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There is no universal mechanism for the cleavage of RNA model compounds in the presence of metal ion catalysts†

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The transesterification of uridine 3′-phosphodiesters with a wide range of leaving group alcohols has been studied in the presence of monometallic and bimetallic complexes. The catalysis of isomerization of the phosphodiester bond was studied with a nucleoside 3′-phosphonate as a substrate. The results obtained are consistent with a step-wise mechanism, where metal ions are able to enhance both the nucleophilic attack and the departure of the leaving group. The mechanism of the catalysis depends on the acidity of the catalyst and of the leaving group alcohol: a change from general base catalysis to general acid catalysis is proposed. Catalysis of the isomerization requires efficient stabilization of the phosphorane by strong interactions with the catalyst. Catalytic strategies utilised by bimetallic complexes are also briefly discussed.

Introduction

Metal ion promoted cleavage of phosphodiesters has been extensively studied over the last three decades and a number of review articles covering different aspects of metal ion catalysis have been published recently.[1–7] The two main objectives of such studies are the need to understand the role of metal ions in the catalysis by metal ion dependent ribozymes or enzymes and the need to develop efficient catalysts for the cleavage of RNA.

In the absence of metal ions RNA is cleaved by intramolecular transesterification, where 2′-OH attacks the phosphate resulting in the formation of a pentacoordinated phosphorane (Scheme 1).[8] The fate of the phosphorane depends on the conditions: under neutral and acidic conditions cleavage and isomerization of a phosphodiester bond are observed. Under alkaline conditions cleavage is the only reaction. The difference results from the properties of the phosphorane under the experimental conditions. While neutral and monoanionic species are stable enough to pseudorotate, which is a prerequisite for isomerization,[9] pseudorotation of a dianionic species has not been observed. The exact status of the dianionic phosphorane was unknown for long, but in 2004 two independent papers were published proposing that the alkaline cleavage is a step-wise reaction with phosphorane being an intermediate.[10,11] The absence of isomerization results, most probably from slow pseudorotation, since according to Westheimer's rules for pseudorotating oxyphosphorane intermediates,[9] placing a negatively charged oxyanion in an apical position, which is inevitable with a dianionic phosphorane, is energetically unfavourable.

Studies with small molecular weight substrates, such as bis(*p*-nitrophenyl)phosphate (**1**), 1-(2-hydroxypropyl)-*p*-nitrophenylphosphate (**2**) and nucleoside 3′-aryl (**3**) and alkyl esters (**4**), have revealed the basic features of metal ion dependent reactions of phosphodiesters. Reviews discussing these studies can be found for example in ref. 3 and 4. References to more specific articles are found in the Results and discussion sections. Zn^{2+} and Cu^{2+} complexes of nitrogen ligands have commonly been used as catalysts. Mononuclear complexes studied include Zn^{2+} complexes with cyclic and acyclic nitrogen ligands (**8–13**), such as azacrowns, 1,5,9-triazacyclododecane (**8**), 1,4,7-triazacyclononane (**9**) and cyclen (**10**) as well as Cu^{2+} complexes with bipyridine (**14**) and terpyridine (**15**) and their derivatives. The shapes of the pH-rate profiles are usually sigmoidal or bell-shaped, depending on the system, and a catalytically important deprotonation at pH close to the pK_a of a metal bound aquo ligand has been observed with a wide variety of catalyst–substrate combinations. Furthermore, metal ion catalysts with acidic aquo ligands, such as Zn^{2+}–**8** with a pK_a of 7.3, are generally the best catalysts. Electrophilic catalysis, nucleophilic catalysis and general acid and base catalysis have all been proposed as the catalysis mechanisms in the cleavage of various types of substrates.

The catalysis by monometallic complexes is fairly modest, and in catalyst development a new era began with the

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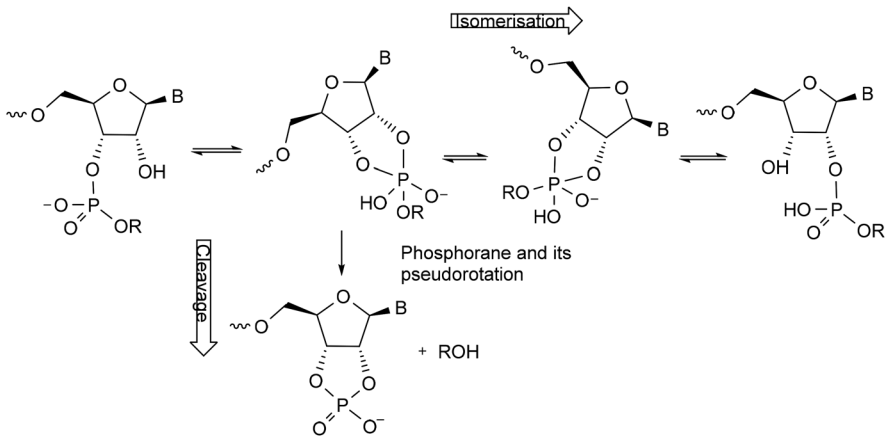
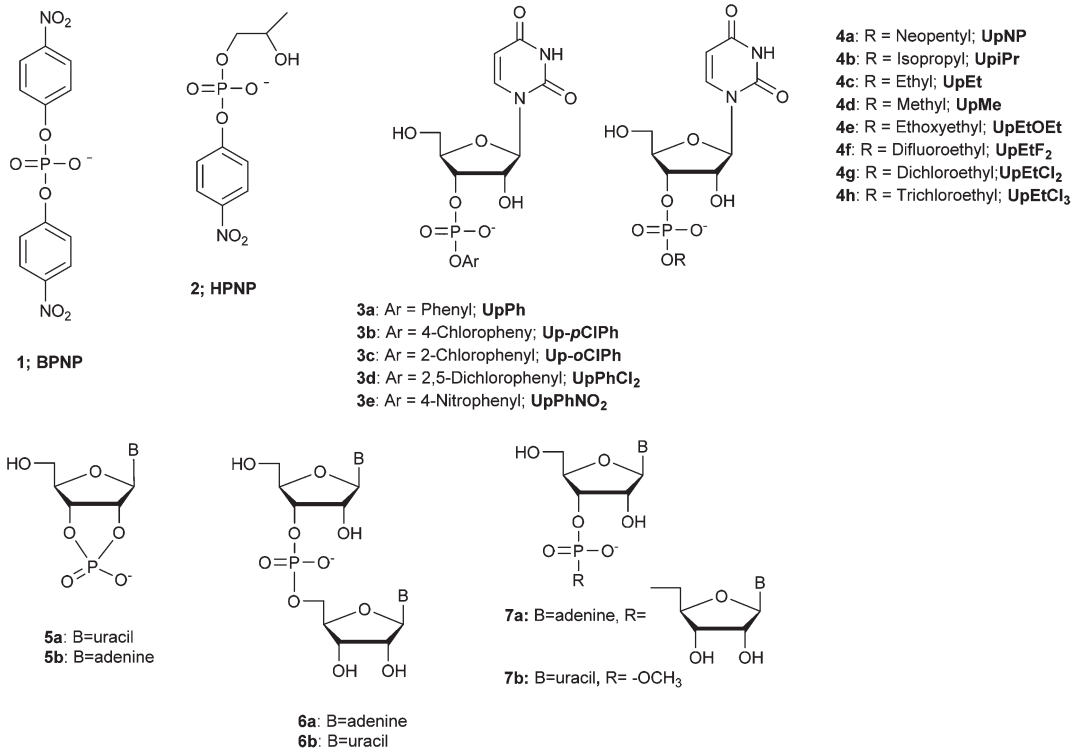
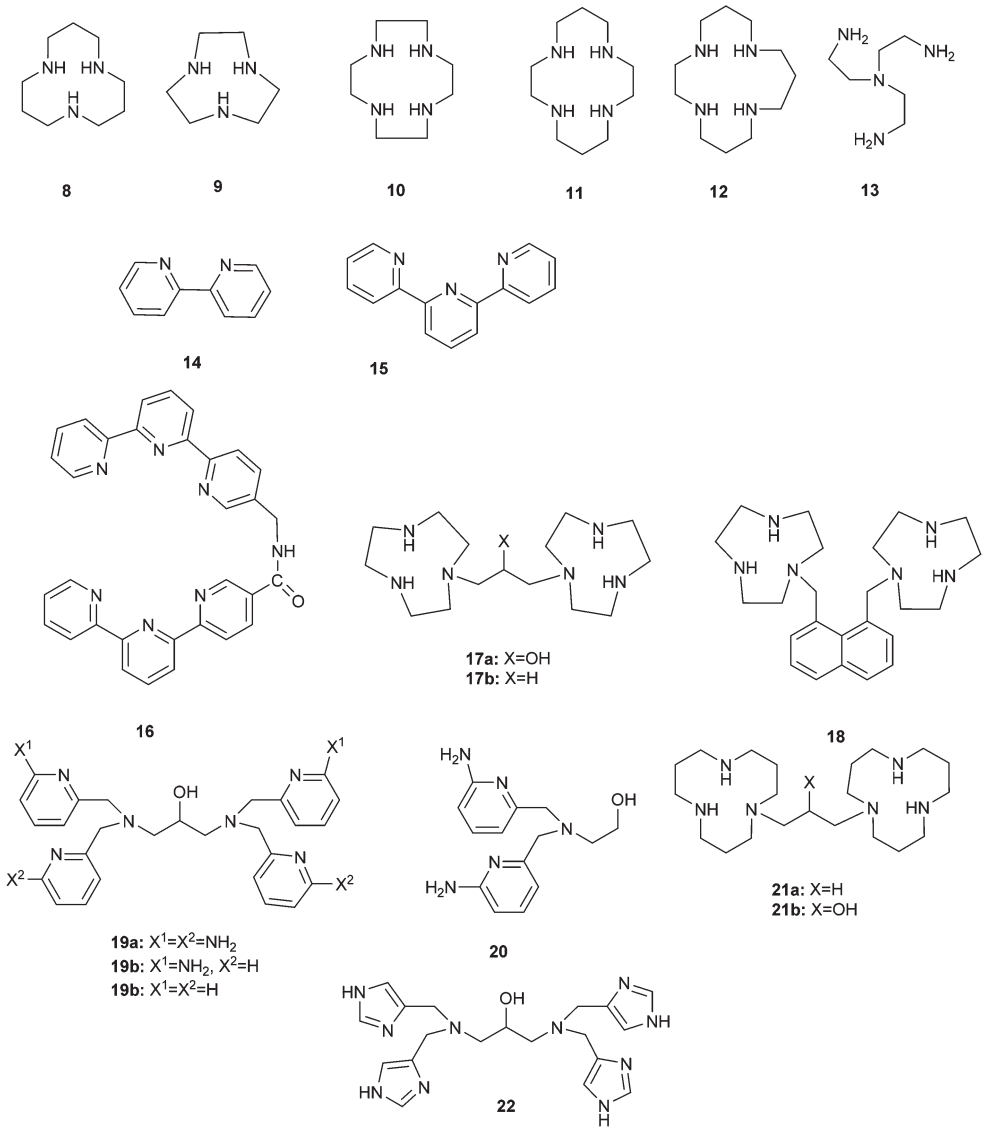


Scheme 1 Intramolecular transesterification reactions of phosphodiester bonds of RNA.



observation that bimetallic complexes can be hundreds of times more efficient as catalysts than their monometallic counterparts. For example, the rate enhancement by the Cu^{2+}–TerPy dimer $Cu^{2+}{}_2$–**16** in comparison to Cu^{2+}–TerPy is 51-fold for the cleavage of HPNP (**2**)[12] and 285-fold for the cleavage of uridine 2′,3′-cyclic monophosphate (**5a**).[13] A Zn^{2+}–**9** dimer $Zn^{2+}{}_2$–**17a** is a 120 times more efficient catalyst for the cleavage of **2** than Zn^{2+}–**9**.[14] $Cu^{2+}{}_2$–**18** is a more than 500 times more efficient catalyst than Cu^{2+}–**9** for the cleavage of 3′,5′-ApA (**6a**), while the difference with 2′,3′-cAMP (**5b**) is 287-fold.[15] An even more significant rate enhancement has been observed with (2-pyridylyl)methylamine based complexes: dimeric $Zn^{2+}{}_2$–**19a** is a more than 1000 times as efficient catalyst than the corresponding monomer Zn^{2+}–**20**. Second-order rate constants of 53 M^{-1} s^{-1} and 0.046 M^{-1} s^{-1} have been reported for reactions catalyzed by $Zn^{2+}{}_2$–**19a**[16] and Zn^{2+}–**20**,[17] respectively. The rate increase can be further enhanced by using an organic medium: the dimer $Zn^{2+}{}_2$–**21a** in methanol is 1.5×10^4 times more efficient as a catalyst for the cleavage of **2** than Zn^{2+}–**8**.[18]

In previous papers we have reported on a very efficient cleavage of 3′,5′-UpU (**6b**)[19] and a series of uridine 3′-alkyl phosphates (**4b**,**4d**–**4h**)[20] by the bimetallic Zn^{2+} complex $Zn^{2+}{}_2$–**19a**. Interestingly this catalyst promoted also the isomerization of phosphodiester bonds. The catalysis on isomerization was



modest in comparison to that for the cleavage reaction, but it was confirmed using a nucleoside 3′-alkylphosphonate **7a** as a substrate.[19] In the absence of competing cleavage, the rate-enhancement could be observed clearly. This was a first observation of a sufficiently stable phosphorane intermediate along the reaction route in a metal ion dependent reaction.

Despite extensive research, a universally accepted mechanism for the metal ion promoted cleavage of RNA model compounds does not exist. It is generally accepted that the strength of interaction between the catalyst and the substrate is essential, but the role of the coordinated metal ion catalyst is not clear. Electrophilic catalysis by coordinated metal ion catalysts is widely supported,[21–24] but general acid[25] and base[26,27] catalyses by metal ion coordinated aquo and hydroxo ligands have also been proposed. Direct coordination to the attacking nucleophile is well established with simpler model compounds.[28–30] A difficulty in mechanistic research is that similar kinetic evidence can be proposed to support different mechanisms. For example pH-rate profiles for the cleavage of a wide variety of phosphodiester substrates are fairly similar. In the present paper we approach the question of the catalysis mechanism from a different angle. The catalytic activity of various mono- and bimetallic complexes in the cleavage of a range of uridine 3′-aryl (**3a–e**) and alkyl phosphates (**4a–h**) has been studied. By contrasting the catalytic effect of metal ion complexes against the nature of the background reaction that varies depending on the acidity of the leaving group, we have gathered information that complements the literature data obtained by studying pH-dependence and solvent isotope effects. Furthermore, the catalytic effect of various metal ion complexes on the isomerization of nucleoside 3′-phosphonate **7b** was studied. In addition to providing new insights into the mechanisms utilised by metal ion complexes in the catalysis of cleavage of different phosphodiesters, the factors

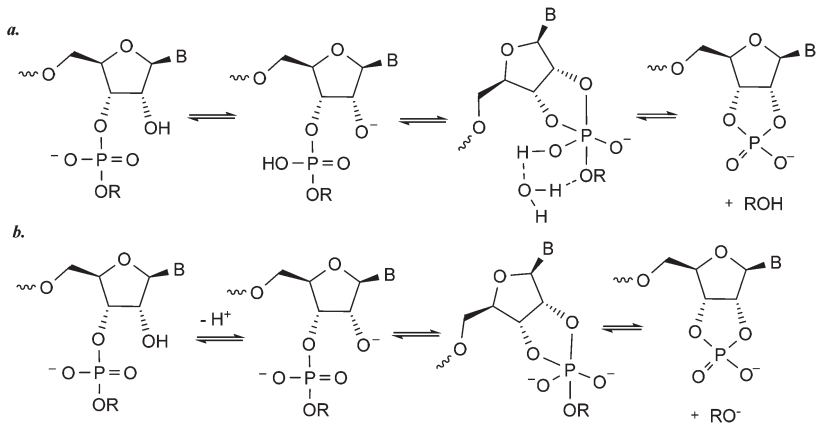
that influence the catalytic efficiency of bimetallic complexes can be evaluated.

Results

Analysis of the uncatalysed reaction

As the first step of the analysis of the kinetic data, the nature of the background reaction under the experimental conditions was identified. According to results of Kosonen *et al.*,[31] spontaneous and base-catalysed reactions (Scheme 2a and 2b, respectively) are the predominant cleavage routes under neutral conditions. The poorer the leaving group is, the higher is the proportion of spontaneous cleavage under the given conditions, as is shown by the shape of pH-rate profiles reported for **4c**, **4e**, **4g** and **4h**.[31] The proportions of these reactions under the experimental conditions, and the rate constants for the background reaction for all alkyl substrates studied in the present work were calculated using parameters reported by Kosonen *et al.*[31] The procedures are explained in the ESI.† According to such an analysis, the reaction of the trichloroethyl ester (**4h**; **UpEtCl$_3$**) at pH 6.5 and 90 °C takes place almost entirely *via* the base-catalysed route, whereas the predominant reaction of the isopropyl ester (**UpiPr**; **4b**) is spontaneous cleavage. Information on the nature of the background reaction is included in Table 1 recording the catalytic activity of mononuclear metal ion complexes for the cleavage of uridine 3′-alkylphosphates.

Mechanistically the two background reactions are fairly different. The spontaneous cleavage is a two-step process with a monoanionic phosphorane intermediate (Scheme 2a). The reaction is believed to involve a concerted proton transfer from



Scheme 2 Predominant mechanisms for the cleavage of phosphodiester bonds under neutral conditions in the absence of metal ions. ***a***. Spontaneous cleavage, ***b***. alkaline cleavage. From ref. 31.

Table 1 Catalysis of the cleavage of uridine 3′-alkyl phosphates 4 by mononuclear Zn^{2+} and Cu^{2+} complexes as measured by relative rate constants $k_{rel} = k_{obs}/k_0$

	UpEtCl$_3$ (4h)	**UpEtCl$_2$ (4g)**	**UpEtF$_2$ (4f)**	**UpEtOEt (4e)**	**UpMe (4d)**	**UpEt (4c)**	**UpiPr (4b)**	**UpnPe (4a)**
pK_a (LG)[a]	12.2	12.9	13.0	14.8	15.5	15.8	17.1	17.3
k_0/s^{-1} pH 6.6 at 90°[b]	7.3×10^{-5}	1.2×10^{-5}	9.5×10^{-6}	1.3×10^{-7}	2.9×10^{-8}	1.8×10^{-8}	2.1×10^{-9}	1.6×10^{-9}
Background reaction[b,c]	3%/97%	6%/94%	7%/93%	37%/63%	62%/37%	65%/35%	90%/10%	89%/6%
k_{rel} 10 mM Zn^{2+}–**8**	29	146		1873		3000	809	
k_{rel} 2 mM Zn^{2+}–**8**	6	26	28	410	(2116)[d]		52	140
k_{rel} 2 mM Zn^{2+}–**9**	1.5	3.3		42		37	81	
k_{rel} 2 mM Zn^{2+}–**9**	1	2.4	2.8	26	(181)[d]		119	110
k_{rel} 2 mM Zn^{2+}–**10**	3	5.7		40		28	20	
k_{rel} 2 mM Zn^{2+}–**11**	1.4	1.6		1.3		2.4	4.5	
k_{rel} 2 mM Zn^{2+}–**12**	1	1.6		1.3		3.3	8.5	
k_{rel} 2 mM Zn^{2+}–**13**	1.1	1.6		1.3		1.8	7.5	
k_{rel} 10 mM Cu^{2+}–**14**	11	15	16	291	1502		1429	1900
k_{rel} 10 mM Cu^{2+}–**15**	61	171	80	2164	2048		4333	13 000
k_0/s^{-1} pH 5.6 at 90°[b]	9.0×10^{-6}	1.9×10^{-6}	1.5×10^{-6}	5.9×10^{-8}	2.2×10^{-8}	1.3×10^{-8}	2.0×10^{-9}	2.0×10^{-9}
Background reaction[c]	22%/78%	39%/41%	41%/58%	84%/14%	88%/7%	94%/5%	96%/1%	69%/1%
k_{rel} 10 mM Zn^{2+}–**8**	18	50		989		870	448	
k_{rel} 10 mM Zn_{aq}[e]	12	25		594		1231	1524	
k_{rel} 2 mM Zn_{aq}	1.5	5	8	44				

[a] From ref. 33, except for that for **4a** that was determined kinetically in 1 M NaOH at 25 °C using the known β_{LG} value of −1.28 from ref. 31. [b] Rate constant for the cleavage in the absence of a metal ion catalyst calculated using the data in ref. 31 as explained in the ESI. [c] Percentage of spontaneous and alkaline reactions in the background reaction calculated using data reported in ref. 31. [d] Catalysis of the cleavage of **UpMe** (**4d**) was exceptionally high and the values should be regarded with caution. [e] Rate constants have been reported in ref. 25.

the phosphate to the leaving group, and it is characterized by a moderately negative β_{LG} of −0.59.[31] The alkaline cleavage involves a nucleophilic attack by a 2′-oxyanion, the formation of a dianionic phosphorane, and the departure of the leaving group as an alkoxy anion (Scheme 2b). Consistent with a negative charge on the leaving group oxygen, the alkaline cleavage is characterized by a highly negative β_{LG} of −1.28.[31] In other words, the analysis of the background reaction shows that while the better alkyl leaving groups, *e.g.* trichloroethoxy and dichloroethyoxy groups, can depart as alkoxy anions at pH 6.6, protonation of the poorer leaving groups is required to allow the cleavage.

As mentioned in the introduction, alkaline cleavage is also a step-wise reaction with a change in the rate-limiting step at a pK_a of 12.6. Accordingly, with **UpEtCl** (**4h**) and with aryl esters **3a–e** the formation of the phosphorane is the rate-limiting step, whereas with esters with pK_a(LG) > 12.6 the departure of the leaving group determines the rate of the cleavage reaction. The difference in kinetic solvent isotope effects determined previously[20] for the spontaneous cleavage of trichloroethyl (3.8) and dichloroethyl (6.0) esters at pH 6.5 is consistent with the change. Values 4.9 and 7.2 have been reported for the alkaline cleavage of nucleoside 3′-phenyl and -methoxyethyl esters, respectively.[32]

Catalysis of the cleavage of uridine 3′-alkyl esters by monomeric Zn^{2+} complexes

The cleavage of a number of uridine 3′-alkyl and aryl esters was studied in the presence of various Zn^{2+} complexes. Table 1 shows the results obtained with the alkyl esters by recording the rate-enhancement observed in the presence of the catalysts. Actual rate constants are given in Table S2 in the ESI.† Examples of catalysis by different Zn^{2+} complexes are also shown in Fig. 1, where the logarithmic rate constants for the cleavage of alkyl esters in the presence of 2 mM Zn^{2+}–**8**, Zn^{2+}–**9** and Zn^{2+}–**11** are shown as a function of pK_a of the leaving group. It is clear on the basis of the results shown that none of the Zn^{2+} based mononuclear catalysts significantly enhance the cleavage of the trichloroethyl ester (**4h**; **UpEtCl$_3$**). At 2 mM concentration only Zn^{2+}–**8** and Zn^{2+}–**10** showed any observable effect. An experiment at a higher catalyst concentration confirms that Zn^{2+}–**8** does enhance the cleavage. A more significant rate-enhancement is observed with the less reactive substrates bearing a more basic leaving group. The largest effect among mononuclear Zn^{2+}-catalysts is observed with 10 mM Zn^{2+}–**8** that at pH 6.6 promotes the cleavage of the ethyl ester **UpEt** (**4c**) by a factor of 3000. As the leaving group becomes poorer, the catalytic effect of Zn^{2+}–**8** decreases, as is clearly shown by the results in Fig. 1. The same trend is observed with Zn^{2+}–**10**.

In contrast to the behaviour of Zn^{2+}–**8**, Zn^{2+} aquo ions at pH 5.6 exhibit the most efficient catalysis with the least reactive substrates as is shown by the results in Table 1. The catalysis by Zn^{2+}_{aq} on the cleavage of the reactive esters **4e–h** is more modest than that by Zn^{2+}–**8**, but with **4b** and **4c**, it is actually the better of the two catalysts. The catalysis by Zn^{2+}–**9** resembles closely that by Zn^{2+}_{aq} but the behavior is less pronounced. Results obtained with Zn^{2+}–**9** are shown in Fig. 1. Other Zn^{2+} complexes tested were Zn^{2+}–**11**, Zn^{2+}–**12** and Zn^{2+}–**13** that showed a very modest catalytic effect and only with phosphodiesters with the poorest leaving groups. Results obtained with Zn^{2+}–**11** are included in Fig. 1 as an example of these inefficient catalysts.

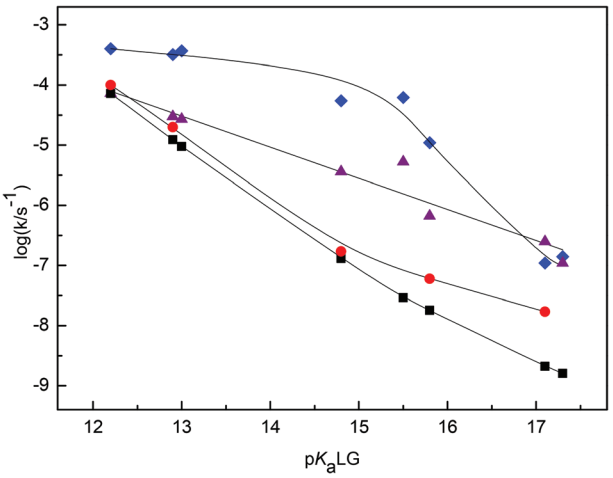


Fig. 1 Rate constants for the cleavage of uridine 3′-alkylesters in the presence of Zn^{2+} complexes of different acidity at pH 6.6 and 90 °C. Notation: ***squares*** – uncatalysed cleavage; rate constants calculated as explained in the ESI, ***circles*** – 2 mM Zn^{2+}–**11** (pK_a = 9.9), ***triangles*** – 2 mM Zn^{2+}–**9** (pK_a = 9.0), ***diamonds*** – 2 mM Zn^{2+}–**8** (pK_a = 7.5). The pK_a values given refer to the deprotonation of a metal aquo ligand at 25 °C. They are discussed in more detail in the Results section. Lines are manually drawn to emphasise the trend.

Zn^{2+} based catalysts can, hence, be roughly divided into three categories. The first group (group A in the following discussion) includes catalysts Zn^{2+}–**8** and Zn^{2+}–**10** which were the only ones with any observable catalytic activity at 2 mM concentration for the cleavage of the trichloroethyl ester **UpEtCl$_3$**. These catalysts show the highest catalytic activity with ethoxyethyl **UpEtOEt**, ethyl **UpEt** or methyl **UpMe** esters. As the acidity of the leaving group further decreases, the catalytic activity decreases. The second group of catalysts consists of Zn^{2+}_{aq} and Zn^{2+}–**9** (group B) that only modestly enhance the cleavage of **UpEtOEt** with a moderately acidic leaving group, but which show their highest catalytic activity with the least reactive substrates **UpiPr** or **UpnPe**. There is no drop in the catalytic activity of Zn^{2+}_{aq}. The third group of catalysts consists of Zn^{2+}-complexes Zn^{2+}–**11**, Zn^{2+}–**12** and Zn^{2+}–**13** (group C) which show only a very modest catalytic activity and only with the least reactive uridine 3′-alkyl esters. The difference between the groups is clearly seen in Fig. 1, where the logarithmic rate constants for the cleavage of alkyl esters in the presence of 2 mM Zn^{2+}–**8**, Zn^{2+}–**9** and Zn^{2+}–**11** as representatives of group A, B and C catalysts are shown as a function of the pK_a of the leaving group.

The difference between the catalysts is in their acidity. pK_a's of 7.5[34] and 8.0[28] have been reported for group A catalysts Zn^{2+}–**8** and Zn^{2+}–**10**, respectively, whereas the pK_a values of group B catalysts are slightly higher. The pK_a of the Zn^{2+} aquo ion is

9.0.[35] The information on the acidity of Zn^{2+}–**9** is slightly ambiguous: according to Zompa[34] and Kimura *et al.*[28] the pK_a cannot be determined by potentiometric titration because of solubility problems. A kinetic value of 9.2 has been proposed on the basis of the pH-dependence of the Zn^{2+}–**9** promoted cleavage of HPNP (**2**).[36] Despite a concern for solubility, this value seems reliable. The shape of the pH-rate profile is similar to those obtained with other catalysts, and generally pK_a values obtained from pH-dependence agree well with values obtained by potentiometric titration. Group C catalysts are even more basic: pK_a values of 9.8 and 9.9 have been reported for Zn^{2+}–**13** and Zn^{2+}–**11**, respectively.[37] The pK_a of Zn^{2+}–**12** is not known, but the similarity of its catalytic inactivity to that of Zn^{2+}–**11** suggests that the pK_a is of the same order.[38]

The pK_a values given above refer to 25 °C, but the temperature dependence of the pK_a values is most probably steep. pK_a values of 7.30 and 7.89 have been determined for the deprotonation of the water ligand of Zn^{2+}–**8** at 25 and 0 °C, respectively.[28] Corresponding pK_a values of Zn^{2+}–**10** bound water are 8.02 and 8.54. Assuming that the temperature dependence is linear, pK_a values of 5.8 and 6.7 at 90 °C can be calculated for aquo ligands of Zn^{2+}–**8** and Zn^{2+}–**10**, respectively. The assumption of a linear dependence is very crude, but the large drop in pK_a values is consistent with the steep temperature dependence of the water autoprotolysis constant pK_W.[39] The 0.3 unit drop in the pK_a value of the Zn^{2+}–**9** bound alcohol group as the temperature increases from 25 to 40 °C is also of the same order.[30]

Complexes with a fairly low pK_a value exist as equilibrium mixtures of aquo and hydroxo forms under neutral conditions. Provided that the estimated pK_a values are correct, at pH 6.6 and 90 °C Zn^{2+}–**8** exists predominantly in the hydroxo form, whereas with Zn^{2+}–**10** the concentrations of the aquo and hydroxo forms are approximately equally large. Assuming that the temperature dependence of the pK_a values of other Zn^{2+} complexes of the same type is similar, pK_a values of 7.2 and 8.1 at 90 °C can be estimated for Zn^{2+}–**9** and Zn^{2+}–**11**, respectively. Accordingly, at pH 6.6, group A catalysts exist to a large extent in their hydroxo form, while with group B catalysts the hydroxo form is a minor component and the aquo form predominates. Group C catalysts exist almost entirely in their aquo form.

Monomeric Zn^{2+} complexes as catalysts of the cleavage of uridine 3′-aryl esters

Table 2 presents catalytic data obtained with mononuclear complexes and uridine 3′-aryl esters **3a–e**. As can be seen, the rate-enhancement by $Zn^{2+}{}_{aq}$ and Zn^{2+} complexes is very modest. Zn^{2+}–**8** is again a better catalyst than Zn^{2+}–**9** or $Zn^{2+}{}_{aq}$ and experiments carried out at a higher catalyst concentration confirm the rate-enhancing effect. The results show also that even though the effects are modest, the rate-enhancement increases as the acidity of the leaving group increases on going from the phenyl ester **3a** to the *p*-nitrophenyl compound **3e**, which is the total opposite of the situation with uridine 3′-alkyl phosphates. It is possible that the effect is actually even larger, but the increasing electronegativity of the leaving group may influence the observed catalytic activity in conflicting ways. While the increasing acidity of the leaving group enhances the reaction, the affinity of the metal ion towards the phosphate may decrease. Consistent with this, phenyl phosphate has been shown to bind the catalyst Zn^{2+}–**8** 2.5 times as strongly as *p*-nitrophenylphosphate.[29]

Catalysis by bipyridine and terpyridine complexes of Cu^{2+}

The bipyridine complex Cu^{2+}–**14** and the terpyridine complex Cu^{2+}–**15** at 10 mM concentration show good catalytic activity in the cleavage of alkyl esters, with Cu^{2+}–**15** being up to 10 times better as a catalyst than Cu^{2+}–**14** (Table 1). The catalytic activity of Cu^{2+}–**15** is approximately the same as that of Zn^{2+}–**8** with the more reactive alkyl esters as a substrate (**4e–4h**; 12 < pK_a(LG) < 15). However, the catalytic activity of Cu^{2+}–**15** or Cu^{2+}–**14** does not drop as the acidity of the leaving group decreases. The higher catalytic activity of Cu^{2+}–**15** is inconsistent with the trend observed with Zn^{2+} catalysts for the pK_a of

Table 2 Catalysis of the cleavage of uridine 3′-aryl phosphates 3 by mononuclear Zn^{2+} and Cu^{2+} complexes as measured by relative rate constants $k_{rel} = k_{obs}/k_0$

	UpPh (3a)	**Up-*p*ClPh (3b)**	**Up-*o*ClPh (3c)**	**$UpPhCl_2$ (3d)**	**$UpPhNO_2$ (3e)**
pK_a[a]	9.95	9.38	8.48	7.51	7.14
$k_{uncat}/10^{-6}$ s^{-1}, pH 6.5, 25 °C	0.39 ± 0.1	1.33 ± 0.02	1.84 ± 0.04	13.2 ± 0.3	43.1 ± 0.6
k_{rel} 2 mM Zn^{2+}–**8**	6.6	5.6	7.6	12	18
k_{rel} 10 mM Cu^{2+}–**14**	26	36	57	108	116
k_{rel} 10 mM Cu^{2+}–**15**	114	167	205	250	179
$k_{uncat}/10^{-4}$ s^{-1}, pH 6.5, 90 °C	3.65 ± 0.05	9.1 ± 0.2	11.7 ± 0.4	51 ± 3	106 ± 5
k_{rel} 2 mM Zn^{2+}–**9**	1.7	1.9	1.8	2.6	5.7
k_{rel} 2 mM Zn^{2+}–**8**	13	10	11		
k_{rel} 10 mM Cu^{2+}–**14**	2.8	4.4	4.4		
k_{rel} 10 mM Cu^{2+}–**15**	22	31	46		
$k_{uncat}/10^{-6}$ s^{-1}, pH 5.9, 25 °C[b]	0.25 ± 0.02	0.68 ± 0.02	0.95 ± 0.01	5.06 ± 0.04	18.9 ± 0.1
k_{rel} 10 mM $Zn^{2+}{}_{aq}$[b]	2.8	3.5	4.6	12	33
k_{rel} 10 mM Zn^{2+}–**8**	10	17	24	51	58
$k_{uncat}/10^{-5}$ s^{-1}, pH 7.5, 25 °C[a]	0.618	1.40	1.92	10.4	
k_{rel} 10 mM Zn^{2+}–**8**	15	32	72	99	

[a] From ref. 40. [b] Rate constants reported in ref. 25.

Cu^{2+}–**15** (8.2) is higher than that of Cu^{2+}–**14** (7.8).[41] A similar difference has been observed before and has been explained by the formation of a catalytically inactive Cu^{2+}–**14** dimer under the experimental conditions.[41] In comparison with the 100-fold difference between 10 mM Cu^{2+}–**15** and 10 mM Cu^{2+}–**14** in the cleavage of 3′,5′-ApA (**6a**), the difference observed in the present work is modest, but it most probably results from different conditions. The previously reported 100-fold difference has been obtained by comparing maximal rate constants obtained at optimal pH, whereas our values refer to a fixed pH value of 6.5.

Cu^{2+}–**15** is also a fairly good catalyst for the cleavage of uridine 3′-aryl esters (**3a–e**), while the catalysis by Cu^{2+}–**14** is again more modest (Table 2). Similar to the catalysis by Zn^{2+} complexes the rate-enhancing effect increases as the acidity of the leaving group increases. The increase is slightly more prominent with Cu^{2+}–**14**, and as a result of this, the difference between the two catalysts decreases as the acidity of the leaving group increases. Another curious feature of the catalysis by the Cu^{2+} complexes is the drop in the activity as the temperature increases. This is clearly seen when the catalytic activity of Cu^{2+}–**15** is compared to that of Zn^{2+}–**8**. At 25 °C Cu^{2+}–**15** is even a better catalyst than Zn^{2+}–**8**, but at 90 °C the situation is the opposite (note that [Zn^{2+}–**8**] is lower than [Cu^{2+}–**15**].

Dimerisation of the catalysts offers most likely an explanation also for the illogical behavior of Cu^{2+} complexes with the aryl esters. While most of Cu^{2+}–**14** is dimerized under neutral conditions,[41] the Cu^{2+}–**15** dimer has been reported as a minor species under neutral conditions at a total Cu^{2+}–**15** concentration of 1.5 mM.[42] We can hence assume that under the experimental conditions of the present study, Cu^{2+}–**14** exists mostly as a dimer, but a monomer is the predominant form of Cu^{2+}–**15** at 90 °C. However, the Cu^{2+}–**15** concentration at 10 mM favours the dimerization, and at 25 °C, the proportion of the Cu^{2+}–**15** dimer may be more significant. The changes in the relative catalytic activities at 25 °C and 90 °C could then be explained by changes in the concentration of monomeric and dimeric species.

The results obtained with aryl esters (Table 2) show also that the difference in catalytic activity of Cu^{2+}–**14** and Cu^{2+}–**15** decreases as the acidity of the leaving group increases. With the *p*-nitrophenyl ester **3e**, Cu^{2+}–**15** and Cu^{2+}–**14** are approximately as good catalysts. This is consistent with the results obtained with HPNP (**2**),[12] where Cu^{2+}–**14** is actually a better catalyst than Cu^{2+}–**15** at pH 6.5. This may suggest a change in the catalytic mechanism as the nucleophilic attack becomes more clearly rate-limiting.

Catalysis by bimetallic complexes

The catalysis by several dinuclear metal ion complexes was also studied. Results obtained with alkyl and aryl esters are presented in Tables 3 and 4, respectively. As the catalysis by dinuclear complexes is generally more efficient than that observed with monomeric complexes, the experiments have been carried out at lower temperatures, where the rate constants of the uncatalysed reactions are available only for the aryl esters and **UpEtCl$_3$** (**4h**). Unfortunately, data obtained with **UpEtCl$_3$** cannot be extended to other alkyl esters, since, as discussed above, the proportion of the spontaneous cleavage increases with less reactive esters, and the temperature dependence of this reaction is not known. Rate-enhancement can hence be only estimated in most of the cases.

Similar to the situation with monomeric complexes, the poorest catalysis is observed with **UpEtCl$_3$** (**4h**) as the substrate. Rate-enhancing effects observed in this case are larger, consistent with the known activity of bimetallic complexes. However, none of the other complexes studied in the present work were even nearly as efficient catalysts as $Zn^{2+}{}_2$–**19a** that at 25 °C and pH 6.5 promoted the cleavage of **UpEtCl$_3$** by a factor of 240 000. Even though the rate constants for the uncatalysed reaction of the other alkyl esters at 25 °C are not known, it can be deduced that similar to the situation with monometallic complexes, the catalytic activity increases as the acidity of the

Table 3 Catalysis of the cleavage of uridine 3′-alkyl phosphates 4 by binuclear Zn^{2+} complexes and a comparison to the activity of corresponding mononuclear complexes

	UpEtCl$_3$ (**4h**)	k_{obs}/k_0 (**UpEtCl$_3$**)[a]	**UpEtCl$_2$** (**4g**)	**UpEtF$_2$** (**4f**)	**UpEtOEt** (**4e**)
pK_a[b]	12.2		12.9	13.0	14.8
2 mM Zn^{2+}–**9** at 50 °C, pH 6.5	$(1.06 \pm 0.1) \times 10^{-6}$	2	$(2.83 \pm 0.5) \times 10^{-7}$	$(3.51 \pm 0.05) \times 10^{-7}$	$(1.5 \pm 0.1) \times 10^{-8}$
2 mM Zn^{2+}–**8** at 50 °C, pH 6.5	$(8.8 \pm 0.3) \times 10^{-6}$	19	$(5.74 \pm 0.07) \times 10^{-6}$		$(6.02 \pm 0.04) \times 10^{-7}$
1 mM $Zn^{2+}{}_2$–**17a** at 50 °C, pH 6.5	$(6.43 \pm 0.03) \times 10^{-4}$	1397	$(1.65 \pm 0.01) \times 10^{-4}$	$(1.96 \pm 0.06) \times 10^{-4}$	$(7.5 \pm 0.4) \times 10^{-6}$
k ($Zn^{2+}{}_2$–**17a**)/k (Zn^{2+}–**9**)[c]	607		583	558	500
1 mM k (Zn^{2+}–**17b**) at 50 °C, pH 6.5	$(1.12 \pm 0.01) \times 10^{-6}$	2	$(4.25 \pm 0.05) \times 10^{-7}$	$(5.91 \pm 0.07) \times 10^{-7}$	$(4.9 \pm 0.7) \times 10^{-8}$
k (Zn^{2+}–**17b**)/k (Zn^{2+}–**9**)[c]	1.06		1.61	1.74	1.02
1 mM ($Zn^{2+}{}_2$–**21b**) at 50 °C, pH 6.5	$(1.30 \pm 0.01) \times 10^{-6}$	3	$(3.55 \pm 0.05) \times 10^{-7}$	$(4.41 \pm 0.04) \times 10^{-7}$	$(3.6 \pm 0.2) \times 10^{-8}$
k ($Zn^{2+}{}_2$–**21b**)/k (Zn^{2+}–**8**)[c]	0.15		0.06		0.06
1 mM $Zn^{2+}{}_2$–**22** at 50 °C, pH 7.0	$(1.34 \pm 0.04) \times 10^{-4}$	89[d]	$(4.7 \pm 0.4) \times 10^{-5}$	$(3.67 \pm 0.06) \times 10^{-5}$	$(1.6 \pm 0.2) \times 10^{-6}$
1 mM $Zn^{2+}{}_2$–**19c** at 50 °C, pH 6.5	$(3.3 \pm 0.5) \times 10^{-4}$	717	$(5.6 \pm 0.3) \times 10^{-5}$	$(5.0 \pm 0.3) \times 10^{-5}$	$(1.40 \pm 0.03) \times 10^{-6}$
1 mM $Zn^{2+}{}_2$–**19a** at 25 °C, pH 6.5[e]	$(5.03 \pm 0.06) \times 10^{-3}$	239 524	$(3.8 \pm 0.1) \times 10^{-4}$	$(4.5 \pm 0.1) \times 10^{-4}$	$(4.4 \pm 0.4) \times 10^{-6}$

[a] Rate constant for the cleavage of **UpEtCl$_3$** (**4h**) in the presence of a catalyst relative to that of the uncatalysed reaction under the same conditions. k_0 values of 2.1×10^{-8} s^{-1} and 4.6×10^{-7} s^{-1} at 25 °C and 50 °C and pH 6.5 were calculated as explained in ESI. [b] From ref. 33. [c] Rate constant for the cleavage in the presence of a bimetallic complex relative to that obtained in the presence of the corresponding monomeric catalyst. [d] A rate constant of 1.5×10^{-6} for the uncatalysed reaction was calculated assuming a first-order dependence on [HO^-]. [e] From ref. 20.

Table 4 Catalysis of the cleavage of uridine 3′-aryl phosphates 3 by binuclear Zn^{2+} complexes and a comparison to the activity of corresponding mononuclear complexes

	UpPh (3a)	**Up-*p*ClPh (3b)**	**Up-*o*ClPh (3c)**	**$UpPhCl_2$ (3d)**	**$UpPhNO_2$ (3e)**
pK_a[a]	9.95	9.38	8.48	7.51	7.14
k_0/s^{-1} pH 6.6 at 25°	$(3.92 \pm 0.09) \times 10^{-7}$	$(1.33 \pm 0.02) \times 10^{-6}$	$(1.84 \pm 0.04) \times 10^{-6}$	$(1.32 \pm 0.03) \times 10^{-5}$	$(4.31 \pm 0.06) \times 10^{-5}$
k/s^{-1}, 1 mM $Zn^{2+}{}_2$–**19c**	$(1.19 \pm 0.02) \times 10^{-3}$	$(4.6 \pm 0.1) \times 10^{-3}$	$(1.28 \pm 0.02) \times 10^{-2}$		
k_{rel} $Zn^{2+}{}_2$–**19c**[b]	3036	3459	6957		
k/s^{-1}, 1 mM $Zn^{2+}{}_2$–**17a**	$(4.14 \pm 0.09) \times 10^{-4}$	$(8.4 \pm 0.2) \times 10^{-4}$	$(2.15 \pm 0.08) \times 10^{-3}$	$(1.58 \pm 0.07) \times 10^{-2}$	
k_{rel} $Zn^{2+}{}_2$–**17a**	1056	632	1168	1197	
k/s^{-1}, 1 mM $Zn^{2+}{}_2$–**22**[c]	$(9.0 \pm 0.1) \times 10^{-5}$	$(3.01 \pm 0.03) \times 10^{-4}$	$(6.5 \pm 0.2) \times 10^{-4}$	$(7.7 \pm 0.4) \times 10^{-3}$	$(1.11 \pm 0.7) \times 10^{-2}$
k_{rel} $Zn^{2+}{}_2$–**22**[b,d]	230	226	353	583	257
k_0/s^{-1} pH 6.5, 50 °C[e]	7.5×10^{-6}	2.2×10^{-5}	3.0×10^{-5}	1.7×10^{-4}	4.7×10^{-4}
k/s^{-1}, 2 mM Zn^{2+}–**9**	$(1.18 \pm 0.01) \times 10^{-5}$	$(4.17 \pm 0.08) \times 10^{-5}$	$(7.3 \pm 0.2) \times 10^{-5}$	$(7.3 \pm 0.2) \times 10^{-4}$	
k/s^{-1}, 2 mM Zn^{2+}–**8**	$(1.23 \pm 0.02) \times 10^{-4}$	$(3.94 \pm 0.05) \times 10^{-4}$	$(7.4 \pm 0.3) \times 10^{-4}$	$(7.6 \pm 0.2) \times 10^{-3}$	
k/s^{-1}, 1 mM Zn^{2+}–**17b**	$(1.76 \pm 0.04) \times 10^{-5}$	$(5.0 \pm 0.3) \times 10^{-5}$	$(1.05 \pm 0.03) \times 10^{-4}$	$(1.30 \pm 0.06) \times 10^{-3}$	$(5.5 \pm 0.6) \times 10^{-3}$
k_{rel} Zn^{2+}–**17b**[b]	2.3	2.3	3.5	7.6	11.7
k Zn^{2+}–**17b**/k Zn^{2+}–**9**[f]	1.5	1.2	1.5	1.8	
k/s^{-1} $Zn^{2+}{}_2$–**21b**	$(1.73 \pm 0.01) \times 10^{-5}$	$(5.76 \pm 0.05) \times 10^{-5}$	$(1.06 \pm 0.08) \times 10^{-4}$	$(1.05 \pm 0.03) \times 10^{-3}$	$(2.9 \pm 0.4) \times 10^{-3}$
k_{rel} $Zn^{2+}{}_2$–**21b**[b]	2.3	2.6	3.5	6.2	6.2
k $Zn^{2+}{}_2$–**21b**/k Zn^{2+}–**8f**	0.14	0.15	0.14	0.14	

[a] From ref. 40. [b] Rate constant relative to that for the uncatalysed reaction. [c] Determined at pH 7.0. [d] Rate constant for the uncatalysed cleavage calculated assuming a first-order dependence on [HO^-]. [e] Calculated by interpolation on the basis of rate constants determined at 25 and 90 °C. [f] Rate constant for the cleavage in the presence of a bimetallic complex relative to that obtained in the presence of the corresponding monomeric catalyst.

leaving group decreases: the β_{LG} value for $Zn^{2+}{}_2$–**19a** promoted reaction is less negative than that for the uncatalysed reaction under the experimental conditions.[20] Assuming that the shape of the log k *vs.* pK_a plot for the uncatalysed reaction is the same at 25 °C and 90 °C, a nearly 10^6 fold rate-enhancement by $Zn^{2+}{}_2$–**19a** could be expected for the cleavage of **UpEtOEt** (**4e**). This is consistent with our previous estimation of approximately 10^6-fold rate-enhancement with 3′,5′-UpU (**6b**) as a substrate.[19] Complex $Zn^{2+}{}_2$–**19c**, the non-amino analogue of $Zn^{2+}{}_2$–**19a**, and complex $Zn^{2+}{}_2$–**17a** showed a more modest activity: at pH 6.5 and 50 °C, rate-enhancements of 717 and 1400 were observed, respectively (Table 3).

The catalysis on the cleavage of uridine 3′-aryl esters is again fairly modest, but in this case the dependence on the acidity of the leaving group is not quite as obvious. The catalysis by the most efficient catalyst $Zn^{2+}{}_2$–**19a** could not be studied because the reactions were too fast to follow using HPLC even at 25 °C. The complex $Zn^{2+}{}_2$–**19c**, a less active analogue, showed a fairly good catalysis (Table 4). In this case, the catalytic activity seems to increase as the acidity of the leaving group increases, but no firm conclusions on a trend should be drawn on the basis of just three data points. Unlike the situation with alkyl esters, the catalytic activity of the complex $Zn^{2+}{}_2$–**17a** is poorer than that of $Zn^{2+}{}_2$–**19c**: an approximately 1000-fold rate enhancement by $Zn^{2+}{}_2$–**17a** was observed with no clear dependence on the acidity of the leaving group. The results obtained with the complex $Zn^{2+}{}_2$–**22** suggest a drop in catalytic activity with the most reactive arylphosphate substrate **3e**.

Results obtained with the complex $Zn^{2+}{}_2$–**17a** confirm the earlier reports according to which the dimer is a significantly better catalyst than monomeric Zn^{2+}–**9**.[14] Our results show also that the difference depends on the substrate. With **$UpEtCl_3$** (**4h**) as a substrate the difference between the catalytic activity of dimeric $Zn^{2+}{}_2$–**17a** and monomeric Zn^{2+}–**9** is 600-fold, but it seems to decrease as the acidity of the leaving group decreases. For the cleavage of aryl esters, rate constants at the same temperature are not available, but assuming that monomeric Zn^{2+}–**9** is equally inactive at 25 °C and 90 °C, the dimeric complex $Zn^{2+}{}_2$–**17a** is approximately 400–500 times as efficient a catalyst for the cleavage of the aryl esters as Zn^{2+}–**9** is. The difference decreases as the acidity of the leaving group increases. In other words, the difference between the dimer and the monomer decreases as the nucleophilic attack becomes more clearly rate-limiting. Consistent with this, the difference between the dimer and the monomer in the reaction of HPNP (**2**) has been reported to be 120-fold.[14]

A comparison of the results obtained with complex $Zn^{2+}{}_2$–**17a** and its analogue with ligand **17b** shows that in this case the formation of a bimetallic complex depends on the hydroxyl group on the linker: the complex with **17b** without the hydroxo group is catalytically approximately as active as the monomer. In this case the lack of enhanced catalysis is to be attributed to coordination chemistry of the ligand: the ligand binds only one Zn^{2+} ion.[43] The results obtained in the present study show also that linking two active catalysts with the hydroxyl containing linker does not necessarily result in an efficient dinuclear catalyst. A surprisingly poor performance was shown by the Zn^{2+}–**8**-based dimer $Zn^{2+}{}_2$–**21b** whose catalytic activity was approximately only one-tenth of that of the Zn^{2+}–**8**. In contrast to results obtained in aqueous solutions, $Zn^{2+}{}_2$–**21b** catalyses the cleavage of **2** in methanol, but even then the catalytic effect is very modest in comparison to that obtained with $Zn^{2+}{}_2$–**21a**.[44]

Catalysis on the isomerization of phosphodiester bonds

Several complexes were also tested as catalysts for the isomerization of phosphonate substrates (**7**). As reported previously,[20] the complex $Zn^{2+}{}_2$–**19a** promotes also the isomerization of the phosphodiester bond. The rate enhancement with other complexes was, however, very modest. At 25 °C the rate constant of the $Zn^{2+}{}_2$–**19a** promoted isomerization of uridine 3′-methyl phosphonate (**7b**) was 2.1×10^{-7} s^{-1}, whereas a rate constant of 0.15×10^{-7} s^{-1} was obtained for the reaction in the presence of 1 mM $Zn^{2+}{}_2$–**19b**. $Zn^{2+}{}_2$–**17a** (1 mM) showed no detectable activity in one month. Assuming that a 1% change in the concentration of the starting material could be reliably observed, a limiting value of 4×10^{-9} s^{-1} can be estimated.

$Zn^{2+}{}_2$–**19c** promoted isomerization of **7b** was studied at 50 °C, where a 3-fold rate enhancement was observed in the presence of a 1 mM complex. Monomeric Zn^{2+}–**9** and Zn^{2+}–**8**, as well as Cu^{2+}–**14** and Cu^{2+}–**15**, showed a barely observable catalytic activity at 90 °C; 1.5-fold rate-enhancements were obtained in the presence of a 10 mM complex. Increasing pH had no observable effect on the isomerization in the presence of Cu^{2+}–**15**, whereas increasing complex concentration slightly enhanced the reaction.

Discussion

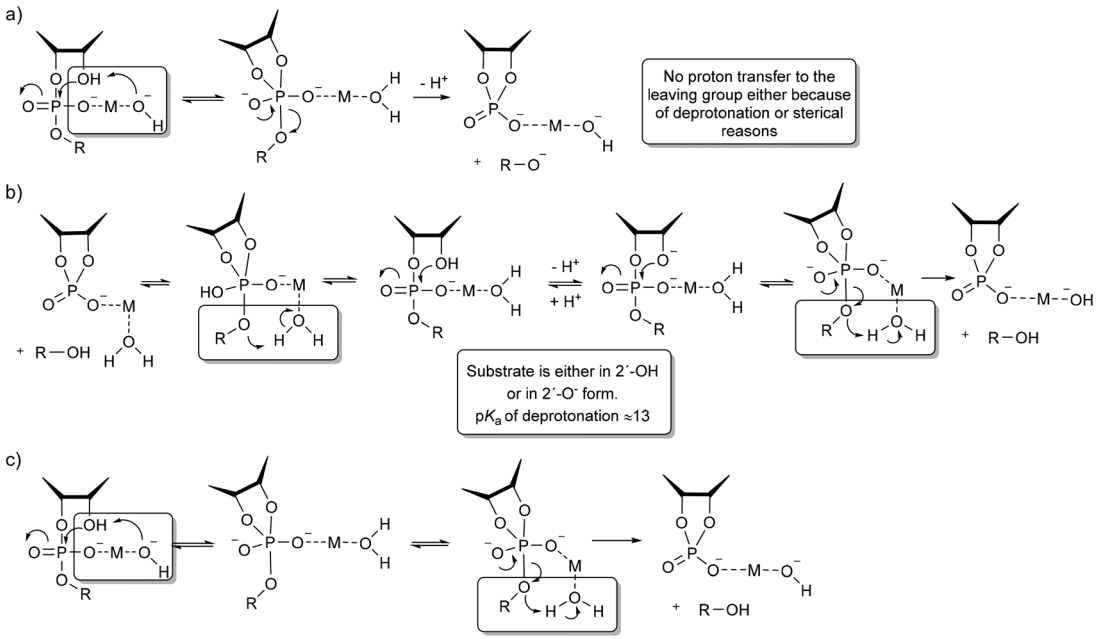
The results discussed above can be understood by considering a step-wise transesterification reaction, where the pentacoordinated phosphorane is an intermediate even in the presence of a metal ion catalyst. As was described above, the existence of an intermediate in the uncatalysed reaction has been proven by a Brønstedt plot with a breakpoint at a pK_a of 12.6, where the pK_a of the leaving group equals that of the attacking nucleophile.[10,11] At this point the energy profile for the cleavage is symmetrical, and as the energy minimum is probably very shallow, a nearly concerted reaction can be proposed. According to our results, this is also approximately the same point where the poorest catalytic activity was observed. With any catalyst studied, the lowest rate-enhancement was observed with **$UpEtCl_3$** (**4h**)‡. However, as the acidity of the leaving group either increases or decreases, the energy profile becomes increasingly asymmetrical. At the same time the observed catalytic activity increases, as we have experimentally shown. Accordingly, metal ion catalysts can enhance both steps of the cleavage reaction. The lack of catalysis on the isomerization reaction in the presence of most metal ion complexes most probably results from the instability of the phosphorane. Similar to alkaline cleavage, a dianionic phosphorane is formed. Apparently, in most of the cases, the interactions with the catalyst are not strong enough for a metal ion to act as a substitute for a proton neutralizing the charge. The phosphorane is, hence, too unstable to pseudorotate, and catalysis on isomerisation is observed[19,20] only with catalysts with particularly strong interactions with the phosphorane, such as $Zn^{2+}{}_2$–**19a**.[16]

Another conclusion that can be drawn on the basis of the results discussed is that there is no universal catalysis mechanism covering all the substrate–catalyst combinations, but the mechanism depends on the substrate and possibly also on the catalyst utilized. We suggest that minimum catalysis observed for the cleavage of **$UpEtCl_3$** (**4h**) is mainly of electrostatic nature: the catalysts bind the phosphate, enhance thereby the nucleophilic attack, stabilize the phosphorane and enhance thereby the overall reaction. The fact that catalysis is almost non-existent for monometallic complexes, even for the most efficient among them, is not surprising considering the low proportion of Zn^{2+}-bound substrate molecules under the experimental conditions. According to Koike and Kimura,[29] the binding constant for complexation between Zn^{2+}–**8** and phosphodiesters cannot be accurately determined, but a limiting log K value of 0.5 has been proposed. Using this value, it can be estimated that not more than 3% of the substrate molecules are bound as Zn^{2+}–**8** complexes in our experiments at room temperature. In fact, this is a crude estimate and several factors, such as temperature, pH and the structure of the substrate, influence the binding.

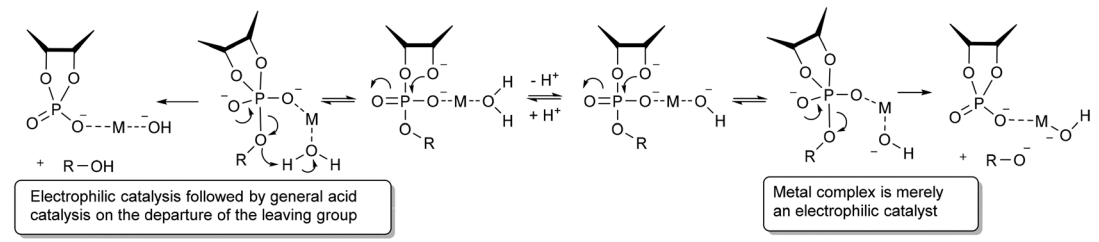
Catalysis of the cleavage when the departure of the leaving group is rate-limiting

The pK_a values show that the complexes exist as equilibrium mixtures of hydroxo and aquo forms, and the proportion of these forms is different for different complexes. General acid and base catalysis is often proposed for the metal ion promoted reactions of RNA model compounds: a hydroxo ligand can act as a general base that deprotonates the attacking nucleophile (Scheme 3a), whereas an aquo ligand can facilitate the reaction by protonating the leaving group oxygen (Scheme 3b). A sequential general base–general acid catalysis (Scheme 3c) is also possible in the presence of complexes with both hydroxo and aquo forms present under experimental conditions. As mentioned in the Results section, three different catalyst groups were identified on the basis of experimental data and they are characterized by different acidity and catalytic properties. Group A complexes are acidic with a high proportion of the hydroxo form. They are good catalysts as long as protonation of the leaving group is not essential, but the catalytic activity decreases abruptly for substrates with more basic leaving groups as is shown with Zn^{2+}–**8** in Fig. 1. This behavior is easily attributed to the reaction route involving general base catalysis only (Scheme 3a). Similarly, the very modest catalysis by complexes belonging to group C, only observed with the most basic leaving groups, can be explained by the reaction route involving only general acid catalysis (Scheme 3b). Apparently, only the most basic leaving groups are able to abstract a proton from these inefficient catalysts. Group B catalysts show a fair and consistently increasing catalytic activity as the leaving group becomes poorer. As both aquo and hydroxo forms are available (though the aquo form is predominant),

‡It is to be noted that the pK_a of 12.6 indicated in ref. 10 and 11 refers to kinetics at 25 °C, whereas our experiments with monometallic complexes were carried out at 90 °C, where the pK_a's can be expected to be lower.



Scheme 3 a. General base catalysis by a coordinated metal complex in the hydroxo form on the nucleophilic attack of 2′-OH on the phosphate. b. Possible reaction routes involving general acid catalysis by a coordinated metal complex in the aquo form for the departure of the leaving group. c. Sequential general base–general acid catalysis by a coordinated metal complex.



Scheme 4 Catalysis mechanisms showing the metal ion complex as an electrophilic catalyst.

they can be proposed to act as bifunctional catalysts following the general base–general acid route in Scheme 3c. Quite possibly the system is a continuum with a gradual change from one mechanism to another depending on the acidities of the aquo ligand and the leaving group alcohol.

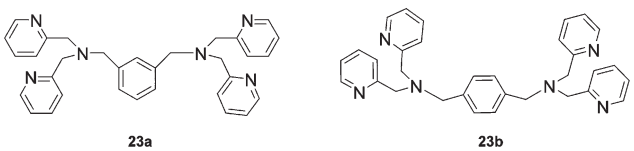
The general acid catalysis on the departure of the leaving group is easy to confirm, for it is clearly seen in the results: for the least reactive esters (**4a**, **b**), catalysts with higher proportion of the acidic aquo form, such as group B catalysts $Zn^{2+}{}_{aq}$ and Zn^{2+}–**9**, retain their catalytic activity, whereas the catalytic activity of group A catalysts decreases abruptly (Fig. 1). As the analysis of the uncatalysed reaction showed, these are substrates that under the experimental conditions react through a mechanism that involves a concerted proton transfer to the leaving group. It is hence a logical conclusion that metal ion complexes with an aquo ligand provide the required proton, assisting hence the cleavage of these unreactive esters. This is consistent with the previously reported β_{LG} value of −0.32 for $Zn^{2+}{}_{aq}$ promoted reaction that is even less negative than the value for spontaneous cleavage.[25] Group A complexes with only hydroxo ligands, such as Zn^{2+}–**8**, cannot provide the required proton and hence a drop in the catalytic activity is observed.

General base catalysis on the nucleophilic attack is more controversial. Even though the catalytic activity of the complexes on the more reactive alkyl esters ($12 < pK_a < 15$), where protonation of the leaving group is not essential, seems to correlate with the proportion of the hydroxo form, the situation is by no means clear; a pK_a value is also a measure of the electrophilicity of a metal ion center. Electrophilic catalysis would enhance the reaction by assisting the nucleophilic attack on the phosphate. Even if the second step was rate limiting, the reaction would be enhanced, because the equilibrium concentration of the phosphorane intermediate would increase. Experimental evidence could, hence, be explained also by mechanisms shown in Scheme 4. The mechanism described is essentially metal ion catalysis on the alkaline cleavage. The fact that the catalysis decreases when the proportion of the

alkaline cleavage increases argues, however, against such a mechanism. Furthermore, a metal complex in its hydroxo form could be expected to be a poorer electrophilic catalyst than the aquo form because of the less positive net charge. The reaction system is, however, complex and these arguments cannot be taken as conclusive evidence against electrophilic catalysis.

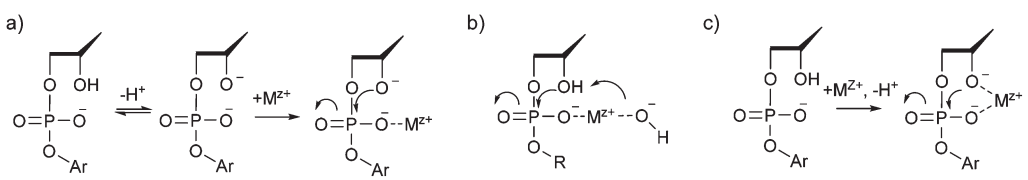
As has been pointed out,[21] electrophilic catalysis by the metal catalyst aquo form on the alkaline cleavage and general base catalysis of the cleavage of a monoanionic substrate are kinetically equivalent and difficult to distinguish from each other. As will be discussed later, several reports supporting the electrophilic catalysis have been published. Most of these, however, concentrate on reactions where the nucleophilic attack is the rate limiting step. We believe that at least in cases where the departure of the leaving group is rate limiting, general base catalysis is more feasible as a part of the catalytic system than electrophilic catalysis. This suggestion is based on results obtained with Zn^{2+}–**8** catalyzed cleavage of phosphodiester bonds in short oligonucleotides. The results show clearly that Zn^{2+}–**8** binds only one phosphate group, but this phosphate is not necessarily the scissile phosphodiester bond.[45] As evidence of this, phosphodiester bonds in 3′-phosphorylated oligonucleotides are cleaved more rapidly than oligonucleotides with no such good coordination site.

On the basis of the observations discussed above, we propose, hence, a general acid–base catalyzed reaction system for the cleavage of nucleoside 3′-alkyl esters where the departure of the leaving group is rate determining (Scheme 3a–c). In principle, both general acid and base catalysis are possible, but the preference depends on the acidity of metal aquo ligand and on the acidity of the leaving group. It would be logical to assume that there is no clear breakpoint, but a gradual change from general base catalysis to general acid catalysis through systems where both are involved. This suggestion is consistent with pH-rate profiles of metal ion promoted cleavage of RNA substrates (pK_a of 14.4 has been determined for 3′,5′-UpU (**6b**) by kinetic experiments under alkaline conditions[20]). Both bell-shaped and sigmoidal pH-rate profiles have been observed. The cleavage of a dinucleoside diphosphate by Zn^{2+}–**9**,[46] the cleavage of oligonucleotides by Cu^{2+}–**15**[47,48] and the cleavage of dinucleoside monophosphates by bimetallic complexes $Zn^{2+}{}_2$–**23a**[49] and $Zn^{2+}{}_2$–**23b**[50] are characterized by bell-shaped pH-dependence. Sigmoidal pH-rate profiles have been observed with the cleavage of poly-U in the presence of Zn^{2+}–**8**,[51] the cleavage of UpU by bimetallic complex $Zn^{2+}{}_2$–**17a**[21] and the cleavage of ApA (**6a**) by bimetallic complex $Zn^{2+}{}_2$–**19c**.[49] At a qualitative level the results obtained with monometallic complexes suggest that bell-shaped dependence is observed with more basic complexes, whereas a sigmoidal dependence refers to catalysis by more acidic complexes. In the case of Zn^{2+}–**8** promoted cleavage of poly-U, the rate increase levels off at around pH 6.6. On the basis of the discussion on temperature dependence of the pK_a values, it seems that this is close to the pK_a of the aquo ligand. Consistent with different catalysis mechanisms, Yashiro *et al.*[49,50] have compared the pH-rate profiles of the cleavage of 3′,5′-ApA (**6a**) to species distribution curves. According to their analysis the bell-shaped dependence is observed when the catalyst is present in a form with both an aquo and a hydroxo ligand, as with $Zn^{2+}{}_2$–**23a** and $Zn^{2+}{}_2$–**23b**, whereas a sigmoidal dependence refers to the presence of a species with only hydroxo ligands, such as $Zn^{2+}{}_2$–**19c**.



Catalysis on the cleavage when the nucleophilic attack is rate limiting

The results obtained with nucleoside 3′-aryl esters show that the catalysis by monometallic complexes is very modest, but it seems to increase, as the acidity of the leaving group increases. This can again be explained by a step-wise mechanism, where the catalysis increases as the difference between the energy barriers of the individual steps increases. As we proposed a general acid–base catalysis (Scheme 3a–c) for the reactions of substrates with a poor leaving group, a general base catalysis (Scheme 3a) would be a logical choice here, but the situation is not straightforward. In addition to general base catalysis (Scheme 5b),[26,27] specific base catalysis of the cleavage of the metal bound substrate (Scheme 5a)[21,22] and nucleophilic catalysis (Scheme 5c)[30] have all been proposed for metal ion promoted cleavage of RNA models. Sigmoidal pH-rate profiles[12,21,26,30,52,53] and a correlation between a pH-rate profile and a species distribution curve[26,27] have been attributed to a kinetically significant deprotonation of a metal bound water ligand. For a wider perspective, studies with different substrates are discussed below together with our own experimental results.



Scheme 5 *a*. Electrophilic + specific base catalysis. *b*. General base catalysis. *c*. Nucleophilic catalysis.

Catalysis on the cleavage of non-nucleosidic substrates with an aryl leaving group

On the basis of their extensive research, Morrow and coworkers have considered two kinetically equivalent mechanisms, intracomplex general base catalysis by a metal hydroxo ligand (Scheme 5b) and specific base catalyzed cleavage of a coordinated substrate (Scheme 5a), and have ended up favouring the latter.[21] This mechanism is supported by the absence of a significant solvent isotope effect in the Zn^{2+}_2–**17a** promoted cleavage of **UpPhNO$_2$** (**3e**)[22] and the inhibition of the catalysis by metal ion complexes observed upon addition of phosphate anions.[53,54] According to the results obtained, the strongest inhibition of the HPNP (**2**) cleavage is observed at a lower pH where the catalyst is present in the protonated form, suggesting that this is the species involved in the catalysis.

In contrast, Bonfa *et al.*[30] have interpreted the sigmoidal pH-dependence and the absence of a significant solvent isotope effect in the cleavage of **2** as evidence for nucleophilic catalysis, *i.e.* direct interaction of the attacking nucleophile and the metal ion catalyst (Scheme 5c). A large drop in the solvent isotope effect was observed in the presence of the metal ion catalyst. While the cleavage of **2** in the absence of a catalyst was characterized by a solvent isotope effect of 4.01 reflecting the difference in equilibrium deprotonation of the attacking nucleophile, the value observed in the presence of a mononuclear Zn^{2+} complex was 1.43. The difference suggests that the catalyst has a more active role than just that of a Lewis acid. However, according to Morrow and coworkers[55] heavy atom isotope effects are more in favour of a reaction where there is no interaction between the nucleophilic OH and the metal ion catalyst.

Nucleophilic catalysis for the cleavage of **2** receives further support from the similarity of the metal ion promoted reactions of **2** and BNPP (**1**). Even though **1** and **2** are fundamentally different in that one reacts by intramolecular transesterification and the other is cleaved by hydrolysis involving an intermolecular nucleophile, the basic kinetic observations for metal ion promoted reactions are similar: the reactions of BNPP are generally characterized by very clearly sigmoidal pH-rate profiles[28,29,56,57] and a hydroxo species has been identified as the catalytically active form. In the case of **1** the hydroxo ligand acts as the attacking nucleophile. An essential observation is that with both **1** and **2** the catalytic activity of metal ion complexes depends not only on the acidity of an aquo ligand, but also on the coordination geometry.[29,30] The analysis by Bonfa *et al.*[30] shows that while logarithmic rate constants obtained with complexes of different tridentate ligands depend linearly on the pK_a, those obtained using tetradentate ligands fall below the line. The trend is more pronounced with **1** than with **2**, but the difference between the tridentate and tetradentate complexes can be seen with both **1**[29,30] and **2**.[30,53] Zn^{2+}–**10** (pK_a 8.0[28]) is in fact even poorer as a catalyst for the cleavage of **2** than Zn^{2+}–**9** (pK_a 9.2)[36] is. Second-order rate constants of 2.1×10^{-3} M^{-1} s^{-1}, 0.016 M^{-1} s^{-1} and 6.3×10^{-4} M^{-1} s^{-1} have been reported for the cleavage of **2** in the presence Zn^{2+}–**9**, Zn^{2+}–**8** and Zn^{2+}–**10**, respectively.[14,53]

Results obtained with Cu^{2+}–**15** and Cu^{2+}–**14** as catalysts support also the suggestion that the reaction of **2** could involve nucleophilic catalysis by the coordinated metal ion. These catalysts show a very different preference for substrates. As was discussed earlier, Cu^{2+}–**15** is a better catalyst for the cleavage of uridine 3′-alkyl esters (**4**) and dinucleoside monophosphate **6b** than Cu^{2+}–**14** is,[40] but with cyclic monophosphate **5b**[13] and **2**[12] the situation has been reported to be the opposite. While the dimerisation of Cu^{2+}–**15** can explain the inactivity observed with nucleoside 3′-alkyl phosphates, the fact that with simpler systems Cu^{2+}–**14** actually is a better catalyst suggests that the catalysis mechanisms required are different. Results reported in the literature show that Cu^{2+}–**14** is an active catalyst in reactions where the nucleophile is a Cu^{2+} bound hydroxo ligand, such as in the cleavage of **1**, where Cu^{2+}–**15** is inactive as a catalyst.[58] Cu^{2+}–**14** is also a far better catalyst than Cu^{2+}–**15** for cleavage of dinucleoside oligophosphates, where an intramolecular nucleophilic attack is not possible.[59] This indirect evidence suggests that in cases where Cu^{2+}–**14** is a better catalyst than Cu^{2+}–**15**, a nucleophilic catalysis should be considered.

Catalysis on the cleavage of nucleosidic substrates with an aryl leaving group

While nucleophilic catalysis (Scheme 5c) may be a feasible catalysis with **2**, with nucleoside based phosphodiesters it most probably is of minor importance. Theoretically thinking, the flexible structure of **2** might be able to allow the simultaneous interaction to the phosphate and to the attacking nucleophile. With nucleoside based phosphodiesters this could be expected to be more difficult because of the more rigidly positioned nucleophile and constraints posed by the ribose ring. The results obtained in the present study may be taken as experimental evidence for the suggestion of different mechanisms: with all the phosphodiesters studied Cu^{2+}–**15** is a more efficient catalyst than Cu^{2+}–**14** is, while the opposite is true with **2** as a substrate. Even if the suggestion of nucleophilic catalysis for the cleavage of **2** is not correct, the different preferences suggest different catalysis mechanisms for different substrates. The fact that the difference in the catalytic activity between Cu^{2+}–**15** and Cu^{2+}–**14** decreases as the acidity of an aryl leaving group decreases suggests that there may be a slight change in the preferred catalysis mechanism on going to more reactive nucleoside 3′-aryl esters.

As mentioned above, specific base catalyzed cleavage of the metal bound substrates (Scheme 5a) has been preferred over the kinetically equivalent mechanism, where a metal bound hydroxo ligand acts as a general base catalyst (Scheme 5b). The absence of a significant solvent isotope effect on the cleavage of **UpPhNO$_2$** (**3e**) has been taken as an indication of a mechanism where the catalysis is based merely on the increased electrophilicity of a phosphate group upon coordination.[22] However, as has been pointed out above, the solvent isotope effect allows different interpretations. When the

reactions are carried out at the same p*L*, the pre-equilibrium deprotonation in the alkaline cleavage is observed as a significant difference in rate constants in H_2O and D_2O.[32] The results obtained in Zn^{2+}–**17a** catalyzed cleavage of **UpPh** (**3a**) show that a normal SIE is observed until p*L* 8.5; above p*L* 8.5 the reaction in D_2O is slightly faster (k_H/k_D = 0.8).[22] Assuming that the kinetically observed pK_a refers to deprotonation of the catalyst, k_H/k_D of 0.8 refers to conditions where the catalyst is in its lyoxo form both in H_2O and in D_2O. If the reaction involved a specific base catalysis on the metal bound substrate, equilibrium deprotonation of the substrate HO was the only proton transfer process, and the same equilibrium isotope effect of approximately 5 should be observed. In contrast to this, intracomplex general base catalyzed reaction might well be characterized even by an inverse solvent isotope effect. The general base catalysis involves a proton transfer from the 2′-OH which is characterized by a normal isotope effect, but this is compensated for by the inverse effect resulting from the protonation of the catalyst. This is consistent with the very modest solvent isotope effects observed for the reactions of phosphodiesters under neutral and acidic conditions, which involve intramolecular proton transfer reactions.[32] The normal solvent isotope effect observed at a lower pH can be explained by the concentration of the hydroxo form of the complex, which is lower in D_2O under conditions where the deprotonation is incomplete.

The discussion above does not offer a clear-cut answer to the question about the mechanism of the metal ion promoted cleavage of nucleoside 3′-aryl phosphates. Three different mechanisms have been proposed, and often the same experimental evidence has been taken to support two different mechanisms: pH-rate profiles and solvent isotope effects allow different interpretations. Similarly, both general base catalysis (Scheme 5b)[60] and specific base catalysis (Scheme 5a)[23,24] as mechanisms of metal complex promoted cleavage of **2** have been supported on the basis of theoretical calculations.

As the discussion on the reactions of nucleoside 3′-alkyl esters showed, there is most probably not a single mechanism covering all the substrate–catalyst combinations studied. It is, hence, quite possible that this is the case also with aryl esters (nucleoside derivatives and **2**). Particularly, when the nature of the substrates is different, caution should be exercised when results obtained with one type of substrate are extended to the reactions of another type of compound. The possibility of different catalysis mechanisms utilized by different complexes should also be considered. As mentioned above, we are inclined to believe that metal ion promoted reactions of nucleoside 3′-aryl esters generally involve a general base catalysis by a hydroxo ligand of the coordinated metal ion. While the experimental evidence obtained with these substrates is fairly limited, none of it argues explicitly against the general base catalyzed reaction. The mechanism of reactions of HPNP may be different, at least with some catalysts, and results obtained with this substrate should not be used as evidence against a given mechanism for another substrate.

Catalysis by bimetallic complexes

The catalysis by bimetallic complexes follows the same trends as those observed with monometallic complexes: The catalysis by any metal complex is the weakest with **UpEtCl$_3$**, and it increases as the asymmetry of the energy profile becomes more pronounced. As numerous reports have shown before, the catalysis by bimetallic complexes can be significantly more efficient than that by their monometallic counterparts, and there is no doubt that enhanced interactions play a significant role in the enhanced catalysis. The effect has been described in different terms: simultaneous interaction with two positively charged metal ions increases the equilibrium concentration of bound substrate molecules, the double Lewis acid activation makes the phosphate increasingly electrophilic and the negatively charged phosphorane is more strongly stabilized by the enhanced interactions. Williams and his coworkers have previously shown that the interactions can be further enhanced by hydrogen bonding.[16,17,61] In the absence of the hydrogen bonding amino groups $Zn^{2+}{}_2$–**19c** binds less strongly, and a less efficient catalysis is observed: $Zn^{2+}{}_2$–**19a** is 726 times as efficient a catalyst for the cleavage of HPNP as $Zn^{2+}{}_2$–**19c**.[16] The results obtained in the present work fit well with this: comparing the results obtained with $Zn^{2+}{}_2$–**19a** at 25 °C and with $Zn^{2+}{}_2$–**19c** at 50 °C, a 370-fold difference in catalytic activity can be calculated.

The efficiency of the binding is determined by the shape of the complex and consequently by the structure of the ligand and the coordination geometry of metal ions. Morrow and her co-workers have shown that in the case of $Zn^{2+}{}_2$–**17a**, the linker hydroxyl group is involved in interactions with the two Zn^{2+} centers.[14] Apparently, this interaction induces a structure that allows a productive binding of a substrate with two metal ions and an efficient catalysis. In the absence of the linker alkoxy group, the catalytic advantage of a bimetallic system is lost nearly completely. In the case of ligand **17b** the inactivity results from the fact that the ligand forms only 1 : 1 complexes with Zn^{2+}.[43] The results obtained in the present study show also that linking two active catalysts with the hydroxyl containing linker does not necessarily result in an efficient dinuclear catalyst. The catalytic activity of the Zn^{2+}–**8** -based dimer $Zn^{2+}{}_2$–**21b** was only approximately one-tenth of that of the Zn^{2+}–**8**. It could be tentatively suggested that intracomplex interactions prevent the binding to the substrate. The importance of the correct coordination geometry is shown for example by the fact that a Cu^{2+} complex, $Cu^{2+}{}_2$–**17a**, has been reported to be nearly inactive as a catalyst for the cleavage of **2**.[14]

While the complexes studied in the present work utilize the linker with a hydroxyl ligand to achieve an overall structure favouring the productive substrate binding, this is by no means the only strategy. Several ligands with a more rigid aromatic linker such as **18**,[15] or **23a**[49] and **23b**[50] have been reported to form efficient bimetallic catalysts. Rigidity in ligand **16** is brought about by the amide linker.[13] The interactions between the catalyst and the substrate can also be

enforced by using a less polar medium. $Zn^{2+}{}_2$–**21b** shows a particularly large rate-enhancing effect in methanol or ethanol, whereas those with aromatic linkers seem to be less prone to the enhancing effect of alcohol solvent.[44]

The discussion above emphasises the importance of enhanced binding, but it is also clear that, similar to catalysis by mononuclear complexes, the bimetallic catalysts play a more active role when the acidity of the leaving group increases. The difference in catalytic activity between $Zn^{2+}{}_2$–**19a** and $Zn^{2+}{}_2$–**19c**, for example, decreases, as the leaving group becomes poorer. We have previously proposed a general acid catalysis on the basis of solvent isotope effect values ranging from 2 to 3.[20] As has been pointed out above, the values are clearly different from those of the uncatalysed reactions and hence consistent with a catalyst induced proton transfer process, even if the interpretation is not straightforward at all with such a complicated system. The decrease in the difference between the catalytic activities of the Zn^{2+}–**9** monomer and the bimetallic complex $Zn^{2+}{}_2$–**17a** can most probably be attributed to different pK_a values: a value of 7.8[52] determined for $Zn^{2+}{}_2$–**17a** is clearly lower than the value of 9.2[35] estimated for the monomer. Therefore $Zn^{2+}{}_2$–**17a** is likely to be a general base catalyst, whereas Zn^{2+}–**9** with an aquo ligand under neutral conditions may act as a general acid catalyst as well.

Conclusions

Transesterification of uridine 3′-phosphodiesters in the presence of metal ion catalysts can be understood as a two-step reaction, where phosphorane is an intermediate. Metal ion catalysts can enhance both the nucleophilic attack and the departure of the leaving group. No universal catalysis mechanism exists, but the mechanism depends on both the catalyst and the substrate. A change from general base catalysis (Scheme 3a) to general acid catalysis (Scheme 3b) through bifunctional general base–general acid catalysis (Scheme 3c) is suggested as the acidity of the metal aquo ligand and of the leaving group alcohol decrease. Catalysis on the isomerization depends on the strength of the binding: strong interactions stabilize the phosphorane allowing the pseudorotation. Pseudorotation is, however, rate-limiting, and only a modest catalysis is observed.

Catalytic advantage achieved with bimetallic complexes depends on interactions within the complex and interactions between the catalyst and the substrate. Different strategies can be used to induce the cooperative binding with two metal ions. The basic catalytic mechanisms utilized by monometallic and bimetallic complexes are the same.

The results discussed underline the difficulty of mechanistic research: any change in reaction conditions may change several parameters at the same time, and all changes should be considered. Furthermore, the kinetic data obtained with different substrates are often similar, but they can be interpreted in different ways. As the discussion above shows, different reaction mechanisms are actually probable, and because of this, caution should be exercised when any results are extended to another system, no matter how closely it is related. The same problems are encountered in the design of bifunctional catalysts: the catalytic activity of a monomer together with design that is successful elsewhere is not a guarantee of an efficient bimetallic catalyst.

Experimental

Synthetic procedures

Phosphodiester substrates and ligands were synthesized using known methods. Synthesis and characterization are described in detail in the ESI.†

Kinetic measurements

The pH of the reaction solutions was adjusted using MOPSO [3-(*N*-morpholino)-2-hydroxypropanesulphonic acid]. pK_a values of MOPSO at higher temperatures were calculated using the data found in the literature[62] and NaOH was used to adjust the buffer ratio. Reactions were carried out in Eppendorf tubes at 25 and 50 °C and in glass tubes at 90 °C. The temperature was controlled using a thermostated water bath. Aliquots were withdrawn at suitable intervals and were kept in an ice bath until the analysis using HPLC. An excess of EDTA was added to samples to quench metal ion catalyzed reactions.

Analysis was carried out with RP HPLC using a Waters Atlantis™ dC_{18} column (150 × 4.6 mm, 5 μm particle size) or a Supelcosil™ LC-18 column (250 × 4 mm, 5 μm particle size). Mixtures of acetic acid buffer ([AcOH] = 0.045 M, [AcONa] = 0.015 M, [NH_4Cl] = 0.1 M]) and acetonitrile were used as eluents. Isocratic elution with 5–20% acetonitrile or gradient elution (0 → 20% acetonitrile) was used. With the methylphosphonate compound **7b**, an isocratic elution with 0.025 M triethylammonium acetate containing 0.2 M tetramethylammonium chloride was used. UV detection was done at 260 nm.

Rate constants of the cleavage were calculated by following the decrease of the signal area of the substrate and by applying the integrated rate-law of first order reactions. The pK_a values of the leaving groups were drawn from the literature.[33,40] The pK_a of neopentanol (2,2-dimethylpropan-1-ol) was determined by comparing the reactivity of **4a** and a series of alkyl esters in 1.00 M NaOH at 25 °C, where the rate of the cleavage is reported to be strongly dependent on the nature of the leaving group, β_{LG} being −1.28.[31] At 1.00 M NaOH and 25 °C, **4a** was cleaved at a rate of (0.46 ± 0.03) × 10^{-6} s^{-1} which refers to a pK_a value of 17.3.

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