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PERSPECTIVE

Cleavage of RNA phosphodiester bonds by small molecular entities: a mechanistic insight

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RNA molecules participate in many fundamental cellular processes either as a carrier of genetic information or as a catalyst, and hence, RNA has received increasing interest both as a chemotherapeutic agent and as a target of chemotherapy. In addition the dual nature of RNA has led to the RNA-world concept, *i.e.* an assumption that the evolution at an early stage of life was based on RNA-like oligomers that were responsible for the storage and transfer of information and as catalysts maintained primitive metabolism. Accordingly, the kinetics and mechanisms of the cleavage of RNA phosphodiester bonds have received interest and it is hoped they will shed light on the mechanisms of enzyme action and on the development of artificial enzymes. The major mechanistic findings concerning the cleavage by small molecules and ions and their significance for the development of efficient and biologically applicable artificial catalysts for RNA hydrolysis are surveyed in the present perspective.

Introduction

RNA molecules play a key role in several fundamental cellular events, serving both as a carrier of genetic information and as a catalyst. In more detail, RNA components of spliceosome, an RNA protein complex, participate in splicing of transcribed pre-messenger RNA to messenger RNA,[1] and the latter transfers the genetic information to ribosomes, *i.e.* to another RNA protein complex, where the RNA component catalyzes the assembly of proteins from amino acids brought to the complex by small transfer RNA molecules.[2] In addition, internally synthesized short micro RNA molecules and short double helical RNA molecules formed enzymatically from external double stranded RNA participate in regulation of gene expression.[3] Accordingly, it is only natural that RNA has recently received extensive attention both as a chemotherapeutic agent and as a target of chemotherapy.[4] But the dual nature of RNA has received even wider interest. The ability of RNA to both store genetic information and catalyze organic reactions has led to the RNA-world concept, according to which the evolution at an early stage of life was based on RNA-like oligomers that were responsible for the storage and transfer of information and as catalysts maintained primitive metabolism.[5]

Chemically RNA is, like DNA, a polymer constructed from monomeric nucleosides by 3′,5′-phosphodiester bonds, but the presence of the 2′-hydroxy function that may serve as an intramolecular nucleophile makes the phosphodiester bonds both chemically and enzymatically less stable in RNA than in DNA.[6] Owing to the obvious desire to understand the chemical behavior of RNA for the reasons indicated above, the kinetics and mechanisms of the cleavage of RNA phosphodiester bonds have received extensive interest, besides synthetic and structural chemistry of RNA. Catalysis by various small molecular entities has been studied to get insight into how the enzymes, either protein enzymes or catalytic nucleic acids, promote RNA processing reactions.[7] Additional motivation for such mechanistic studies has been provided by the striving to obtain artificial ribonucleases, *i.e.* small molecule cleaving agents that match their enzyme counterparts.[8] Such artificial nucleases, when conjugated to a structure recognizing the base sequence, can be envisaged as tools for structural studies and site selective tailoring of large RNA molecules and as chemotherapeutics with which to inhibit gene expression by selective targeting either to messenger RNA or micro RNA.

Many sequence-selective artificial nucleases have actually been introduced, but the efficiency is still low compared to protein enzymes or even efficient ribozymes.[9] Kinetic studies on the cleavage of RNA phosphodiester bonds by hydronium, hydroxide and metal ions, however, suggest that a markedly higher catalytic activity could be achieved by proper combination of various factors affecting the cleavage rate. The cleavage is initiated by a nucleophilic attack of the neighboring 2′-hydroxy group on the phosphorus atom, as known since the early studies of Brown and Todd,[10] and it proceeds *via* a phosphorane intermediate obeying the rules of Westheimer (Scheme 1).[11] The cyclic 2′,3′-phosphate obtained is subsequently hydrolyzed to a mixture of 2′- and 3′-phosphates. Often the accumulation of the cyclic phosphate is hardly detectable.

The half-life for the cleavage of an individual phosphodiester bond is of the order of 100 years at 25 °C at pH 5–6, where the spontaneous hydrolysis predominates. It has been estimated that deprotonation of the attacking nucleophile (2′-OH) and

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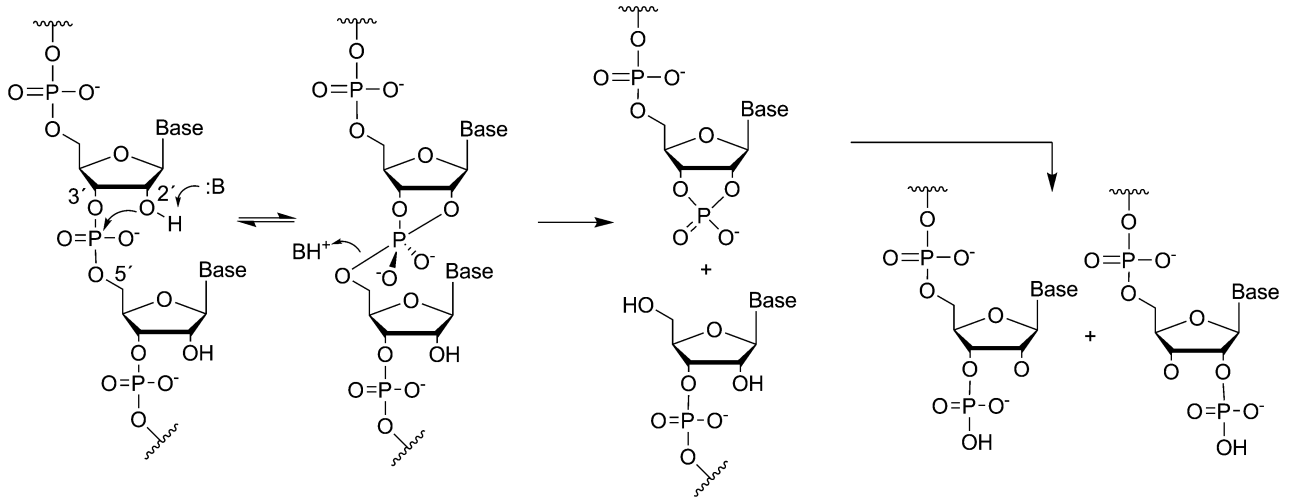


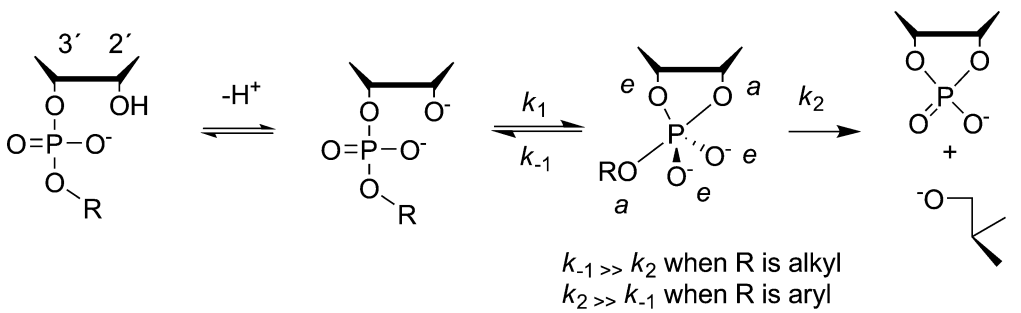
Scheme 1 Cleavage of RNA phosphodiester bond.

protonation of the departing nucleophile (5′-O$^-$) both accelerate the reaction by a factor of up to 10^6.[12] Protonation of the non-bridging phosphoryl oxygen may result in 10^5-fold acceleration and constraint of the molecule in a conformation that allows co-linear attack of 2′-O and departure of 5′-O results in a 100-fold acceleration. Up to 10^5-fold acceleration may be achieved by simultaneous engagement of both of the non-bridging oxygen atoms in metal ion coordination.[13] While enzymes obviously are able to exploit several of these rate-enhancing factors, if not all, in a co-operative manner, the small molecular cleaving agents described so far make use of only a subset of the catalytic repertoire utilized by enzymes. Detailed mechanistic understanding obviously is a prerequisite for development of multifunctional small molecular catalysts that are able to benefit, similarly to enzymes, from cooperative action of several rate-accelerating elementary processes. The present review is a survey of the major mechanistic findings and achievements on the way to efficient and biologically applicable small molecule cleaving agents for RNA.

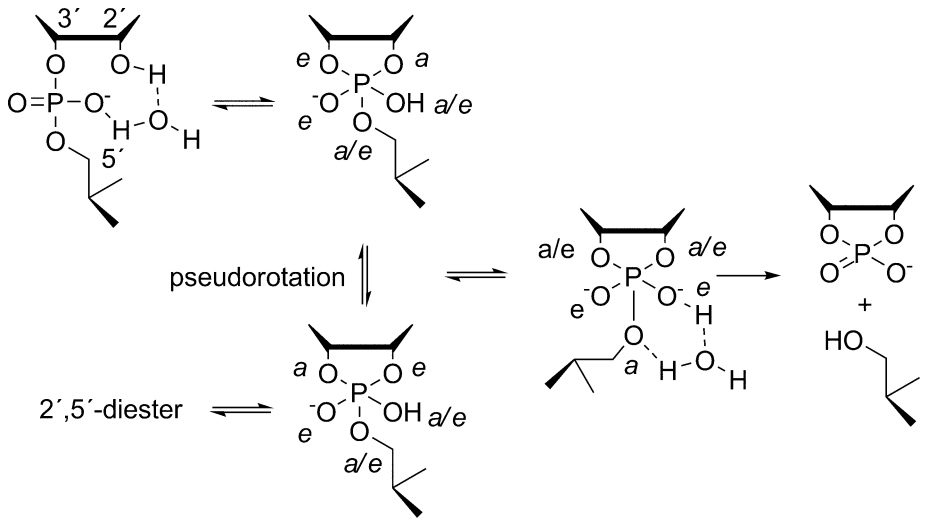
Specific base-catalyzed and spontaneous cleavage of RNA phosphodiester bonds

The predominant reaction of RNA phosphodiester bonds at pH >7 is hydroxide ion catalyzed transesterification to a 2′,3′-cyclic phosphate with concomitant release of the 5′-linked nucleoside.[14] This reaction proceeds by a rapid initial deprotonation of the 2′-hydroxyl group, followed by the attack of the 2′-oxyanion on the phosphorus atom and breakdown of the resulting phosphorane intermediate by departure of the 5′-linked nucleoside as an oxyanion. The fact that the susceptibility of this transesterification reaction to the basicity of the departing oxyanion is with ribonucleoside 3′-alkylphosphates much higher ($\beta_{lg} = -1.28$)[15] than with their aryl counterparts ($\beta_{lg} = -0.59$)[16] lends support for the change in the rate-limiting step on going from alkyl to aryl esters.[17] In other words, the dianionic phosphorane derived from the alkyl esters has a finite life-time and the breakdown of this intermediate *via* a late transition state is the rate-limiting step of their transesterification to a cyclic phosphate (Scheme 2). With aryl esters, the rate-limiting step, in turn, is formation of this intermediate. This difference in mechanism is important to note, since 2-hydroxypropyl *p*-nitrophenyl phosphate (HpNP) is often used as a model compound in mechanistic studies of RNA cleavage.

Consistent with the late transition state of the breakdown of the dianionic phosphorane derived from 3′-alkylphosphates, the primary ^{18}O isotope effect for the alkaline cleavage of uridine 3′-(3-nitrobenzyl)phosphate is as large as 1.0272 ± 0.0001, suggesting that the PO bond is almost entirely cleaved in the transition state.[18] According to DFT calculations with a continuum solvation model, the attack of methoxide ion on ethylene phosphate monoanion gives a metastable phosphorane intermediate, the attack of the methoxide ion being rate-limiting. The implication for RNA hydrolysis, hence, is that a marginally stable dianionic phosphorane is formed in a pre-equilibrium step and the rupture of the PO5′



Scheme 2 Specific base-catalyzed cleavage of ribonucleoside 3′-phosphodiesters.



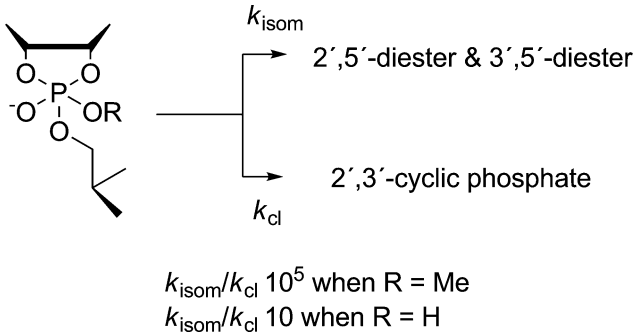
Scheme 3 pH-independent cleavage and isomerization of RNA phosphodiester bonds.

bond is rate-limiting.[19] Calculations with tetrahydrofuran-3,4-diol cyclic phosphate have given consistent results.[20]

In principle, a dianionic intermediate having a finite life time might be expected to allow a hydroxide ion catalyzed 3′,5′- to 2′,5′-isomerization of phosphodiester bonds, but this reaction has never been detected with internucleosidic phosphodiester bonds, only with 3′-O–P-CH(OH)-5′ phosphonate linkages, where the dianionic phosphorane may be stabilized by intramolecular hydrogen bonding.[21] Evidently, the two negatively charged non-bridging oxygen atoms are as electron rich ligands locked to equatorial positions within the phosphorane.[11] This prevents pseudorotation around phosphorus and, hence, isomerization, since the 3′-O cannot adopt an apical position required for the departure. Accordingly, isomerization could only take place *via* a temporary kinetically invisible protonation/deprotonation of one of the non-bridging oxyanions, which would enable pseudorotation. Evidently this route is not utilized, in spite of the fact that thermodynamically favored protonation to a monoanionic phosphorane takes place under rather basic conditions; the latest estimations for the second pK_a value of phosphoranes range from 13.5 to 14.3.[22]

Less attention has been paid to the fact that RNA phosphodiester bonds also undergo a pH-independent cleavage. The reaction is slower than pH-independent isomerization and it is detected only over a narrow pH-range around pH 5.[14] Its existence has, however, been verified with ribonucleoside 3′-alkylphosphates derived from alcohols that are more acidic than the 5′-OH of nucleosides.[18,23] This pH-independent cleavage may even be a more appropriate model than the hydroxide ion catalyzed reaction for the action of small molecular cleaving agents. The reaction most likely proceeds by water mediated proton transfer from 2′-OH to the phosphoryl oxyanion concerted with the attack of the developing 2′-oxyanion on the phosphorus atom (Scheme 3). The monoanionic phosphorane obtained then undergoes rate-limiting breakdown to a 2′,3′-cyclic phosphate by water-mediated proton transfer from the phosphorane oxygen to the departing 5′-oxygen atom concerted with the PO bond cleavage. Owing to the latter proton transfer, the susceptibility to the polar nature of the departing alcohol is much lower (β_{lg} = –0.59) than with the hydroxide ion catalyzed reaction.[23] Concurrently, the monoanionic phosphorane may undergo pseudorotation and subsequent isomerization to a 2′,5′-diester.

The primary ^{18}O isotope effect for the pH-independent cleavage of 3′-(3-nitrobenzyl)phosphate is 1.009 ± 0.001, consistent with a monoanionic phosphorane intermediate, the PO bond cleavage of which is considerably advanced in the transition state.[18] Comparison of the breakdown of the monoanionic phosphoranes derived from phosphodiesters and -triesters lends further support to the importance of the proton transfer to the departing oxygen atom (Scheme 4). Triester-derived phosphoranes, where proton transfer is not possible, give isomerization products up to 10^5 times faster than cleavage products, indicating that endocyclic oxygen atoms depart as oxyanions much more readily than the exocyclic one.[24,25] With diester-derived phosphoranes, the endocyclic fission is only one order of magnitude faster than the exocyclic one, evidently due to intramolecular proton transfer.[26]



Scheme 4 Breakdown of monoanionic phosphorane intermediates derived from a phosphotriester (R = Me) and phosphodiester (R = H).

Recent DFT calculations lend further support for the mechanism depicted in Scheme 3. They, for example, highlight the importance of proton transfer between the attacking/departing nucleophile and a nonbridging phosphoryl oxygen atom upon formation/breakdown of a monoanionic phosphorane.[20,27] The requirement for water as a mediator of these proton transfers, however, seems to remain open to various interpretations. No

isomerization takes place in anhydrous *tert*-butyl alcohol, suggesting that in the absence of water, a stable monoanionic phosphorane is not formed.[18] According to recent DFT calculations, direct proton transfer from the 2′-OH to the phophoryl oxygen is, however, as easy as the water mediated one,[28] while earlier calculations have underlined the importance of water molecules as mediators of the proton transfer.[27] Application of the polarizable continuum model to the cleavage and isomerization of tetrahydrofuran-3,4-diol methylphosphate *via* a monoanionic phosphorane shows that, consistent with experimental results, the barrier for the exocyclic PO bond fission (14.1 kcal mol^{-1}) is considerably higher than that for the endocyclic fission (3.2 kcal mol^{-1}).[29] The latter barrier is comparable to the barrier of the pseudorotation of the monoanionic phosphorane and, hence, the pseudorotation may partially limit the rate of isomerization in the pH range 4–6.

General acid/base-catalyzed cleavage of RNA phosphodiester bonds

RNA phosphodiester bonds are also subject to general acid/base-catalyzed cleavage, although this catalysis is rather inefficient.[7a,b] In buffers having pK_a values close to 7, the formation of the phosphorane intermediate has been reported to be catalyzed by general acids and its breakdown by general bases. The mechanistic interpretation for the former reaction is rapid initial protonation of the anionic phosphodiester and general base catalyzed deprotonation of the 2′-OH (Scheme 5). The observed general base catalyzed breakdown of the intermediate has, in turn, been attributed to rapid initial deprotonation of the phosphorane intermediate accompanied by general acid protonation of the departing 5′-O.[30] While the consistency of this mechanistic interpretation with that described above for the pH- and buffer-independent reaction is obvious, alternative mechanistic descriptions cannot be excluded, either. It has been suggested that under neutral conditions two buffer catalyzed reactions occur in parallel: (i) a simple general base catalyzed deprotonation of the attacking 2′-OH accompanied by a more or less concerted breakdown of the dianionic phosphorane predominates and (ii) general acid catalyzed breakdown of the monoanionic phosphorane obtained without assistance of any buffer.[31] According to this mechanistic explanation, the isomerization reaction is not buffer catalyzed. Undeniably, the buffer catalysis observed for the isomerization is weak and may be attributable to medium effects. In more basic buffers and with ribonucleoside 3′-phosphodiesters having a better leaving group, the former of these reactions predominates.[32]

Cleavage of RNA phosphodiester bonds by multifunctional organic agents

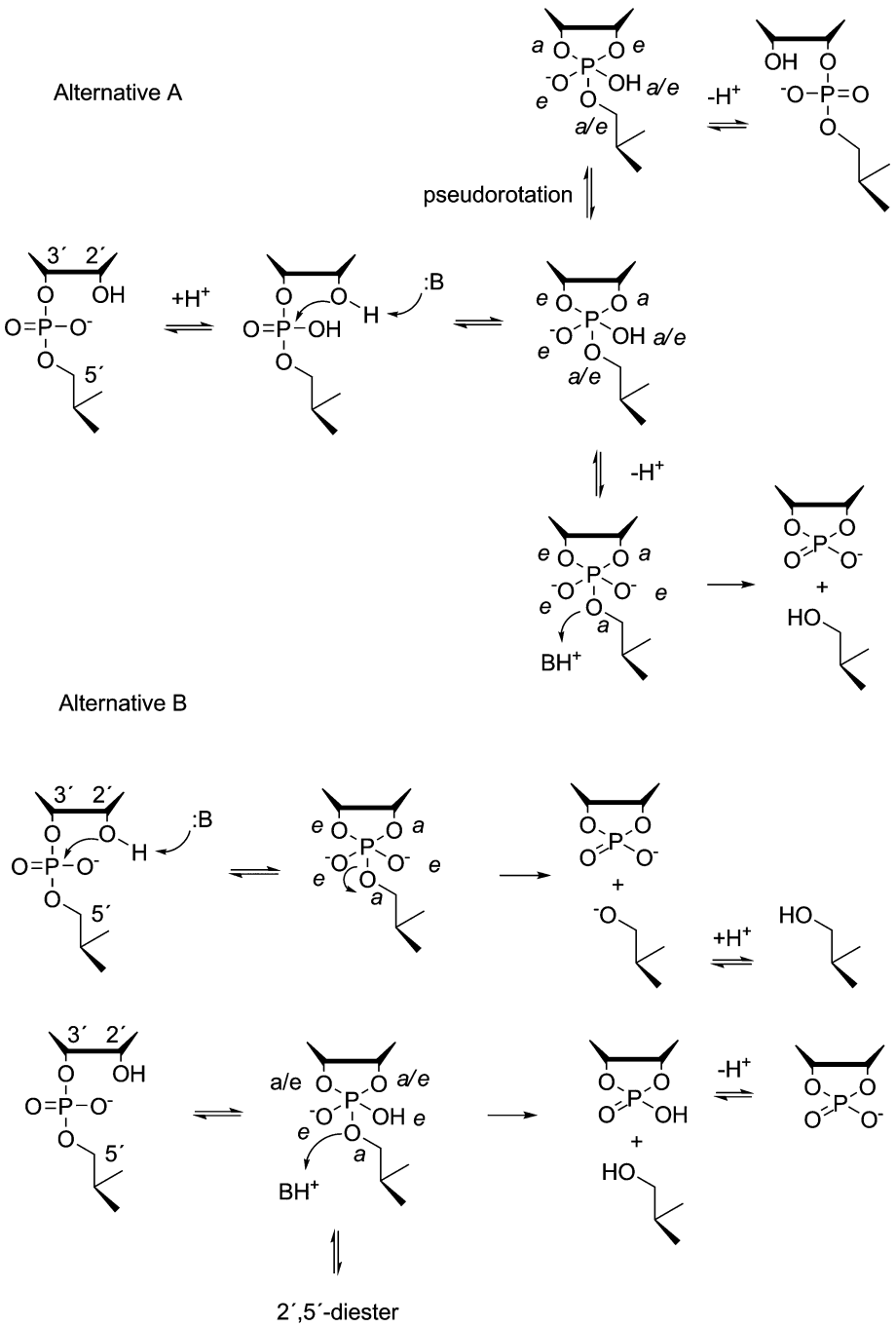
While several ribonucleases[33] and ribozymes[34] are metal-ion-dependent, metal-ion-independent protein enzymes and ribozymes are also known. The most thoroughly studied example of such protein enzymes is RNase A,[35] the catalytic mechanism of which appears quite simple: deprotonation of 2′-OH by a His residue, protonation of the departing 5′-O by a protonated His residue and stabilization of the dianionic phosphorane intermediate by hydrogen bonding with a protonated Lys residue. Among small ribozymes, at least the hairpin ribozyme seems to exploit simple general acid/base-catalysis by nucleobases.[36] Several attempts have been made to mimic the catalytic center of these enzymes by constructing multifunctional organic molecules or ions that are aimed at benefiting from the synergic action of more than one catalytic function.

Such constructs are attractive building-blocks for construction of chemotherapeutic agents, such as catalytically active antisense oligonucleotides which could sequence selectively hydrolyze their target RNA. Unfortunately, the efficiency of the non-metallic catalysts developed so far is generally rather low, although some particular structural motives of RNA, such as the loop and bulge structures within the TAR element, are quite effectively recognized and cleaved even by purely organic constructs.[37]

Non-metallic agents that cleave RNA phosphodiester bonds independently of the secondary structure or base composition often incorporate guanidinium groups. The most efficient among such catalysts is tris[2-(benzimidazol-2-yl)ethyl]amine (**1**).[38] The first-order rate constant for the cleavage of an individual phosphodiester bond within a 29-mer oligoribonucleotide has been reported to be 3.3×10^{-6} s^{-1} in 1.0 mmol L^{-1} solution of **1** at pH 6.0 and 37 °C. The reaction, however, takes place within an aggregate consisting of several catalyst molecules and oligomeric substrates. Nevertheless, a considerable part of the catalytic activity is retained in a nonaggregated state and tethering of **1** to an oligodeoxyribonucleotide has afforded rather efficient sequence selective artificial RNases.[39] The pH-rate profile of the cleavage reaction suggests that the catalyst is most active in the deprotonated state. Accordingly, the high activity compared to other guanidinium-derived agents may result from reduced acidity of **1**, having a pK_a value as low as 7. The mechanistic details of the reaction remain obscure. Tentatively, guanidinium groups may shuttle the proton from the attacking 2′-OH to one of the non-bridging oxygen atoms of the phosphorane intermediate and/or from the monoanionic intermediate to the departing 5′-oxygen. Consistent with this assumption, proton inventory studies with an aryl ester model (**2**) have shown that two protons are transferred in the rate-limiting step.[40] It has been suggested that the guanidinium group protonates a non-bridging oxygen of the developing phosphorane intermediate concerted with deprotonation of the 2′-OH by an external base. The rate-accelerating effect of this proton transfer has been estimated to be two orders of magnitude. One should, however, bear in mind that the ease of the breakdown of the phosphorane intermediate plays a decisive role in the cleavage of RNA phosphodiester linkages in striking contrast to their aryl ester models.

Another exceptionally efficient non-metallic cleaving agent is a quaternized 1,4-diazabicyclo[2,2,2]octane conjugate of histidine (**3**) that has been shown to cleave a 10-mer RNA oligonucleotide (UUCAUGUAAA) in 4 h at pH 7.0 and 37 °C, when present at 0.5 mmol L^{-1} concentration. This means that the first-order rate constant for the cleavage of an individual phosphodiester bond should be greater than 10^{-5} s^{-1}. On using larger RNAs, single stranded regions are preferably cleaved.[41] The hydrophobic tail is an essential component of the construct.[42] Conjugate **3b** has been shown to form aggregates in solution, but the maximal efficacy of RNA cleavage is observed already at a concentration well below that required for micelle formation.[43]

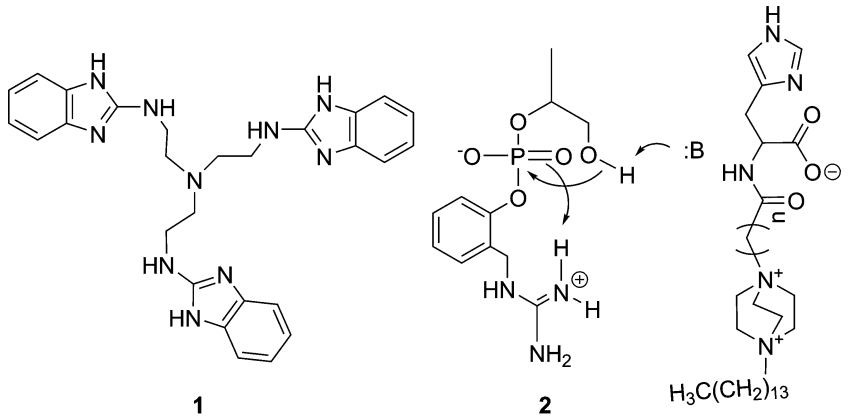
Simple di- and oligo-amines exhibit only modest catalytic activity towards RNA. The first order rate constant for the



Scheme 5 Mechanistic alternatives proposed for the buffer-catalyzed reactions of RNA phosphodiester bonds. For A see *Ref.* 30, for B *Ref.* 31.

cleavage of 3′,5′-ApA in 1.0 mol L^{-1} solution of ethane-1,2-diamine is 1.2×10^{-6} s^{-1} at pH 8 and 50 °C.[44] The neutral diamine is 6.6 times as efficient a catalyst as the monocation, but the mole fraction of this species is under neutral conditions so low that the cleavage is still predominantly catalyzed by the monocation. The reaction has been suggested to proceed by H-bond anchoring of the ammonium group to phosphate and general base catalyzed deprotonation of 2′-OH by the amino group. This mechanism does not, however, explain the higher catalytic activity of the neutral diamine. 3-Azapentane-1,5-diamine and 3,6-diazaoctane-1,8-diamine are catalytically slightly less active than ethane-1,2-diamine. By contrast, the tetracation of macrocyclic 1,4,16,19-tetraoxa-7,10,13,22,25,28-hexaazacyclotriacontane (**4**) is two orders of magnitude more active, although a structurally related 1,4-dioxa-7,10,13-triazacyclopentadecane (**5**) is catalytically inactive.[45] This data does not allow any detailed mechanistic conclusions. Stabilization of the anionic phosphorane intermediate by hydrogen-bonding and/or electrostatic interaction, together with assistance in protonation of the departing 5′-O appear the most attractive alternatives.

As mentioned above, several non-metallic agents have been reported to cleave selectively some particular phosphodiester



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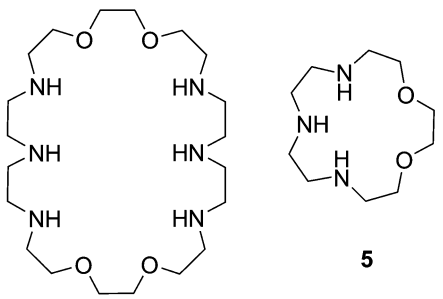

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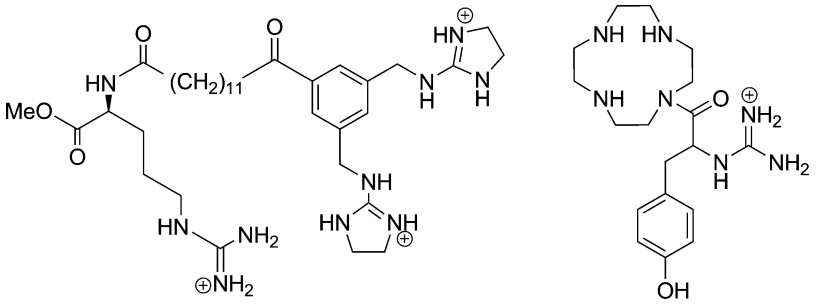

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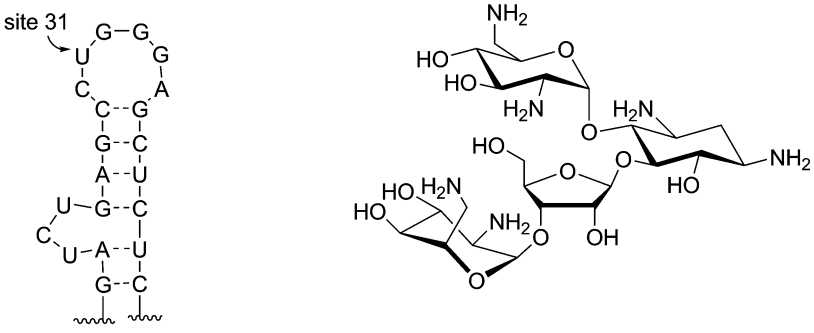

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bonds within RNA, although their catalytic activity towards RNA phosphodiester bonds in general is much lower or even nonexistent. For example, the tris(guanidinium) construct **6**[46] and the guanidinium modified cyclen conjugate of tyrosine (**7**)[47] both cleave at a millimolar concentration the TAR element (**8**) within the loop at U31 in a few hours under physiological conditions, while none of the constituents of **6** or **7** exhibit alone a comparable activity.

In addition to compounds **6** and **7**, antibiotic aminoglycosides, such as neomycin B (**9**), cleave the TAR element. They bind to

the UCU-bulge and induce the cleavage within the neighboring hairpin loop at the 5′-side of U31. Pseudo first-order rate constant for the reaction promoted by **9** at a 50 μmol L^{-1} concentration is as high as 6.1×10^{-5} s^{-1} at pH 6.5 and 21 °C ($I = 0.1$ mol L^{-1}), which means that the reaction is 10^6 times faster than the cleavage of 3′,5′-ApA by the same agent.[48] It has been suggested that binding of neomycin B to the UCU-bulge induces a conformational change that accelerates the intramolecular transesterification at the[31] U-site. The acceleration, however, appears too large to be entirely attributed to favorable mutual orientation of the attacking and departing nucleophile.

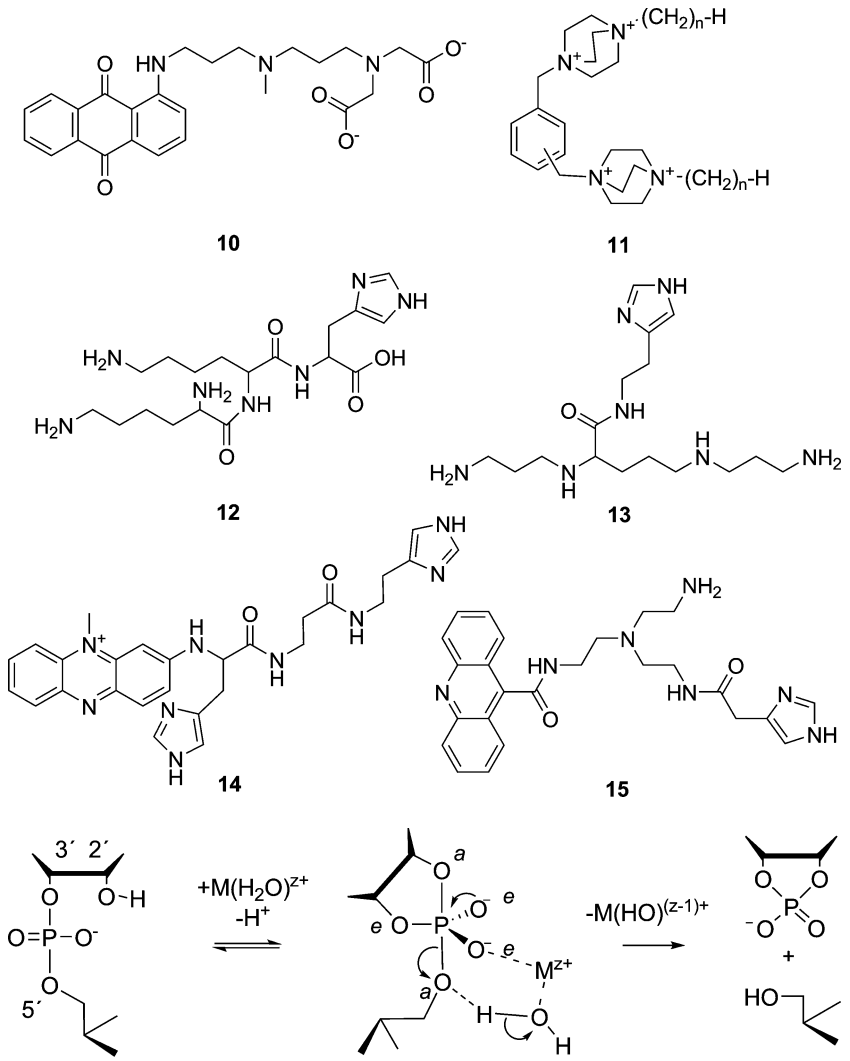
While the compounds discussed above cleave a relatively small hairpin RNA at a single phosphodiester bond, a wider spectrum of selectivity is found on using larger tRNA molecules as substrates. Many constructs containing several amino and/or imidazolyl groups cut the RNA chain at some preferred sites, usually most readily at 5′-CpA-3′ sites within single stranded regions. These include antraquinone conjugates of aza carboxylic acids (**10**),[49] dimers of quaternized 1,4-diazabicyclo[2.2.2]octane bearing a hydrophobic tail (**11**)[50] and a variety of conjugates containing, in addition to amino, amido and carboxy functions, one or two imidazole residues (**12,13**),[51] and possibly an intercalator to enhance the binding (**14,15**).[52] With these kind of conjugates, the reaction usually exhibits a pH-optimum around 7, which has been taken as an indication of the participation of both imidazole and imidazolium ion in the catalytic step. In other words, the cleaving agent is assumed to bind in the vicinity of the scissile bond by stacking with base moieties or by H-bonding and electrostatic interactions with phosphate groups, and concerted deprotonation of the attacking 2′-OH by imidazole and protonation of the departing 5′-*O* by imidazolium ion then take place.[53] With conjugates containing only one imidazole group, external imidazolium ion has been suggested to take the role of the general acid. The experimental evidence for the mechanism is, however, still meagre. On the basis of the preceding discussion, a rapid initial formation of the phosphorane intermediate and subsequent facilitation of the P-O5′ bond cleavage by a general acid also appears feasible. Accordingly, the hydroxide ion catalysis observed at pH <7 may be attributed to pre-equilibrium formation of a dianionic phosphorane, stabilized by H-bonding and/or electrostatic interaction with the cleaving agent. The rate-limiting breakdown of the intermediate is then subject to general acid catalysis of an imidazolium residue. On going to higher pH, deceleration takes place since deprotonation of the cleaving agent weakens the H-bonding/electrostatic stabilization of the phosphorane intermediate and reduces the concentration of the imidazolium group that serves as the general acid.

Cleavage of RNA phosphodiester bonds by metal ions and mononuclear metal ion complexes

It has been known since the early 1950s that metal ions promote the cleavage of RNA phosphodiester bonds.[54] Since the single stranded regions are cleaved much more rapidly than the double helical parts, even one nucleotide bulge allowing the cleavage,[55] metal ions have received extensive attention as probes in structural studies of RNA.[56] In spite of this, the mechanistic details of the metal ion promoted reactions still seem to remain open to various interpretations. Usually only transesterification to a 2′,3′-cyclic phosphate, not isomerization to a 2′,5′-diester, is accelerated.[57] Lanthanide ions apart, only one metal ion participates in the catalysis, the rate of the reaction being proportional to the concentration of the hydroxo complex of the metal ion. With lanthanide ions, the catalytically most active species are polynuclear.[58] 3d transition metal ions are more efficient catalysts than less acidic alkaline earth metal ions,[57] in spite of the fact that catalytic nucleic acids prefer Mg^{2+} ion.[34] The cleavage of ribonucleoside 3′-alkylphosphates is insensitive to the acidity of the esterified alcohol ($\beta_{lg} = -0.32 \pm 0.04$ for the Zn^{2+} promoted cleavage), which suggests that the leaving group departs as an alcohol rather than an alkoxide ion.[59] In other words, the reaction appears to proceed by pre-equilibrium formation of a dianionic phosphorane stabilized by a coordinated metal aqua ion that in the subsequent rate-limiting step protonates the departing 5′-oxygen atom (Scheme 6). Evidently the dianionic phosphorane, although stabilized by the metal ion, is not sufficiently long-lived to undergo pseudorotation and, hence, only the cleavage reaction is accelerated. Consistent with the involvement of the metal ion in the breakdown of the intermediate, Co(III) complexes have been shown to accelerate more markedly the cleavage of 3′,5′-ApA than the cleavage of adenosine 3′-phenylphosphate, which expectedly proceeds by rate-limiting formation of the phosphorane intermediate.[60] The pH–rate constant profile for the cleavage of 3′,5′-ApA is bell-shaped with a maximum around pH 7, and a notable D_2O solvent isotope effect (2.0) is observed. By contrast, no such maximum occurs with the 3′-phenylphosphate, but the reaction is continuously accelerated on passing pH 7. While a Co^{3+}-bound aqua ligand is required as a general acid to promote the departure of the 5′-linked nucleoside, phenoxide ion may depart without protonation and, hence, acceleration instead of deceleration is observed also at pH >7.

The results of DFT calculations utilizing a conductor-like solvation model (COSMO) for approximation of the solvent effect are consistent with this mechanism. The preferred reaction path involves a marginally stable phosphorane intermediate having both the entering and leaving oxygen atoms H-bonded to the aqua ligands of the $Mg^{2+}(H_2O)_5$ ion that, in turn, is bound to one of the non-bridging phosphorane oxyanions. This intermediate undergoes rate-limiting breakdown by PO bond cleavage concerted with a proton transfer from the Mg^{2+} aqua ligand to the departing oxygen.[61] It should be, however, noted that direct inner sphere coordination of Mg^{2+} ion to the attacking and departing oxygen atom, instead of phosphorane oxyanion, has also received support by DFT calculations.[27]

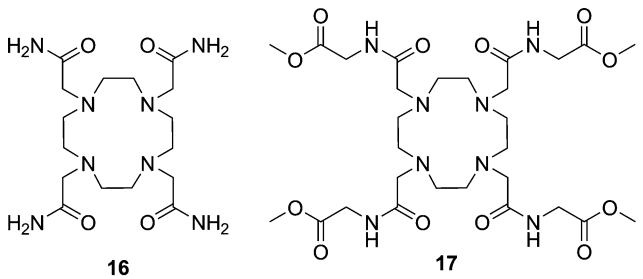
Metal ion promoted cleavage of RNA has received extensive interest as the basis of artificial ribonucleases, agents that cleave the RNA chain at a single desired phosphodiester linkage.[9,62,63] For this purpose, the metal ion must be tightly bound to a ligand structure that allows its attachment to various sequence-recognizing carriers. Since tri- and tetravalent lanthanide ions exhibit a much higher cleaving activity than divalent metal ions, their complexes have received wide attention as potential cleaving agents. Complexing with anionic ligands often gives highly stable complexes, but the catalytic activity is largely lost. Among neutral ligands, *N*-substituted azacrowns have given encouraging results. 2,2′,2″,2‴-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetamide (**16**), for example, has been reported to bind



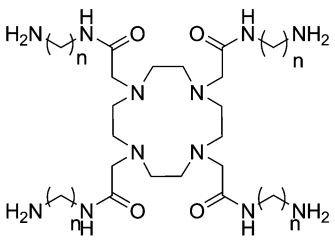
Scheme 6 Metal ion promoted cleavage of RNA phosphodiester bonds.

lanthanide and Th^{4+} ions so tightly that no release of the metal ion can be detected in several weeks under physiological conditions.[64] On using oligo$(A)_{10}$ as a substrate, the pseudo first-order rate constant for the cleavage of an individual phosphodiester bond was 9×10^{-5} s^{-1} at 1 mmol L^{-1} concentration of the Th^{4+} complex at pH 7.4 and 37 °C, and the La^{3+} complex was one order of magnitude less active. Luminescence spectroscopic studies on the Eu^{3+} complex of 2,2′,2″-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetamide suggests that the Eu^{3+} ion bears two aqua ligands over the pH range 7–9.[65] Since no aqua ligand ionizations take place, the catalysis may be attributed to stabilization of the dianionic phosphorane by Eu^{3+} binding and protonation of the departing 5′-oxygen atom by the remaining aqua ligand (*cf*. Scheme 6). Interestingly, conversion of **16** to its tetrakis-*N*-(2-methoxy-2-oxoethyl) (**17**) or tetrakis-*N*-(ω-aminoalkyl) (**18a–c**) analogs markedly increases the catalytic efficiency of the La^{3+} complex on using an aryl ester, HpNP, as a substrate. The rate acceleration is 7-fold with **17**[66] and 7800-, 1400- and 260-fold with **18a**, **b** and **c**,[67] respectively. The catalytic activity disappears upon removal of the primary amino groups from **18a–c**. The acceleration has been attributed to facilitation of the substrate binding by H-bonding or intracomplex deprotonation of the 2′-hydroxy group, but most likely stabilization of the phosphorane by intracomplex H-bonding also plays a role.

Mononuclear Zn^{2+} and Cu^{2+} complexes have also received extensive attention as catalytic groups of sequence selective artificial ribonucleases,[9] although their catalytic activity usually is low compared to lanthanide chelates. The most efficient catalysts of this category are Zn^{2+} complexes of triazacrowns, above all 1,5,9-triazacyclododecane and 1,4,7-triaza-10-oxacyclodocane.[68] The catalytic efficiency of mononuclear Zn^{2+} complexes may, however, be markedly enhanced by appropriately situated H-bond donors. Complexes **19b** and **c** bearing two guanidiniummethyl or ammoniummethyl groups, respectively, offer an example.[69] The cleaving activity of **19b** towards 3′,5′-ApA reaches the maximum value at pH 7.3, *i.e.* around the pK_a value of the complexed Zn^{2+} aqua ion. The reaction is first-order in the concentration of **19b**, the pseudo first-order rate constant being 2.2×10^{-5} s^{-1} at [**19b**] = 5 mmol L^{-1}, pH 7.4 and 37 °C (10 mmol L^{-1} HEPES). In other words, the activity of **19b** is comparable to that of mononuclear lanthanide chelates. The contribution of the H-bond donors to the catalytic activity is remarkable, since **19b** is 3300 times and **19c**



16 **17**



18a: n = 2
b: n = 3
c: n = 4

370 times as efficient cleaving agent as their dimethyl counterpart **19a**. It has been suggested that the H-bond donors stabilize the dianionic phosphorane intermediate by H-bonding, while the hydroxo/aqua ligands of the phosphate anchored Zn^{2+} ion shuttle the proton from the attacking 2′-OH to the departing 5′-O.

Another example of the synergic action of metal ions and H-bond donors is afforded by the effect of amino substitution on the cleaving activity of the Zn^{2+} complex of tris(pyridine-2-ylmethyl)amine (**20a**): the Zn^{2+} complex of tris(6-aminopyridine-2-ylmethyl)amine (**20b**) cleaves HpNP two orders of magnitude faster than the unsubstituted **20a**.[70] The rate-enhancing effect of the amino groups is, in fact, comparable to the influence of an additional metal ion: the mononuclear Zn^{2+} complex of *N*,*N*-bis(6-aminopyridine-2-ylmethyl)-2-aminoethanol (**21**) cleaves HpNP as rapidly as the dinuclear Zn^{2+} complex of *N*,*N*,*N*′,*N*′-tetrakis(pyridine-2-ylmethyl)-1,3-diaminopropan-2-ol (**22**).[71] The affinity of the dinuclear complex to the substrate is higher, but the mononuclear complex once bound to the substrate is a more efficient catalyst. In other words, H-bonding appears to be superior to an additional metal ion in stabilizing the phosphorane structure. DFT calculations on the cleavage of HpNP by **20b** have been carried out to shed light on the role of the amino groups, but the results are somewhat controversial.[72a,b] On one hand, the amino groups have been argued to mediate proton transfer from the attacking 2-hydroxy group to phosphate bound Zn^{2+} hydroxo ligand, which then becomes H-bonded to the departing oxygen.[72a] On the other hand, water-assisted proton transfer has been preferred, the zinc aqua complex being the catalytically active form.[72b] According to this model, the rate-limiting step consists of a concerted double-proton transfer, concomitant with the nucleophilic addition of the deprotonated 2′-OH group to form a five-coordinated phosphorus intermediate. The amino groups in the second coordination sphere further accelerate the reaction by stabilizing the proton transfer transition state. Somewhat unexpectedly, the rate-accelerating effect of the 6-amino group is much weaker with bis-*N*,*N*-(pyridine-2-ylmethyl)propylamine, only about one order of magnitude.[73]

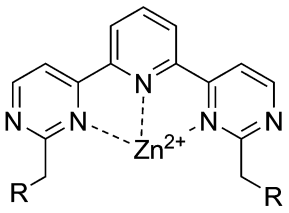
Cleavage of RNA phosphodiester bonds by di- and tri-nuclear metal ion complexes

Several enzymes catalyzing phosphate transfer contain two metal ions in their catalytic center. One of these metal ions, if not both, is often Zn^{2+}, the other one being Mg^{2+}, Fe^{2+}, Fe^{3+} or Mn^{2+}.[33] Accordingly, dinuclear Zn^{2+} complexes have received extensive attention both as enzyme mimics and as potential artificial nucleases.[74] The most attracting feature of the dinuclear complexes is the possible double Lewis acid activation. Studies with substitution inert Co(III) complexes have shown that while metal ion binding to only one of the non-bridging phosphoryl oxygen atoms accelerates the cleavage by less than two orders of magnitude, a 10^5-fold acceleration may be achieved by simultaneous engagement of both of the non-bridging oxygens in the metal ion coordination.[75] Substitution-labile metal ions may also exhibit double Lewis acid activation when bound to a common ligand at an appropriate 3–4 Å distance from each other. This is the case, for example, when both of the metal ions of a dinuclear complex share a common donor atom within the ligand structure. Table 1 records the cleaving activity of several such complexes (**22–25**). In most studies, HpNP has been used as a substrate instead of a dinucleoside-3′,5′-phosphate. On drawing mechanistic conclusions, one should bear in mind that with aryl esters, formation rather than breakdown of the phosphorane

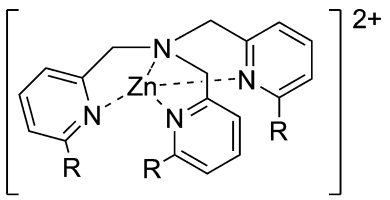
Table 1 Catalytic activities of dinuclear metal ion complexes towards 3′,5′-dinucleoside monophosphates and 2-hydroxypropyl *p*-nitrophenyl phosphate (HpNP)

Catalyst	Substrate	[catalyst]/mmol L^{-1}	pH	*T*/C°	*I*/mol L^{-1}	k/s^{-1}	*Ref*
22	3′,5′-ApA	2.0[a]	7.0	50	not adjusted[c]	1.9×10^{-5}	76
22	HpNP	1.0	7.4	25	not adjusted[c]	6.6×10^{-5}	71
23	HpNP	2.0[a]	>8	25	0.1 ($NaNO_3$)	1.4×10^{-3}	77
24	3′,5′-UpU	2.0[a]	7.0	35	0.1 ($NaNO_3$)	2.2×10^{-6}	80
25	3′,5-UpU	1.0[b]	6.5	25	not adjusted[d]	2.8×10^{-5}	82
25	HpNP	[e]	7.4	25	not adjusted[c]	1.7×10^{-2}[f]	81
26a	3′,5′-ApA	5.0[a]	7.3	50	0.1 (NMe_4NO_3)	1.2×10^{-6}	83
26b	3′,5′-ApA	5.0[a]	>10	50	0.1 (NMe_4NO_3)	2.0×10^{-6}	83
28	3′,5′-UpU	2.0	7.0	30	0.1 ($NaNO_3$)	2.0×10^{-7}	84
29	3′,5′-ApA	2.5	7.0	50	not adjusted[c]	5.1×10^{-5}	85
32	3′,5′-UpU	1.0[a]	7.6	25	0.1 ($NaNO_3$)	2.1×10^{-5}	65

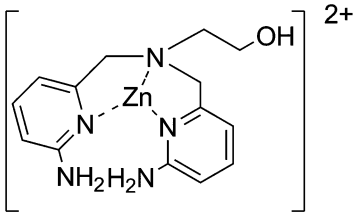
[a] First-order in catalyst concentration, [b] Independent of the catalyst concentration, [c] 50 mmol L^{-1} HEPES, [d] 50 mmol L^{-1} MOBS, [e] Michaelis–Menten kinetics obeyed, $K_M = 0.32$ mmol L^{-1}, [f] k_{cat} when Michaelis–Menten kinetics applied.



19a: R = H
b: R = NH-C(NH_2)=NH_2^+
c: R = NH_3^+



20a: R = H
b: R = NH_2



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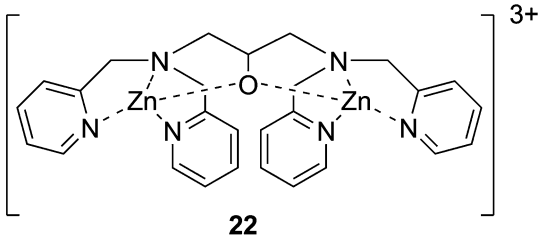


22

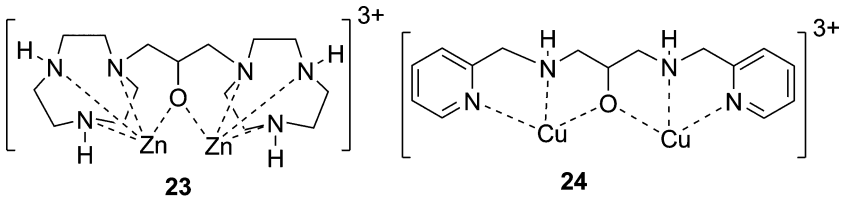
intermediate is rate-limiting and, hence, the results referring to cleavage of HpNP cannot necessarily be extrapolated to cleavage of RNA phosphodiester bonds.

The dinuclear Zn^{2+} complex **22** discussed above offers an illustrative example of the efficiency of double Lewis acid activation with substitution labile metal ions; HpNP is cleaved 200 times as fast as the mononuclear complex **21**.[71] The same complex also cleaves 3′,5′-dinucleoside monophosphates rather efficiently, but the cooperativity is less prominent than on using HpNP as a substrate.[76] The pH-rate profile referring to cleavage of 3′,5′-ApA is sigmoid, indicating that the cleavage rate correlates with the mole fraction of the monohydroxo form of **22**. The related dinuclear Zn^{2+} complex of 1,3-bis(1,4,7-triazacyclononan-1-yl)propan-2-ol (**23**), in turn, cleaves HpNP only 12 times as fast as the mononuclear complex of 2-(1,4,7-triazanonan-1-yl)ethanol.[77] Omission of the hydroxyl group on the tether of **23** entirely abolishes the cooperativity between the two metal ions.[78] The corresponding Cu^{2+} complex is a poor catalyst, but the Cd^{2+} complex is even 4 times as efficient as the Zn^{2+} complex. This high rate is, however, achieved only at high pH (pH>9).[79] Among Cu^{2+} complexes, the dinuclear Cu^{2+} complex of *N*,*N*′-bis(pyridine-2-ylmethyl)-1,3-diaminopropan-2-ol (**24**), however, exhibits rather high cleaving activity. The pH-rate-profile obtained for the cleavage of 3′,5′-UpU is sigmoid and the plateau value is 2.2×10^{-6} s^{-1} at [**24**] = 2.0 mmol L^{-1}, pH >7 and 35 °C.[80] The assumed binding of both non-bridging phosphate oxygens to Cu^{2+} receives support from the crystal structure of the corresponding diphenyl phosphate complex.

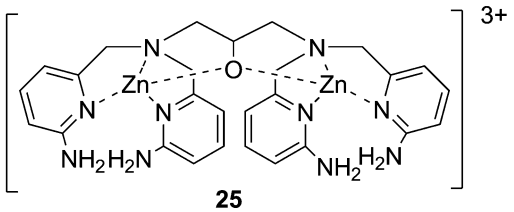
As with mononuclear Zn^{2+} complexes, cooperative hydrogen bonding and metal ion coordination greatly increases the catalytic activity of dinuclear complexes. The dinuclear Zn^{2+} complex of *N*,*N*,*N*′,*N*′-tetrakis(6-aminopyridine-2-ylmethyl)-1,3-diaminopropan-2-ol (**25**) cleaves HpNP 700 times as efficiently as its structural analog **22** lacking the amino substituents.[81] Approximately half of the acceleration results from enhanced ground state binding, and half from enhanced breakdown of the substrate catalyst complex to products. At pH 7.4 and 25 °C, k_{cat} is 0.017 s^{-1} and K_M = 0.32 mmol L^{-1}. **25** also catalyzes the cleavage of 3′,5′-UpU as efficiently as the cleavage of HpNP.[82] The first-order rate constant for the cleavage is 2.6×10^{-5} s^{-1} at pH 6.5 and 25 °C, corresponding to a 10^6-fold acceleration compared to the uncatalyzed cleavage at this pH. The rate is independent of the catalyst concentration, indicating high affinity binding. Somewhat unexpectedly, the phosphate migration is also accelerated, although to much lesser extent than the cleavage, 150-fold compared to the uncatalyzed migration. It has been concluded that coordination of the Zn^{2+} ions to the non-bridging oxygens stabilizes the phosphorane intermediate sufficiently to allow pseudorotation, which is a prerequisite for isomerization. The role of the amino groups remains somewhat obscure. One might speculate that they also participate in stabilization of the intermediate by H-bonding, and possibly somehow mediate proton transfer to the departing 5′-O, as discussed in the context of the catalytic action of **20b** above.

Macrocyclic oxa-aza ligands enable construction of dinuclear complexes, where the two metal ions are bridged by an aqua or hydroxo ligand, instead of an alcoholic hydroxy group. The double Lewis acid activation achieved by this kind of construct remains, however, modest. Monohydroxo and dihydroxo forms of the dinuclear Zn^{2+} complex of 1,4,16,19-tetraoxa-7,10,13,22,25,28-hexaazacyclotriacontane (**26**) catalyze the cleavage of 3′,5′-ApA 4 times as efficiently as the mononuclear Zn^{2+} hydroxo complex of 1,4-dioxa-7,10,13-triazacyclopentadecane (**27**).[45] The dinuclear Zn^{2+} complex of 1,13-dioxa-4,7,10,16,19,22-hexaazacyclotetracosane (**28**) cleaves 3′,5′-UpU less efficiently, but the enhancement of the catalytic activity over a mononuclear counterpart, the Zn^{2+} complex of 1,5,9-triazacyclododecane, is greater than with **26**, up to one order of magnitude.[83,84]

A common donor atom shared by two metal ions is not, however, the only way to achieve double Lewis acid activation. In



23 **24**



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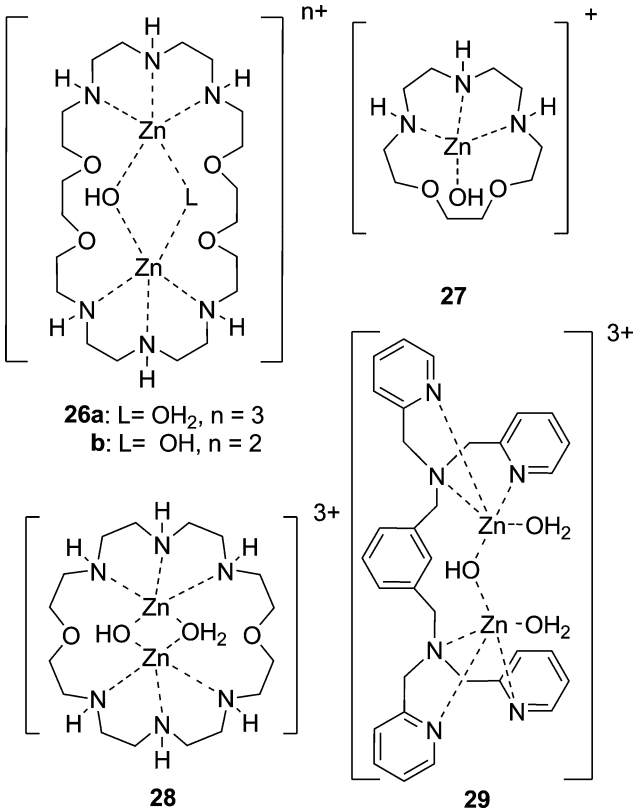
fact, the dinuclear Zn^{2+} complex of *N*,*N*,*N*′,*N*′-tetrakis(pyridine-2-ylmethyl)-*m*-xylenyldiamine (**29**) has been reported to be catalytically even more active than **22**.[85] The underlying structural difference seems to be that **29** dissociates three protons on going to pH 10.7, in striking contrast to **22** which dissociates only one proton. Evidently, the Zn^{2+} ions in **29** both bear a non-bridging aqua ligand in addition to the bridging one, while the metal ions in **22** have no such aqua ligands in addition to the two bridged oxygen ligands. The rate of the **29** promoted cleavage correlates with the mole fraction of the monohydroxo form, as with **22**. Owing to formation of a dihydroxo complex at high pH, the pH-rate profile is, however, bell-shaped, not sigmoid as with **22**. The higher catalytic activity of **29** has been accounted for by the presence of the non-bridging aqua ligands in **29**, which may serve as intracomplex general acids, protonating the departing 5′-O. Compared to the mononuclear bis(pyridine-2-ylmethyl)amine complex, the acceleration is 15-fold. Complex **29** binds to 3′,5′-ApA less tightly than **22** (K_M 12 mmol L^{-1} and 4.6 mmol L^{-1}, respectively), but exhibits a higher k_{cat} value (1.9×10^{-4} s^{-1} and 2.8×10^{-5} s^{-1}, respectively).

Many enzymes catalyzing phosphoryl transfer reactions actually contain two different metal ions in their catalytic center. This suggests that heterodinuclear complexes might be superior to their homodinuclear counterparts, possibly for the reason that the two metal ions play a different role in the catalytic event. For example, one metal ion could participate in formation of the phosphorane intermediate and the other one in its breakdown. The data on heterodinuclear cleaving agents is scarce, but some hints of the superiority of such species have been observed. The dinucleating ligand **30** catalyzes the cleavage of 3′,5′-ApA one order of magnitude more efficiently in the presence of Zn^{2+} and Fe^{3+} than in the presence of only one of these ions. The maximal rate is achieved between pH 4.5 and 5.5. It has been tentatively suggested that a Zn^{2+}-bound hydroxo ligand deprotonates the attacking 2′-OH, while Fe^{3+} stabilizes the departing 5′-oxyanion by inner sphere coordination.[86] The experimental data is, however, so meagre that several alternative mechanisms appear as plausible. Additional indications of the superiority of heterodinuclear complexes over the homodinuclear ones are afforded by cleavage of the HpNP model. The dinuclear $Zn^{2+}Fe^{3+}$ complex **31a** has been reported to be a 10 times more efficient catalyst than its $Fe^{2+}Fe^{3+}$ counterpart **31b**.[87]

The dinuclear Eu^{3+} complex **32** cleaves 3′,5′-UpU 46 times as fast as its mononuclear analog **33**. The reaction is first-order in

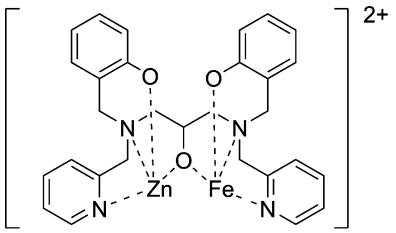


26a: L= OH_2, n = 3
b: L= OH, n = 2

27

28 **29**

the catalyst concentration, the second-order rate constant being 0.021 L mol^{-1} s^{-1} at pH 7.6 and 25 °C.[65] According to luminescence spectroscopic studies, both Eu^{3+} ions bear two aqua ligands at pH 7.0, and no aqua ligand ionizations take place on going to pH 9. In this respect the Eu^{3+} complexes differ from the dinuclear Zn^{2+} or Cu^{2+} complexes which readily form complexes containing a bridging hydroxo ligand. For this reason, **32** retains high affinity to the dianionic phosphorane even at high pH. Owing to pre-equilibrium deprotonation of the 2′-OH, the reaction is first-order in hydroxide ion concentration up to pH 9 (the highest pH studied). Under neutral conditions, the cleaving activity is comparable to that of the most efficient dinuclear Zn^{2+} complex, **25**, but at higher pH, it becomes an even more efficient catalyst. Studies with a septadentate Eu^{3+} complex (**34**) have indicated that dimerization *via* a hydroxo ligand takes place at pH >8, but this does not



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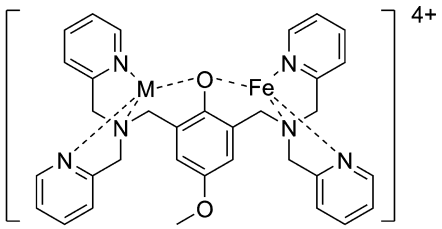
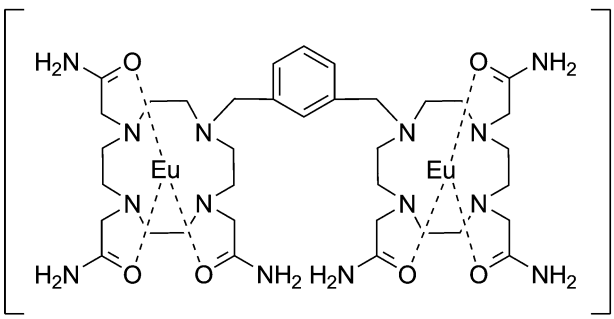


31a: M = Zn(II)
b: M = Fe(II)



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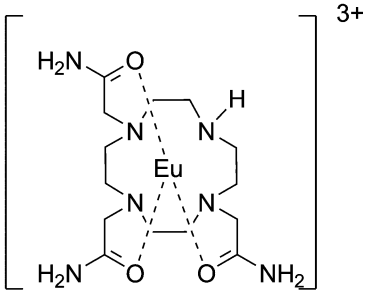

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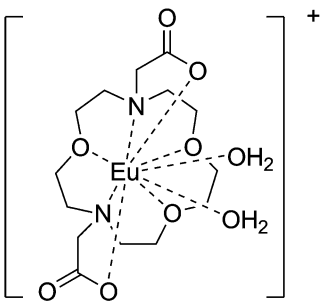

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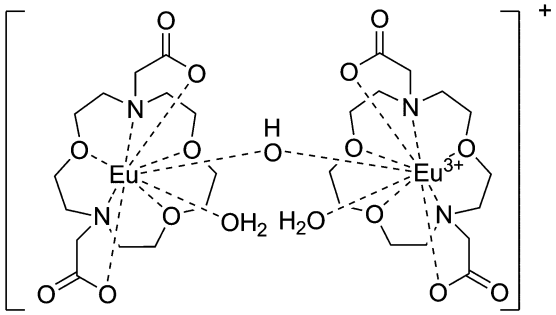


35

bring any marked increase in the catalytic efficiency.[88] A bridging hydroxide ion (**35**) does not promote cooperative catalysis between the Eu^{3+}, but the two Eu^{3+} centers work independently, as also indicated above.

As indicated above, dinuclear metal ion complexes usually exhibit higher cleaving activity than their mononuclear counterparts. The main origin of this double Lewis acid activation appears to be stabilization of the phosphorane intermediate.[89] The cleavage of uridine 3′-(4-nitrophenyl phosphate) catalyzed by **23** does not exhibit a marked kinetic solvent deuterium effect, suggesting that no proton transfer takes place in the rate-limiting stage, but the rate acceleration is due to electrostatic stabilization of the phosphorane intermediate, the breakdown of which does not require protonation of the leaving group.[90] Kinetic heavy atom isotope effects measured in the attacking hydroxyl group (^{18}O) and departing 4-nitrophenoxy group (^{15}N) show that the cleavage of HpNP catalyzed by **23** proceeds through a late transition state with greater leaving group bond fission and greater nucleophilic bond formation than observed for the uncatalyzed cleavage.[91] Accordingly, the dianionic phosphorane seems to be stabilized by complexing with **20b** so markedly that the breakdown rather than formation of the phosphorane becomes rate-limiting, as with alkyl esters. Departure of the aryloxy ion does not require assistance by a general acid, which in all likelihood is the case with RNA. DFT calculations on the cleavage of HpNP by the dinuclear Zn^{2+} complex of 2,6-bis[bis(pyridin-2-ylmethyl)aminomethyl]-4-methylphenoxide ion lend additional support for the stabilization of the phosphorane by the dinuclear Zn^{2+} coordination.[92] However, the metal ions have been argued to bind to the non-bridging oxygen atoms already at the initial state and the bridged hydroxide ion is argued to serve as an intracomplex general base that deprotonates the attacking hydroxyl function. Accordingly, both the phosphorane stabilization and facilitation of the deprotonation of the attacking nucleophile are assumed to contribute to the rate acceleration. These mechanisms should not, however, be automatically extrapolated to alkyl esters, such as RNA. With alkyl esters, the breakdown of the phosphorane intermediate still is more difficult than with aryl esters and structural factors that facilitate protonation of the departing oxygen are important.

Another common feature of dinuclear metal ion catalysts is that the cleavage rate correlates with the mole fraction of the monohydroxo complex. This means that one proton is removed on going from the predominant ionic form of the substrate and the catalyst to the transition state: either (i) the metal aqua ion binds to monoanionic phosphodiester and the attacking nucleophile (2′-OH in RNA) is deprotonated by an external base either prior to or concerted with the formation of the P-O2′ bond, or (ii) the catalyst is bound to the substrate as a hydroxo complex and the proton transfer from the 2′-OH to the hydroxo ligand of the phosphate bound catalyst takes place within the substrate-catalyst complex. As discussed above, the former alternative appears to be more widely accepted, in spite of the fact that the latter mechanism has also been supported by some DFT studies.[93] Independently of which one of these mechanisms is obeyed, formation of the phosphorane intermediate constitutes a rapid base catalyzed pre-equilibrium step of the cleavage of RNA, followed by rate limiting departure of the 5′-linked nucleoside. The latter reaction is subject to general acid catalysis by the phosphorane bound aqua complex, as depicted in Scheme 6 for simple metal aqua ions.

The cleaving activity of some dinuclear complexes of potentially trinucleating ligands has been shown to be subject to modulation by a third metal ion, which by coordination to the remaining binding site markedly alters the distance between the two catalytically active metal ions. The trinuclear Cu^{2+} complex **36**, for example, is 3 times as efficient catalyst as the $Ni^{2+}Cu^{2+}_2$ complex and 10 times as efficient as the $Pd^{2+}Cu^{2+}_2$ complex.[94] The two Cu^{2+} ions coordinated to the terminal bipyridyl sites are responsible for the catalytic effect, while tetradentate binding of the third metal ion, either Cu^{2+}, Ni^{2+} or Pd^{2+}, to the internal bipyridyl site modulates the geometry of the catalyst and, hence, affects the catalytic activity. When Co^{2+} is used as the structural metal ion, oxidation of Co^{2+} to Co^{3+} considerably enhances the catalytic activity.[95] The sigmoidal dependence of the catalytic activity of the trinucleating ligand **37** on the concentration of Zn^{2+} or Cu^{2+} ion may similarly be accounted for by allosteric activation. The di(pyridin-2-ylmethyl)amino groups bind the first two metal ions, and binding of the third one induces rotation of the 2,2′-bipyridine moiety from *trans* to *cis* conformation, which allows cooperative action of the two metal ion centers.[96]

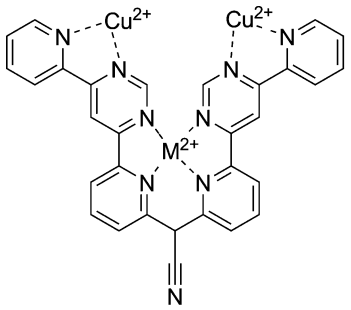
Very recently, an astonishing observation has been made: $[V_4O_{12}]^{4-}$ catalyzes the cleavage of HpNP.[97] No solvent kinetic isotope effect occurs, arguing against proton transfer in the rate-limiting step. Otherwise the mechanistic details remain obscure. It has been tentatively suggested that the substrate becomes as a phosphate diester incorporated into the cyclic tetrameric vanadate structure and this somehow increases the susceptibility to hydrolysis.

Base moiety selective cleavage of RNA phosphodiester bonds

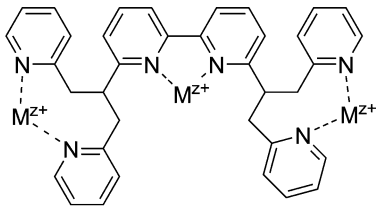
Some di- and tri-nuclear metal ion complexes exhibit as cleaving agents marked base moiety selectivity. The first indication of such selectivity was offered by a dinuclear Cu^{2+} complex **38** containing both a terpyridine and phenanthroline residue as a nucleating moiety.[98] This complex cleaved adenine containing dinucleoside monophosphates one order of magnitude more readily than other dimers. The selectivity was attributed to stacking of the adenine base with the bipyridine moiety of the complex. More marked selectivity has recently been obtained with the Zn^{2+} complexes incorporating two or three 1,5,9-triazacyclododecane groups ([12]aneN$_3$) as nucleating moieties. The dinuclear complexes, such as **39**, cleave the RNA chain at both sides of uridine, since one of the azacrown ligands anchors the complex to the uracil base[99] and the other serves as a catalyst.[100] The UpU sites are not, however, cleaved by **39**, since both azacown moieties are engaged in base moiety binding. The trinuclear complex **40**, in turn, cleaves these sites. It is worth noting that although the base moieties of both the 3′- and 5′-linked nucleosides are bound to **40**, this does not prevent the 2′- and 5′-oxygen atoms adopting apical positions around phosphorus, but the reaction readily proceeds by an in-line type mechanism. In other words, the base moiety binding does not pose similar conformational constraints as double helix formation, which highly retards the cleavage of phosphodiester bonds. Besides uracil, guanine base offers a site of anchoring for **39** and **40**, but binding to N1 of guanine base is much weaker than binding to N3 of uracil. Hence, cleavage at guanosine sites takes place at higher catalyst concentrations than the cleavage at uridine sites.[101] Consistent with the preceding discussion, the dinuclear Zn^{2+} complex of 1,4-bis[(1-oxa-4,7,10-triazacyclododecan-7-yl)methyl]benzene (**41**) catalyzes under saturating concentration the cleavage of uridine 3′-(4-nitrophenyl)phosphate one order of magnitude more efficiently than the mononuclear 1-oxa-4,7,10-triazacyclododecane complex.[102] The trinuclear Cu^{2+} complex **42**, in turn, catalyzes the cleavage of 2′,5′-UpU 57 times as efficiently as the cleavage of 3′,5′-UpU.[103] With GpG no similar selectivity is observed, and with ApA the 3′,5′-isomer is cleaved 48 times more rapidly.

Cone calix[4]arenes bearing three nucleating 2,6-bis(dimethylaminomethyl)pyridine residues at the upper rim (**43a**) constitute another class of ligands, the metal ion complexes of which exhibit base moiety selective cleavage.[104] The homotrinuclear $(Zn^{2+})_3$ and heterotrinuclear $(Zn^{2+})_2Cu^{2+}$ complexes cleave GpG sites from one to two orders of magnitude more rapidly than the other dimers, among which UpU is most labile and ApA most stable. With the dinuclear Zn^{2+} complex bearing the nucleating ligands in 1,3-position, no base moiety selectivity occurs. It has been suggested that one metal ion is involved in base moiety binding, while the other two are engaged in double Lewis acid activation. As well the situation may be analogous to that discussed above: two of the metal ions participate in base moiety anchoring and one is catalytically active. This alternative better explains why GpA and ApG are cleaved only slowly. The trinuclear Zn^{2+} complex is only slightly more active than the dinuclear complex on using HpNP as the substrate.[105]

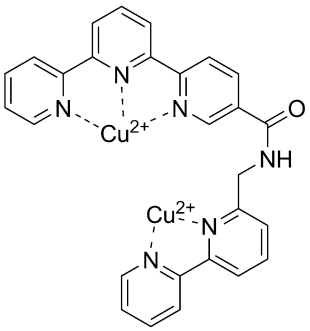
Trinuclear Cu^{2+} complex of *cone* calix[4]arene bearing [12]aneN$_3$ groups (**43b**) instead of the 2,6-bis(dimethylaminomethyl)pyridine groups cleaves UpG and UpU 10–40 times as fast as the other dimers studied. The dinuclear 1,2-complex behaves similarly.[106] The di- and trinuclear Cu^{2+} complexes bearing the [12]aneN$_3$ groups in 1,2-, 1,3-, or 1,2,3-positions cleave oligonucleotides preferably at CpA sites.[107] It should be, however, noted that the oligonucleotides studied have actually been their 5′-phosphates, and hence, binding of the of the metal ion centers to this functionality may well affect the cleavage selectivity.[108]



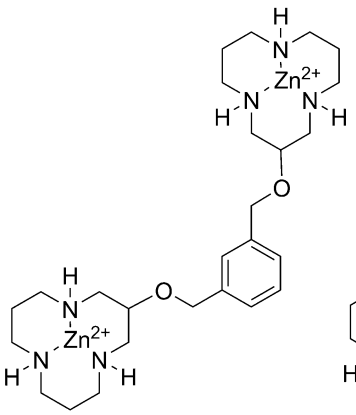
36



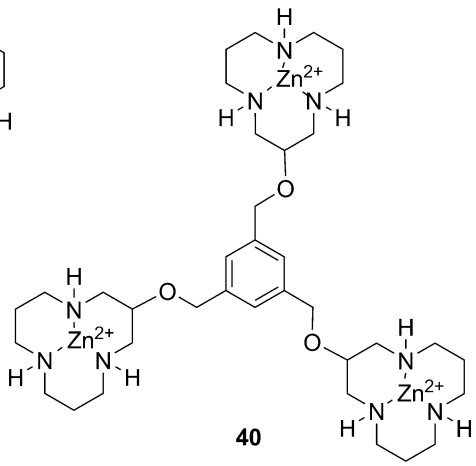
37



38



39



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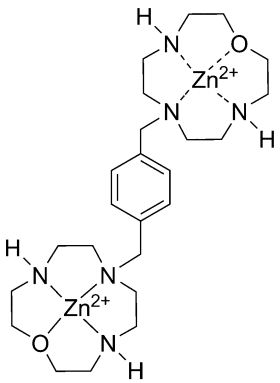
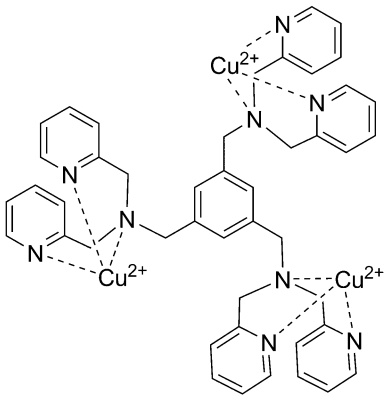
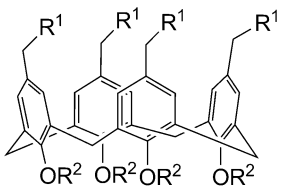
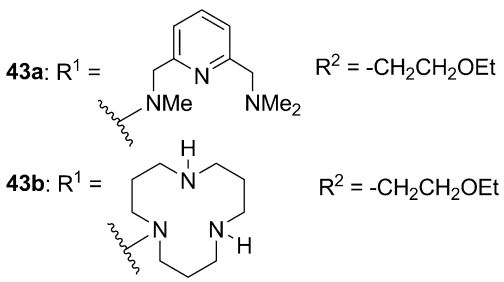


41



42





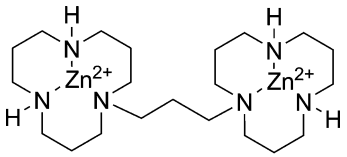
43a: R^1 = R^2 = -CH_2CH_2OEt

43b: R^1 = R^2 = -CH_2CH_2OEt

Solvent effects on the cleavage rate

Structurally flexible dinuclear metal ion complexes, such as the dinuclear Zn^{2+} complex of 1,3-bis(1,5,9-triazacyclododenan-1-yl)propane (**44**), that do not exhibit double Lewis acid activation in aqueous solution, are surprisingly good catalysts for the cleavage of HpNP and related aryl phosphates in MeOH or EtOH.[109] The second-order rate constant for the cleavage of HpNP in MeOH is 2.75×10^5 L mol^{-1} s^{-1}, *i.e.* several orders of magnitude faster than the reactions promoted by any related system in water. The β_{lg} value for the reaction (–0.97), is slightly more negative than that for the methoxide ion catalyzed cleavage (–0.72), suggesting that in the

transition state of the rate-limiting formation of the phosphorane intermediate, the POAr bond is approximately half-cleaved. It has additionally been argued that when the leaving group is very good, such as 4-nitrophenyl group, binding of the catalyst may even become rate-limiting.[110] In EtOH, where the reaction is even slightly faster than in MeOH, a clear break in the plot of log *k vs.* pK_a of the departing phenol is observed; the β_{lg} value experiences an abrupt change from –1.12 to 0 on passing pK_a 14.3.[111] Almost as high rate-accelerations are achieved by the corresponding dinuclear Cu^{2+} complex.[112] Consistent with the proposed concerted rate-limiting formation of the phosphorane, the corresponding aryl phosphonates that cannot undergo cleavage, do not undergo phosphate migration either.[113]



44

Conclusions and outlook

Studies on the cleavage of ribonucleoside 3′-phosphodiesters and their congeners by small molecules, ions and complexes have shed light on the factors that most profoundly affect the stability of RNA phosphodiester bonds under physiological conditions. It has turned out that stabilization of the phosphorane intermediate obtained by the attack of 2′-oxygen on the phosphorus, together with facilitation of the rupture of the P-O5′ bond by donation of a proton to the departing oxygen atom, is of the utmost importance. Deprotonation of the 2′-hydroxy function evidently forms the bottleneck in cases where the departure of the 5′-oxygen is very efficiently enhanced by proton donation. Then formation of the phosphorane intermediate becomes rate-limiting. Studies with dinuclear metal ion complexes, in particular with those that are additionally able to serve as H-bond donors, have given useful hints about the structure of a receptor optimal for binding the phosphorane intermediate, and DFT calculations have still refined the picture.

The improved mechanistic understanding of RNA phosphodiester cleavage has impact on several fields in chemistry and biology. It helps to critically evaluate the mechanisms proposed for the action of protein ribonucleases and ribozymes and provides a solid basis for development of artificial restriction enzymes with which large RNA molecules may be manipulated *in vitro* or a desired mRNA may be cut *in vivo*. Detailed mechanistic knowledge may also serve as a source of inspiration for scientists who try to find out methods for selective manipulation of the intracellular pool of non-coding micro RNA hairpins or try to create credible concepts for the role of RNA during the early stage of life.

As mentioned above, studies on metal ion based cleaving agents have not only produced good cleaving agents for *in vitro* purposes, but they have also given invaluable information about the structural requirements for efficient catalysts of RNA cleavage. This information undoubtedly helps to generate covalently constructed structures that exhibit a catalytic activity comparable to that achieved so far by the best metal ion based catalysts. This most likely is a prerequisite for really successful *in vivo* applications. For example, small molecule entities that exhibit selectivity to both a particular motif of a secondary structure and the base moieties present in this motif might one day lead to a novel type of chemotherapy targeted to small non-coding RNAs.

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