

Synthesis, Structure, and DNA Cleavage Activity of New Trinuclear Zn₃ and Zn₂Cu Complexes of a Chiral Macrocycle: Structural Correlation with the Active Center of P1 Nuclease

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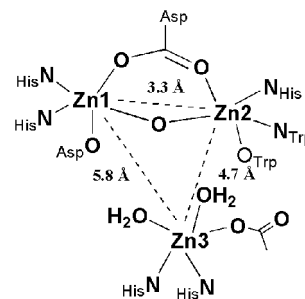
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New homo trinuclear Zn(II) complexes [Zn₃L¹(μ-OAc)](ClO₄)₂·3CHCl₃·H₂O, **1**, and [Zn₃L¹(μ-OAc)]·ClO₄·PF₆·5CH₃OH·H₂O, **2**, and hetero trinuclear complex [Zn₂CuL¹(μ-OAc)](ClO₄)₂·3CHCl₃·H₂O, **3**, of optically active hexaaza triphenolic macrocycle H₃L¹ were synthesized and crystallographically characterized. The cation [Zn₃L¹(μ-OAc)]⁺ structure of **1** and **2** closely resembles the trinuclear Zn(II) active site of P1 nuclease. The distorted tetrahedral geometry of Zn₃ was successfully reproduced at Cu₁ in complex **3**. The complexes **2** and **3** cleave CT DNA at 37 and 50 °C.

Trinuclear complexes with bridging oxo and carboxylato motifs have received considerable attention due to the isolation and crystallographic characterization of trinuclear metal constellations in the active sites of metalloenzymes such as P1 nuclease¹ (Zn₃), phospholipase C² (Zn₃), and alkaline phosphatase³ (Zn₂Mg) (Scheme 1). Attempts at reproducing these enzymatic environments have resulted in several structural models of type LZn–OH–Zn,⁴ LZn–(μ-RCOO)–Zn,⁵ LZn–(μ-O)(μ-RCOO)–Zn,⁶ and LZn–(μ-

Scheme 1. A Schematic Illustration of the Trinuclear Zinc Active Site of P1 Nuclease^a



^a Zn1···Zn2, 3.3 Å; Zn3···Zn1, 5.8 Å; Zn3···Zn2, 4.7 Å.

OH)(μ-R₂PO₂)–Zn⁷ (L = ligand) corresponding to the dinuclear part of the trinuclear active site.^{4–8} Spurred by the catalytic activity of the mononuclear and dinuclear zinc complexes,^{4–8} a few recent studies have reported trinuclear zinc complexes from self-assembled molecules¹⁰ and calixarenes¹¹ to be functional models.^{9,12} For instance, greater efficiency has been noted for a heteronuclear Zn₂Cu sandwiched calixarene complex than for the Zn₃ complexes.¹¹ The self-assembly of small ligands can produce a trinuclear constellation, though the essential coordination environment

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is not certain. Hence, there is a need for a multinucleating macrocyclic ligand capable of binding three metal ions in close proximity, in desired fashion even with proportional variations in the metal ions and anions. We report herein the first structurally characterized chiral homo trinuclear Zn₃ complexes, **1** and **2**, and hetero trinuclear Zn₂Cu complex, **3**, of the optically active, 3 + 3 macrocyclic hexaamino triphenolic ligand¹³ H₃L¹. The ligand H₃L¹ ensures the facilitation of three metal ions in close proximity, despite the proportional variations of metals. The Cu^I ion in complex **3** possesses a significantly similar geometrical environment to the Zn³ ion of **1** and **2** and provides an opportunity to spectroscopically examine the DNA hydrolytic activity. The 5 and/or 4 coordination of Zn/Cu metal ions in these complexes enables substrate binding and cleavage activity. Preliminary studies on the nuclease activity of complexes **2** and **3** with CT DNA are also discussed.

Complexes **1–3** were synthesized in good yields and characterized by satisfactory elemental analyses and spectral data. The FAB mass spectral, analytical data along with IR and NMR spectra of complexes **1–3** propose the trinuclear composition [M^a₃L¹(μ-OAc)]ClO₄·H₂O·X (M^a = M^b = Zn (**1, 2**); M^a = Zn, M^b = Cu (**3**); X = ClO₄/PF₆). The 4.96% (calcd 5.35%) copper content in complex **3** and its magnetic moment of μ = 1.60 μ_B, a shoulder d–d transition band (in CH₃CN) at 632 nm, and a reversible cyclic voltammetric redox couple at –1.00 V (Cu^{II}/Cu^I) are consistent with the presence of one distorted tetrahedral copper atom.

The molecular structure of complex **1** was established crystallographically.¹⁴ Complex **1** crystallizes in the orthorhombic P2₁2₁2₁ (No. 19) space group. The trinuclear cation [Zn₃L¹(μ-OAc)]⁺ with atom numbering is shown in Figure 1. The trans geometry of the cyclohexyldiamine moiety and the flexibility offered by the –CH₂–NH– linkage together with the large size of the macrocyclic ring influences the ligand to twist to accommodate three metal ions in its cavity, in contrast to its rigid Schiff base analogue.¹⁵ The complex has a central Zn₃O₃ coordination core where zinc and phenoxo oxygen atoms are alternate to each other, constituting a cyclic six-membered ring. Each Zn(II) ion is coordinated by two bridging phenoxo oxygens and two amino nitrogens of the macrocycle. Zn1 and Zn2 are bridged vertically by an external acetato group. The geometry around Zn1 and Zn2 can best be described as distorted trigonal bipyramidal. For Zn1, the atoms N1, O1, O2 of the ligand constitute the basal trigonal plane and N2, O4 (acetato) atoms (N2–Zn1–O4 = 175.6°) occupy the axial positions. In the case of Zn2, the basal plane constituting O2, O5 (acetate

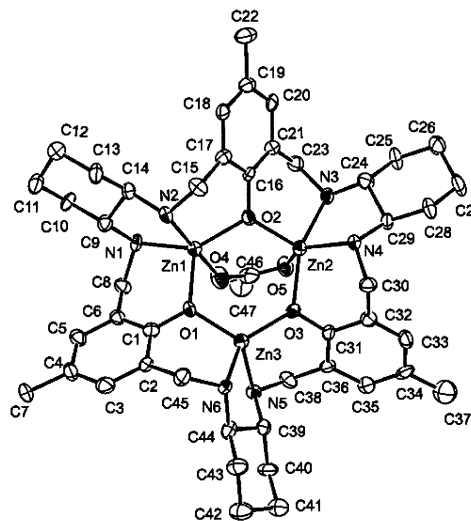


Figure 1. Molecular structure (50% thermal ellipsoids) of the cation [Zn₃L¹(μ-OAc)]⁺ of **1**. Selected bond lengths (Å) and angles (deg): Zn(1)–Zn(2), 3.3480(10); Zn(1)–Zn(3), 3.2980(10); Zn(2)–Zn(3), 3.5680(10); Zn(1)–O(1), 1.985(6); Zn(1)–O(2), 1.973(6); Zn(1)–O(4), 2.147(7); Zn(1)–N(1), 2.040(7); Zn(1)–N(2), 2.123(7); Zn(2)–O(2), 1.961(6); Zn(2)–O(3), 2.070(6); Zn(2)–O(5), 2.057(7); Zn(2)–N(3), 2.125(7); Zn(2)–N(4), 2.122(7); Zn(3)–O(1), 1.910(6); Zn(3)–O(3), 1.905(6); Zn(3)–N(5), 2.077(8); Zn(3)–N(6), 2.036(8); O(1)–Zn(1)–O(2), 120.8(2); O(1)–Zn(1)–O(4), 81.7(3); O(2)–Zn(1)–O(4), 88.5(3); O(2)–Zn(2)–O(3), 107.6(2); O(2)–Zn(2)–O(5), 102.8(3); O(3)–Zn(2)–O(5), 88.1(3); O(1)–Zn(3)–O(3), 117.7(3); O(1)–Zn(3)–O(3), 117.7(3); O(1)–Zn(3)–N(5), 127.5(3); O(3)–Zn(3)–N(5), 98.8(3); O(1)–Zn(3)–N(6), 96.7(3); O(3)–Zn(3)–N(6), 129.8(3); N(5)–Zn(3)–N(6), 85.5(3); Zn(1)–O(1)–Zn(3), 115.7(3).

group), N4 atoms and O3, N3 atoms (O3–Zn2–N3 = 157.5°) occupies the axial positions. Zn1 and Zn2 are separated at a distance of 3.348 Å. The Zn3–N (2.03, 2.07 Å) and Zn3–O (1.90, 1.91 Å) distances are smaller than the corresponding distances of Zn1 and Zn2 metal ions. The bond angles between the coordinated atoms distinctly indicate distorted tetrahedral geometry around the Zn3 metal ion. The interatomic distances between Zn3 and Zn1 and between Zn3 and Zn2 are 3.298 and 3.568 Å, respectively. The basic cationic structure of the complexes **2** and **3** is very similar to complex **1**, with a meager variation in bond angles and distances, and metal ions have identical geometry. In complex **3**, the replacement of Zn3 with Cu^I produced the same distorted tetrahedral geometry around the Cu(II) ion and the Cu^I–N (2.026, 2.083 Å) and Cu^I–O (1.916, 1.923 Å) distances varied marginally compared to the Zn3 metal ion of **1** and **2**. The Cu(II) ion is separated from Zn1 and Zn2 by 3.327 and 3.535 Å, respectively (Figure S1).

The molecular structures of complexes **1–3** show that the Zn1–Zn2 coordination environment with an oxo and acetato bridge is very similar to that of the dinuclear zinc site of the P1 nuclease trinuclear active site. Structural similarities include the 3.348–3.386 Å between Zn1 and Zn2, the two coordinating nitrogens and three oxygens around the zinc ions, and the distorted trigonal bipyramidal geometry of Zn1 and Zn2. Complexes having the distorted tetrahedral third metal ion (Zn³/Cu^I) in close proximity at an average distance to Zn1 of 3.3 Å and to Zn2 of 3.53 Å are also at an advantage in comparison with the trigonal bipyramidal Zn³ ion at average distances of 4.7 and 5.8 Å, respectively.

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(14) Crystal data for **1**: molecular formula = Zn₃N₆O₁₄C₅₀H₆₅Cl₁₁; fw = 1560.22; orthorhombic, P2₁2₁2₁ (No. 19); a = 9.9732(5) Å; b = 17.1315(2) Å; c = 37.4953(5) Å; T = 173 °C; V = 6406.3 Å³; Z = 4; D_{calcd} = 1.154 g/cm³; μ(Mo Kα) = 14.01 cm⁻¹; λ = 0.71070 Å; R = 0.059, R_w = 0.160. Crystal data for **3**: molecular formula = Zn₂CuN₆O₁₄C₅₀H₆₅Cl₁₁; fw = 1558.38; orthorhombic, P2₁2₁2₁ (No. 19); a = 9.9294(2) Å; b = 17.1354(6) Å; c = 37.6763(11) Å; T = 173 °C; V = 6410.4 Å³; Z = 4; D_{calcd} = 1.615 g/cm³; μ(Mo Kα) = 15.94 cm⁻¹; λ = 0.71070 Å; R = 0.086, R_w = 0.260.

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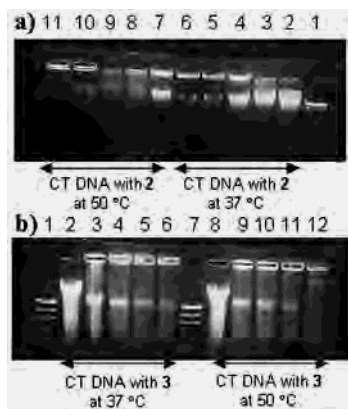


Figure 2. Nuclease activity of (a) complex **2** and (b) complex **3**. Marker: lanes 1 (a, b) and 7 (b). CT DNA in DMSO: lanes 2 (a, b), 7 (a), and 8 (b). CT DNA with complex 100 μM ($\times 1, 3, 5, 7$) incubated at 37 and 50 $^{\circ}\text{C}$: lanes 3–6 (a, b), 8–11 (a), and 9–12 (b).

The stability of the complexes toward water was investigated by NMR spectra. Retention of ^1H NMR (d_6 -DMSO) signals corresponding to the ligand and acetate bridge of complex **2** in 0.5 μL of water indicates the stability of the complex in DMSO/ H_2O solvent medium. Preliminary investigations into mimicking the hydrolytic activity of the complexes **2** and **3** (in DMSO) were conducted on calf thymus (CT) DNA. Both of the complexes were found to be effective in the cleavage of CT DNA. The effects of complex concentration and temperature on DNA cleavage

were significant, as shown in Figure 2a,b. Our results suggest that DMSO solvent does not induce any changes in DNA, as shown in lanes 2 and 7 of Figure 2a (complex **2**) and lanes 2 and 8 in Figure 2b (complex **3**). At 37 $^{\circ}\text{C}$, low (100 μM) concentrations of the complexes (**2/3**) did not cleave DNA as effectively as in lane 3, whereas 3- or 5-fold concentrations of the complex completely hydrolyzed the DNA, as seen in lanes 5 and 6. At an elevated temperature (50 $^{\circ}\text{C}$), DNA was completely hydrolyzed irrespective of the concentrations of complex **2**, as seen in lanes 8–11, whereas complex **3** was found to be highly active only at high concentrations (lanes 11 and 12). The high activity of complex **2** indicates the active role of the Zn^{3+} ion in catalytic processes.

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Supporting Information Available: The synthesis, characterization, ligand structure, X-ray crystallographic CIF files and Figure S1 of the complexes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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