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An enzyme mimic that hydrolyzes an unactivated ester with catalytic turnover

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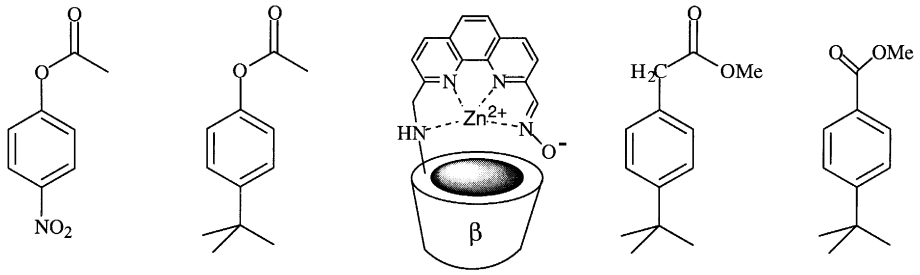
Abstract

The Cu(II) complex of a cyclodextrin dimer linked by a bipyridyl unit catalyzes the hydrolysis of an unactivated doubly-bound benzyl ester.

Keywords: cyclodextrin dimer; copper.

We have described a number of enzyme mimics that hydrolyze phenyl and nitrophenyl esters with good rates and good catalytic accelerations,[1–4] and others have done similar work.[5–10] However, getting effective catalytic hydrolysis of unactivated alkyl esters and amides is more challenging. For instance, we recently described the cleavage of nitrophenyl acetate **1** and *tert*-butylphenyl acetate **2** by the zinc oxime catalyst **3**, but found that **3** showed no detectable acceleration of the hydrolysis of methyl esters **4** and **5**.[4] The problem has to do with the partitioning of tetrahedral intermediates.



1 **2** **3** **4** **5**

In the intermediate **6**, when R is a phenyl group the phenoxide ion departs preferentially, so formation of **6** can be rate limiting (Scheme 1). That is, k_2 is greater than k_{-1}. However, when R is an alkyl group the zinc oxime anion group departs rather than the more basic alkoxide ion, reversing the formation of the intermediate. That is, k_{-1} is greater than k_2.

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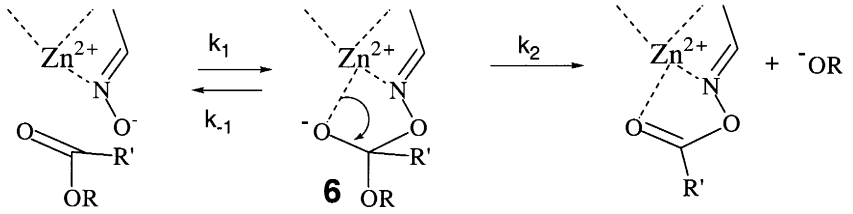
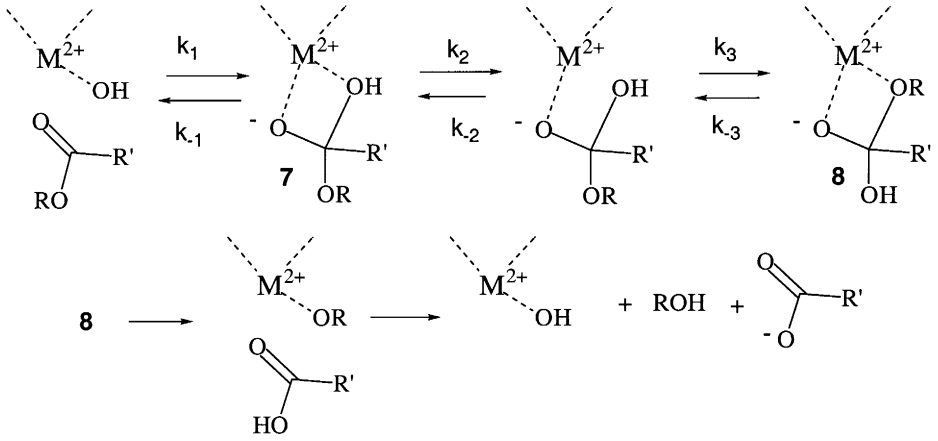


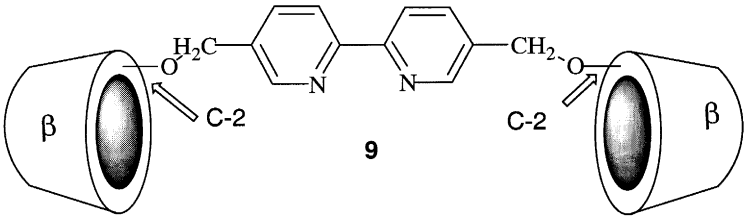
Scheme 1.

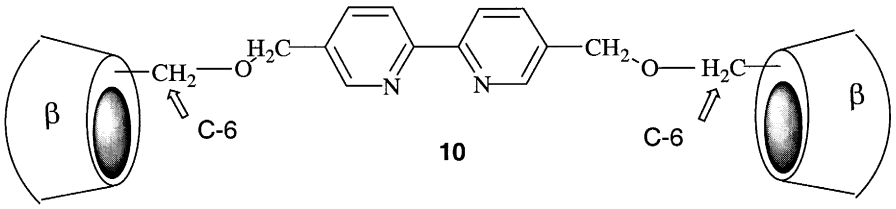
It seemed to us that one solution to this problem is to use a metal ion to deliver a water molecule, as a bound hydroxide ion, instead of delivering a linked ligand anion. Then in the tetrahedral intermediate **7** there would be a choice of losing a metal-assisted hydroxide ion, step k_{-1}, reversing the process of forming a tetrahedral intermediate, or of equilibrating via an open intermediate to give intermediate **8** (Scheme 2). This can lose a metal-assisted alkoxide ion to complete the hydrolysis. If equilibration between intermediates **7** and **8** is rapid, the two processes should have similar rates, so 50% of the tetrahedral intermediate could go forward. If equilibration is not instantaneous, the reverse partitioning will be favored over the forward one.

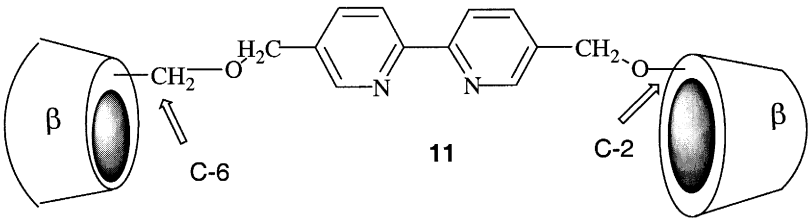


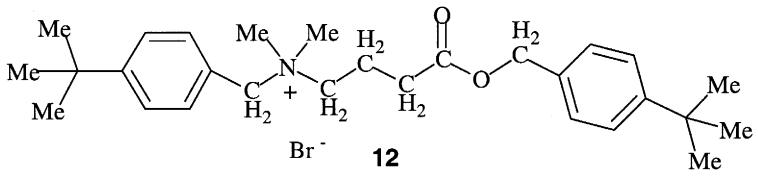
Scheme 2.

We have tested these ideas with a cyclodextrin dimer linked by a metal binding group, compound **9**. This was prepared by alkylation of β-cyclodextrin (β-CD) with 5,5′-bis(bromomethyl)-2,2′-bipyridine and NaH. This afforded **9**, doubly alkylated on the CD C-2 positions, along with an isomer **10** doubly linked at C-6 and another **11** linked between C-2 of one CD and C-6 of the other. The desired isomer **9** was isolated in 11% yield by Lobar chromatography, and characterized by FAB MS: 2475.26 (M+Na) and ^{1}H NMR. The critical point is that in the ^{1}H NMR in DMSO-d_6 solution the secondary face OH signals of β-CD appear at 5.9–5.6 ppm while the primary face OH signals are at 4.6–4.4 ppm. In **9** the area of the 5.9–5.6 signal corresponds to 26 protons, in **10** to 28 protons, and in **11** to 27 protons. At the same time, the multiplet at 4.5–4.43 in **9** corresponds to 14 protons, that in **10** to 12 hydroxyl protons plus four benzylic protons, and that in **11** to 13 hydroxyl protons plus two benzylic protons. The benzylic protons attached to the secondary face oxygens are seen at 5.01–4.81 in both **9** and **11**, but the benzylic protons attached to the primary face appear at 4.65–4.45 in both **10** and **11**. On the secondary face, alkylation under basic conditions is expected from previous studies[11,12] to go to the more acidic C-2 OH, not C-3. All this confirms our structural assignments.









For a substrate that would be adequately water soluble and able to bind both ends into the β-CDs of **9**, we selected compound **12**. Molecular models indicate that when **12** binds into the Cu(II) complex of **9** the carbonyl group is held next to the metal ion, with the carbonyl oxygen at the side of the Cu(II) as needed for square planar coordination. The *t*-butylphenyl groups are strong ligands for β-CD. Also, cleavage of **12** produces two fragments that are poorly bound by the catalyst. Thus, as expected, we saw slow but real fourfold turnover catalysis with an excess of the substrate.

Rates of hydrolysis of **12** were followed by HPLC analysis, and pseudo-first-order rate constants were determined by plotting ln $ester_0/ester_t$ vs time. At 25°C and pH 7.00 with 0.1 M KNO_3 and 40 mM HEPES buffer in 30 volume% DMSO in water, the hydrolysis of **12** without catalyst had $k_{obs}=2.1\pm0.3\times10^{-8}$ s^{-1}, while with 1.50×10^{-3} M of both $Cu(ClO_4)_2$ and **9** k_{obs} was $2.0\pm0.1\times10^{-5}$ s^{-1} so the rate increased by 1000-fold. From a Lineweaver–Burk plot we obtained K_m for **9** plus **12** as $6.0\pm0.1\times10^{-4}$ M and $k_{cat}=2.7\pm0.2\times10^{-5}$ s^{-1}, so k_{cat}/k_{uncat} is 1350. The 30% DMSO was needed to assure full solubility of **12**, but it diminishes the hydrophobic binding.

We also examined the activity of the Cu(II) complexes of catalysts **10** and **11** under the same conditions. The all-primary linked **10** gave a rate acceleration of only 277, while the unsymmetrical dimer **11** gave a rate acceleration of 736, compared with the 1000-fold acceleration for **9**. Thus, linkage on the secondary face is preferable to linkage on the primary face in the β-CD dimers. A catalyst related to **9** with only one β-CD group was sixfold less effective than **9** in the hydrolysis of **12**. Also, substitution

of Zn(II) for Cu(II) reduced the catalytic acceleration from 1000-fold to 340-fold, and with Ni(II) to only 100-fold.

As a function of pH, the uncatalyzed reaction showed base hydrolysis beginning at pH 9. The rate of catalyzed hydrolysis of **12** by **9** rose from pH 6.5 to an apparent plateau between pH 7 and 9 (and then the uncatalyzed reaction added to it). Thus the transition state involves a Cu–OH complex of **9**, or its kinetic equivalent, but without loss of a second proton.

Various controls showed that neither the catalyzed nor the uncatalyzed reaction showed rate increases with higher buffer concentrations. The hydrolysis of **12** showed only modest seven to 13-fold rate increases with buffer plus added Cu(II), Cu(II) with bipyridine, and that mixture with simple β-CD. Without added Cu(II), dimer **9** showed only a 4.8-fold rate increase in the hydrolysis of **12**, similar to the 6.5-fold increase with simple β-CD.

With the Cu(II) complex of a cyclodextrin dimer linked on the primary faces by a bipyridinedithiol, we had observed an 18 300-fold acceleration in the hydrolysis of a phenyl ester (although with Zn(II) and an additional pyridineoxime ligand the acceleration was 1 400 000-fold).[3] Our 1000-fold acceration in the hydrolysis of the non-phenyl ester **12** with the Cu(II) complex of **9** is thus less than a related reaction for a phenyl ester, whose carbonyl group is intrinsically more reactive, but still respectable. Apparently by the use of a metal hydroxide catalytic group we have indeed overcome the need for an unusually reactive phenyl ester for hydrolysis by an enzyme mimic.

Acknowledgements

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References

1. Breslow, R.; Chipman, D. *J. Am. Chem. Soc.* **1965**, *87*, 4195.
2. Breslow, R.; Overman, L. E. *J. Am. Chem. Soc.* **1970**, *92*, 1075.
3. Zhang, B.; Breslow, R. *J. Am. Chem. Soc.* **1997**, *119*, 1676.
4. (a) Breslow, R.; Nesnas, N. *Tetrahedron Lett.* **1999**, *40*, 3335. (b) Nesnas, N. PhD thesis, Columbia University, 1999.
5. Liu, S.; Luo, Z.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2678.
6. Bhattacharya, S.; Snehalatha, K.; George, S. K. *J. Org. Chem.* **1998**, *63*, 27.
7. Molenveld, P.; Engbersen, J. F. J.; Kooijiman, H.; Spek, A. L.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1998**, *120*, 6726.
8. Koike, T.; Inoue, M.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 3091.
9. Ragunathan, K. G.; Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1219.
10. Young, M. J.; Wahnon, D.; Hynes, R. C.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 9441.
11. For example: Breslow, R.; Czarnik, A. W. *J. Am. Chem. Soc.* **1983**, *105*, 1390–1391.
12. Rong, D.; D'Souza, V. T. *Tetrahedron Lett.* **1990**, *31*, 4275–4278.