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CATALYSIS OF PHOSPHODIESTER TRANSESTERIFICATION BY DINUCLEAR Cu(II) COMPLEXES: THE ROLE OF THE SECOND Cu(II) ION

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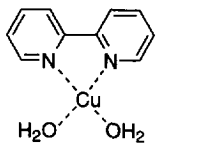
Abstract: Two novel dinuclear Cu(II) complexes are prepared and shown to be highly active catalysts for phosphodiester transesterification. Comparative studies on the dinuclear complexes and corresponding mononuclear complexes reveal that one dinuclear complex achieves high activity by double Lewis acid and double general base catalysis while the other exploits single Lewis acid and double general base catalysis.

Cu(II) complexes **1** and **2** are able to catalyze RNA hydrolysis both individually and as catalytic sites of ribozyme mimics.[1] However, these catalysts are far less active than natural ribonucleases. We recently showed that in neutral media **3** is significantly more active than **2** in catalyzing transesterification of 2-hydroxypropyl-*p*-nitrophenylphosphate (HPNPP), a widely used model compound for RNA.[2,3] This rate increase is due to the general base catalysis provide by the pendent amino groups in **3**. Inspired by phosphodiesterases that are activated by two or more metal ions,[4] we have joined **1** and **2** together with a simple amide spacer to make dinuclear Cu(II) complexes **4** and **5**. In this communication we show that **4** and **5** are much more active than **1**-**3** in catalyzing HPNPP transesterification. A number of highly active dinuclear metal complexes have also been developed by other researchers.[5-8]



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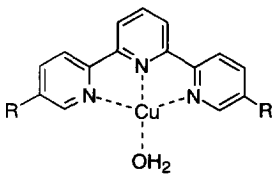
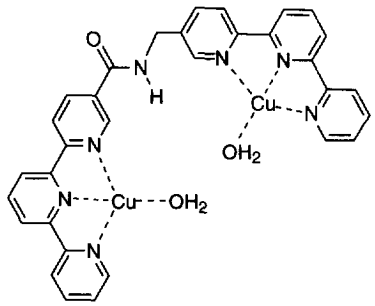


2, R = H
3, R = $CH_2N(CH_3)_2$



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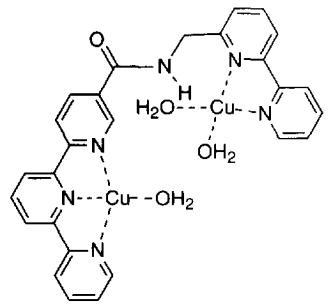


5

The two ligands[9] for **4** and **5** were prepared by reactions of the terpyridine acid chloride with the corresponding amines. The terpyridine acid chloride was prepared by refluxing thionyl chloride with the corresponding acid, which was obtained from oxidation of 5-methyl-2,2':6',2"-terpyridine[10] by potassium permanganate.[11] The aminomethylterpyridine was synthesized as described previously.[10] Preparation of the aminomethylbipyridine began with monomethylation of 2,2'-bipyridine at the 6-position.[12] Subsequent

bromination of the methyl group with NBS followed by Gabriel amine synthesis gave the aminomethylbipyridine in good yield. Dinuclear complexes **4** and **5** were freshly generated from the corresponding chlorides,[13] which were prepared by mixing methanolic solutions of the ligands and Cu(II) chloride.[5a] Mononuclear complex **1** was prepared from 2,2'-bipyridine and Cu(II) chloride by the method of Chin.[14] The values of the first and second pK_a for dinuclear complex **4**, as determined by potentiometric titration, are 7.4 and 8.2, respectively. Similarly, the values of the first and second pK_a for dinuclear complex **5** are 5.3 and 7.4, respectively. These pK_a values are what one would expect based on those of mononuclear complexes **1** (pK_{a1} = 6.6) and **2** (pK_{a1} = 8.1). The pseudo first-order rate constants (k_{uncat} and k_{obs}) for transesterification of HPNPP in the absence and presence of **2** and **3** have been determined previously.[2] The same procedure was used to obtain the values of k_{obs} for **1**, **4**, and **5**.[15] The k_{obs} values are reproducible with an error of less than 5%.

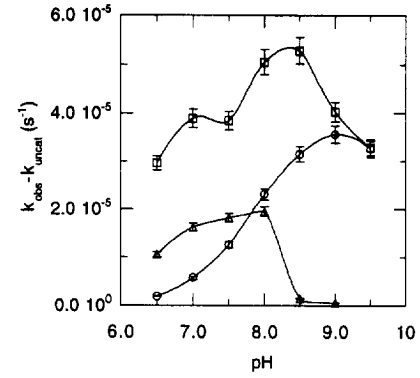
Table 1 summarizes the observed and relative values of k_{obs}-k_{uncat} for transesterification of HPNPP at neutral pH catalyzed by **1**-**5**. The values of k_{rel} for **4** and **5** represent the rate enhancements afforded by the second Cu(II) ion in the two catalysts. As can be seen from Table 1, the second Cu(II) ion increases the activities of **4** and **5** by 51- and 67-fold, respectively, relative to **2**. The second Cu(II) ion increases the activities of **5** by 24-fold relative to **1**. These levels of rate enhancement are comparable to those observed for the highly active dinuclear metal complexes reported previously.[5-8] Very recently, Chin and coworker showed that a La(III) dimer formed at pH > 9 hydrolyzes RNA 10^4 times faster than monomeric Ln(III) ions.[16] However, the La(III) dimer only exists in solution and its exact structure remains to be firmly established.

Table 1. Observed and relative values of k_{obs}-k_{uncat} for transesterification of HPNPP catalyzed by mono- and dinuclear complexes **1**-**5**.[a]

catalyst	**1**	**2**	**3**	**4**	**5**
k_{obs}-k_{uncat} (s^{-1})[b]	1.62×10^{-5}	5.74×10^{-6}[c]	3.89×10^{-5}[c]	2.93×10^{-4}	3.86×10^{-4}
k_{rel}	2.8	1	6.8	51	67(24[d])

[a]In pH 7.0 HEPES buffer (0.05 M) and at 25 °C; [HPNPP] = 0.2 mM, [**1**-**5**] = 2.0 mM. [b]$k_{uncat} = 1.13 \times 10^{-7}$ s^{-1}. [c]Data taken from ref 7. [d]Relative to **1** (k_{rel} = 1).

Two metal ions may cooperate in a number of different ways to promote rapid hydrolysis of phosphodiesters, as is seen in enzymes[4] as well as in model systems.[5-8] To gain some insight into the mechanisms by which the two Cu(II) ions in **4** and **5** cooperate in promoting transesterification of HPNPP, pH-rate profiles for dinuclear complexes **4** and **5** as well as for mononuclear complexes **1**-**3** were obtained (Figure 1). Figure 2 shows the pH dependence of the rate enhancement (k_{rel}) by the two amino groups in **3** and by the second Cu(II) ion in **4** and **5**. As can be seen from Figure 1, the pH-rate profiles for dinuclear complex **4** and corresponding mononuclear complex **2** are quite different. First, the activity of **4** reaches a maximum at a significantly lower pH than that of **2** does. Second, two base groups, one having a pK_a around 6.8 and the other around 7.6, provide general base catalysis in **4**-catalyzed transesterification of HPNPP but only one base group with a pK_a around 8.0 provides general base catalysis in the **2**-catalyzed transesterification of HPNPP. These differences are due to the presence of two Cu(II)-bound water molecules in **4** but only one in **2**, with the



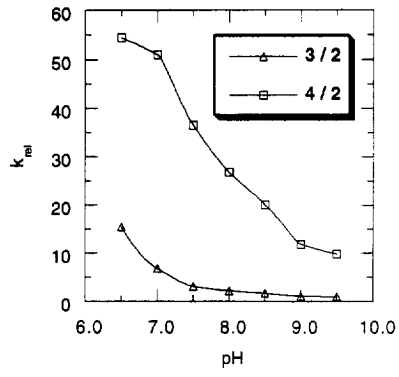
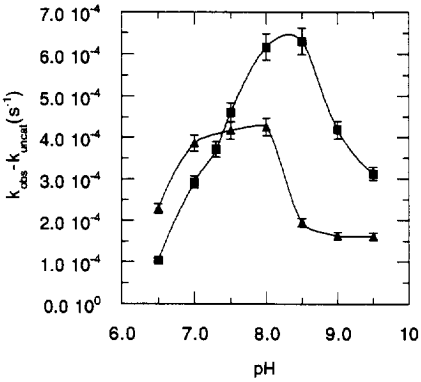


Figure 1. pH-rate profiles for transesterification of HPNPP catalyzed by **1-5**. △, **1**; ○, **2**; □, **3**; ■, **4**; ▲, **5**. The curves for **2** and **3** are taken from ref 7.



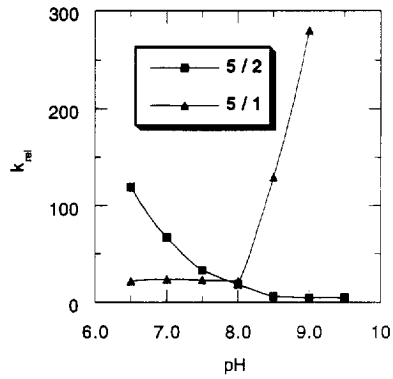
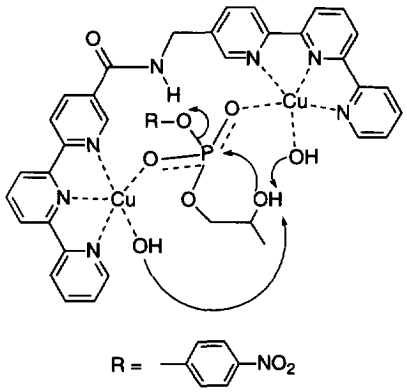


Figure 2. pH dependence of the rate enhancement by the amino groups in **3** and the second Cu(II) ion in **4** and **5** relative to **1** and **2**. The curve for **3/2** is taken from ref 7.

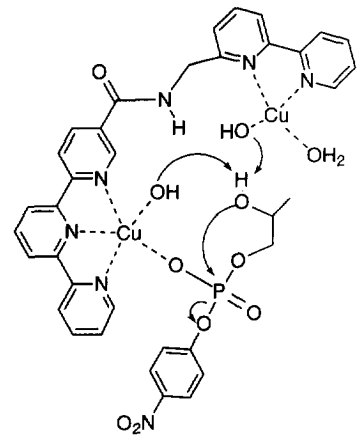
Conditions for Figures 1 and 2: [HPNPP] = 0.2 mM, [**1-5**] = 2.0 mM. Temperature: 25 °C. The reaction media with pH 7.0, 7.5, and 8.0 are HEPES buffers (0.05 M); those with pH 8.5, 9.0, and 9.5 are AMPSO buffers (0.05 M); the one with pH 6.5 is MES buffer (0.05 M).

former being more easily deprotonated than the latter. Indeed, the estimated pK_a values for the two base groups that provide general base catalysis in **4**-catalyzed transesterification of HPNPP are quite close to the values of the first and second pK_a for **4**. The pH-rate profiles for **3**- and **4**-catalyzed transesterifications of HPNPP are somewhat similar in shape since in both cases two base groups with different pK_a's can provide general base catalysis. However, the rate enhancement by the second Cu(II) ion in **4** is much larger than that by the two amino groups in **3** over the entire pH range studied (Figure 1). Furthermore, the rate enhancement by the two amino groups in **3** decreases rapidly with increasing pH and levels off at high pH but the effect of the second Cu(II) ion in **4** decreases with increasing pH over the entire pH range studied (Figure 2). These differences

suggest that the second Cu(II) ion in **4** provides not just general base catalysis through its coordinated hydroxyl group but also Lewis acid activation of the P=O bond for nucleophilic attack.[17] Scheme 1 shows a possible mechanism for **4**-catalyzed transesterification of HPNPP based on the above analysis. This mechanism is similar to that proposed by Chin and coworkers for catalysis of hydrolysis of RNA by a highly active dinuclear Cu(II) complex.[5a] However, in Chin's mechanism only one Cu(II) ion-bound hydroxyl group provides general base catalysis, since the activity of the catalyst decreases when the second Cu(II) ion-bound water molecule is deprotonated. It is likely that this second water molecule acts as a general acid catalyst to facilitate departure of the 5'-hydroxyl group. Transesterification of HPNPP does not require general acid catalysis.[3] Therefore, deprotonation of the second Cu(II) ion-bound water molecule in **4** increases instead of decreasing the activity of the catalyst because it provides additional general base catalysis (Scheme 1).



Scheme 1. Proposed mechanism for **4**-catalyzed traneseterification of HPNPP



Scheme 2. Proposed mechanism for **5**-catalyzed transesterification of HPNPP

The pH-rate profiles for **1**- and **5**-catalyzed transesterification of HPNPP are very similar,[18] as can be seen from Figure 1. This suggests that in **5**-catalyzed transesterification of HPNPP general base catalysis is mainly provided by the Cu(II) ion-bound hydroxyl group in the bipyridine subunit. The Cu(II) ion in the bipyridine part of the catalyst may also provide Lewis acid activation. However, the rate enhancement afforded by the Cu(II)-bipyridine in **5** (Figure 2, **5/2**) shows a pH dependence very similar to that observed for the two amino groups in **3** (Figure 2, **3/2**), suggesting that the two functionalities play similar roles, namely as general base catalysts. Nevertheless, the Cu(II)-bipyridine seems to be more effective than the two peripheral tertiary amino groups in providing general base catalysis (Table 1 and Figure 2). The rate enhancement afforded by the terpyridine subunit in **5** is almost constant from pH 6.5 to 8.0 (Figure 2, **5/1**). The general base catalysis provided by the terpyridine Cu(II)-OH is thus insignificant over this pH range. The large increase in the rate enhancement at pH > 8.0 is probably a combined effect of increased activity of **5** due to increased general base catalysis and decreased activity of **1** (Figure 1).[18] Scheme 2 shows a possible mechanism for **5**-catalyzed transesterification of HPNPP. This mechanism is similar to one Breslow proposed for transesterification of HPNPP catalyzed by a dinuclear Zn(II) complex, involving combined Lewis acid and general base catalysis.[6]

In summary, two dinuclear Cu(II) complexes (**4** and **5**) were prepared by joining the Cu(II)-bipyridine complex (**1**) and the Cu(II)-terpyridine complex (**2**) through an amide group. Both dinuclear complexes exhibit much higher activity than the corresponding mononuclear complexes in catalyzing transesterification of HPNPP. Moreover, the two dinuclear complexes operate by different mechanisms. The bis-terpyridine complex (**4**) promotes transesterification of HPNPP by providing double Lewis acid activation as well as double general base catalysis. On the other hand, the terpyridine-bipyridine complex (**5**) operates by a mechanism of single Lewis acid activation and double general base catalysis.

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3. How the activity of a metal complex for transesterification of a *p*-nitrophenyl phosphodiester like HPNPP relates to its activity for transesterification of RNA has not been systematically evaluated. A review of the recent work by Breslow and coworkers reveals that most Zn(II) complexes show parallel activities for HPNPP and UpU.[6] On the other hand, Chin and coworkers found that a dinuclear complex that cleaves HPNPP with unprecedented efficiency does not cleave ApA, probably because the catalyst is unable to bind to the bulky RNA dimer.[5a] For comparison of the behaviors of RNA and *p*-nitrophenyl phosphodiesters similar to HPNPP as substrates for ribonuclease A and its mutants, see (a) Thompson, J. E.; Raines, R. T. *J. Am. Chem. Soc.* **1994**, *116*, 5467. (b) Breslow, R.; Chapman, W. H. Jr. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 10018.

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9. The two ligands gave satisfactory spectroscopic data. Ligand for **4** (white solid, mp 249-250 °C): ^{1}H NMR (CDCl3/DMSO-d_6) δ 9.25 (s, 1H), 8.98 (t, 1H), 8.68 (m, 4H), 8.62 (m, 2H), 8.46 (m, 6H), 7.95 (m, 5H), 7.37 (m, 2H), 4.75 (d, 2H); ^{13}C NMR (CDCl3/DMSO-d_6) δ 165.4, 157.7, 155.3, 154.9, 154.7, 148.6, 148.5, 148.1, 148.0, 137.6, 136.8, 135.9, 134.6, 129.2, 123.6, 121.2, 121.1, 120.8, 120.6, 120.1, 40.8; HRMS *m/e* for $C_{32}H_{23}N_7O$ calcd 521.1951, obsd 521.1955. Ligand for **5** (white solid, mp 209-210 °C): ^{1}H

NMR ($CDCl_3$) δ 9.21 (d, 1H), 8.73 (m, 3H), 8.63 (d, 1H), 8.51 (m, 2H), 8.38 (m, 3H), 7.97 (t, 1H), 7.87 (m, 4H), 7.36 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 165.4, 158.6, 155.9, 155.6, 155.5, 154.9, 154.3, 149.4, 149.2, 147.5, 138.0, 137.9, 137.1, 136.9, 136.1, 129.7, 123.9, 122.2, 121.6, 121.5, 121.2, 120.9, 120.8, 120.0, 44.7; HRMS *m/e* for $C_{27}H_{20}N_6O$ calcd 444.1698, obsd 444.1693.

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13. The chlorides were characterized by electron spray mass spectrometry which gave major peaks at 753.7 (M^+-35[loss of Cl]) and 640.7 (M^+-70[loss of Cl_2]) for the chlorides corresponding to **4** and **5**, respectively.

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15. The hydrolysis was followed by a Hewlett-Parkard UV-visible spectrophotometer which recorded the absorbance at 400 nm (due to the formation of the *p*-nitrophenoxide ion) as a function of time and $k_{obs} = b/\varepsilon[S]_o$ where b is the slope of the initial linear portion of the absorbance-time trace, ε is the molar extinction coefficient of the *p*-nitrophenoxide ion, and $[S]_o$ is the initial HPNPP concentration.

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17. The rate enhancement afforded by the two amino groups in **3** decreases with increasing pH because the concentration of the Cu(II)-bound hydroxyl group that provides even more effective general base catalysis increases.[2] This rate enhancement levels off at high pH because the concentration of the Cu(II)-bound hydroxyl group reaches a maximum (all Cu(II)-bound water molecules being deprotonated). If the second Cu(II) ion in **4** plays the same role as the two amino groups in **3** then the rate enhancement afforded by this Cu(II) ion should also level off at high pH. That this rate enhancement still decreases at high pH is more consistent with the second Cu(II) ion in **4** also providing Lewis acid activation which is then inhibited at high pH by the competition between the substrate and OH^- ions for coordination to the Cu(II) ion.[6]

18. These two pH-rate profiles are unusual in that the rate increases only slightly from pH 7.0 to 8.0 and decreases sharply from pH 8.0 to 8.5. Complex **1** is known to dimerize when one of the Cu(II) ion-bound water molecules become deprotonated (the first pK_a of **1** is 6.6) and the dimer is catalytically inactive.[14] The unusual flatness of the pH-rate profiles from pH 7.0 to 8.0 is probably due to increased dimerization of the catalysts which slows the rate increase by decreasing the concentration of the active Cu(II)-OH forms. On the other hand, the unusually large rate decrease from pH 8.0 to 8.5 is probably due to inhibition of the activity of the catalysts by AMPSO in the buffer solution. The Cu(II) ion in **1** is more accessible than that in **2** to other potential ligands. Also, the secondary amino group in AMPSO is not as steric hindered as the tertiary amino group in HEPES. Therefore, it is possible that the amino group in AMPSO binds to the Cu(II) ion in **1** and in the bipyridine subunit of **5**. As a result, the Cu(II) ion becomes inaccessible to the substrate and the activities of **1** and **5** are diminished. The above explanation is supported by our unpublished observation that **1** and **5** also show much lower activities in the TAPSO (also a secondary amine) buffers than in the HEPES buffer at the same pH (7.5-8.0).

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