If complex **2** had shown a similar rate enhancement for base catalysed dissociation as for the hydrolysis reaction, the half life for release of dimethyl phosphate would be reduced to around 10 minutes at pH 7 and 25 °C. Even if this were to be the rate limiting step, this would be quite respectable for an artificial catalyst catalysing the reaction of such unreactive substrates.

In conclusion, the activity towards phosphate diester hydrolysis of the highly reactive dinuclear core of **1** is enhanced ≥30 fold by substituting the ligand tacn with tame. This acceleration alters the nature of the transition state as predicted for the relief of strain in the high energy transition states involved. However, there is still substantial bond cleavage to the leaving group at phosphorus. pH independent dissociation of the diesters from the complex is also accelerated, but base catalysed dissociation is reduced. This suggests that the nucleophilic reactivity of the complex may be separated from the turnover properties, allowing separate tuning of turnover and rate acceleration properties.

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References

1 (*a*) N. Sträter, W. N. Lipscomb, T. Klabunde and B. Krebs, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 2024; (*b*) D. E. Wilcox, *Chem. Rev.*, 1996, **96**, 2435.
2 (*a*) P. Hendry and A. M. Sargeson, *Prog. Inorg. Chem.*, 1990, **38**, 201; (*b*) J. Chin, *Acc. Chem. Res.*, 1991, **24**, 145; (*c*) T. Bruice, *Acc. Chem. Res.*, 1999, **32**, 494.
3 A. J. Kirby, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 707.
4 (*a*) N. H. Williams, B. Takasaki, M. Wall and J. Chin, *Acc. Chem. Res.*, 1999, **32**, 485; (*b*) P. Molenveld, J. F. J. Engberson and D. N. Reinhoudt, *Chem. Soc. Rev.*, 2000, **29**, 75.
5 N. H. Williams and P. Wyman, *Chem. Commun.*, 2001, 1268.
6 D. W. Wahnon, A.-M. Lebuis and J. Chin, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 2412.
7 N. H. Williams, W. Cheung and J. Chin, *J. Am. Chem. Soc.*, 1998, **120**, 8079.
8 J. Chin, M. Banaszczyk, V. Jubian and X. Zou, *J. Am. Chem. Soc.*, 1989, **111**, 186.
9 M. Komiyama, J. Sumaoka, K. Yonezawa, Y. Matsumoto and M. Yashiro, *J. Chem. Soc., Perkin Trans. 2*, 1997, 75.
10 R. Schenker, H. Weihe and H. U. Gudel, *Chem. Phys. Lett.*, 1999, **303**, 229.
11 D. Ventur, K. Wieghardt, B. Nuber and J. Weiss, *Z. Anorg. Allg. Chem.*, 1987, **551**, 33.
12 (*a*) G. P. Haight, Jr., *Coord. Chem. Rev.*, 1987, **79**, 293; (*b*) refs. 6, 7 and 13; (*c*) N. H. Williams and J. Chin, *Chem. Commun.*, 1996, 131; (*d*) N. H. Williams, A.-M. Lebuis and J. Chin, *J. Am. Chem. Soc.*, 1999, **121**, 3341; (*e*) N. H. Williams, *J. Am. Chem. Soc.*, 2000, **122**, 12023.
13 J. Connolly, J. H. Kim, M. Banaszczyk, M. Drouin and J. Chin, *Inorg. Chem.*, 1995, **34**, 1094.
14 D. A. Buckingham and C. R. Clark, *Inorg. Chem.*, 1994, **33**, 6171.
15 (*a*) A. Williams, *Acc. Chem. Res.*, 1984, **17**, 425; (*b*) A. Williams, *Chem. Soc. Rev.*, 1986, **15**, 125; (*c*) N. Bourne and A. Williams, *J. Org. Chem.*, 1984, **49**, 1200.
16 (*a*) J. D. Edwards, S.-W. Foong and A. G. Sykes, *J. Chem. Soc., Dalton Trans.*, 1973, 829; (*b*) K. L. Scott, M. Green and A. G. Sykes, *J. Chem. Soc. A*, 1971, 3651.
17 M. L. Tobe, *Adv. Inorg. Bioinorg. Mech.*, 1983, **2**, 1.
18 P. A. Lay, *Inorg. Chem.*, 1987, **26**, 2144.