Ester Hydrolysis by a Catalytic Cyclodextrin Dimer Enzyme Mimic with a Metallobipyridyl Linking Group

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Abstract: A β-cyclodextrin dimer with a linking bipyridyl group is synthesized as a catalyst precursor, a holoenzyme mimic. It binds both ends of potential substrates into the two different cyclodextrin cavities, holding the substrate ester carbonyl group directly above a metal ion bound to the bipyridyl unit. The result is very effective ester hydrolysis with good turnover catalysis. For example, a Cu(II) complex accelerates the rate of hydrolysis of several nitrophenyl esters by a factor of 10^4-10^5, with at least 50 turnovers and no sign of product inhibition. In the best case, with an added nucleophile that also binds to the metal ion, a rate acceleration of 1.45×10^7 over the background reaction rate was observed. Hydrolysis by a catalyst with only one cyclodextrin binding group is significantly slower than in the bidentate binding cases. As expected, the binding of a transition state analogue to these catalysts is stronger with the metal ion present than without. This and kinetic evidence point to a mechanism in which the metal ion plays a bifunctional acid−base role, enforced by the binding geometry that holds the substrate functionality right on top of the catalytic metal ion.

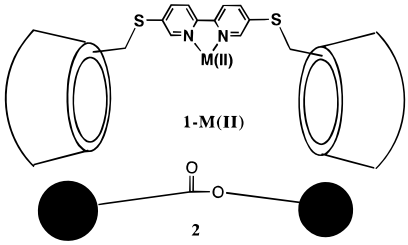
Introduction

Since metal ions are often very effective catalysts, and many enzymes take advantage of this, it has been attractive to synthesize enzyme mimics that combine metal ion catalysis with substrate binding. In fact, the first compound called an "artificial enzyme" in the literature was such an example, with a metal ion bound to a ligand that was attached as an ester to β-cyclodextrin.[1] This catalyst was able to use the metal ion to catalyze the hydrolysis of substrates that would bind into the cyclodextrin cavity but would not otherwise bind to the metal ion. However, the rate accelerations seen were not high in this rather flexible case.

In true enzymes a substrate is rigidly held in the active site, by multiple binding interactions with the enzyme. The catalytic groups of the enzyme are held next to the substrate, so that little entropy must be expended in approaching the transition state and little conformational enthalpy. In order to achieve this in an enzyme mimic, the catalyst−substrate complex must be reasonably stable, and pre-organized into the reactive geometry.

It seemed to us that such requirements could be achieved if the catalyst was a cyclodextrin dimer, for double binding of the substrate, in which the linker in the dimer carried an effective rigidly held catalytic group. The substrate would have to have hydrophobic groups at both ends, to bind into the two cyclodextrin groups, with an ester or other potentially reactive group in the middle so it would end up next to the catalytic group. Our previous work indicated that various cyclodextrin dimers could show very strong binding of suitable ditopic substrates that could occupy both cyclodextrin cavities, and that, once the substrate was cut in half by the catalytic group, the products would bind much more weakly.[2−4] Thus product inhibition was not expected to be a problem.

Computer and solid models indicated that compound **1** would fit the requirements, its metal complex being able to place the metal ion right on top of the ester group of various substrates of the general form **2**. Thus we synthesized **1** and examined



the binding and catalytic properties of some of its metal ion complexes. The complexes indeed proved to be outstanding catalysts, showing large rate accelerations with high catalytic turnover and no evidence of inhibition by the product fragments. Detailed studies indicate the mechanism of the catalytic process.[5]

Results and Discussion

Syntheses. The synthesis of the cyclodextrin bipyridyl dimer **1** is outlined in Scheme 1. The reduction of 2-chloro-5-nitropyridine by iron and ammonium chloride in a water−methanol mixed solvent gave 5-amino-2-chloropyridine (**3**),[6] whose amino group was protected with benzaldehyde to form *N*-benzylidene-5-amino-2-chloropyridine (**4**). The coupling of **4** with Ni(II)−Zn[7] gave the protected bipyridine **5**. The deprotection of **5** formed the desired product **6**. After conversion of 5,5′-diamino-2,2′-bipyridine (**6**) into the bis-diazonium compound, and reaction of the bis-diazonium salt of 5,5′-diamino-2,2′-bipyridine with potassium ethyl xanthate following

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(1) Breslow, R.; Overman, L. E. *J. Am. Chem. Soc.* **1970**, *92*, 1075−1077.

(2) Breslow, R.; Greenspoon, N.; Guo, T.; Zarzycki, R. *J. Am. Chem. Soc*. **1989**, *111*, 8296−8297.

(3) Breslow, R.; Chung, S. *J. Am. Chem. Soc*. **1990**, *112*, 9659−9660.

(4) Breslow, R.; Halfon, S. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6916−6918.

(5) For a preliminary report of some of this work see the following: Breslow, R.; Zhang, B. *J. Am. Chem. Soc*. **1992**, *114*, 5882−5883.

(6) Ramadas, K.; Srinivasan, N. *Syn. Commun.* **1992**, *22*, 3189−3195.

(7) Iyoda, M.; Otsuka, H.; Sato, K.; Nisato, N.; Oda, M. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 80−87.

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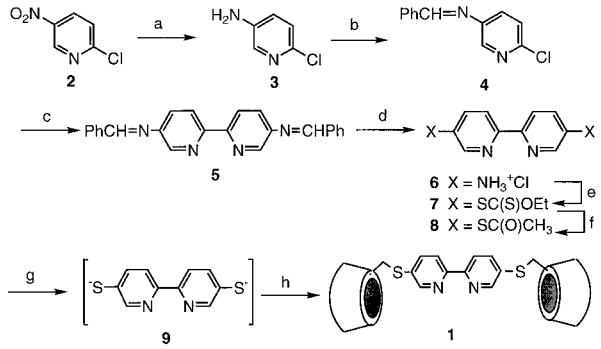
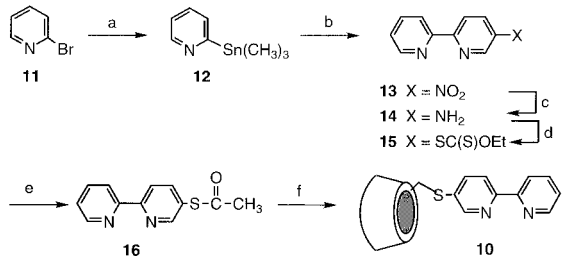
Scheme 1



(a) Fe/NH_4Cl, MeOH, H_2O,rt; (b) PhCHO, $MgSO_4$, Et_3N, CH_2Cl_2, rt, 24 h; (c) $NiBr_2(PPh_3)_2$, Zn/$Et_4N^+I^-$/THF, 50-80 °C, 20 h; (d) 1 N HCl, reflux; (e) (1) $NaNO_2/H_3^+O$, (2) KSC(S)OEt, H_2O, 65-70 °C; (f) (1) 20% KOH/EtOH, reflux, (2) CH_3COCl, 0-5 °C; (g) NH_3/MeOH, rt, 1 h; (h) mono-6-iodo-beta-cyclodextrin, DMF, 60-65 °C, 3 h.

Scheme 2

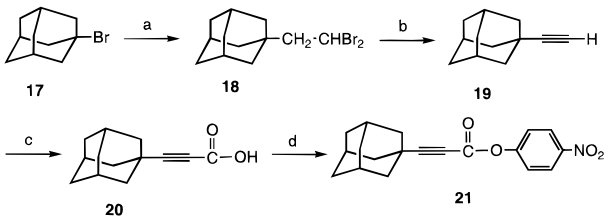


(a) (1) BuLi/Et_2O, -78 °C, (2) $(CH_3)_3SnCl$, THF, -78 °C; (b) 2-chloro-5-nitropyridine, $Pd(Ph_3P)_2Cl_2$, THF, reflux, 24 h; (c) Pd^0 on activated carbon(10%), $NaBH_4$, MeOH, rt, 5 h; (d) (1) $NaNO_2/H_3^+O$, (2) KSC(S)OEt, H_2O, 65-70 °C; (e) (1) 20% KOH/EtOH, reflux; (2) CH_3COCl, 0-5 °C; (f) (1) NH_3/MeOH, rt, 1 h; (2) mono-6-iodo-beta-cyclodextrin, DMF, 60-65 °C, 3 h.

Katz's procedure,[8] 2,2′-bipyridine-5,5′-bis(ethyl xanthate) (**7**) was obtained in 35% yield. The bis(ethyl xanthate) **7** was refluxed with 20% ethanolic potassium hydroxide, then treated with acetyl chloride to give 2,2′-bipyridyl-5,5′-bisthioacetate **8**. Compound **8** was deprotected by ammonia saturated methanol solution into dianion **9**, which was treated *in situ* with 6-deoxy-6-iodo-*β*-cyclodextrin[9] in DMF to afford the cyclodextrin dimer **1**. The dimer **1** was purified by reverse-phase chromatography, eluted with a gradient ranging from water to 40% methanol−water.

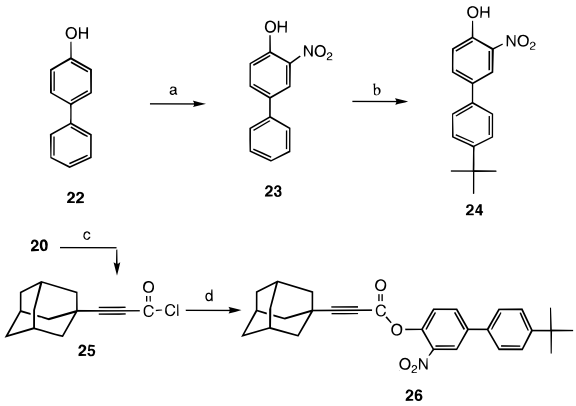
We also synthesized a bipyridyl cyclodextrin monomer **10**, for comparison with dimer **1** in binding and catalytic studies. The synthesis of monomer **10** is described in Scheme 2. The key step is the coupling of two pyridine rings by Pd(II) catalyst. 2-Bromopyridine (**11**) was treated with *n*-butyllithium and then with trimethyltin chloride in THF.[10] The coupling reaction of 2-trimethylstannylpyridine (**12**) with 2-chloro-5-nitropyridine was catalyzed[11] by $Pd(Ph_3P)_2Cl_2$ to give 5-nitro-2,2′-bipyridine (**13**) with 76% yield. Compound **13** was reduced by sodium borohydride with catalysis by 10% palladium on activated carbon to convert it to 5-amino-2,2′-bipyridine (**14**). The following steps to prepare bipyridyl cyclodextrin monomer **10** were by essentially the same method as was described for the bipyridyl dimer **1**. The 5-amino-2,2′-bipyridine (**14**) was converted to 2,2′-bipyridine-5-ethyldithiocarbonate (**15**) through the diazonium intermediate. The dithiocarbonate **15** was hydrolyzed and treated with acetyl chloride to give 2,2′-bipyridyl-5-thioacetate (**16**), which was deprotected by NH_3, then directly reacted with 6-deoxy-6-iodo-*β*-cyclodextrin to give the desired bipyridyl cyclodextrin monomer **10**.

Scheme 3



(a) Vinyl bromide, $AlBr_3$, -65 °C; (b) diethylene glycol, KOH, reflux; (c) (1) MeLi/Ethyl ether; (2) CO_2, -78 °C; (3) HCl (aqueous); (d) DCC, DMAP, *p*-nitrophenol, DMF.

Scheme 4



(a) HNO_3, $CHCl_3$, 0 °C; (b) $(CH_3)_3CCl$, $AlCl_3$, 100 °C; (c) oxalyl chloride, benzene, DMF (4 drops); (d) **24**, benzene, Et_3N.

Ester **21** was synthesized as shown in Scheme 3. 1-Bromoadamantane (**17**) was treated with vinyl bromide; heating the product with potassium hydroxide yielded 1-adamantylacetylene (**19**). Then **19** was converted to 1-adamantylpropiolic acid (**20**) by treatment with base, trapping the anion with carbon dioxide, then acidifying with HCl. Subsequently, carboxylic acid **20** was coupled with *p*-nitrophenol, to afford ester **21** in 67% yield.

The synthesis of *p*-(4-*tert*-butylphenyl)-*o*-nitrophenyl 1-adamantylpropiolate (**26**) is shown in Scheme 4. 4-Phenylphenol (**22**) was employed as the starting material, which was nitrated in the ortho position to give compound **23**; subsequent Friedel−Crafts alkylation furnished *p*-(4-*tert*-butylphenyl)-*o*-nitrophenol (**24**) in good yield. 1-Adamantylpropiolic acid (**20**) was converted to the acyl chloride **25**, which was then reacted with nitrophenol **24** to afford ester **26**.

Detailed procedures for these syntheses, and data on characterization of the compounds, are given in the supporting information section.

Binding Energy of Dimer 1 with Substrates and with Transition State Analogues. We have investigated the binding of **1** with the guests **27**−**29** (Scheme 5) with and without Zn(II). The results for the binding of dimer **1** with guests are listed in Table 1. Compounds **28** and **29** were selected as transition state analogues; phosphodiester anions are often used as analogues of the tetrahedral transition state for ester and amide hydrolysis in antibodies and protein enzymatic studies. The binding constants of dimer **1** with carbonate **27** and phosphate **28** in HEPES buffer at pH 7.0 are very similar in the absence of Zn(II), 1.43×10^6 M^{-1} and 1.0×10^6 M^{-1}, respectively. The binding of dimer **1** and bis(1-adamantylethyl) phosphate (**29**) with Zn(II) is 50-fold stronger than without Zn(II), and reaches a binding constant as high as 10^9 M^{-1}. A similar binding enhancement (55-fold) was obtained with phosphate **28** binding into dimer **1** in the presence of zinc ion, but the constant for the neutral carbonate **27** binding into dimer **1** is only 5-fold higher with zinc ion than that without zinc ion.

(8) Katz, L.; Schroeder, W.; Cohen, M. *J. Org. Chem.* **1954**, *19*, 710.
(9) Tabushi, I.; Kuroda, Y.; Mochizuki, A. *J. Am. Chem. Soc.* **1980**, *102*, 1152−53.
(10) Jutzi, P.; Gilge, U. *J. Organomet. Chem.* **1983**, *246*, 163.
(11) Bailey, T. R. *Tetrahedron Lett.* **1986**, *27*, 4407.

Scheme 5

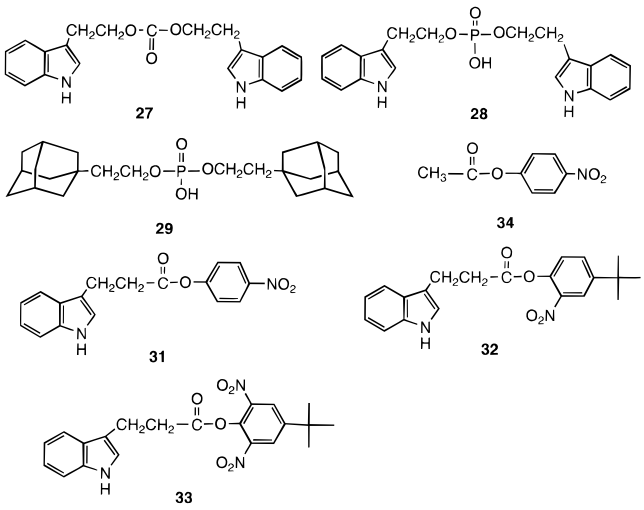


Table 1. Binding Constants of Guests with Dimer **1** in Water[a]

guest	K_a with Zn(II) (M^{-1})	K_a (M^{-1})	K_a(Zn(II))/K_a
carbonate **27**	$(7.70 \pm 0.10) \times 10^6$	$(1.43 \pm 0.20) \times 10^6$	5
phosphate **28**	$(5.47 \pm 0.17) \times 10^7$	$(1.00 \pm 0.34) \times 10^6$	55
phosphate **29**	$(1.02 \pm 0.05) \times 10^9$	$(2.05 \pm 0.24) / x \times 10^7$	50

[a] Values determined by competition with DiTNS **30** (see refs 2−5) in 10 mM HEPES buffer solution (pH 7.0) at ambient temperature (25 °C); average of more than three independent runs.

These results suggest that the two hydrophobic ends of the ditopic guests bind strongly into the two cyclodextrin cavities of dimer **1**, and that for phosphodiesters there is an extra interaction of the phosphate anion with the metal cation. This artificial metalloenzyme is thus much like a true enzyme, in that binding with substrate in the transition state is tighter than in the ground state. This is the first example of an artificial metalloenzyme effectively binding a tetrahedral, negatively charged phosphate transition state analogue for hydrolysis of an ester, and indicates that the bipyridyl dimer **1** should have catalytic power for ester hydrolysis with metal ions. However, the difference of the binding constants for the carbonate **27** and the transition state analogue **28** with the Zn(II) complex of dimer **1** greatly underestimates the magnitude of the rate accelerations we observed.

Ester Hydrolysis. The first investigation of ester hydrolysis employed *p*-nitrophenyl 3-indolepropionate (**31**) under various conditions in 10 mM HEPES buffer (pH 7.0 or pH 8.0) at 37 °C; the results are listed in Table 2. Comparison of the catalyzed and uncatalyzed (entry 5) reaction rates obtained at pH 7.0 indicated enhancements of 18 300 for dimer **1** with Cu(II) (entry 11) and 60 for dimer **1** without Cu(II) (entry 10). When the reaction catalyzed by **1** and Cu(II) (entry 4) was conducted at pH 8.0, the rate enhancement was 10 400. With simple β-cyclodextrin, the rate enhancement observed for the hydrolysis of substrate **31** was only 25 (entries 8 and 9) with and without Cu(II) at pH 7.0. With 2,2′-bipyridine (entry 7), the hydrolysis rate of ester **31** increased by a factor of only 12 with Cu(II).

The large rate acceleration by our enzyme mimic clearly comes from the combination of the binding sites (two hydrophobic cavities) and the catalytic site (metal ion ligand). The two hydrophobic cavities bind the substrate and juxtapose the ester functional group and the catalytic metal ion of the artificial metalloenzyme. The Cu(II) complex of dimer **1** is a successful catalyst for the hydrolysis of this ester and others (*vide infra*) under physiological conditions of temperature and pH, in contrast to many enzyme model studies that have employed extreme conditions.

Table 2. Hydrolysis of *p*-Nitrophenyl 3-Indolepropionate (**31**) at 37 °C[a]

entry	catalyst	M(II)	pH[b]	k_{obs} (s^{-1})[c]	k_{rel}[d]
1			8.0	1.0×10^{-7}	1
2		Cu(II)	8.0	3.2×10^{-7}	3
3	bipyridine	Cu(II)	8.0	3.7×10^{-7}	4
4	bipyridyl dimer **1**	Cu(II)	8.0	$(1.04 \pm 0.02) \times 10^{-3}$	10 400
5			7.0	3.0×10^{-8}	1
6		Cu(II)	7.0	2.5×10^{-7}	8
7	β-cyclodextrin		7.0	7.1×10^{-7}	24
8	β-cyclodextrin	Cu(II)	7.0	$(7.39 \pm 0.09) \times 10^{-7}$	25
9	bipyridyl dimer **1**		7.0	$(1.81 \pm 0.42) \times 10^{-6}$	60
10	bipyridyl dimer **1**	Cu(II)	7.0	$(5.49 \pm 0.40) \times 10^{-4}$	18 300

[a] Reactions were performed at 37 °C in 10 mM HEPES buffer solution, in 1.0×10^{-4} M bipyridyl dimer **1**, cyclodextrin, and bipyridine; 5.0×10^{-5} M *p*-nitrophenyl 3-indolepropionate (**31**), 5.0×10^{-4} M $CuCl_2$. [b] The pH was checked at the beginning and the end of each run to hold to within ±0.1 unit. [c] The rate constants were obtained by analyzing the data using the Kore program and are averages of at least two runs. All correlation coefficients were ≥0.9999. [d] Rate constants relative to background (no catalyst).

Table 3. Hydrolysis of Esters Catalyzed by Bipyridyl Cyclodextrin Dimer **1** in the Presence of Cu(II) at 37 °C[a]

entry	substrate	pH	k_{obs} (s^{-1})[b]	k_{un} (s^{-1})[c]	k_{rel}[d]
1	ester **34**	8.0 ± 0.1	9.61×10^{-5}	1.42×10^{-5}	7
2	ester **32**	8.0 ± 0.1	1.35×10^{-4}	1.50×10^{-7}	900
3	ester **33**	8.0 ± 0.1	1.74×10^{-4}	1.00×10^{-7}	1 740
4	ester **31**	7.0 ± 0.1	5.49×10^{-4}	3.00×10^{-8}	18 300
5	ester **31**	8.0 ± 0.1	1.04×10^{-3}	1.00×10^{-7}	10 400
6	ester **21**	7.0 ± 0.1	6.76×10^{-3}	3.00×10^{-8}	225 000
7	ester **21**	8.0 ± 0.1	1.20×10^{-2}	1.80×10^{-7}	66 700

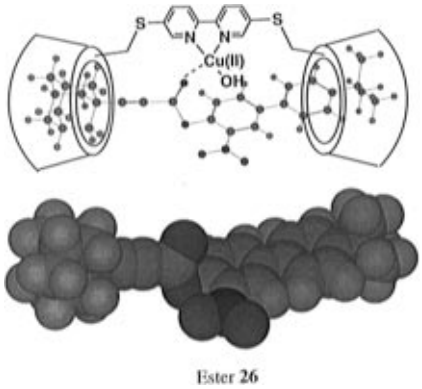
[a] All solutions were 1.0×10^{-4} M bipy-dimer **1**, 2.0×10^{-4} M $CuCl_2$, and 6.0×10^{-5} M substrates. Reactions were conducted in 10 mM HEPES buffer solution for substrates **21**, **31**, and **34**. Reactions were carried out in 60% 10 mM HEPES buffer and 40% DMSO solution for substrates **32** and **33**. Rate constants were obtained by averaging two or more kinetic runs. Standard deviations were less than 8% of the rate constants in the table. [b] Reactions were monitored to >95% completion. The rate constants were obtained by analyzing the data using the Kore program; the correlation coefficients were ≥0.9999. [c] k_{un} were obtained by calculating the initial rate constant without **1-Cu(II)**. [d] $k_{rel} = k_{obs}/k_{un}$.

We have synthesized some other esters (Scheme 5) to study their hydrolysis catalyzed by bipyridyl dimer **1** with Cu(II); their pseudo-first-order rate constants are shown in Table 3. The hydrolysis of **34** showed only a 7-fold rate enhancement (entry 1); it can bind into only one cyclodextrin unit of dimer **1**. We expected that ester **21**, containing an adamantane group, should bind more strongly to **1** than **31**. In fact, the hydrolysis rate of ester **21** with **1** and $CuCl_2$ at pH 7.0 and 37 °C was 225 000 times (entry 6) faster than the rate of uncatalyzed hydrolysis. When an excess of substrate **21** was employed, at least 50 turnovers were seen in the ester hydrolysis catalyzed by dimer **1** and $CuCl_2$.

Esters **32** and **33** contain a *tert*-butylphenyl group which should bind strongly with dimer **1**. However, the solubility of substrates **32** and **33** is poor in aqueous solution, so the hydrolyses of esters **32** and **33** were performed in 40% DMSO−buffer solution. This solvent system decreases the binding ability of these esters with bipyridine dimer **1** (*vide infra*), and decreases k_{obs}.

Ester **26** is long enough for binding into two cavities of dimer **1** (Scheme 6). Since the solubility of ester **26** in water is poor, the hydrolytic experiments of ester **26** were performed in 70% methanol−aqueous buffer or DMSO−aqueous buffer solvent systems. The results are listed in Table A (Supporting Information) and plotted in Figure A (Supporting Information). The data show that the rate constant for the Cu(II) complex of dimer

Scheme 6



Ester **26**

Table 4. Pseudo-First-Order Rate Constants for Catalytic Hydrolysis of Ester **26** by Dimer **1** with Cu(II) in Different Solvents[a]

entry	solvent	k_{obs} (s^{-1})[b]	log k_{obs}	E_T(30) (kcal/mol)[c]
1	water	5.10×10^{-2}[d]	−1.2924	63.0
2	ethylene glycol	$(2.47 \pm 0.05) \times 10^{-3}$	−2.6073	55.9
3	MeOH	$(3.40 \pm 0.06) \times 10^{-3}$	−2.4672	55.5
4	formamide	$(5.75 \pm 0.30) \times 10^{-4}$	−3.2403	55.2
5	EtOH	$(3.03 \pm 0.15) \times 10^{-4}$	−3.5185	51.9
6	DMSO	$(6.78 \pm 1.00) \times 10^{-5}$	−4.1688	45.0
7	DMF	$(3.10 \pm 1.50) \times 10^{-6}$[e]	−5.5086	43.8

[a] Reactions were run at 30 ± 1 °C in 80% organic solvent and 20% 20 mM Hepes buffer (pH ~ 7.0). Product formation was monitored by absorbance at 445 nm. [b] k_{obs} was calculated using the Kore program, with product ≥95% and the correlation coefficients ≥0.9999. [c] E_T(30) values from ref 20. [d] k_{obs} was calculated by extrapolating to 0 % volume of methanol from different % volumes of methanol. [e] k_{obs} was calculated from the initial rate using the Passage fitting program.

1 is ca. 100 times higher than that with monomer **10**. Since the solvents used decrease hydrophobic binding, the difference could be even larger in water solution.

Solvent Effects on Catalysis. We have carried out kinetic studies in various organic solvents, employing the Cu(II) complex of dimer **1** as catalyst and **26** as the substrate in 20 mM HEPES (pH 7.0) at 25 °C. Water is the best solvent for inducing strong association between apolar binding partners.

The k_{obs} of ester **26** hydrolysis catalyzed by 1-Cu(II) decreases with increasing methanol content (Figure B, Supporting Information). Methanol is less polar than water and interferes with hydrophobic binding of the substrate into the cyclodextrin cavities.

Apolar complexation is not limited to water or alcohols but occurs in solvents of all polarity.[12,13] However, a dramatic solvent dependence was observed for the catalytic hydrolysis of ester **26** by dimer **1-Cu(II)**. When the solvent was changed from water, the most polar solvent, to DMF, the least polar solvent, the k_{obs} of catalytic hydrolysis of ester **26** by **1-Cu(II)** decreased from 5.10×10^{-2} s^{-1} to 3.10×10^{-6} s^{-1} (Table 4), a factor of over 10^4.

A strong linear relationship exists between the k_{obs} and the solvent polarity parameter E_T(30) of the various solvents (Figure 1). The results are in excellent agreement with some binding data in the literature.[14] Strong correlation between E_T(30) and binding free energies in related host−guest systems have also been found for binary aqueous solvent mixtures.[14−16] Although the added solvents may also affect the behavior of the catalytic groups, such a large solvent effect reflects primarily the strength of hydrophobic binding.

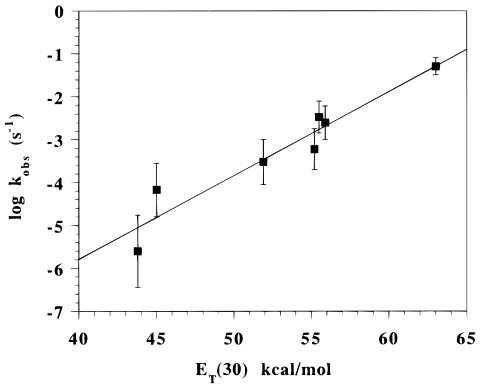


Figure 1. Organic solvent effects on the catalytic hydrolysis of ester **26** with bipy-dimer **1** and copper(II) at 30 °C (data from Table 4).

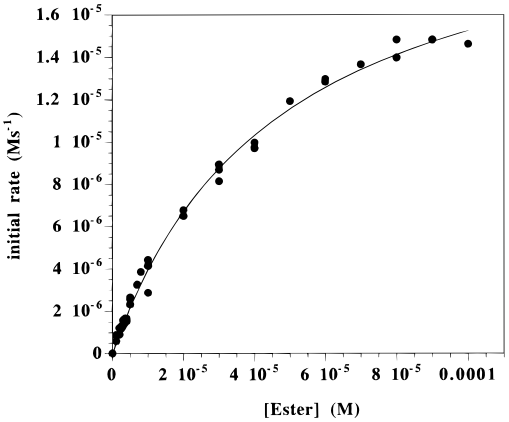


Figure 2. Catalytic hydrolysis of ester **26** by bipy-dimer **1** (0.05 mM) with Cu(II) in 80%(v/v) DMSO/20 mM HEPES (pH 7.0) at 25 °C. Best-fit values of V_{max} and K_m were obtained by fitting the data to the Michaelis−Menten equation by the KaleidoGraph program. The continuous line corresponds to initial rate = V_{max}[ester]/(K_m + [ester]) with $V_{max} = 2.24 \times 10^{-5}$ M s^{-1} and $K_m = 4.69 \times 10^{-5}$ M.

Kinetic Characteristics. The dependence of the initial rate of **1-Cu(II)**-catalyzed hydrolysis of the ester substrate **26** on the substrate concentration for a typical set of data is shown in Figure 2 as a v vs [S] plot and in Figure C (Supporting Information) as a $1/v$ vs 1/[S] plot. In each case the continuous line corresponds to the best fit. Values of the parameters V_{max} and K_m were obtained by fitting the initial rate vs [S] data to the Michaelis−Menten equation. The value of K_m for the **1-Cu(II)**-catalyzed hydrolytic reaction is 47 μM, a value consistent with strong double binding. The reactions were carried out in 80% DMSO solvent, and in water the binding affinity would be much higher. The k_{cat} for the ester hydrolysis catalyzed by **1-Cu(II)** is 0.45 s^{-1}. The rate enhancement (k_{cat}/k_{uncat}) for ester **26** is 1.45×10^7.

Mechanism. We have studied ester hydrolysis by dimer **1** with copper(II) at 37 °C, using ester **31** in 10 mM buffer at various pH's. The pH vs rate profiles in Figure 3 are for the hydrolysis of *p*-nitrophenyl 3-indolepropionate (**31**) by bipyridyl dimer **1** with Cu(II) and by buffer only. The difference in the hydrolysis rates between the reaction catalyzed by dimer **1** and Cu(II) and the reaction run with only buffer is the greatest in the pH range of 6.5 to 8.0. With respect to rate, the buffer

(12) Siegel, B.; Breslow, R. *J. Am. Chem. Soc.* **1975**, *97*, 6869.
(13) Smithrud, D. B.; Diederich, F. *J. Am. Chem. Soc.* **1990**, *112*, 339.
(14) Diederich, F.; Smithrud, D. B.; Sanford, E. M.; Wyman, T. B.; Ferguson, S. B.; Carcanague, D. R.; Chao, I.; Houk, K. N. *Acta Chem. Scand.* **1992**, *46*, 205−215.
(15) Schneider, H.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442−6448.
(16) Ferguson, S. B.; Seward, E. M.; Sanford, E. M.; Hester, M.; Uyeki, M.; Diederich, F. *Pure Appl. Chem.* **1989**, *61*, 1523−1528.

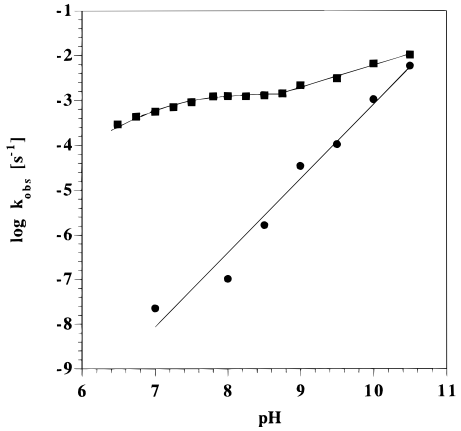
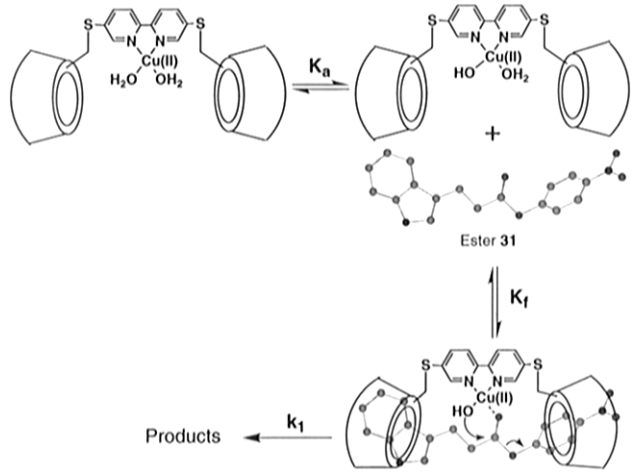


Figure 3. pH−rate (log k_{obs}) profile for the hydrolysis of *p*-nitrophenyl 3-indolepropionate (**31**) by bipyridyl cyclodextrin dimer **1** (■) and buffer only (●). Reactions were run at 37 °C in buffer solutions using 10 mM MES for pHs between 5.5 and 6.5, 10 mM HEPES for pHs between 6.70 and 8.25, 10 mM CHES for pHs between 8.50 and 9.50, and 10 mM CAPES for pHs between 10.00 and 10.50.

Scheme 7



hydrolysis of ester **31** shows a linear pH dependence, whereas the **1-Cu(II)**-catalyzed ester hydrolysis shows a different pH dependence.

The pH profile in Figure D (Supporting Information) is expanded from Figure 3 at pH 6.5 to 9.0 for the dimer catalyzed hydrolysis reaction. This pH profile shows a pH-independent plateau and a pH-dependent range. The experimental data fit the theoretical curve described by eq 1, where K_f is the binding constant of **1-Cu(II)** with ester **31**, K_a is the acid dissociation constant for the coordinated water molecule, and k_1 is the rate constant of the rate-determining step. The best fitting of the data gave pK_a = 7.15, $k_1 = 2.05 \times 10^{-4}$ s^{-1}, and $K_f = 7.0 \times 10^4$ M^{-1}.

$$k_{obs} = k_1 K_f \frac{K_a}{K_a + [H^+]} \quad (1)$$

We propose the mechanism shown in Scheme 7, in which the substrate binds to the dimer and is attacked by hydroxide bound to copper, from a bound water with pK_a = 7.15.[17] There are other kinetically equivalent mechanisms that seem less likely.

Effects of Metal Ions and Ligand. In 1965 we reported that the zinc or nickel ion complexes of (*E*)-2-pyridine-carbaldehyde oxime (PAO, **35**) are remarkably active catalysts for the hydrolysis of 8-acetoxyquinoline-5-sulfonate.[18] Thus we have examined the cleavage of ester **31** with added PAO **35** bound to the dimer **1**−metal ion complex (Figure E, Supporting Information). The cleavage of ester **21** by dimer **1** with various metal ions in the presence of PAO **35** was examined in 20 mM HEPES buffer (pH 7.0 ± 0.1) at 37.0 ± 0.2 °C and followed by monitoring the formation of *p*-nitrophenoxide by UV at 400 nm. The results are listed in Table 5 (and displayed in Figure F of Supporting Information). A rate enhancement of 1.7×10^6-fold over the background reaction rate was observed for cleavage of ester **21** by **1**-Zn(II) with PAO **35** (entry 14). In line with our previous work, we suggest the mechanism shown in Scheme 8. As can be seen from Table 5, the relative catalytic activities of metal ions in the presence of PAO **35** ligand were different from those in the absence of PAO. The order of catalytic activity with PAO **35** is Zn(II) > Ni(II) > Eu(III) > Tb(III) > Cu(II) > Co(II). The order of catalytic activity without PAO **35** is Cu(II) > Ni(II), Zn(II). As Scheme 8 shows, we propose that the Zn(II) becomes pentacoordinate in the transition state, which is less likely for Cu(II). Without PAO, four-coordination by the metal ion is enough. In our previous work,[18] we showed that the acylated catalyst that is formed by the mechanism of Scheme 8, with an acyl group on the oxygen of PAO, is then hydrolyzed by the metal ion. Thus turnover catalysis was seen with such systems, but we have not examined this question in the present case.

Table 5. Catalytic Hydrolysis of Esters by Dimer **1** in pH 7.0 Buffer at 37 °C[a]

entry	ligand[b]	metal ion	k_{obs} (s^{-1}) × 10^3 [c]	k_{rel}
		Ester **31**		
1			0.000030[d]	1
2		Ni(II)	0.30	10 000
3		Cu(II)	0.55	18 000
4	PAO **35**	Co(II)	0.14	4 700
5	PAO **35**	Cu(II)	0.27	0 900
6	PAO **35**	Tb(III)	0.55	18 000
7	PAO **35**	Eu(III)	0.62	21 000
8	PAO **35**	Ni(II)	1.77	59 000
9	PAO **35**	Zn(II)	8.89	300 000
		Ester **21**		
10			0.000030[d]	1
11		Zn(II)	1.2	40 000
12		Cu(II)	6.8	220 000
13	PAO **35**	Ni(II)	11.2	371 000
14	PAO **35**	Zn(II)	51.2	1700 000

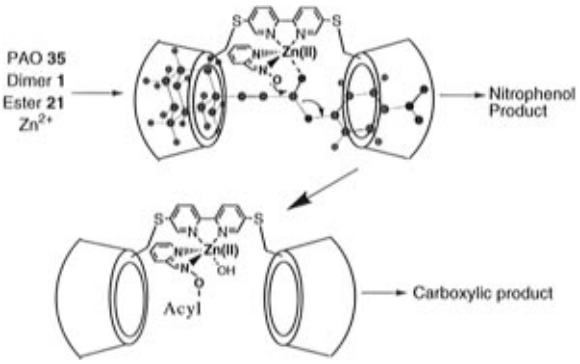
[a] All solutions were 1.0×10^{-4} M in bipy-dimer **1**, 1.0×10^{-3} M in metal ion and 6.0×10^{-5} M in substrate. Reactions were carried out in 10 mM HEPES buffer solution (pH 7.0) at 37 °C. [b] PAO **35** is 2-pyridinecarbaldehyde oxime. [c] Reactions were monitored to >95% completion. The rate constants were obtained by analyzing the data using the Kore program; the correlation coefficient s were ≥0.9999, and are the average of two or more kinetic experiments. The errors in k_{obs} were <8%. [d] Uncatalyzed rate constants of esters.

Summary. The catalysts that can bind a substrate at both ends so as to hold a substrate ester group right above a catalytic functionality are the most effective. They perform ester hydrolysis with turnover and very high rate accelerations. When the catalytic group is only a metal ion, Cu(II) is more effective than Zn(II) or Ni(II). However, when the catalyst also carries a nucleophilic bound oxime ligand, the best metal ion is Zn(II). The differences are reasonable in terms of the mechanisms involved and the coordinating abilities of the different metal ions.

(17) The pK_a of the Cu(bipy)$(OH_2)_2$ complex is 7.8: Rosch, M. A., Trogler, W. C. *Inorg. Chem.* **1988**, *29*, 2409−2416.

(18) Breslow, R.; Chipman, D. *J. Am. Chem. Soc.* **1965**, *87*, 4195−4196.

Scheme 8



Experimental Section

General. ^{1}H-NMR spectra were recorded on a Varian VXR 200 or 400 MHz spectrometers. ^{13}C-NMR spectra were recorded on a 75 MHz spectrometer. All spectra were with the residual solvent peaks as reference signals. Mass spectra were recorded on a Nermag R-1010 instrument (for chemical ionization(CI) or electron impact ionization(EI) spectra) or a Jeol JMS-DX-303 HF instrument (for FAB spectra). pH values were measured on an Orion 701A ionalyzer with a glass electrode after calibration to standard buffer solutions. Solvents, drying agents, and inorganic salts were obtained from Fisher Scientific Co., Amend Drug and Chemical Co., Sigma Chemical Co., or Aldrich Chemical Co. unless otherwise indicated. β-Cyclodextrin was obtained from the American Maize-Product Co. Diethyl ether (Et_2O) was dried by distillation under argon from K^0-Na^0 amalgam/benzophenone, tetrahydrofuran (THF) was dried by distillation under argon from K^0/benzophenone, and benzene and methylene chloride (CH_2Cl_2) were dried by distillation from calcium hydride. Anhydrous *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide, pyridine, and acetonitrile were obtained in Sure/Seal™ bottles from Aldrich Chemical Co. Most starting reagents were obtained from Aldrich.

Kinetics. Aqueous buffers were prepared with deionized water and pH value were adjusted with 1 N NaOH or 1 N HCl solution. Stock solutions of metal ions, substrates, and catalysts were prepared in the appropriate buffer solution, except for water insoluble substrates which were prepared in CH_3OH or DMF. The pH was checked at the beginning and the end of each run to hold to within $\pm$0.1 unit.

All reactions for *p*-nitrophenol product were monitored by following absorbance change against time at 400 nm. A kinetic run was initiated by adding 1.00 mL of 100 μM bipy-dimer **1** dissolved in 10 mM HEPES solution and 10 μL of metal ion stock solution to make the solution 500 μM in metal ion and brought to 37 $\pm$ 0.1 °C in the spectrophotometer chamber. A 6 μL sample of substrate solution was injected (to make the solution 60 μM in substrate), shaken vigorously, and the absorbance at 400 nm monitored as a function of time.

For the catalytic reactions (fast reactions), the pseudo-first order rate constants were obtained by analyzing the data using the Kore program.[19] For the uncatalytic reactions (slow reactions), the rate constants were obtained by an initial rate treatment. The final product absorbance was determined by making a solution with the same concentrations of products and catalyst, and with the same buffer solution used as the kinetic runs.

Syntheses. The synthetic sequences are outlined in the paper. The detailed procedures are available in Supporting Information.

Acknowledgment. This paper is dedicated to the memory of Paul Dowd. This work has been supported by grants from the National Institutes of Health and the Office of Naval Research.

Supporting Information Available: Full experimental details on the syntheses, product characterization, and kinetic studies, including one table and six figures (18 pages). See any current masthead page for ordering and Internet access instructions.

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(19) Swain, C. G.; Swain, S. M.; Berg, F. L. *J. Chem. Inf. Comput. Sci.* **1980**, *20*, 47.
(20) Reichardt, C. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 490.