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Notes

Hydrolysis of Phosphate Esters Mediated by New Unsymmetrical Binuclear Cu Complex

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There have been considerable research efforts in developing efficient catalysts carrying two or three metal ions for the hydrolysis of phosphate esters.[1] It might be unnecessary to place two metal ions in close proximity by using metal binding functional groups. Recently some Zn and Cu complexes of binuclear ligands have shown moderate to enormous rate enhancements in cleaving phosphate esters.[2] Binuclear Cu and Zn complexes of 1,3-di(1,5,9-triazacyclododecan-1-yl)propane in methanol turned out to be far the most efficient catalyst in cleaving 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP) through the substrate bridging to both metal ions.[3]

We have previously reported that a binuclear Cu_2L2 complex (L2: 1,3-bis(1,4,7-triaza-1-cyclononyl)propane) efficiently hydrolyzed 4-nitrophenyl phosphate (NPP) but showed no reactivity in hydrolyzing 2,4-dinitrophenyl phosphate (BDNPP), compared to those of the corresponding mononuclear CuL3 complex (L3: tacn).[4] We are interested in evaluating how the subtle changes in coordination environments can affect the reactivity of the catalyst and in this study, we report the binuclear Cu complex of 1-(3-(1,4,7-triazonan-1-yl)propyl)-1,4,7,10-tetraazacyclo-dodecane (L1), hydrolyzed BDNPP and HPNPP more than 10 times efficiently than the Cu_2L2 complex under mild conditions.

Experimental Section

Instruments. ^{13}C NMR were taken on a Varian Unity-Inova 300 MHz. Kinetic studies were carried out using PerkinElmer Lambda 25 spectrometer. Mass spectrum of the ligands and the intermediates were recorded on a Thermo Finnigan AQA LC-Mass.

Materials. BDNPP(bis(2,4-dinitrophenyl)-phosphate) and HPNPP were synthesized by the known method.[5] 1,4,7-tri-*tert*-boc-1,4,7,10-tetraazacyclododecane and 1,4-di-*tert*-boc-1,4,7-triazacyclononane were synthesized by literature methods.[6] Most chemicals and solvents were purchased from Sigma-Aldrich Co. and used without further purification.

Synthetic Procedure. 1,4,7-Tri-*tert*-boc-1,4,7,10-tetraazacyclododecane **1** was reacted, in the presence of excess 1,3-dibromopropane, to give mono substituted **2**,[7] which was then reacted with 1,4-di-*tert*-boc-1,4,7-triazacyclononane, to give fully protected bicyclic ligand **3**. Deprotection of boc groups in **3** by HCl/MeOH and following base work-up gave **L1**.

Tri-*tert*-butyl-10-(3-bromopropyl)-1,4,7,10-tetraazacyclo-dodecane-1,4,7-tricarboxylate 2: To a solution of 3.0 g (6.34 mmol) 1,4,7-tri-*tert*-boc-1,4,7,10-tetraazacyclododecane **1** and 1.5 mg (14.1 mmol) of anhydrous Na_2CO_3 in 100 mL of dry acetonitrile was added 11.0 g (54.5 mmol) of 1,3-dibromopropane. The reaction mixture was refluxed for 3 d, cooled to room temperature, filtered, and evaporated in *vacuo*. After column chromatography (CH_2Cl_2; MeOH = 100:2), 2.5 g of transparent syrup was obtained (66.5%). ESI MS: *m/z* 593.8, for $[MH]^+$ (Calcd. 592.28 for $C_{26}H_{49}BrN_4O_6$).

Di-*tert*-butyl-10-(3-(4,7-bis(tert-butoxycarbonyl)-1,4,7-triazonan-1-yl)-propyl)-1,4,7,10-tetraazacyclo-dodecane-1,4,7-tricarboxylate 3: To a solution of 2 g (3.37 mmol) of **2** and 408 mg (4.02 mmol) of triethylamine in 100 mL of dry acetonitrile was added 1.2 g (3.64 mmol) of 1,4-di-*tert*-boc-1,4,7-triazacyclononane. The reaction mixture was refluxed for 3d, cooled to room temperature, filtered, and evaporated in *vacuo*. After column chromatography (CH_2Cl_2; MeOH = 100:5), 2.0 g of syrupy solid was obtained (70%). ESI MS: *m/z* 842.59 for $[MH]^+$ (Calcd. 841.59 for $C_{42}H_{79}N_7O_{10}$).

1-(3-(1,4,7-Triazonan-1-yl)propyl)-1,4,7,10-tetraazacyclo-dodecane L1: To 1.3 g (1.54 mmol) of **3** in 2 mL MeOH was added HCl/MeOH solution (generated from acethyl chloride and MeOH) slowly. After stirring at room temperature for overnight, a white precipitate was formed, filtered, washed with cold ether, and dried in *vacuo*. The **L1**·xHCl was dissolved in small amount of water and the pH of the solution was adjusted to 12 by adding solid NaOH. The solution was extracted by $CHCl_3$ and dried with anhydrous Na_2CO_3. Evaporation to dryness gave **L1** as yellow syrup in quantitative yield. ^{13}C NMR (**L1**·xHCl in D_2O): δ 53.15, 52.28, 50.40, 47.65, 44.32, 43.96, 43.71, 43.36, 42.49,

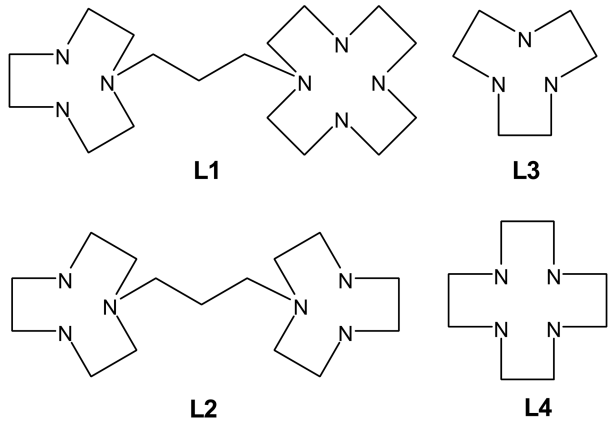


Figure 1. Chemical structures of the ligands.

19.16. ^{13}C NMR (**L1** in $CDCl_3$): δ 55.56, 52.62, 52.23, 51.50, 46.82, 46.42, 46.30, 45.96, 44.90, 25.30. ESI MS: *m/z* 342.33 for $[MH]^+$ (Calcd. 341.33 for $C_{13}H_{39}N_7$). Cu(II) complexes of **L1**-**L4** were prepared according to the known method by mixing ethanolic solution of the ligand and 1.0-2.0 equivalents of $Cu(NO_3)_2$, respectively.[4]

Cu_2L1 Complex: 117 mg (0.34 mmol) of **L1** in 5 mL of EtOH was added to 167 mg (0.72 mmol) of $Cu(NO_3)_2{\cdot}H_2O$ in EtOH 5 mL and the solution was stirred overnight. The blue precipitate was filtered, washed with EtOH, ether and dried. ESI MS: *m/z* 529.2, 531. 2, 533.1 [M-3(NO_3)] (Calcd. 529. 17 for $C_{17}H_{39}Cu_2N_8O_3$).

Kinetics. Cu complexes promoted hydrolysis of BDNPP and HPNPP was monitored by following the UV absorbance change at 400 nm (assigned to 2,4-dinitrophenolate, and 4-nitrophenolate) at 30(± 0.5) °C with I = 0.10 M ($NaNO_3$). All the reactions were carried out under pseudo-first order conditions with large excess of the Cu complexes over the phosphate esters. The rate constants were calculated by the initial rate (< 5%) method (correlation coefficient > 0.99). Buffered reaction solutions were prepared with MES (pH 6-6.5), HEPES (pH 7-8), TAPS or EPPS (pH 8-9), CHES (pH 9-10), CAPS (pH 10-11) respectively. In a typical kinetic run, to a 2 mL of 0.5-1.5 mM of Cu complex prepared in a 20 mM buffer and equilibrated at 30(± 0.5) °C for 10 min, was added a 5 μL of 0.01 M stock solution of BDNPP or HPNPP.

Results and Discussion

The unsymmetrical binucleating ligand **L1** was prepared initially by synthesizing **2**. Under the experimental conditions used, an attempt to isolate di-*tert*-butyl-7-(3-bromopropyl)-1,4,7-triazacyclononane-1,4-dicarboxylate was not successful. It is probably due to remarkably different reactivities of boc-protected azamacrocycles often observed in the course of the synthetic manipulations. Reaction of **2** with 1,4-di-*tert*-boc-1,4,7-triazacyclononane gave the fully protectected ligand **3**, which was subsequently reacted with methanolic HCl. Base work up of the **L1**·HCl salt produced ligand **L1** as a free amine (Scheme 1). Cu complexes were prepared by mixing ethanolic solutions of **L1**-**L4** and 1-2 equivalents of $Cu(NO_3)_2$ respectively.

Hydrolysis of BDNPP was performed under pseudo-first order conditions with the Cu complexes in excess. The Cu complex catalyzed hydrolysis of BDNPP produced 1 equivalent of 2,4-dinitrophenol. The hydrolysis of BDNPP was first order in the complex concentration.[8] The second order rate constants for the hydrolysis of BDNPP and HPNPP were listed in Table 1.

Table 1. Second-order rate constants ($M^{-1}s^{-1}$)[a] of phosphate hydrolysis mediated by Cu complexes at pH 9.0, 30 °C[a]

Substrate	Cu_2L1	Cu_2L2	CuL3	CuL4
BDNPP	4.53	0.349	0.733	0.018
HPNPP	0.075	0.006	0.021	-[b]

[a][Cu] = 0.5-1.3 mM, [S] = 2.5 × 10^{-5} M, pH 9 (20 mM CHES, I = 0.1 N $NaNO_3$) and hydrolysis by buffer has been subtracted. [b]too slow to be measured under experimental conditions.

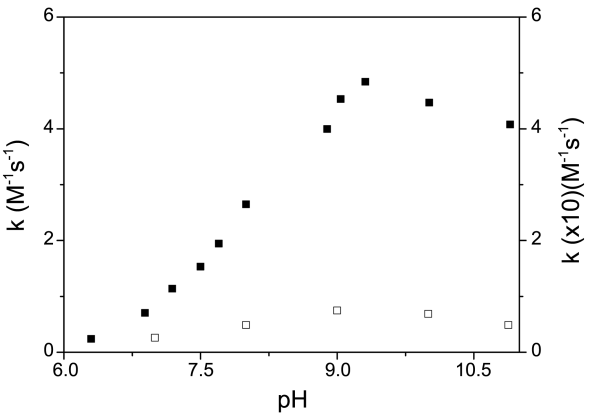
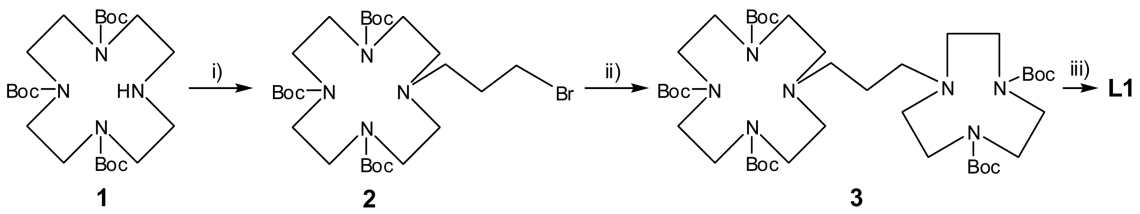


Figure 2. pH rate profile for the Cu_2L1 complex catalyzed hydrolysis of BDNPP (■: left) and HPNPP (□: right) at 30 °C, [Cu] = 0.3-0.5 mM, MES, HEPES, EPPS, CHES, and CAPS (20 mM), I = 0.1 N $NaNO_3$.



Scheme 1. Synthesis of ligand L1; i) 1,3-dibromopropane, Na_2CO_3, CH_3CN, reflux, ii) 1,4-di-boc-1,4,7-triazacyclononane, Et_3N, CH_3CN, reflux, iii) HCl/MeOH, NaOH/H_2O/$CHCl_3$.

The rate constants were measured at the pH near the maximum rate obtained from the pH-rate profiles shown in Figure 2. The maximum rates for the hydrolysis of BDNPP and HPNPP by the Cu_2L1 complex are reached at pH 9.0-9.3.

The Cu_2L1 complex was 6-200 times more efficient than the corresponding mononuclear CuL3 and CuL4 complexes and 10 times faster than the binuclear Cu_2L1 complex in hydrolyzing BDNPP. The similar pattern was observed for HPNPP hydrolysis with smaller rate enhancements. It is evident that two Cu ions in the Cu_2L1 complex interacted cooperatively. The observed rate enhancements over the symmetrical Cu_2L2 complex could be attributed to the differences of substrate binding ability and nucleophilicity.[9] Michaelis constant K_M of BDNPP to Cu_2L1 and Cu_2L2 complexes were determined as 6.9 mM and 2.4 mM, respectively.[10] The pKa of the bound water for Cu_2L1 complex is much higher than that of the Cu_2L2 complex.[11] Nucleophilic reactivity difference due to pKa of the bound water molecules, however, might be negligible under the experimental conditions where the rates were measured at the pH near its maximum rate (pH 6.4 for the Cu_2L2 and pH 9 for the Cu_2L1 complexes).[12] Since BDNPP binds better to the Cu_2L2 complex, intramolecular attack by a nucleophile are facilitated much more efficiently by the Cu_2L1 complex ($k_{cat} = 1.2 \times 10^{-2}$ s^{-1}) than the Cu_2L2 complex ($k_{cat} = 2.4 \times 10^{-4}$ s^{-1}) to achieve the rate enhancement observed in the hydrolysis of BDNPP. At present, whether coordination environments of two Cu ions in the Cu_2L1 and Cu_2L2 complexes would influence the hydrolysis rate is not clear. Although Cu ions could have six-coordinated structures, the isolated crystal structures of the CuL3 and CuL4 complexes are known to have five-coordinated square pyramidal structures[13] that available substrate binding sites might not be the same in the Cu_2L1 and Cu_2L2 complexes.

The Cu_2L1 complex also efficiently cleaves HPNPP. It is well known that HPNPP undergoes hydrolysis *via* an transesterification by the intramolecular attack of 2-hydroxy functional group of the substrate.[14a] The pH-rate profiles for BDNPP and HPNPP cleavages by the Cu_2L1 complex are quite similar, suggesting the same active species, probably the aquahydroxy form of the complex or its kinetic equivalent, could be involved in the reactions.[14] The difference in the reactivity of the Cu_2L1 and Cu_2L2 complexes could be attributed from the different binding modes. Phosphate diesters might bind to the Cu_2L1 complex in bidentate fashion (double Lewis acid type activation), while in monodentate fashion to the Cu_2L2 complex. The similar type of activation has been reported in the reaction mediated by the binuclear Cu complex of [12]aneN_3 analog of **L2**, where HPNPP was proposed to bind to both metal ions.[3]

In conclusion, the unsymmetrical Cu_2L1 complex efficiently hydrolyzes phosphate diester BDNPP and HPNPP. Two Cu ions interacts cooperatively, where more than 10 fold rate enhancements is observed over its symmetrical binuclear Cu_2L2 complex. The detailed mechanistic studies including X-ray structure determinations of the Cu complexes are further to be investigated.

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