Edited by Ronald Breslow

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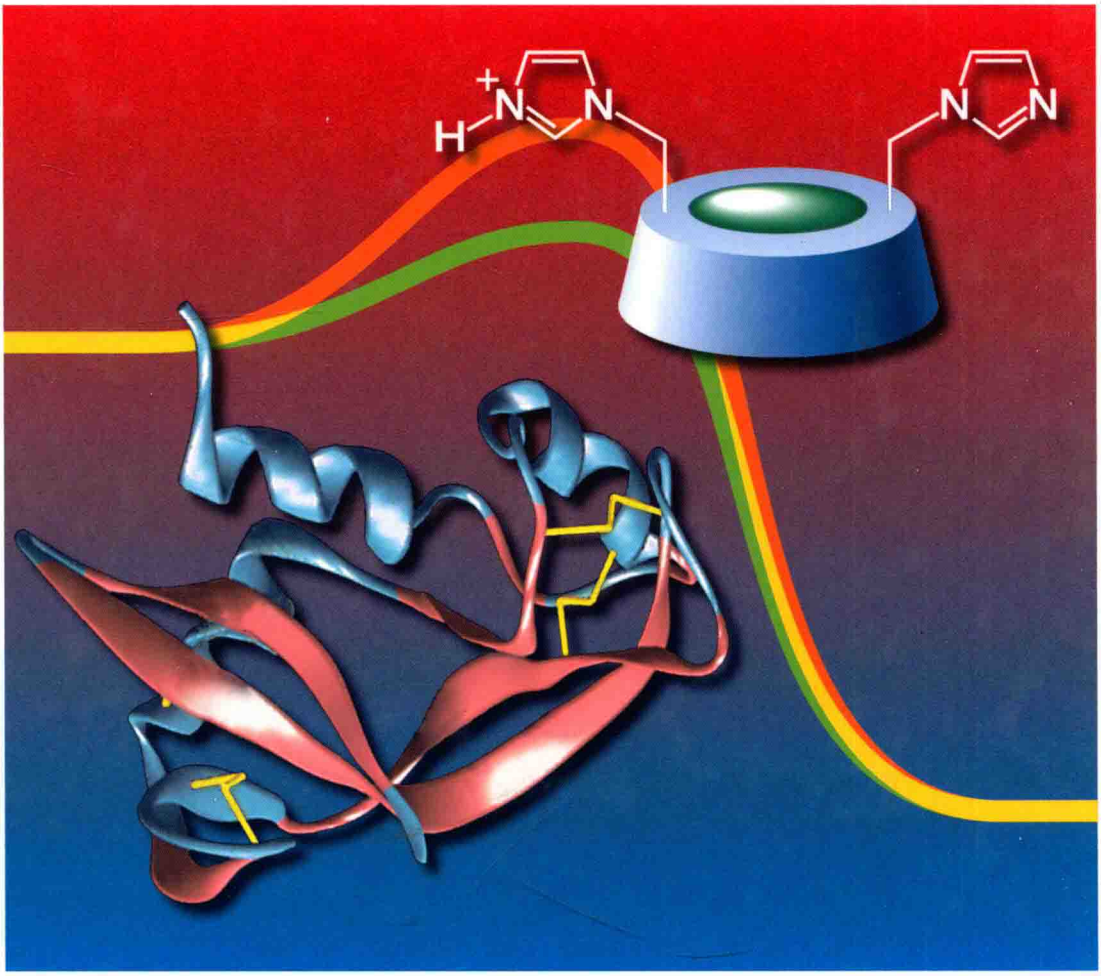
Artificial Enzymes



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Artificial Enzymes

WILEY-VCH Verlag GmbH & Co. KGaA

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Library of Congress Card No.: Applied for.

British Library Cataloguing-in-Publication Data:
A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at http://dnb.ddb.de

Printed in the Federal Republic of Germany

Printed on acid-free paper

Cover Design Gunter Schulz, Fußgönheim
Typesetting Mitterweger & Partner, Kommunikationsgesellschaft mbH, Plankstadt
Printing Strauss GmbH, Mörlenbach
Bookbinding Litges & Dopf Buchbinderei GmbH, Heppenheim

ISBN-13: 978-3-527-31165-1
ISBN-10: 3-527-31165-3

Ronald Breslow
Artificial Enzymes

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Preface

Chemists have been extending Nature for hundreds of years. This has generally taken the form of creating new compounds that did not exist in Nature, including new medicines, new polymers, and simply new interesting compounds such as novel aromatics, for instance. At this point well over 90% of all known chemical substances are unnatural products. They were often developed by imitating the general style of natural substances – a good example is synthetic polymers, which differ from but are intellectually related to the polymers of biology. The motivation for extending Nature is often simple curiosity about what else is possible, but there is always the hope that a useful new property might emerge.

I have coined the term Biomimetic Chemistry to describe novel chemistry that is inspired by that done in living systems. In that sense modern aviation is biomimetic, inspired by the principles we see in birds and flying insects (wings) but not the exact details of how they are used in living creatures (we gave up on efforts to flap wings after some disastrous tries in early airplanes). Artificial Enzymes are part of the world of Biomimetic Chemistry, in which we are imitating the *catalytic processes* that occur in living systems, not just the substances of biology. We hope to achieve the rapid and selective reactions typical of enzymatic conversions by imitating the general principles – in particular the well-defined geometry in enzyme–substrate complexes – but not every detail of natural enzymes. This will greatly increase the power of synthetic chemistry.

There is another important function of biomimetic chemistry – increasing our understanding of natural chemistry by putting it in a larger context. Thus, the properties of artificial enzymes help us understand and appreciate the special properties of Nature's enzymes. In particular, the great challenge in achieving, with artificial enzymes, the huge rate accelerations that the best natural enzymes can achieve makes us think more deeply about what is special in natural enzymes. As one example, the work on polymeric artificial enzymes described in Chapters 2 and 3 indicates a special role that the macromolecular structures of enzymes can play in excluding water solvent from the reaction zone.

The seven chapters in this book describe various approaches to the synthesis and study of artificial enzymes. In Chapter 1, I describe work in my laboratory over the past almost 50 years creating enzyme models and enzyme mimics. A major theme is the use of hydrophobic binding of substrates into cyclodextrins carrying catalytic groups,

Artificial Enzymes. Edited by R. Breslow

ISBN: 3-527-31165-3

to imitate the reversible formation of enzyme–substrate complexes. In Chapter 2, Lei Liu and I describe a particular aspect of our and related work in more detail, namely the creation and study of artificial transaminases and other enzyme mimics based on co-factors in the pyridoxal/pyridoxamine family. In this work we have taken advantage of the pioneering studies, by Irving Klotz, of artificial enzymes based on ethylenimine polymers. Then, in Chapter 3, Klotz and Suh describe other work on these fascinating polymers, which show both the ability to hide a reaction zone away from water solvent and also the intrinsic effective acid–base catalysis by the amino groups of the polymers.

Since catalysis can be achieved by selective binding of the reaction's transition state (binding is simply an interaction between two components that lowers the free energy of the system), there is much interest in the use of antibodies as artificial enzymes – antibodies raised against structures resembling the transition states of reactions. When they bind the transition states, the reaction is accelerated. In Chapter 4, Hilvert describes the remarkable advances in this field, including his own ground-breaking creation of antibody-based artificial enzymes that can substitute in a living organism for the natural enzyme. Antibody-based artificial enzymes are, of course, proteins, and in Chapter 5 Duckworth and Distefano describe another approach to artificial enzymes – modifying natural proteins to impart novel enzymatic activity.

Many natural enzymes use metal ions for their principal catalytic functions, so artificial enzymes have been created using such metal ion catalysis. Chin and Kim describe this area, and their own exciting work, in Chapter 6. One of the areas in which natural enzymes play an important role is in molecular biology; restriction enzymes can hydrolyze DNA at specific target sites and facilitate genetic engineering. In Chapter 7, Yamamoto and Komiyama describe trail-blazing work on the construction of artificial restriction enzymes. They use metal ion catalysis, but with site selectivity to cleave both single stranded and double stranded DNA.

I hope that this book, a sampling of work on artificial enzymes, conveys the flavor and some of the achievements of the field. As in the past, chemists have not simply admired nature, they have set about to learn from it and extend it. The result is novel catalysts that can be tailored to achieve selective reactions previously available only in enzymatic processes. With such a promising start, I believe that the field of Artificial Enzymes, as part of Biomimetic Chemistry, will continue to grow into a major area of modern chemistry. The methods can be attractive in the synthesis and manufacture of medicinal compounds, and artificial enzymes themselves may become useful as therapeutics and in molecular devices. Thus I urge young students to join the effort to revolutionize chemical catalysis by applying the principles of enzymatic reactions to the invention of artificial enzymes, and their applications. There is still much to be done.

Columbia University
December 2004

Ronald Breslow

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ISBN: 3-527-31165-3

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Table of Contents

Artificial Enzymes. Edited by R. Breslow

ISBN: 3-527-31165-3

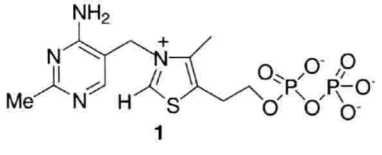
1
Artificial Enzymes

Ronald Breslow

Biomimetic Chemistry, including that involved in the synthesis and study of artificial enzymes, has grown to enormous proportions. Even the part of the field using cyclodextrins as binding groups in synthetic catalysts that mimic enzymes has been the subject of a large review article [1]. Thus in this chapter I will focus mainly, but not exclusively, on work from our own laboratory. Other chapters will help make up for this somewhat narrow focus. I have published several reviews of our work elsewhere [2–51].

1.1
Mimics of Enzymes that use Thiamine Pyrophosphate as a Coenzyme

I have been pursuing enzyme mimics, artificial enzymes that perform biomimetic chemistry, since starting my independent career in 1956. In the first work [52–59] my co-workers and I studied models for the function of thiamine pyrophosphate **1** as a coenzyme in enzymes such as carboxylase. We discovered the mechanism by which it acts, by forming an anion **2** that we also described as a stabilized carbene, one of its resonance forms. We examined the related anions from imidazolium cations and oxazolium cations, which produce anions **3** and **4** that can also be described as nucleophilic carbenes. We were able to explain the structure–activity relationships in this series, and the reasons why the thiazolium ring is best suited to act as a biological



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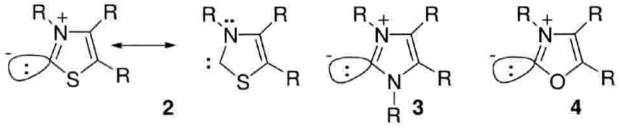

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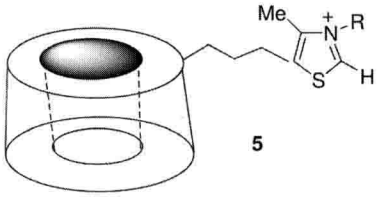

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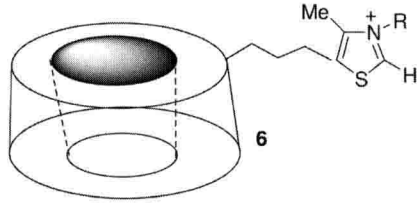
β-Cyclodextrin



6

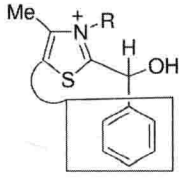
γ-Cyclodextrin

catalyst. Later, we confirmed [60] the thiamine mechanism proposed earlier, for which an alternative had subsequently been proposed [61, 62].

We synthesized artificial enzymes **5** and **6** that incorporated the thiazolium ring of thiamine into a cyclodextrin binding unit [63, 64]. The cyclodextrin imitated the hydrophobic binding pocket typical of many enzymes, but these mimics did not incorporate the catalytic groups that enzymes also use. Thus **5** and **6** showed the substrate selectivity that enzyme binding also achieves, and there was some rate acceleration from binding the substrates in proximity to the catalytic coenzyme group, as in enzymes. However, the rate accelerations were not nearly as large as those in artificial enzymes (vide infra) that incorporated more features of natural enzymes.

With β-cyclodextrin, consisting of seven glucose units in a ring, benzaldehyde bound into the cyclodextrin cavity of **5** and was converted into thiazolium adduct **7**, similar to a cyanohydrin [63]. This readily formed the benzylic anion **8** that underwent deuterium exchange and easy oxidation. However, this β-cyclodextrin ring was too small to bind both benzaldehydes – so this artificial enzyme did not catalyze the formation of benzoin **9** significantly better than did a simple thiazolium salt without the attached cyclodextrin binding group.

When the thiazolium unit was attached to the larger γ-cyclodextrin in **6**, with eight glucose units in the ring, benzoin condensation of two benzaldehydes was indeed well



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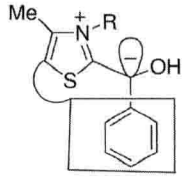

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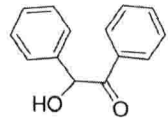

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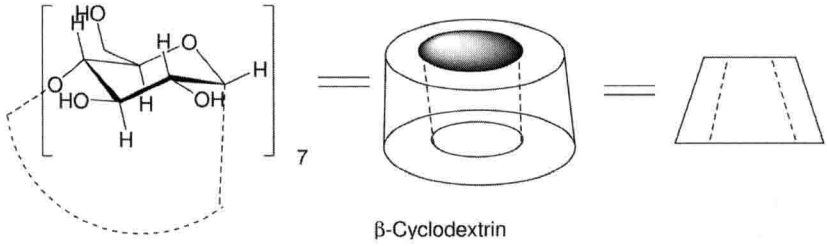


β-Cyclodextrin

catalyzed, with a rate 150-fold higher than that for a thiazolium salt lacking the cyclodextrin. Interestingly, in this benzoin condensation the rate-determining step – addition of anion **8** to the second benzaldehyde – allowed the benzaldehyde units to bind next to each other in the cavity, but in the product benzoin **9** the extended geometry does not permit this. Thus the benzoin product did not bind strongly to the artificial enzyme **6**, and did not inhibit the process. Our other studies on the benzoin condensation [65, 66] revealed geometries of the transition state and product that support this interpretation.

1.2
Mimics of Enzymes that use Pyridoxamine and Pyridoxal Phosphates as Coenzymes

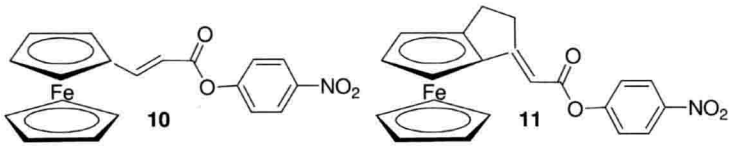
We also attached pyridoxamine to a cyclodextrin and saw that the resulting enzyme mimics showed good substrate selectivity in the conversion of keto acids into amino acids [67–71]. With a pyridoxamine doubly-linked to the cyclodextrin there was a preference for the hydrophobic *t*-butylphenylpyruvic acid relative to pyruvic acid of at least 15 000-fold. We also made a related system, in which a synthetic macrocycle was attached to the coenzyme mimic [72], that also showed substrate selectivity. In other work we synthesized molecules in which base groups attached to the pyridoxamine could perform transaminations with good stereoselectivity [73–75]. We also made others in which the geometry of the attached base groups could promote different catalyzed processes for pyridoxal, selecting among the various enzymatic processes for which pyridoxal phosphate is a coenzyme [76–80].

These and subsequent artificial enzymes that perform transaminations are described in Chapter 2.

1.3
Artificial Hydrolytic Enzymes

1.3.1
Chymotrypsin Mimics

The field of artificial enzymes has been greatly concerned with mimicking *hydrolytic* enzymes. Since the enzyme chymotrypsin was one of the first to be extensively studied and understood, many laboratories have created artificial peptidases and esterases, including those that use the nucleophilic mechanism like that in chymotrypsin. (However, one chymotrypsin mimic from other laboratories did not have the reported mechanism [81].) The critical requirement is bifunctional catalysis, which in chymotrypsin involves imidazole acting first as a general base, then as a general acid, and the serine hydroxyl group serving as a nucleophile. I have pointed out the special kinetic situation this mechanism implies [82].



We have made several artificial enzymes that use cyclodextrin to bind a substrate and then react with it by acylating a cyclodextrin hydroxyl group. This builds on earlier work by Myron Bender, who first studied such acylations [83]. We added groups to the cyclodextrin that produced a flexible floor, capping the ring [84]. The result was to increase the relative rate of cyclodextrin acylation by *m-t*-butylphenyl acetate from 365 relative to its hydrolysis rate in the buffer to a $k_{complex}/k_{buffer}$ of 3300. We changed the substrate to achieve better geometry for the intracomplex acylation reaction, and with a *p*-nitrophenyl ester of ferroceneacrylic acid **10** we achieved a relative rate for intracomplex acylation of ordinary β-cyclodextrin vs. hydrolysis of over 50 000 and a V_{max} comparable to that for hydrolysis of *p*-nitrophenyl acetate by chymotrypsin [85].

Our best combination of the flexible capped cyclodextrin with the well-fitting substrate *p*-nitrophenyl ester **10** gave an acceleration – relative to hydrolysis in the same medium – of over one-million fold, exceeding that achieved by chymotrypsin with *p*-nitrophenyl acetate [86]. An even better fitting substrate (**11**) afforded an acceleration of ca. 80 000 000-fold, and saw a 62-fold increase in enantioselectivity as well [87, 88]. This is an enantiomeric excess of 98.4%.

Substrate binding into the cyclodextrin cavity, which ordinarily is studied in water solution, also occurs in highly polar organic solvents such as DMSO [89]. Furthermore, kinetic studies of our reactions at high pressure were consistent with the geometries proposed for these acylation processes [90]. Molecular modeling showed geometries of the bound substrates and the tetrahedral intermediates that helped explain some of the large rate effects [91].

In the acylation of a cyclodextrin hydroxyl group by a nitrophenyl ester, the preferred geometry requires that the oxyanion of the cyclodextrin attack perpendicular to the plane of the ester carbonyl, so as to form the tetrahedral intermediate. However, the product cyclodextrin ester has the cyclodextrin oxygen *in* the plane of the carbonyl group. Thus, a rapid reaction requires enough flexibility to be present to permit this geometric change to occur rapidly. With very rigid substrates the conversion of the tetrahedral intermediate into the product can be rate determining, and slow. In a study of this question we used substrate **12** in which the ester carbonyl can freely rotate, and saw that this made the formation of the tetrahedral intermediate rate-limiting, and rapid [92].