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Final Technical Report
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The Synthesis of Artificial Enzymes

Professor Ronald Breslow
Department of Chemistry, Columbia University
New York, NY 10027

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This is an edited version of the Progress Report included in the Proposal for a new Contract, later approved as a new Grant. What has been removed is simply all material referring to the future proposals that are in the Research Proposal itself. The publications—listed at the end of the report—were also described in technical reports that were submitted.

In the past three-year contract period, we have had 18 publications and 5 Ph.D. theses with ONR support. I will first review what is in this published work, then discuss unpublished progress.

A previously reported paper with Chung (1) showed that there can be such a thing as too much rigidity in an enzyme model, and that some freedom of movement must be maintained even though in general flexibility is deleterious in catalysis. The point here is that hydrolysis of an ester by an enzyme such as chymotrypsin requires an interesting geometric change: the attacking hydroxyl group of the enzyme must approach in one direction, but then there must be a rotation to permit the second step leading to product. In a cyclodextrin enzyme mimic we had very effective imitation of the first step when there was complete rigid geometric control, but then the second step was slowed. When we incorporated one flexible bond to permit the altered geometry the first step slowed a bit but now the entire process was faster. This gives insight into enzymes themselves, and into optimal design for synthetic catalysts as well.

The thesis of Hans Thiem [20] describes some detailed molecular mechanics calculations that support this, and that were published (2) and reported in a previous contract period. This is one of the first examples of using molecular mechanics calculations to explain rate effects, not just geometries of stable molecules.

One of our principal proposed lines of research involves the use of metal ions in synthetic organic ligands as mimics of metallo-enzymes.

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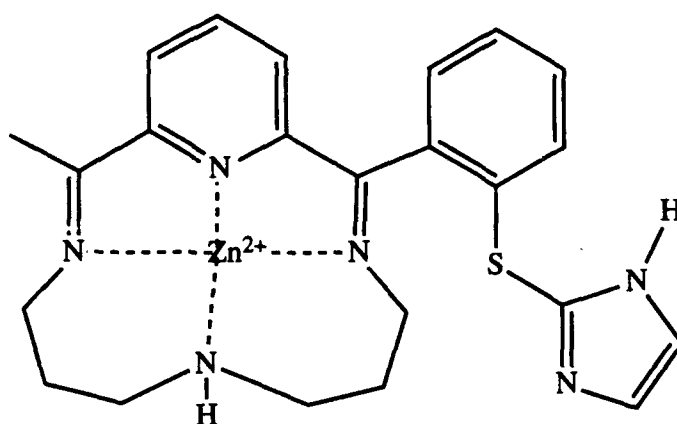
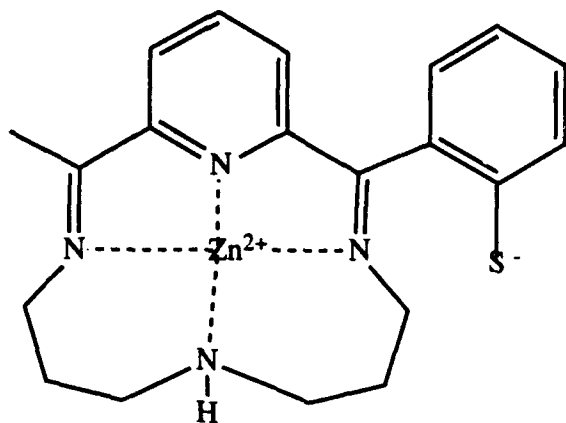
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particularly hydrolytic enzymes. The paper with Jim Light [1] and part of his Ph.D. thesis [21] describes catalyzed cleavage of a peptide bond by a metal ion and a base, related to the catalytic mechanism of the enzyme carboxypeptidase A and many other proteases. In the enzyme a carboxylate group is used as a base, but we had found (3) that it was not very effective in a model system. However, in the enzyme the carboxylate ion is in an environment that raises its basicity quite a bit. When we used a phosphonate group—whose basicity is like that of the carboxyl in the enzyme—we now saw much more effective bifunctional catalysis. The lesson from this is that slavish imitation of the details of Nature's catalysts may be a mistake, and that some analogs can be more effective than the exact mimics.

A paper with Dan Berger and D. Huang [3] describes some other metal-base combinations and their catalytic properties. These are the first examples of systems in which a metal ion and a base are rigidly held so that they cannot "short-circuit", but must interact through the substrate they are hydrolyzing.

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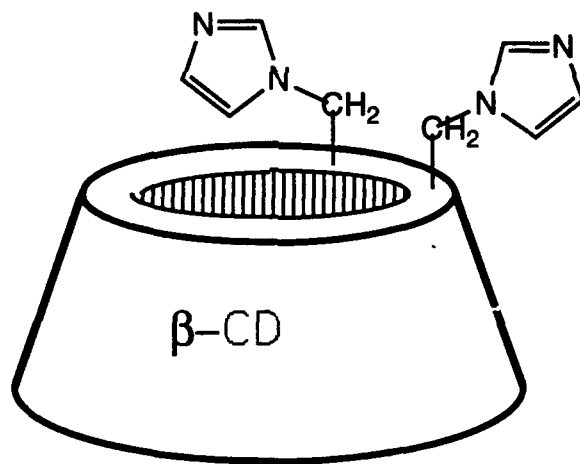


A paper with Huang [8] describes the cleavage of RNA catalyzed by metal ions along with bases; some of this is relevant to the mode of action of "ribozymes", Nature's own enzyme mimics in which RNA is a catalyst. We found reasonable catalysis by the metal ion that ribozymes use, magnesium ion, and very good catalysis by lanthanides. Again, using catalytic groups not easily available to biological systems can lead to even better enzyme mimics than does the slavish imitation of natural details. This work is also described in Huang's Ph.D. thesis [23].

Some of the studies on RNA cleavage involved non-metal catalysts, but were relevant to the overall goals of this project. For instance, the paper with Huang [4] was related to the mechanism of RNA cleavage. In this paper we showed that the cleavage of RNA and its isomerization must proceed through a common intermediate; this demonstrated that the cleavage mechanism was not the obvious one, but a new process that is probably also used in modified form by the enzyme ribonuclease itself. In this short paper

we introduced the evidence that increasing the concentration of a catalyst slowed one of the reactions. Although our use of the term "negative catalytic experimental rate constant" to describe this slowing has stimulated a lot of heated discussion in the literature, the scientific observation was clear and only the terminology is really at issue.

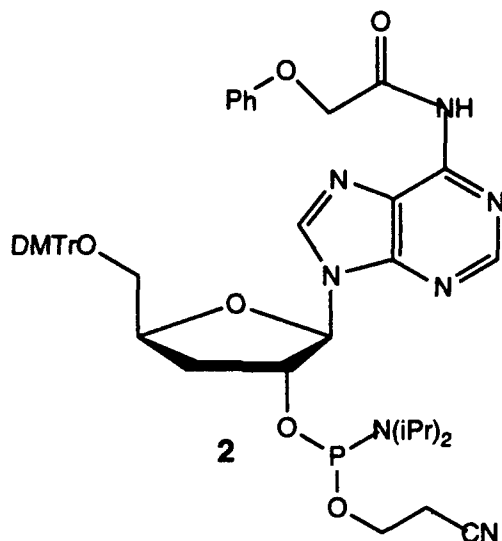
The science revealed by these studies was reviewed in the paper with Anslyn and Huang [6], and in a Breslow paper [9]. Further evidence supporting the mechanistic conclusions was published in a paper with Xu [17], and in a theoretical kinetics paper by Breslow [18]. This work, and some work currently being refereed by JACS, clearly establishes the novel mechanism. In a paper previously reported with Anslyn (4), we showed that a novel catalyst (1) that had been designed taking account of our new mechanism indeed uses the simultaneous bifunctional catalytic mechanism characteristic of the enzyme itself. This work also strengthened the case for a mechanistic tool—proton inventory—that is widely used in enzyme mechanism studies.



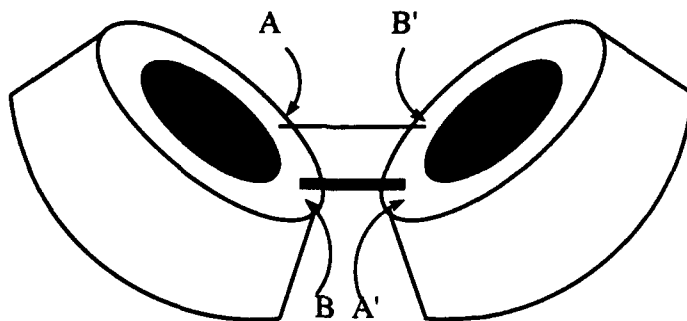
(1)

Two studies [13] and [14] on an unusual DNA isomer also have RNA cleavage as an eventual goal. What has been done here is to remove the 3-oxygen from ribose, making a DNA in which the linkage is to carbon 2. The synthetic intermediate used is (2), for instance. The unusual DNA isomer produced from 2 shows some properties related to natural DNA, but it is inferior to the natural material with respect to its ability to function as a genetic molecule. This gives insight into the reason that our natural DNA is

as it is, but the properties of the DNA isomer may well have interesting applications. These have been outlined in the Research Proposal.

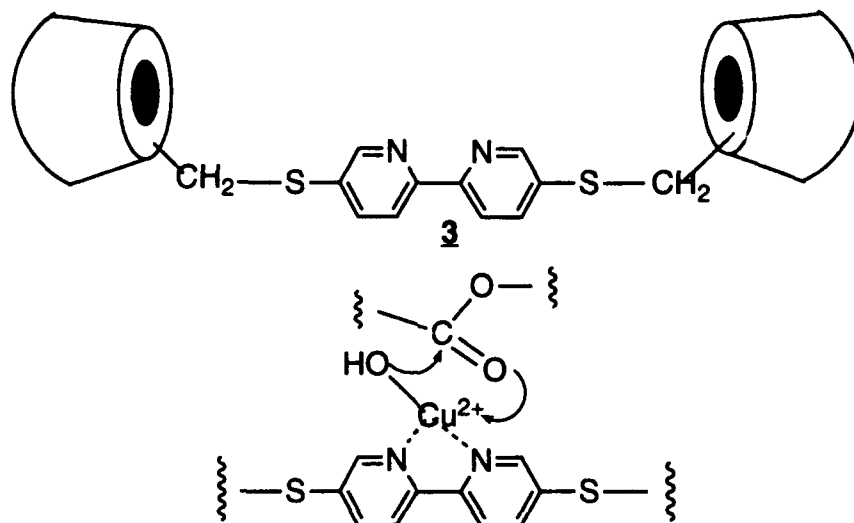


Much work has been done on the cyclodextrin dimers that were proposed for this contract period. The previously reported paper with Greenspoon et. al.(5) described the synthesis of several such dimers, and their very high binding constants for substrates of appropriate geometry. The work by Chung in his paper [5] and his Ph.D. thesis [22] describes dimers with two linking groups. They have very strong geometric preferences, and in the best case have binding constants in water exceeding $100,000,000,000 M^{-1}$! This makes them comparable to the best antibodies.



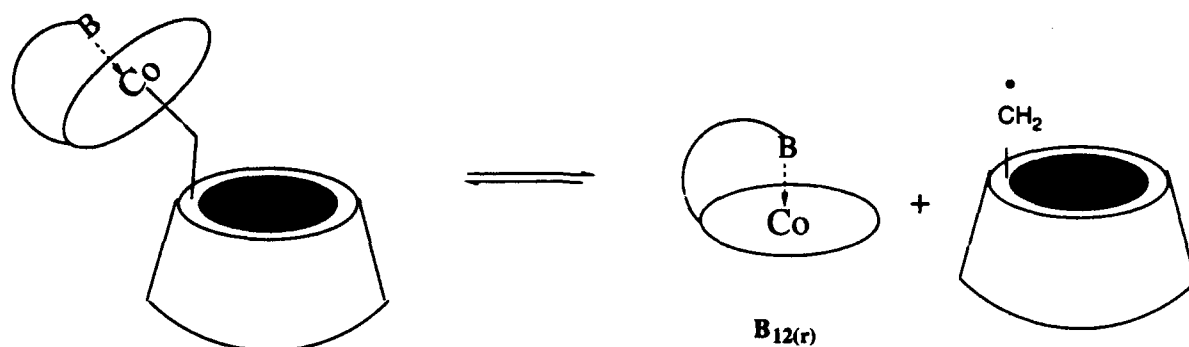
In the paper with Zhang [12] we describe catalysis of ester hydrolysis by a cyclodextrin dimer **3** carrying a metal ion in the linking group, with an acceleration of 225,000-fold. Such catalysts not only bind substrates very

strongly, they also hold groups to be cleaved right on top of the catalytic groups, as in good enzymes. This is an important advance, and it was built upon in our Research Proposal.



Sherin Halfon has published a study of the factors that make binding so strong in such dimers [15]. She has shown how one can get quantitative estimates of the contribution from hydrophobic binding, which is the principal contributor. The quantitative tools developed here will be of great use in designing and understanding new catalysts that operate in water solution.

A particularly interesting catalytic role of metals in biochemistry is seen in the enzymes that use coenzyme B-12 as a cofactor. These promote rearrangement reactions that are essentially unprecedented in ordinary chemistry. A paper with Jim Light and Peter Duggan [10] and the Light thesis [21] describe an interesting model (4) of these processes in which vitamin B-12 is attached to cyclodextrin. The cyclodextrin simultaneously plays the role of enzyme—holding a substrate next to the coenzyme—and of part of the coenzyme itself. That is, the glucose residue of cyclodextrin mimics the ribose residue of the coenzyme. This work is being further pursued.



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A few review articles describing our work supported by ONR have been published [2, 7, 11, 16]. The thesis by Kumabe [19] describes some synthetic advances that are useful in our ONR work.

Good progress has also been made in work that is still unpublished. Thus Chip Chapman has developed improved synthetic procedures to prepare the isomeric DNA molecules, making them more available for detailed studies. Such studies are underway, in part in collaboration with Ken Breslauer of Rutgers. He is experienced in the biophysical chemistry of normal DNA, and is clarifying the novel properties of our isomer. Detailed molecular mechanics calculations on the geometry of our iso-DNA have also been done here; they clarify the reason for the altered properties relative to normal DNA.

Terry Sheppard has prepared some DNA molecules carrying one of our isomeric DNA units along with normal units, and he has also made an RNA molecule that should be cleaved with the assistance of this modified DNA piece. Sherin Halfon has made a very interesting molecule in which a cyclodextrin dimer carries not only a metal binding group but also two well placed acid-base groups.

Chip Chapman and Corinna Grisostomi have finally worked out syntheses of the difficult molecules we proposed in which an effective zinc ion catalyst is held rigidly near appropriate acid-base groups. We hope to finish these syntheses shortly, and to investigate the properties of these promising catalysts.

Alan Graff has initiated a very interesting study using the cyclodextrin bis-imidazole catalysts (e.g. **1**) that we developed as mimics of ribonuclease. We had three catalysts with differing geometries, and found that in the

RNAse mimic putting the two catalyst groups on neighboring glucose residues of cyclodextrin gave the best rate. Now we are examining other reactions that can show bifunctional catalysis, such as carbonyl chemistry involving enolization. Although this work is still in an early stage, we know that a different isomer is the best bifunctional catalyst for carbonyl chemistry. This tool may give us otherwise unavailable information about the preferred geometry of chemical reactions, and about the best design for catalysts of such reactions.

Miroslav Rezac and Julio Medina have initiated the difficult task of preparing mimics of enzymes that use coenzyme B-12 in which the B-12 unit is permanently held in the vicinity of the binding group. There is some evidence that this may be important in the enzymatic processes we hope to imitate. Finally, Roy Xu has made some very interesting bifunctional catalysts based in part on the optimal geometries we have deduced from our mechanistic studies.

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