Imidazole−Zinc Catalysts for RNA Hydrolysis

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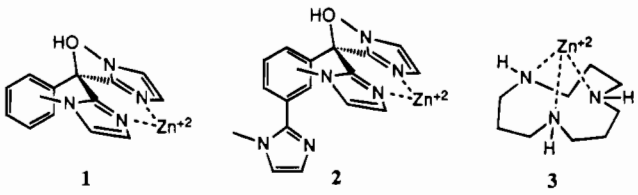
Zinc is often chelated with imidazoles, carboxylates and water at nuclease active sites.[1] The water bound to zinc in this manner is postulated to possess a pK_a near 7,[2] allowing a metal hydroxide to participate in general base delivery of an RNA 2′-OH to an RNA phosphodiester. As a means of studying the Zn−OH mechanism using a system that closely mimics nuclease active sites, we sought an imidazole containing artificial nuclease that would bind zinc resulting in a water pK_a near 7, and that would yield RNA hydrolysis. Herein, we show that compound **1**, first developed by Lippard,[3] meets these criteria. In addition, we find that compound **2**, with a third imidazole as a potential general base or acid, also acts as an RNA hydrolysis catalyst, although the extra imidazole results in only a small increase in rate over **1**. These two structures hydrolyze ApUp at rates comparable to zinc alone.

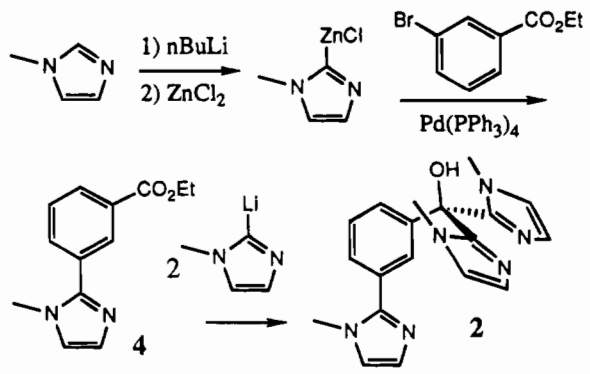


1 **2** **3**

A large number of zinc-polyaza-macrocycles[4] and other metal catalysts[5] (some containing imidazoles) have been investigated for nuclease activity, but of the zinc compounds only **3** has been shown to possess a water pK_a near 7 (specifically 7.3).[6] Kimura, Morrow, and Breslow have all studied its properties and have concluded that it is among the most active artificial zinc containing catalysts for phosphodiester hydrolysis yet produced.[6,7] However, compound **3** does not truly reflect a nuclease's zinc binding site in that zinc is chelated with neither imidazoles nor carboxylates. Hence, compounds **1** and **2** may prove more useful in elucidating the mechanism of enzymatic Zn−OH promoted RNA hydrolysis, eventhough, as discussed herein, both are marginally slower than compound **3** at promoting hydrolysis.

The synthesis of **1** has been previously reported, and involves the addition of 2-lithio-1-methylimidazole to benzoylchloride.[3] The synthesis of **2** (Scheme 1) first involves a Pd catalyzed

Scheme 1. Synthetic Route to **2**



coupling to introduce the third imidazole,[8] followed by steps similar to those used in the synthesis of **1** (see Supporting Information).[9]

The structures and activities of compounds **1** and **2** were determined by several different methods. First, the binding constant of Zn^{+2} to apo-**1** was determined by UV/vis titrations to be $(9 \pm 1) \times 10^4$ M^{-1}, similar to other bis(imidazole) complexes.[10] pH titrations of **1** revealed a zinc bound water pK_a of 7.4 (±0.1), indicating significant zinc hydroxide formation at neutral pH. Therefore, the water molecule bound to **1** is only 0.1 pK_a units less acidic than that bound to **3**. To determine the influence of the third imidazole in **2** on binding and the pK_a, a similar UV/vis binding study and pH titration with **2** were initiated. The binding study, however, was hampered by a lack of isosbestic points indicating more than one zinc per ligand, while the pH titration curve was complex due to zinc precipitation. Nevertheless, a crystal structure of compound **3** reveals that the third imidazole does not interact with the zinc within the complex, but rather with a nearest neighbor zinc (Figure 1). This bridging in the crystal explains the lack of isosbestic points in the binding study, indicating potential oligomerization in solution. The crystal structure also reveals two bridging hydroxides between two zinc atoms, further supporting the existence of Zn−OH species within bis(imidazole) complexes **1** and **2**.

Catalytic activities of **1** and **2** were examined using both denaturing gel electrophoresis on a 300 nucleotide RNA transcript made from the pBM-PRP2 plasmid[11] and HPLC kinetics on ApUp.[12] As comparisons, compound **3**, as well as zinc alone, was incubated with the RNA at the same concentration, pH, temperature, and buffer conditions. Figure 2 shows the results after a 4 h incubation of the RNA with 0.5 mM catalyst at 37 °C, 10 mM tris buffer, and pH = 7.4, followed by gel electrophoresis. The cleavage is essentially sequence independent as shown by the smear of radioactivity on the gel and by the lack of any significant "hot spots". Catalysts **1** and **2** show slower cleavage compared to zinc, but are comparable to **3**. Attempts to quantitate the rates of cleavage using a radiolabeling assay

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(9) Apo-**2**. ^{1}H NMR (300 MHz, CD_3OD): δ 3.37 (s, 6H), 3.53 (s, 3H), 6.65 (d, 2H), 6.82 (d, 1H), 6.92 (d, 2H), 6.97 (d, 1H), 7.32 (m, 4H). $^{13}C\{^1H\}$ NMR (75.0 MHz, CD_3OD): δ 34.93, 35.22, 76.69, 124.12, 125,13, 126.57, 128.09, 128.38, 128.67, 129.38, 130.91, 144.07, 148.83, 149.60. HRMS-CI: calcd for $C_{19}H_{20}N_6O$ + 1, 349.1776; found, 349.1772.
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(12) Special caution has been excercised to prevent contamination by nucleases. Whenever possible, all instruments, containers, and buffers were autoclaved. Solutions were made with autoclaved, deionized water that was pretreated with 0.2% diethyl pyrocarbonate.

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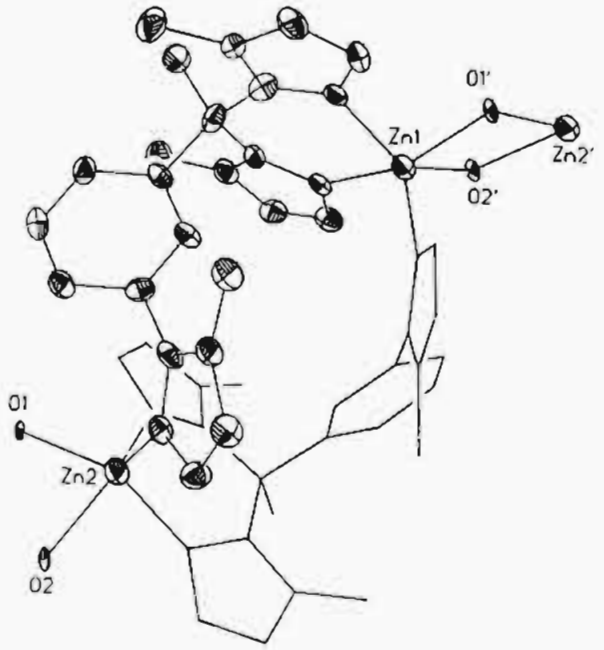

Figure 1. Ortep diagram of the crystal structure of **2**$(BF_4^-)(OH^-)$·MeOH. The BF_4^- and MeOH are not shown for clarity. In addition, one 2OH is shown as a line drawing. The zinc shown in the line drawing is translationally equivalent to the dangling zinc given in the thermal ellipsoid drawing.

failed due to continued RNA−catalyst precipitation at the concentrations required for the assay.[13] Therefore, HPLC kinetics on short RNA oligomers (ApUp) were performed.[14] Again, zinc was the best catalyst, imparting a hydrolysis rate of 1.6×10^{-6} s^{-1} at 0.5 mM. At the same concentrations, compounds **3**, **1**, and **2** gave rates of 1.1×10^{-6} s^{-1}, 5.3×10^{-7} s^{-1}, and 7.8×10^{-7} s^{-1}.[15] The background rate under the same conditions, but with no zinc or amine-based ligands was 6.9×10^{-8} s^{-1}. Therefore, compounds **1** and **2** are only 2.2 and 1.5 times slower than **3**. The third imidazole in **2** only yields a 1.5-fold increase in rate over **1**. As expected, all the ligated forms of zinc impart slower rates than zinc alone because the electrophilicity of the zinc is decreased upon ligation to amines. In contrast, compound **3** has been found to enhance the hydrolysis of a *p*-nitrophenyl−RNA mimic to a greater extend than zinc alone.[7b]

In summary, imidazole ligated zinc (**1** and **2**) can produce metal bound water with pK_as near 7 as is proposed for enzyme

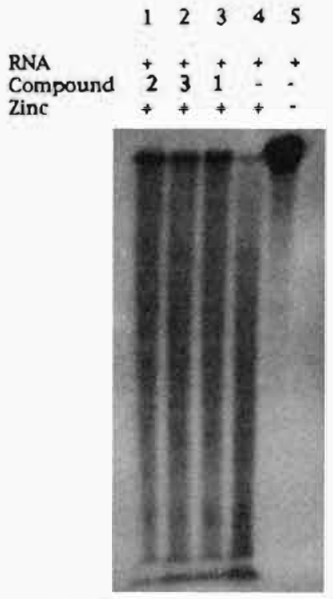

Figure 2. Representative cleavage patterns of ^{32}P-labeled mRNA as shown by autoradiography on a 5% polyacrylamide denaturing gel. $[Zn^{2+}] = 0.5$ mM; [compd] = 0.64 mM.

active sites. Compounds **1** and **2** are easily synthesized and yield RNA cleavage at rates comparable to other zinc complexes and zinc alone. With **1**, the cleavage mechanism likely involves 2′-OH attack on a phosphate promoted by the Zn−OH, whereas with **2** the potential exists for delivery of the 2′-OH by the third imidazole or general acid protonation of the leaving group by imidazolium. Currently, the study of these catalysts is proceeding along two lines. First, the Zn−OH mechanism is being studied in detail. Second, in order to increase the rate enhancements imparted by these synthetic systems, we are now investigating the combination of zinc (such as **1**) and guanidiniums, an extremely common motif for nucleases.[16]

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Supporting Information Available: Text describing the synthetic procedure for **2**, the RNA and ApUp hydrolysis kinetic assays, the potentiometric and UV/vis titration procedures, graphs of hydrolysis kinetics and pH titrations, text describing the X-ray experimental procedures, tables of crystallographic data, fractional coordinates and thermal parameters, and bond lengths and angles, and views showing the atom labeling, as well as complete unit cell packing diagrams (31 pages). Ordering information is given on any current masthead page.

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(14) Pseudo-first-order rate constants were obtained via initial rate kinetics by monitoring the disappearance of ApUp. The reactions were followed until about 30% completion, and the cleavage product Up was identified.

(15) The solutions used for kinetics contained 0.64 mM ligand and 0.5 mM $ZnCl_2$. Under these conditions more than 94% of Zn^{2+} was complexed with the ligand. The 6% free zinc can only account for a maximum rate constant of 9.18×10^{-8} s^{-1} out of the near 10^{-6} s^{-1} rate constants observed.

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