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Cooperativity between Metal Ions in the Cleavage of Phosphate Diesters and RNA by Dinuclear Zn(II) Catalysts

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A series of ligands containing linked 1,4,7-triazacyclononane macrocycles are studied for the preparation of dinuclear Zn(II) complexes including 1,3-bis(1,4,7-triazacyclonon-1-yl)-2-hydroxypropane (L2OH), 1,5-bis(1,4,7-triazacyclonon-1-yl)pentane (L3), 2,9-bis(1-methyl-1,4,7-triazacyclonon-1-yl)-1,10-phenanthroline (L4), and α,α′-bis(1,4,7-triazacyclonon-1-yl)-*m*-xylene (L5). The titration of these ligands with $Zn(NO_3)_2$ was monitored by ^{1}H NMR. Each ligand was found to bind two Zn(II) ions with a very high affinity at near neutral pH under conditions of millimolar ligand and 2 equiv of $Zn(NO_3)_2$. In contrast, a stable mononuclear complex was formed in solutions containing 5.0 mM L2OH and 1 equiv of $Zn(NO_3)_2$. ^{1}H and ^{13}C NMR spectral data are consistent with formation of a highly symmetric mononuclear complex Zn(L2OH) in which a Zn(II) ion is sandwiched between two triazacyclononane units. The second-order rate constant k_{Zn} for the cleavage of 2-hydroxypropyl-4-nitrophenyl phosphate (HPNP) at pH 7.6 and 25 °C catalyzed by Zn_2(L2O) is *120-fold larger* than that for the reaction catalyzed by the closely related mononuclear complex Zn(L1) (L1 = 1,4,7-triazacyclononane). By comparison, the observation that the values of k_{Zn} determined under similar reaction conditions for cleavage of HPNP catalyzed by the other Zn(II) dinuclear complexes are only *3–5-fold larger* than values of k_{Zn} for catalysis by Zn(L1) provides strong evidence that the two Zn(II) cations in Zn_2(L2O) act cooperatively in the stabilization of the transition state for cleavage of HPNP. The extent of cleavage of an oligoribonucleotide by Zn(L1), Zn_2(L5), and Zn_2(L2O) at pH 7.5 and 37 °C after 24 h incubation is 4,10, and 90%. The rationale for the observed differences in catalytic activity of these dinuclear Zn(II) complexes is discussed in terms of the mechanism of RNA cleavage and the structure and speciation of these complexes in solution.

Introduction

Many metalloenzymes that catalyze the hydrolysis of phosphate diesters contain two metal ions in the active site,[1−3] and certain ribozymes that catalyze hydrolysis or transesterification of phosphate diesters also require two metal ions for full catalytic activity.[4,5] The catalytic rate acceleration of protein or RNA catalysts that utilize one metal ion will be at least doubled by recruitment of a second metal ion which acts independently of the first, for example, at a second identical subunit. It is much more difficult to understand how two metal ions might interact cooperatively at a catalyst binding site to produce a catalytic rate acceleration far in excess of that obtained from recruitment of only a single metal ion.

An important challenge to inorganic and bioinorganic chemists is the design of dinuclear metal ion catalysts, where the metal ions act cooperatively to stabilize the transition state for the catalyzed reaction in a manner that models catalysis by proteins and RNA. Extremely impressive and efficient "catalysis" has been observed by exchange inert Co(III) complexes that contain a bridging phosphate diester that shows extraordinary reactivity toward hydrolysis.[6−8] These Co(III) complexes provide important insight into the potential for transition state stabilization by the cooperatively

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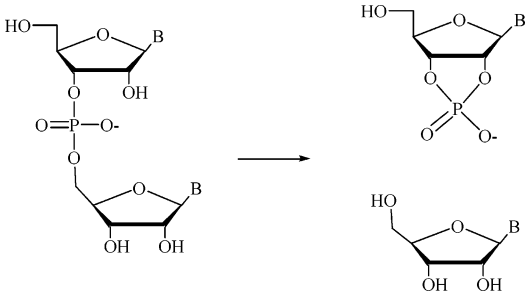
Scheme 1

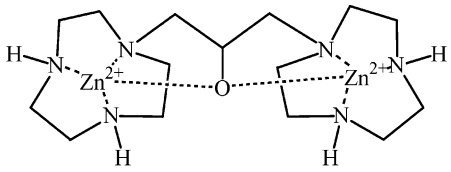


interacting metal ions. However, they do not undergo multiple turnover.

Efficient catalysis can only be observed by metal ion complexes that undergo rapid exchange between free and bound substrate. Many mononuclear Zn complexes have been shown to be effective catalysts for RNA cleavage,[9−11] and a simple strategy for the design of dinuclear catalysts is to connect two mononuclear zinc ligands by a short tether. However, it is not clear that cooperative interaction between metal ions should be obtained for such dinuclear ligands, given the inherent electrostatic repulsion between metal ions in these complexes. Cooperative interaction between metal ions can be promoted by incorporation of functionality within the tether, which will allow it to serve as a scaffold for chelation of the two metal ions. This presents obvious challenges in the design and synthesis of the appropriate tether.

We are interested in designing efficient catalysts for the hydrolysis of RNA (Scheme 1). Varying degrees of success have been achieved with dinuclear catalysts using Zn(II),[12−21] Cu(II),[22−28] and La(III).[29] The 2-fold rate enhancements sometimes observed for dinuclear compared to mononuclear complexes are consistent with catalysis at noninteracting catalyst "subunits", while the larger, up to 500, rate enhancements observed with other catalysts suggest that in some cases it is possible to achieve cooperative stabilization of the transition state for RNA cleavage by two metal ions.

The studies of these metal ion complexes often do not proceed far beyond the determination of kinetic parameters for catalysis of phosphodiester cleavage, so that the underlying explanation for the differences in catalytic activity for different metal ion complexes is not well understood. This is partly because dinuclear metal ion complexes reported to date often show low solubility in water. This limits their applications in the study of biological molecules and requires that their mechanism be characterized in mixed aqueous/organic solvents, whose properties are less well-defined than in water. In addition, the problem of determining the role of the metal ion in catalysis takes on added layers of complexity for dinuclear complexes because of the many roles possible for each metal ion in providing stabilization of the reaction transition state.[30−32]



Zn_2(L2O)

We recently reported kinetic parameters and structural data for Zn_2(L2O), an extremely effective dinuclear Zn(II) catalyst for the cleavage of the model RNA substrate HPNP (HPNP = 2-hydroxypropyl-4-nitrophenyl phosphate).[33] This Zn(II) complex is highly water soluble, binds two Zn(II) ions very strongly, and shows relatively clear and simple speciation in water. In addition, this complex is one of the best dinuclear complex catalysts for HPNP cleavage with the highest reported second-order rate constant in 100% aqueous solution for a Zn(II) catalyst.[33] In this paper we report the synthesis, ^{1}H and ^{13}C NMR spectral data, and speciation for several additional Zn(II) complexes, including those of 1,5-bis(1,4,7-triazacyclonon-1-yl)pentane (L3), 2,9-bis(1-methyl-1,4,7-triazacyclonon-1-yl)-1,10-phenanthroline (L4), and α,α′-bis(1,4,7-triazacyclonon-1-yl)-*m*-xylene (L5), that are structurally related to Zn_2(L2O). A comparison of the second-order rate constants k_{Zn} for catalysis of HPNP cleavage by this series of structurally related mono- and dinuclear complexes provides strong evidence that the metal ions in Zn_2(L3), Zn_2(L4), and Zn_2(L5) act nearly independently of

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one another in catalysis of the cleavage of HPNP but that addition of an alkoxide to the tether is sufficient to provide a scaffold for the cooperative transition state stabilization by the Zn(II) cations in Zn_2(L2O).

Experimental Section

Materials. All reagents and solvents were of analytical reagent grade and were used without further purification, unless otherwise noted. Acetonitrile, dimethylformamide, and methanol were dried over 4-Å molecular sieves. All aqueous solutions were prepared using Millipore MILLI-Q purified water. All reactions were carried out under a N_2(g) atmosphere. Thin-layer chromatography (TLC) plates were silica gel $60F_{254}$, 0.2-mm thickness (Aldrich). Merck grade 9385, 230−400 mesh silica gel, 60 Å (Aldrich), was used in all column flash chromatography. 1,3-Dibromo-2-propanol, 1,5-dibromopentane, and α,α′-dibromo-*m*-xylene were purchased from Aldrich. *N*-(2-Hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) and *N*-(2-hydroxyethyl)piperazine-*N*′-(3-propanesulfonic acid) (EPPS) were purchased from Sigma and/or Aldrich. The barium salt of 2-hydroxypropyl-4-nitrophenyl phosphate (HPNP) was prepared according to the literature procedure.[34] The oligoribonucleotide A_6 was synthesized on an Applied Biosystems DNA synthesizer using phosphoramidites from Glen research and was deprotected following established protocols.

Aqueous stock solutions (40.0 mM) of the ligands were prepared from their respective salts, and concentrations were determined by ^{1}H NMR using *p*-toluenesulfonic acid as internal standard. Solutions of $Zn(NO_3)_2$ (Aldrich) were standardized with EDTA using Eriochrome Black T.[35] Solutions of oligoribonucleotides were prepared using autoclaved triply distilled water, and all standard precautions were taken to avoid ribonuclease contamination.

An Orion Research Digital ion analyzer/501 and an Orion Research Ross Combination pH Electrode 8115BN were used for all pH measurements. An UVIKON-XL spectrophotometer by Bio-Tek instruments equipped with a thermostatic multicell transfer compartment was used for all kinetic measurements. ^{1}H NMR spectra were recorded on a Varian Inova 500, Varian Inova 400, or Varian Gemini 300 spectrometer. ^{13}C NMR spectra were recorded on a Varian Inova 500 or Varian Gemini 300 spectrometer and ^{31}P NMR spectra on a Varian Inova 400 spectrometer. Chemical shifts are reported as parts per million (ppm) downfield from tetramethylsilane (TMS). A VG 70-SE mass spectrometer with fast atom bombardment (FAB) and chemical ionization was utilized for FAB *m*/*z* low-resolution analysis. A ThermoFinnigan Mat 95 XL spectrometer with a Cs ion gun at 20 kV was utilized for FAB *m*/*z* high-resolution analysis. For analysis by FAB-MS, *m*-nitro benzyl alcohol (NBA) was used as a matrix.

Syntheses. *N*,*N*′,*N*″-Tris(*p*-toluenesulfonyl)-diethylenetriamine[36] and 1,2- bis(*p*-toluenesulfonyl)-1,2-ethanediol[37] were prepared according to literature procedures. 1,4,7-Tris(*p*-toluenesulfonyl)-1,4,7-triazacyclononane (Ts_3L1) was deprotected according to established procedures.[38] The free base form of 1,4,7-triazacyclononane (L1) was generated by adjusting the pH of a solution of the trihydrobromide salt (10.9 mmol) to 12.5−13.0 with solid NaOH. The free amine base was extracted with chloroform (6 × 130 mL), and the combined organic fractions were dried over Na_2SO_4. The solvent was removed on a rotary evaporator, and the oily residue was dried at room temperature under high vacuum to give 1.25 g (9.67 mmol) of 1,4,7-triazacyclononane in 89% yield. 1,4,7-Triazatricyclo-[5.2.1.$0^{4,10}$]decane was prepared from L1 by a published method.[39,40] 1,5-Bis(1,4,7-triazacyclonon-1-yl)pentane (L3)[41] and α,α′-bis(1,4,7-triazacyclonon-1-yl)-*m*-xylene (L5)[42] were prepared using the orthoamide derivative of L1 (1,4,7- triazatricyclo-[5.2.1.$0^{4,10}$]decane) according to literature procedures. The protecting group was removed by refluxing for 4 h in water followed by 10−12 h reflux in a basic solution of 4 M NaOH. 2,9-Bis-(bromomethyl)-1,10-phenanthroline was synthesized from neocuproine hydrate (Aldrich) by following a literature procedure.[43]

1,4,7-Tris(*p*-toluenesulfonyl)-1,4,7-triazacyclononane (Ts_3L1). A modification of a reported procedure was used to form this macrocycle.[44] N,N′,N″-Tris(*p*-toluenesulfonyl)-diethylenetriamine (28.3 g, 50.0 mmol) was dissolved in 270 mL of dimethylformamide. Cesium carbonate (34.34 g, 105.4 mmol) was added to this solution, and the resulting suspension was stirred at room temperature for 1 h. 1,2-Bis(*p*-toluenesulfonyl)-1,2-ethanediol (18.65 g, 50.3 mmol) in 125 mL of dimethylformamide was added dropwise to this suspension. The reaction mixture was stirred under N_2 overnight at room temperature, and the resulting turbid-yellow solution was added slowly to 1.5 L of water with rapid stirring to give the product as a light-yellow precipitate. This solid was collected by filtration and washed in 2:1 (v:v) dimethylformamide−water by stirring for several hours. The resulting white solid was collected by filtration, thoroughly washed with water, and dried under vacuum. The product was recrystallized from 80:20 (v:v) dimethylformamide−water. Yield: 85−90%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, ^3J = 8.4 Hz, 6H, Ar), 7.36 (d, ^3J = 8.4 Hz, 6H, Ar), 3.46 (s, 12H, ring-CH_2), 2.47 (s, 9H, CH_3).

2,9-Bis(1-methyl-1-azonia-4,7-diazatricyclo[5.2.1^{4,10}]decane)-1,10-phenanthroline Dibromide. 1,4,7-Triazatricyclo-[5.2.1.$0^{4,10}$]-decane (0.571 g, 4.10 mmol) was dissolved in 9 mL of dry acetonitrile, and 2,9-bis(bromomethyl)-1,10-phenanthroline (0.684 g, 1.89 mmol) in 60 mL of dry acetonitrile was added dropwise, with stirring, over 2 h to give a beige precipitate. The slurry was heated to reflux for 2 h and stirred overnight at room temperature. The solid was collected by filtration, washed with cold acetonitrile and dry ether, and dried at room temperature under vacuum to afford 0.796 g of beige powder. Yield: 66%. ^{1}H NMR (500 MHz, D_2O, see Supporting Information (SI) Figure S1 for labeling): δ 8.51 (d, ^3J = 8.0 Hz, 2H, H(4) and H(7)), 7.94 (s, 2H, H(5) and H(6)), 7.94 (d, ^3J = 8.0 Hz, 2H, H(8) and H(3)), 6.36 (s, 2H, methine H), 4.96 (s, 4H, CH_2 (15)), 4.11−4.09 (m, 4H, ring-CH_2), 3.48−3.37 (m, 12H, ring-CH_2), 3.17−3.15 (m, 4H, ring-CH_2), 3.07−3.05 (m, 4H, ring-CH_2).

HCl Salt of 2,9-Bis(1-methyl-1,4,7-triazacyclonon-1-yl)-1,10-phenanthroline (L4·6HCl). 2,9-Bis(1-methyl-1-azonia-4,7-diazatricyclo[5.2.1^{4,10}]decane)-1,10-phenanthroline dibromide (0.796 g, 1.24 mmol) was added to 60 mL of aqueous 6 M HCl. The

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reaction mixture was heated to reflux for 24 h, the solvent was removed on a rotary evaporator, and the yellow residue was coevaporated with water (2 × 25 mL). This solid was washed with 30 mL of 1:2 (v:v) 12 M aqueous HCl−ethanol by stirring the slurry for 4 h at room temperature. The solid was collected by filtration, washed with ethanol, and dried at room temperature under high vacuum to afford 0.811 mg of a beige powder. Yield: 96%. ^{1}H NMR (500 MHz, D_2O, see SI Figure S1 for labeling): δ 8.75 (d, ^3J = 8.4 Hz, 2H, H(4) and H(7)), 8.08 (s, 2H, H(5) and H(6)), 8.05 (d, ^3J = 8.4 Hz, 2H, H(8) and H(3)), 4.43 (s, 4H, CH_2(15)), 3.63 (s, 8H, ring-CH_2(18)), 3.24 (t, ^3J = 5.5 Hz, 8H, ring-CH_2-(17)), 3.13 (t, ^3J = 5.5 Hz, 8H, ring-CH_2(16)). ^{13}C NMR (500 MHz, D_2O, see SI Figure S1 for labeling): δ 156.11 (C(2) and C(9)), 142.89 (C(11) and C(14)), 137.91 (C(4) and C(7)), 129.67 (C(12) and C(13)), 127.62 (C(5) and C(6)), 126.27 (C(4) and C(8)), 58.214 (C(15)), 48.52, 44.17, 42.65 (ring carbons). FAB *m*/*z*: 463.3 (MH^+). HR FAB *m*/*z*: 463.3297 (calculated for $C_{26}H_{39}N_8$: 463.3292).

***N,N′*-Bis(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (Boc_2-L1).** L1 (2.16 g, 16.72 mmol) and triethylamine (3.38 g, 33.42 mmol) were dissolved in 80 mL of dry chloroform, and the solution was cooled in a 0−3 °C ice bath. Di-*tert*-butyl dicarbonate (6.78 g, 31.06 mmol) in 155 mL of dry chloroform was added slowly with stirring. After the addition was complete, the reaction mixture was allowed to warm to room temperature and then stirred for ca. 20 h. The solvent was removed by rotary evaporator to afford an oily residue. This was dissolved in a minimum amount of hexanes to give a slurry, which was applied to a silica gel column. The product was eluted under flash chromatography conditions with a gradient of 100% hexanes to 30:70 (v:v) hexanes−ethyl acetate. Fractions were collected and analyzed by TLC (30:70 (v:v) hexanes−ethyl acetate, R_f = 0.80 for Boc_3-L1, R_f = 0.28 for Boc_2-L1). Fractions containing Boc_2-L1 were combined, the solvent was removed by rotary evaporation, and the final residue was dried at room temperature under high vacuum to give 3.43 g of a white powder. Yield: 62%. The ^{1}H NMR of this compound is complex due to the presence of two conformers. The two conformers are more readily resolved in the ^{13}C NMR spectrum. ^{13}C NMR (300 MHz, $CDCl_3$): δ 156.49, 156.24 (CO), 80.21, 80.11 (*t*Bu: *C*(CH_3)$_3$), 53.54, 52.96, 52.84, 52.10, 50.89, 50.26, 49.99, 48.74, 48.57, 48.17, 47.78 (ring carbons), 28.97 (*t*Bu: C(*C*H$_3$)$_3$). FAB *m*/*z* (relative intensity): 330.2 (MH^+, 100%), 274.3 (MH^+ − 56, 24%).

1,3-Bis(4,7-di(*tert*-butoxycarbonyl)-1,4,7-triazacyclonon-1-yl)-2-hydroxypropane (Boc_4-L2OH). Boc_2-L1 (3.14 g, 9.53 mmol), 1,3-dibromo-2-propanol (0.98 g, 4.50 mmol), and triethylamine (1.15 g, 11.36 mmol) were added to 110 mL of dry acetonitrile. The solution was heated to reflux for 41 h, and the progress of the reaction was monitored by TLC (30:70 (v:v) hexanes−ethyl acetate, R_f = 0.28 for Boc_2-L1, R_f = 0.72 for Boc_4-L2OH). The solvent was removed by rotary evaporation, and the oily residue was dissolved in 110 mL of chloroform. The resulting solution was washed with 10% aqueous NaOH (3 × 100 mL) and water (3 × 100 mL), and the solution was dried over Na_2SO_4. The solvent was removed under vacuum to afford an oil that was purified by flash chromatography using the same conditions as for Boc_2-L1. The fractions containing the product Boc_4-L2OH (R_f = 0.72) were combined, the solvents were removed by rotary evaporation, and the final residue was dried at room temperature under high vacuum to give 1.63 g of a light-yellow powder. Yield: 50%. ^{13}C NMR (500 MHz, $CDCl_3$, only the chemical shifts of the major isomer were assigned): δ 155.78 (CO), 79.74 (*t*Bu: *C*(CH_3)$_3$), 67.62 (*C*HOH), 63.15 (*C*H_2−CHOH−*C*H_2), 54.70, 50.54, 49.21 (ring carbons), 28.73 (*t*Bu: C(*C*H$_3$)$_3$). FAB *m*/*z*: 715.6 (MH^+). HR FAB *m*/*z*: 715.4953 (calculated for $C_{35}H_{67}O_9N_6$: 715.4964).

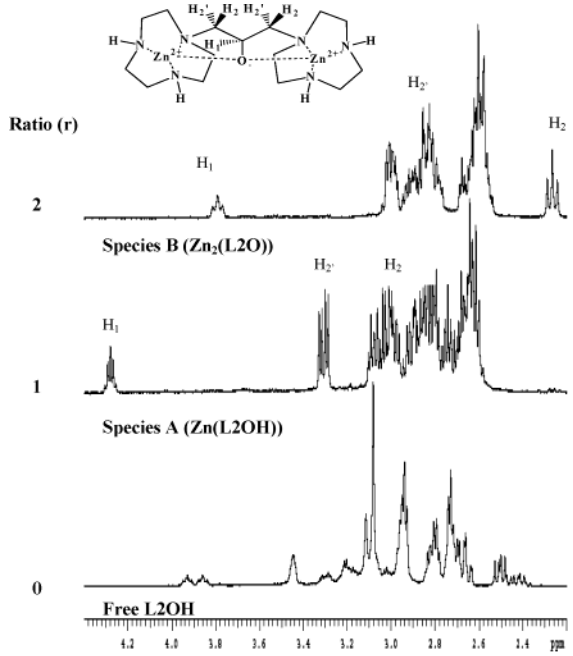

Figure 1. ^{1}H NMR spectra of L2OH as a function of r (r = [$Zn(NO_3)_2$]/[L2OH]) at 5.0 mM L2OH, pD = 6.93, 22 °C and I = 0.10 M, $NaNO_3$.

1,3-Bis(1,4,7-triazacyclonon-1-yl)-2-hydroxypropane HCl Salt (L2OH·6HCl). Boc_4-L2OH (1.23 g, 1.72 mmol) was added to 18 mL of a solution containing 1:1 (v:v) 12 M aqueous HCl−ethanol, and the solution was stirred for 4 h at room temperature. The white solid that formed during this time was collected by filtration, washed with cold ethanol, and washed by stirring in a solution containing a 1:2 (v:v) 12 M aqueous HCl−ethanol. The final white solid was collected by filtration, washed with cold ethanol, and dried at room temperature under vacuum to afford 0.736 g of the pure hydrochloride salt. Yield: 81%. ^{1}H NMR (500 MHz, D_2O, see SI Figure S1 for labeling): δ 4.16 (tt, $^3J_{12}$ = 9.9 Hz, $^3J_{12'}$ = 2.4 Hz, 1H, CH(1)−OH), 3.59−3.53 (m, 8H, ring-CH_2(5)), 3.25 (t, ^3J = 5.6 Hz, 8H, ring- CH_2(4)), 3.08−3.03 (m, 4H, ring-CH_2(3)), 2.98−2.93 (m, 4H, ring-CH_2(3)), 2.70 (dd, $^2J_{2'2}$ = 14.3 Hz, $^3J_{2'1}$ = 2.4 Hz, 2H, H(2′)), 2.59 (dd, $^2J_{22'}$ = 14.3 Hz, $^3J_{21}$ = 9.8 Hz, 2H, H(2)). ^{13}C NMR (300 MHz, D_2O, see SI Figure S1 for labeling): δ 65.96 (C(1)), 58.71 (C(2)), 48.34, 43.94, 42.30 (ring carbons).

Synthesis of the Metal Complexes. The Zn(II) complexes of L1, L2OH, L3, L4, and L5 were prepared in aqueous solution by mixing $Zn(NO_3)_2$ and the corresponding HBr or HCl salt of the ligand in 1:1.1 (mononuclear system) and 2:1.1 (dinuclear system) molar ratios and adjusting the pH to 6.5−7.0. Concentrations of ligand and Zn(II) were determined as described above.

^{1}H NMR Spectroscopic Measurements. The formation and stoichiometry of the Zn(II) dinuclear complexes in D_2O were studied by ^{1}H NMR. ^{1}H NMR spectra were recorded for solutions that contained a constant concentration of ligand and increasing concentrations of $Zn(NO_3)_2$ (I = 0.10 M, $NaNO_3$), using ligand concentrations of 5.0, 4.0, 1.0, and 5.0 mM, respectively, for L2OH, L3, L5, and L4 and increasing $Zn(NO_3)_2$ from 0 to 3.0 equiv with respect to the corresponding ligand. The solution pD was calculated by adding 0.4 to the reading from the pH meter.[45] pDs were adjusted to the desired value using NaOD or DCl. ^{1}H NMR spectra for these compounds are given in Figure 1 and SI Figures S2−5. ^{13}C NMR spectra of the two Zn(II) complexes obtained with the L2OH ligand are given below.

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Zn_2(L2O). ^{13}C NMR (300 MHz, D_2O, pD = 6.93, see Figure 1 for labeling): δ 64.90 (C(1)), 62.44 (C(2)), 52.35, 49.09, 44.96, 43.86, 42.92, 42.48 (ring carbons).

Zn(L2OH). ^{13}C NMR (300 MHz, D_2O, pD = 6.93, see Figure 1 for labeling): δ 64.72 (C(1)), 63.35 (C(2)), 54.40, 53.44, 43.63, 43.56, 43.00, 42.78 (ring carbons).

Kinetics of Transesterification of HPNP (2-Hydroxypropyl 4-nitrophenyl phosphate). The concentration of the Zn(II) complexes examined in these experiments ranged from 0.20 to 2.0 mM. In a typical experiment, a solution of the metal complex in 20.0 mM buffer at $I = 0.10$ M ($NaNO_3$) was adjusted to the desired pH, transferred to a cuvette, and equilibrated at 25 °C in a thermostated spectrophotometer. The reaction was initiated by injection of 10 μL of a solution of HPNP to give a final HPNP concentration of 0.038 mM, and transesterification was monitored by following the increase in absorbance at 400 nm due to the release of 4-nitrophenolate. The pH values of these solutions were determined at the end of each spectrophotometric experiment and were found to be within 0.02 units of the initial value.

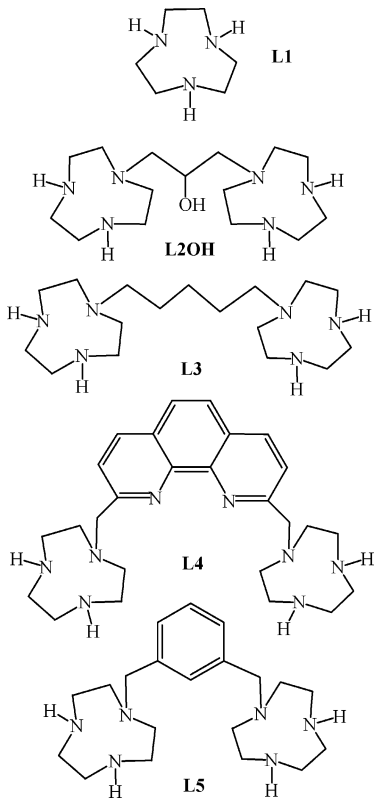
The reactions catalyzed by Zn_2(L2O) were monitored for three half-lives, and pseudo-first-order rate constants k_{obsd} were determined as the slopes of semilogarithmic plots of reaction progress against time. In most other cases, the reaction of HPNP was monitored for the disappearance of the first 5−10% of the substrate, and the reaction endpoint was obtained by heating the reaction to 60 °C. Values of k_{obsd} (s^{-1}) were determined as $k_{obsd} = v_i/[S]_o$, where v_i is the initial reaction velocity and $[S]_o$ is the initial substrate concentration. Second-order rate constants k_{Zn} (M^{-1} s^{-1}) for the reactions catalyzed by the different Zn(II) complexes were determined as the slope of linear plots of k_{obsd} against Zn(II) complex concentration (correlation coefficients >0.997). Rate constants were reproducible to ±6%. The pseudo-first-order rate constants for transesterification of HPNP in the absence of the catalyst k_{uncat} (s^{-1}) were determined by the method of initial rates (5% conversion). At pH 7.6, the background rate constant was 5.2×10^{-8} s^{-1} and this value was subtracted from the pseudo-first-order rate constant k_{obsd} (s^{-1}) in the presence of $Zn(NO_3)_2$.

Cleavage of A_6. The oligonucleotide was purified by TLC on silica gel plates by using a solvent system containing 1-propanol−NH_4OH−H_2O in a 55:35:10 ratio. A_6 was 3′ labeled with [α-^{32}P]-pCp using RNA T4-ligase (Amersham Life Science). The oligonucleotide was purified by polyacrylamide gel electrophoresis on a gel containing 20% acrylamide and 1% bisacrylamide, eluted with water, and desalted by size-exclusion chromatography (PD-10 column containing G-25M Sephadex, Pharmacia). All reaction mixtures contained 1.00 mM complex, 2.5 μM A_6, and 10.0 mM HEPES buffer. Solutions were spiked with ^{32}P-labeled A_6 and then incubated for 24 h at 37 °C, pH 7.5. Following incubation, 10 μL of loading buffer (9 M urea in tris-borate buffer) was added. Samples were loaded on a 20% polyacrylamide gel, and reactions were run alongside RNA ladders obtained from base hydrolysis of the RNA. The gel was run at 10−20 W for 3−4 h. Radioactivity was quantitated by phosphorimaging (Molecular Dynamics). Results are the average of two or more experiments. A control reaction run without catalyst had 2% cleavage after incubation for 24 h.

Results and Discussion

Ligand Synthesis. There are several methods in the literature for the preparation of linked 1,4,7-triazacyclononane macrocycles. Typically, these syntheses use a deprotected derivative of L1[46−48] or, alternately, the orthoamide protecting group is used.[41,42,49,50] Three of the ligands here were prepared from the orthoamide derivative of L1 (1,4,7−triazatricyclo[5.2.1.0^{4,10}]decane) including L3, L4, and L5 (Chart 1, Scheme 2). The synthetic procedure using this protecting group leads to high yields of ligand over a relatively short reaction time if linkers contain aromatic groups adjacent to the methylene group. Such aromatic linkers activate the methylene carbon to nucleophilic attack, facilitating the synthesis of dinuclear ligands. In contrast, the preparation of ligands from the orthoamide derivative of L1 that contain alkyl linkers (L3) requires reaction times of several days.[41] Similarly, the preparation of the L2OH ligand is problematic because the alkyl linker is not activated. In addition, previous work suggests that the alcohol group in the linker needs protection if the orthoamide derivative of L1 is used as a precursor.[51,52] For these reasons, the L2OH ligand was prepared by using Boc protected L1 as a precursor. This route was chosen over use of tosyl protecting groups as reported in the original synthesis of this compound in order to avoid the use of mercury amalgam for the deprotection step.[47] The Boc protecting group is readily removed under mild conditions making this preparative route

Chart 1



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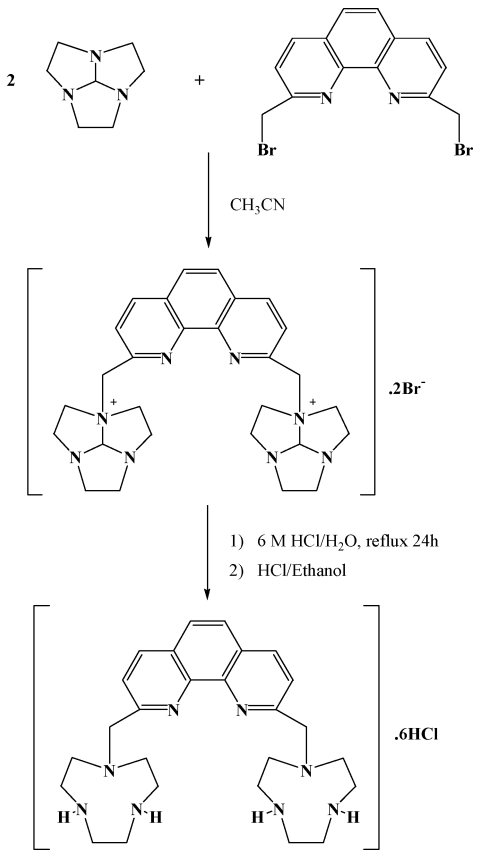
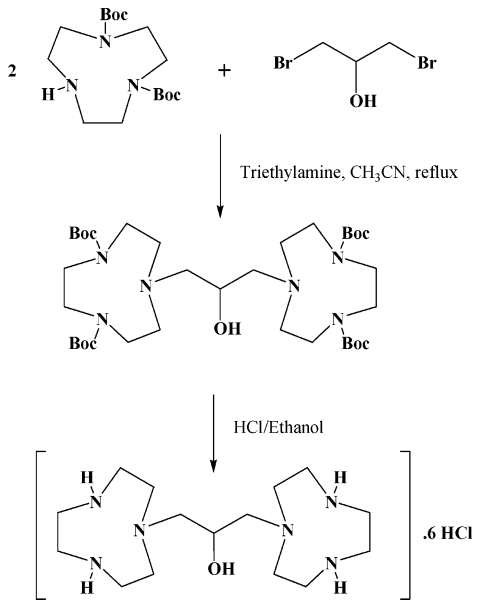
Scheme 2



Scheme 3



the method of choice for linkers containing alcohol groups (Scheme 3).

Zn(II) Binding to Dinuclear Ligands. The macrocyclic ligands L2OH and L3 strongly bind two Zn(II) ions in solution as determined previously in studies using pH−potentiometric titrations.[33,53] The large binding constants of these ligands lead to 97 and 98% of the Zn(II) being bound in dinuclear complexes in solutions containing 10.0 mM $Zn(NO_3)_2$ and 5.0 mM L3 or L2OH, respectively, at pH 6.53. Here we use NMR spectroscopy to further study the structure of the different metal complexes postulated in these titrations and, for cases where the potentiometric data is not available, to confirm that two Zn(II) ions bind to the ligand under conditions similar to those in the kinetics experiments (vide infra). The titration of dinuclear ligands L2OH, L3, L4, and L5 with varying ratios of $Zn(NO_3)_2$ was studied by use of ^{1}H NMR spectroscopy as depicted in Figure 1 for L2OH and Figures S2−5 in SI for L3, L4, L5, and L2OH.

For L2OH, L4, and L5, the ^{1}H NMR spectrum of the free ligand shows different conformers in solution at near neutral pH values. In contrast, at acidic pH values the ^{1}H NMR spectra are consistent with a single conformer. In all sets of experiments, the ^{1}H NMR resonances of the free ligand disappear upon addition of $Zn(NO_3)_2$ to give rise to new resonances corresponding to the formation of new Zn(II) complexes in slow exchange with free ligand. The protons of each CH_2 group in the macrocycle ring become inequivalent to each other upon addition of $Zn(NO_3)_2$, and all ligand resonances shift upon Zn(II) coordination. The resonances corresponding to the protons in the different linkers generally appear in a region of the ^{1}H NMR spectrum relatively free of other signals, facilitating assignments. In cases where they overlap with the resonances of other groups, homonuclear ^{1}H correlation spectroscopy (COSY) NMR experiments were used to make assignments.

For the ligand L2OH, only two Zn(II)-containing species are observed when the ratio of $Zn(NO_3)_2$ to ligand is increased from 0 to 2.0, giving rise to relatively simple ^{1}H NMR spectra (Figure 1 and SI Figure S5). When $r < 1.0$, signals of a new species A are observed in addition to signals due to free L2OH (SI Figure S5). When $r = 1.0$, only resonances attributed to species A are present while the resonances due to free L2OH completely disappear. When more $Zn(NO_3)_2$ is added, $1.0 < r < 2.0$, new resonances are detected corresponding to species B (SI Figure S5) in addition to the resonances of species A. At $r = 2.0$, only resonances attributed to species B are observed and further addition of $Zn(NO_3)_2$ ($r > 2.0$) does not produce any change in the spectrum, confirming that there is no further binding of Zn(II). On the basis of these results, species A is assigned to a mononuclear complex of Zn(II) and L2OH (Zn(L2OH)) and species B to a dinuclear complex (Zn_2(L2O)). These dinuclear and mononuclear complexes of Zn(II) with L2OH were further characterized by ^{13}C NMR, homonuclear ^{1}H COSY NMR, and ^{1}H nuclear Overhauser effect spectrometry (NOESY) 1D NMR spectroscopy. The ^{13}C NMR spectra of both the dinuclear complex Zn_2(L2O) and the mononuclear complex Zn(L2OH) show eight carbon resonances, two corresponding to the linker and six corresponding to the methylene groups of the L1 units. These results suggest that both complexes are highly symmetric. ^{1}H NOESY NMR spectra gave further information about the conformation of the linker protons ($CH_2H_{2'}$−CH_1OH−$CH_2H_{2'}$) in the two complexes. For the dinuclear complex, irradiation of H_1 enhances the intensity of the resonance of $H_{2'}$ but no effect

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is observed for the resonance of H_2. For the mononuclear complex, irradiation of H_1 notably enhances the resonance of $H_{2'}$ and also slightly enhances the intensity of H_2.

These NMR spectral data are consistent with the following solution structures for the mononuclear and dinuclear Zn(II) complexes of L2OH. For the mononuclear complex, the intramolecular cross-relaxation from H_1 to $H_{2'}$ and from H_1 to H_2 observed by use of ^{1}H NOESY NMR spectroscopy is consistent with a relatively flexible linker, suggesting that the alcohol group of the linker is not coordinated to the Zn(II) ion. This species is designated Zn(L2OH) because the alcohol group is in protonated form. In addition, both the ^{1}H and ^{13}C NMR spectra of this complex suggest a highly symmetric structure with two equivalent L1 units. This NMR solution data, together with structural data for complexes of related bis(L1) ligands, suggests that the Zn(L2OH) complex has a sandwich structure with both L1 units binding a single Zn(II) ion to form a six-coordinate complex.[53,54] Such a structural motif, where the alcohol group is not coordinated to the metal ion, has been found for other potentially dinucleating systems under conditions of equimolar amounts of metal ion and ligand.[17,28] An alternate structure we cannot rule out is a dimeric complex with each Zn(II) bound to two L1 units from different ligands. Such a dimeric complex of the form $Zn_2(L2OH)_2$ could give rise to the NMR spectra we observe. However, such dimeric complexes are generally observed for alkyl linkers with four, five, or six methylene units. Ligands containing two L1 units linked by a propyl group form the intramolecular metal ion sandwich structure.[41,53,55] For the dinuclear complex $Zn_2(L2O)$, the linker is relatively rigid as suggested by nuclear Overhauser effect experiments. All the linker protons exhibit a marked upfield shift compared to the free ligand (Figure 1). This is consistent with coordination and deprotonation of the alcohol group to form $Zn_2(L2O)$. On the basis of these observations, the change in the chemical shift observed in our system suggests binding of two Zn(II) to the alcohol group of the linker and its deprotonation to form an alkoxo-bridged dinuclear Zn(II) complex. This result is supported by the X-ray crystal structure obtained for the complex $Zn_2(L2O)$ and the results of pH-potentiometric titrations.[33] These data indicate that the ligand L2OH is able to form both stable mononuclear and dinuclear Zn(II) complexes with L2OH, a result that differs from that of the related ligand 1,3-bis(1,4,7-triaza-1-cyclononyl)-propane, which only forms a very stable mononuclear sandwich complex with Zn(II).[53] This distinct behavior is likely due to the presence of the alcohol group in the linker moiety that favors the opening of the stable sandwich complex to form the dinuclear Zn(II) complex when the ratio of $Zn(NO_3)_2$ to ligand is increased to 2.0.

For the ligands L3, L4, and L5, a mixture of free ligand and different Zn(II)-containing species, most likely mononuclear as well as dinuclear species, is observed as the ratio of $Zn(NO_3)_2$ to ligand is increased from 0 to 2.0 (SI Figures S2−4). For the ligands L3, L4, and L5, the ^{1}H NMR spectra of solutions containing equimolar amounts of $Zn(NO_3)_2$ and ligand do not correspond to the exclusive formation of a highly symmetric mononuclear Zn(II) complex as is observed for the ligand L2OH. However, when the ratio is increased to 2.0, the spectra are consistent with the presence of a single type of complex, corresponding to the dinuclear complexes $Zn_2(L3)$, $Zn_2(L4)$, and $Zn_2(L5)$. Notably, in all cases, the ^{1}H NMR spectrum does not change further when the ratio is greater than 2.0, suggesting that the dinuclear complex is essentially completely formed with 2 equiv of Zn(II) under the conditions of the NMR titration. Also consistent with the formation of a dinuclear complex is the lack of any observed precipitate of free Zn(II) ion when a second equivalent of Zn(II) is added. This is especially significant for the L4 ligand, which contains both L1 and phenanthroline units that may bind Zn(II). That the complexed Zn(II) atoms in $Zn_2(L4)$ are bound to the macrocycle units is supported by the inequivalent ^{1}H NMR resonances of each CH_2 group in the L1 ring. Binding of the first two Zn(II) ions to the macrocycle units is not surprising given the much larger propensity of L1 to bind Zn(II) ($\log K = 11.6$)[56a] compared to substituted phenanthrolines ($\log K = 4.1$ for neocuproine).[56b] What is more surprising is that a third Zn(II) does not appear to bind as suggested by the ^{1}H NMR spectra (compare $r = 2.0$ and $r = 3.0$) and the appearance of a precipitate when a third equivalent of Zn(II) is added. However, the phenanthroline unit may have a reduced affinity for Zn(II) in the highly positively charged $Zn_2(L4)$ complex. Kinetic data also suggest that L4 binds two Zn(II) ions (see below).

Zompa and co-workers used pH-potentiometric titrations to study the solution chemistry of a series of Zn(II) complexes of ligands containing two L1 macrocycles linked by alkyl groups with two to eight carbons.[53] They found that the ligand L3 is able to form stable dinuclear complexes with Zn(II), consistent with our NMR studies. For the ligands L4 and L5, there are no previous studies on the stability of their Zn(II) complexes. However, Zompa and co-workers studied the solution chemistry of the Cu(II) complexes of L5 as well as those of L3 and found that both ligands form stable dinuclear Cu(II)−ligand complexes.[41,57] This is consistent with our NMR studies, which suggest that two units of L1 connected by an aromatic linker (L4 and L5) form dinuclear complexes of Zn(II). In addition, our pH-potentiometric and structural studies on L2OH complexation of Zn(II) are consistent with the results obtained here.[33] Our previous studies suggested that an alkoxy-bridged dinuclear Zn(II) complex was essentially completely formed in solutions containing 1.0 mM L2OH and 2.0 mM $Zn(NO_3)_2$ at pH 6.0. ^{1}H and ^{13}C NMR studies here confirm that there is a single dinuclear Zn(II) complex formed under these conditions. In summary, all the ligands studied here bind two Zn(II) ions

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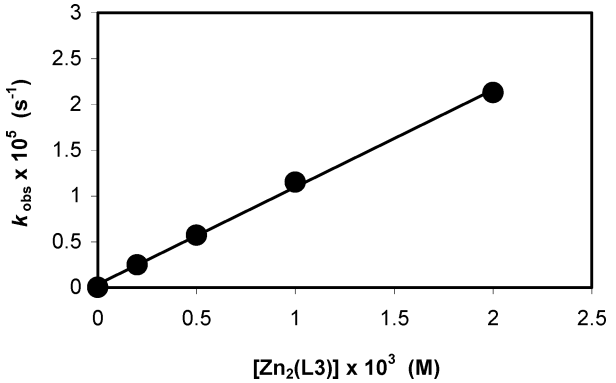


Figure 2. Dependence of the observed pseudo-first-order rate constant on the concentration of Zn_2(L3) at pH = 7.6, 25 °C, and I = 0.10 M $NaNO_3$.

Table 1. Rate Constants for the Cleavage of HPNP by Zn(II) Complexes at pH 7.6, 25 °C, and I = 0.10 M $NaNO_3$

catalyst	k_{Zn} (M^{-1} s^{-1})	relative k^a
Zn_2(L2O)	25×10^{-2}	120
Zn_2(L3)	1.1×10^{-2}	5
Zn_2(L4)	0.89×10^{-2}	4
Zn_2(L5)	0.58×10^{-2}	3
Zn(L1)	0.21×10^{-2}	1

[a] $k_{Zn}/k_{Zn(L1)}$.

to form a dinuclear complex under conditions similar to those in our kinetics experiments described below.

Kinetics of Transesterification of HPNP and RNA. The transesterification of HPNP catalyzed by the different Zn(II) complexes was monitored by following the increase in absorbance at 400 nm due to the release of 4-nitrophenolate. The cyclic phosphate diester was identified by ^{31}P NMR as the sole phosphorus-containing product of each metal ion catalyzed transesterification of HPNP examined in this work. Figure 2 shows the effect of increasing concentrations of Zn_2(L3) on the observed first-order rate constant for cleavage of HPNP. The second-order rate constant for the Zn_2(L3)-catalyzed reaction was determined as the slope of the linear plot of k_{obsd} against [Zn_2(L3)]. The reactions of other catalysts were examined in the same way through [cat] = 2.0 mM, except for Zn_2(L5), which was only soluble up to ≈1.0 mM.

Table 1 summarizes the second-order rate constants (k_{Zn}, M^{-1} s^{-1}) for transesterification of HPNP catalyzed by several different Zn(II) complexes at pH 7.6. The data for dinuclear complexes are also reported as the relative rate acceleration with respect to the mononuclear complex Zn(L1). The catalytic activities of most of these dinuclear Zn(II) complexes are only marginally larger than that for Zn(L1) (3- to 5-fold), with the exception of Zn_2(L2O), which is a 120-fold better catalyst than Zn(L1).

The L4 ligand contains a phenanthroline unit as a part of the linker and may bind a third Zn(II) ion. Cleavage of HPNP at pH 8.0 was studied in the presence of 1.0 mM L4 with 2 and 3 equiv of Zn(II). These reactions were run in parallel, and although precipitation was observed with 3 equiv of Zn(II), the reaction was allowed to proceed until completion. The pseudo-first-order rate constants k_{obsd} (s^{-1}), determined by the method of three half-lives, are identical (1.4×10^{-5} s^{-1}) for experiments conducted with a 2:1 or a 3:1 ratio. This result, along with the ^{1}H NMR data which shows no additional change in the ^{1}H NMR spectrum upon addition of a third equivalent of Zn(II), suggests that the primary species present in solution under these conditions has two Zn(II) ions, not three.

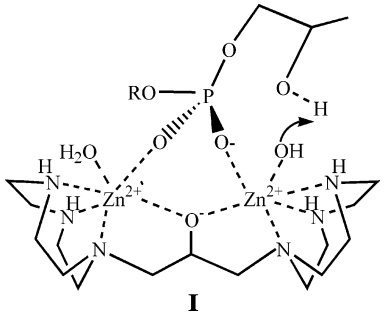
It was not possible to determine values for k_{obsd} when [$Zn(NO_3)_2$] is ≥0.50 mM at pH 7.6 in solutions that contain no ligand because of precipitation of Zn(II) hydroxide. However, the value of $k_{obsd} = 7.2 \times 10^{-7}$ s^{-1} for reaction in the presence of 0.21 mM $Zn(NO_3)_2$ is sufficient to show that the catalytic contribution of free Zn(II) ion toward the cleavage of HPNP by the dinuclear Zn(II) complexes examined in Table 1 is negligible. For example, in the case of the reactions catalyzed by L3 and L2OH at pH 7.6 with 2.0 mM ligand and 4.0 mM $Zn(NO_3)_2$, only 1.14% (0.046 mM) and 0.0017% (6.8×10^{-5} mM), respectively, of the total Zn(II) is present as free metal ion, so that the contribution of this metal ion to k_{obsd} should be much less than the value of 7.2×10^{-7} s^{-1} determined for reaction in the presence of 0.21 mM $Zn(NO_3)_2$. In the case of L4 and L5, the failure to detect free ligand by NMR spectral analysis of solutions that contain a 2-fold-excess concentration of zinc over ligand shows that <5% of Zn(II) under these conditions is present in the free form. We conclude that under the conditions given in Table 1, all dinuclear Zn(II) catalysts examined here are more effective than free Zn(II) in catalysis of transesterification of HPNP.

The catalysis of the cleavage of an oligoribonucleotide containing six adenosines (A_6) by Zn(L1) and by two dinuclear Zn(II) catalysts was also studied. The extent of cleavage of A_6 after 24 h was 4, 10, and 90% for Zn(L1), Zn_2(L5), and Zn_2(L2O), respectively. This represents all cleavage products, and no attempt was made to separately quantify the oligoribonucleotides of different lengths. The oligonucleotide products from the Zn(II) complex catalyzed cleavage comigrate with those produced by hydroxide catalyzed cleavage. The 3′-end-labeled oligonucleotide products of the hydroxide ladder contain 5′-OH as a terminal group. Comigration of the Zn(II) catalyzed cleavage products confirms that the Zn(II) complexes promote the hydrolytic cleavage of RNA. Qualitatively, the extent of cleavage correlates to the relative rate constants observed for the HPNP substrate with the dinuclear complexes being the best catalysts and Zn_2(L2O) by far the more active of the two.

Comments on the Mechanism of Cleavage of RNA and RNA Analogues by Dinuclear Catalysts. Table 1 shows that the catalytic activity of the dinuclear complexes Zn_2(L3), Zn_2(L4), and Zn_2(L5) is not much greater than the sum of their parts, as determined from the activity of Zn(L1). These results provide strong evidence that the subunits of these dinuclear catalysts act nearly independently. The observation that the activity of these dinuclear complexes is 3−5-fold larger than that of the mononuclear Zn(L1) is consistent with weak (<0.4 kcal/mol) stabilization of the transition state for reaction at one tethered complex by long-range electrostatic interactions with the second.

The situation is more interesting for the reaction catalyzed by Zn_2(L2O), for which the overall catalytic activity is 120-fold greater than the mononuclear complex Zn(L1), or 60-

fold greater than the activity expected for a complex in which the tethered complexes react independently. Here, the alkoxy group shields the electrostatic interactions between the Zn(II) ions and allows the cations to be drawn relatively close together in a complex of greatly enhanced activity (**I**). This high density of positive charge at Zn_2(L2O) is ideal for providing electrostatic stabilization of the transition state for cleavage of HPNP relative to the reactant state because there is a net unit increase in negative charge on proceeding from the reactant to transition state.



I

The bridging alkoxide group in Zn_2(L2O) may play a similar role to the bridging ligands in enzymes that utilize two metal ions. Dinuclear functional motifs in the active center of metalloenzymes have the metal ions bridged by an endogenous anionic amino acid, usually Asp or Glu, or by an exogenous ligand such as water or hydroxide.[1−3] Consequently, the electrostatic repulsions between metal ions are reduced by the bridging ligands and they can come in close proximity to function as a catalytic unit.

Mononuclear metal ion complexes that are effective catalysts for cleavage of RNA have a least two coordination sites available for catalysis.[58] These coordination sites are necessary for binding the substrate and catalyzing cleavage. The tridentate ligand L1 was chosen because it binds Zn(II) tightly ($\log K = 11.6$) while leaving two to three coordination sites open for catalysis. Zn_2(L2O) probably has fewer available coordination sites than do the other dinuclear complexes lacking a bridging group and, on this basis, might be expected to be a poorer catalyst. The crystal structure of $[Zn_2(L2O)(Cl)(H_2O)_2](ClO_4)_2$ has one five-coordinate and one six-coordinate Zn(II) center with four coordination sites on each Zn(II) occupied by L2O, including two tridentate L1 units and a bridging alkoxide. [33] This leaves one or two coordination sites available for catalysis on each Zn(II) center, and this is evidently a sufficient number to form a good catalyst. For dinuclear catalysts, the minimum number of available coordination sites for a good catalyst has not been established and could be different from the requirements for mononuclear catalysts if the two metal ion centers cooperatively catalyze the reaction. Of note here is that the dinuclear Cu(II) complex of L2OH, which has two five-coordinate Cu(II) centers with a single available coordination site on each Cu(II),[59] is completely inactive in catalyzing the cleavage of HPNP.[60] This suggests either that the coordination sites on Cu(II) are not suitable for binding and catalysis or that two available coordination sites per metal center are required to form a good dinuclear catalyst. For Zn_2(L3), Zn_2(L4), and Zn_2(L5), a lack of available coordination sites is not the most likely reason that these complexes are not good catalysts. These complexes are expected to have two or three available coordination sites on each Zn(II) center.

Another important feature of metal ion complex catalyzed cleavage of RNA and RNA analogues is that pH−rate profiles frequently track the formation of metal ion hydroxide complexes. For Zn_2(L2O) catalyzed cleavage of HPNP, the pH−rate profile is consistent with a single ionization to form an active catalytic species and the kinetic pK_a of this species matches the thermodynamic pK_a for formation of Zn_2(L2O)-(OH) within experimental error.[33] Although there are alternative mechanisms possible, one interpretation of this observation is that the metal hydroxide ligand participates in RNA cleavage by acting as general base catalyst (**I**). One reason that Zn_2(L2O) is a good catalyst at near neutral pH values is that the Zn(II) hydroxide complex forms at lower pH values compared to the analogous mononuclear complex, making catalysis more effective at neutral pH. Thus knowledge of the pK_a of the Zn(II)−water ligands and a full pH dependence of the catalyzed reaction is important in making comparisons of the catalytic properties of dinuclear and mononuclear Zn(II) complexes.

A second difference observed for dinuclear catalysts compared to mononuclear catalysts is the strength of their interaction with substrate. Zn_2(L2O) binds a phosphate diester 6-fold more strongly than does an analogous mononuclear Zn(II) complex.[33] Strong substrate binding to the Zn(II) complex has the effect of increasing the second-order rate constant for cleavage because more of the Michaelis complex is formed. Binding constants for the other dinuclear complexes were not determined here. However, given that the difference in binding to a dinuclear complex compared to the mononuclear complex is only 6-fold, it is unlikely that binding constants alone would be responsible for the differences observed here in catalytic properties of the dinuclear Zn(II) complexes.

The mechanism of cleavage of HPNP by the Zn(II) complexes of L3, L4, and L1 is not easily interpreted because the solution chemistry of these complexes is not well-defined. For example, the pK_a of water ligands in Zn_2(L3) is not readily measured. This is due to slow equilibria at pH values where hydroxide complex formation is generally observed, possibly due to the formation of hydroxide-bridged complexes of higher order aggregates. Similarly, pH profiles for the cleavage of HPNP by Zn_2(L4) complexes from pH 6 to 10 are not consistent with a single well-defined ionization attributable to a Zn(II)−water ligand (data not shown). The detailed mechanistic study of Zn(L1) as a catalyst is similarly made difficult by its precipitation at pH values greater than 8.0. Dinuclear or mononuclear complexes with complicated

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(60) Manuscript in preparation.

speciation are not amenable to detailed mechanistic analysis. In contrast, the bridging alkoxide in the L2OH ligand facilitates the formation of a dinuclear Zn(II) complex with relatively simple solution chemistry. This solution chemistry can then be correlated to catalytic properties for the elucidation of the mechanism of cleavage of RNA and RNA model substrates. The high activity at neutral pH and straightforward solution chemistry of the Zn_2(L2O) complex facilitates its study as a catalyst.

It is interesting that Zn_2(L2O) also effectively cleaves the oligoribonucleotide A_6. One criticism of work with HPNP is that this substrate has a much better leaving group than does RNA and does not have the ribose ring. It has been pointed out that free metal ions and Zn(II) mononuclear complexes catalyze cleavage of HPNP more effectively than RNA or substrates that more closely resemble RNA.[31] Our initial results here suggest that three of our Zn(II) catalysts promote cleavage of RNA and that the extent of cleavage correlates roughly to that for the HPNP substrate. Further studies are underway using different RNA substrates to better understand why dinuclear complexes in general and Zn_2(L2O) in particular are so effective at promoting the cleavage of RNA.

To date, our scheme for the mechanism of cleavage of RNA by dinuclear Zn(II) complexes has the following features. For effective catalysis, two metal ions are maintained in close proximity. This is accomplished here and in metalloenzymes that utilize two metal ions for phosphate diester cleavage by use of a bridging ligand. The first step in catalysis involves binding of the dinuclear complex to the phosphate diester moiety of the RNA or RNA model substrate. It is possible that the phosphate diester binds by bridging the two Zn(II) centers, but we have no structural or spectroscopic evidence for this binding mode. However, Zn_2(L2O) binds phosphate diesters 6-fold more strongly than does an analogous mononuclear complex, suggesting that the second metal ion interacts with the phosphate diester substrate. In addition, dinuclear Zn(II) and Cu(II) complexes with similar bridging alkoxide groups have been shown to form complexes containing bridging phosphate diesters.[17,22,28] A Zn(II)−hydroxide complex is likely the active catalytic form and may act as a general base catalyst although we cannot rule out other possible mechanisms as discussed previously.[33] The Zn(II) complex also facilitates cleavage through an electrophilic role, most likely through stabilization of a negatively charged phosphorane transition state. Another possible important role for these complexes is in facilitating leaving group departure in the cleavage of RNA or RNA substrates that contain poor leaving groups.[31] Further experiments are underway in our laboratories with the goal of delineating these possible roles for metal ion complexes in the cleavage of RNA.

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Supporting Information Available: Ligand labels for L4 and L2OH and ^{1}H NMR spectra of L3, L5, L4, and L2OH. This material is available free of charge via the Internet at http://pubs.acs.org.

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