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Bioorganic Chemistry

A Chemical Approach to Enzyme Action

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With 82 Figures

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Cover: The green illustration represents the hypothetical mode of binding of a rigid structural analogue of *N*-benzoyl-L-phenylalanine methyl ester at the active site of α-chymotrypsin. The illustration emphasizes the equilibration toward the favored configuration (see text page 224). The background design is taken from a diagrammatic representation of the primary structure of α-chymotrypsin. After *Nature* with permission [B.W. Matthews, P.B. Sigler, R. Henderson, and D.M. Blow (1967), *Nature* **214**, 652–656].

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Series Preface

Springer Advanced Texts in Chemistry

New textbooks at all levels of chemistry appear with great regularity. Some fields like basic biochemistry, organic reaction mechanisms, and chemical thermodynamics are well represented by many excellent texts, and new or revised editions are published sufficiently often to keep up with progress in research. However, some areas of chemistry, especially many of those taught at the graduate level, suffer from a real lack of up-to-date textbooks. The most serious needs occur in fields that are rapidly changing. Textbooks in these subjects usually have to be written by scientists actually involved in the research which is advancing the field. It is not often easy to persuade such individuals to set time aside to help spread the knowledge they have accumulated. Our goal, in this series, is to pinpoint areas of chemistry where recent progress has outpaced what is covered in any available textbooks, and then seek out and persuade experts in these fields to produce relatively concise but instructive introductions to their fields. These should serve the needs of one semester or one quarter graduate courses in chemistry and biochemistry. In some cases the availability of texts in active research areas should help stimulate the creation of new courses.

New York, New York

CHARLES R. CANTOR

Foreword

In the early 1960s, while at the University of Ottawa, my colleagues of the Chemistry Department agreed that the long-term future of organic chemistry lay in its applications to biochemical problems, apart from its eventual rationalization through theoretical modeling. Accordingly, I proceeded with the preparation of an undergraduate general biochemistry course specifically designed for the benefit of graduating chemistry students lacking any background in classical, descriptive biochemistry. The pedagogical approach centered chiefly on those organic chemical reactions which best illustrated at the fundamental level their biochemical counterparts. Effective chemical modeling of enzymatic reactions was still in an embryonic state, and over the last fifteen years or so much progress has been made in the development of biomimetic systems. It came as a surprise, as word spread around and as years went by, to witness the massive invasion of my classes by undergraduates majoring in biochemistry and biology, so that often enough the chemistry students were clearly outnumbered. As it turned out, the students had discovered that what they thought they really knew through the process of memorization had left them without any appreciation of the fundamental and universal principles at work and which can be so much more readily perceived through the appropriate use of models. By the time I moved to McGill in 1971, the nature of the course had been gradually transformed into what is now defined as bioorganic chemistry, a self-contained course which has been offered at the undergraduate B.Sc. level for the past ten years. The success of the course is proof that it fills a real need. Over these years, I never found the time to use my numerous scattered notes and references as a basis to produce a textbook (the absence of which is still a source of chronic complaint on the part of the students). Fortunately, the present authors (H.D. and C.P.) had first-hand experience at teaching the course (when I was on leave of absence) and as a result felt encouraged to undertake the heroic task of organizing my telegraphic notes

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into a framework for a textbook which they are now offering. There is little doubt that what they have accomplished will serve most satisfyingly to fill a very serious need in the modern curricula of undergraduate chemists, biochemists, biologists, and all those contemplating a career in medicinal chemistry and medical research. The field is moving so rapidly, however, that revised editions will have to be produced at relatively short intervals. Nevertheless, the substance and the conceptual approach can only have, it is hoped, lasting value.

Montreal
February 1981

BERNARD BELLEAU
MCGILL UNIVERSITY

Preface

Bioorganic chemistry is the application of the principles and the tools of organic chemistry to the understanding of biological processes. The remarkable expansion of this new discipline in organic chemistry during the last ten years has created a new challenge for the teacher, particularly with respect to undergraduate courses. Indeed, the introduction of many new and valuable bioorganic chemical principles is not a simple task. This book will expound the fundamental principles for the construction of bioorganic molecular models of biochemical processes using the tools of organic and physical chemistry.

This textbook is meant to serve as a *teaching* book. It is not the authors' intention to cover all aspects of bioorganic chemistry. Rather, a blend of general and selected topics are presented to stress important aspects underlying the concepts of organic molecular model building. Most of the presentation is accessible to advanced undergraduate students without the need to go back to an elementary textbook of biochemistry; of course, a working knowledge of organic chemistry is mandatory. Consequently, this textbook is addressed first to final-year undergraduate students in chemistry, biochemistry, biology, and pharmacology. In addition, the text has much to offer in modern material that graduate students are expected to, but seldom actually, know.

Often the material presented in elementary biochemistry courses is overwhelming and seen by many students as mainly a matter of memorization. We hope to overcome this situation. Therefore, the chemical organic presentation throughout the book should help to stimulate students to make the "quantum jump" necessary to go from a level of pure memorization of biochemical transformations to a level of adequate comprehension of biochemical principles based on a firm chemical understanding of bioorganic concepts. For this, most chapters start by asking some of the pertinent questions developed within the chapter. In brief, we hope that this approach will stimulate curiosity.

Professor B. Belleau from McGill University acted as a "catalyst" in promoting the idea to write this book. Most of the material was originally inspired from his notes. The authors would like to express their most sincere appreciation for giving us the opportunity of teaching, transforming, and expanding *his* course into a book. It is Dr. Belleau's influence and remarkable dynamism that gave us constant inspiration and strength throughout the writing.

The references are by no means exhaustive, but are, like the topics chosen, selective. The reader can easily find additional references since many of the citations are of books and review articles. The instructor should have a good knowledge of individual references and be able to offer to the students the possibility of discussing a particular subject in more detail. Often we give the name of the main author concerning the subject presented and the year the work was done. This way the students have the opportunity to know the leader in that particular field and can more readily find appropriate references. However, we apologize to all those who have not been mentioned because of space limitation.

The book includes more material than can be handled in a single course of three hours a week in one semester. However, in every chapter, sections of material may be omitted without loss of continuity. This flexibility allows the instructor to emphasize certain aspects of the book, depending if the course is presented to an audience of chemists or biochemists.

We are indebted to the following friends and colleagues for providing us with expert suggestions and comments regarding the presentation of certain parts of the book: P. Brownbridge, P. Deslongchamps, P. Guthrie, J. B. Jones, R. Kluger, and C. Lipsey. And many thanks to Miss C. Potvin, from the Université de Montréal, for her excellent typing assistance throughout the preparation of this manuscript.

Finally, criticisms and suggestions toward improvement of the content of the text are welcome.

Montreal, Canada
January 1981

HERMANN DUGAS
CHRISTOPHER PENNEY

Contents

Chapter 1
Introduction to Bioorganic Chemistry

"It might be helpful to remind ourselves regularly of the sizeable incompleteness of our understanding, not only of ourselves as individuals and as a group, but also of Nature and the world around us."

N. Hackerman
Science **183**, 907 (1974)

1.1 Basic Considerations

Bioorganic chemistry is a new discipline which is essentially concerned with the application of the tools of chemistry to the understanding of biochemical processes. Such an understanding is often achieved with the aid of *molecular models* chemically synthesized in the laboratory. This allows a "sorting out" of the many variable parameters simultaneously operative within the biological system.

For example, how does a biological membrane work? One builds a simple model of known compositions and studies a single behavior, such as an ion transport property. How does the brain work? This is by far a more complicated system than the previous example. Again one studies single synapses and single synaptic constituents and then uses the observations to construct a model.

Organic chemists develop synthetic methodology to better understand organic mechanisms and create new compounds. On the other hand, biochemists study life processes by means of biochemical methodology (enzyme purification and assay, radioisotopic tracer studies in *in vivo* systems). The former possess the methodology to synthesize biological analogues but often

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fail to appreciate which synthesis would be relevant. The latter possess an appreciation of what would be useful to synthesize in the laboratory, but not the expertise to pursue the problem. The need for the multidisciplinary approach becomes obvious, and the bioorganic chemist will often have two laboratories: one for synthesis and another for biological study. A new dimension results from this combination of chemical and biological sciences; that is the concept of *model building* to study and sort out the various parameters of a complex biological process. By means of simple organic models, many biological reactions as well as the specificity and efficiency of the enzymes involved have been reproduced in the test tube. The success of many of these models indicates the progress that has been made in understanding the chemistry operative in biological systems. Extrapolation of this multidisciplinary science to the pathological state is a major theme of the pharmaceutical industry; organic chemists and pharmacologists working "side-by-side," so that bioorganic chemistry is to biochemistry as medicinal chemistry is to pharmacology.

What are the tools needed for bioorganic model studies? Organic and physical organic chemical principles will provide, by their very nature, the best opportunities for model building—modeling molecular events which form the basis of life. A large portion of organic chemistry has been classically devoted to natural products. Many of those results have turned out to be wonderful tools for the discovery and characterization of specific molecular events in living systems. Think for instance of the development of antibiotics, certain alkaloids, and the design of new drugs for the medicine of today and tomorrow.

All living processes require energy, which is obtained by performing chemical reactions inside cells. These biochemical processes are based on chemical dynamics and involve reductions and oxidations. Biological oxidations are thus the main source of energy to drive a number of endergonic biological transformations.

Many of the reactions involve combustion of foods such as sugars and lipids to produce energy that is used for a variety of essential functions such as growth, replication, maintenance, muscular work, and heat production. These transformations are also related to oxygen uptake; breathing is a biochemical process by which molecular oxygen is reduced to water. Throughout these pathways, energy is stored in the form of adenosine triphosphate (ATP), an energy-rich compound known as the universal product of energetic transactions.

Part of the energy from the combustion engine in the cell is used to perpetuate the machine. The machine is composed of structural components which must be replicated. Ordinary combustion gives only heat plus some visible light and waste. Biological combustions, however, give some heat but a large portion of the energy is used to drive a "molecular engine" which synthesizes copies of itself and which does mechanical work as well. Since these transformations occur at low temperature (body temp., 37°C)

and in aqueous media, catalysts are essential for smooth or rapid energy release and transfer. Hence, apart from structural components, *molecular catalysts* are required.

These catalysts have to be highly efficient (a minimum of waste) and highly specific if precise patterns are to be produced. Structural components have a static role; we are interested here in the dynamics. If bond-breaking and bond-forming reactions are to be performed on a specific starting material, then a suitable specific catalyst capable of recognizing the substrate must be "constructed" around that substrate.

In other words, and this is the fundamental question posed by all biochemical phenomena, a substrate molecule and the specific reaction it must undergo must be translated into another structure of much higher order, whose information content perfectly matches the specifically planned chemical transformation. Only large macromolecules can carry enough *molecular information* both from the point of view of substrate recognition and thermodynamic efficiency of the transformation. *These macromolecules are proteins.* They must be extremely versatile in the physicochemical sense since innumerable substrates of widely divergent chemical and physical properties must all be handled by proteins.

Hence, protein composition must of necessity be amenable to wide variations in order that different substrates may be recognized and handled. Some proteins will even need adjuncts (nonprotein parts) to assist in recognition and transformation. These cofactors are called coenzymes. One can therefore predict that protein catalysts or *enzymes* must have a high degree of order and organization. Further, a minimum size will be essential for all the information to be contained.

These ordered biopolymers, which allow the combustion engine to work and to replicate itself, must also be replicated exactly once a perfect translation of substrate structure into a specific function has been established. Hence the molecular information in the proteins (enzymes) must be safely stored into stable, relatively static language. This is where the nucleic acids enter into the picture. Consequently another translation phenomenon involves protein information content written into a linear molecular language which can be copied and distributed to other cells.

The best way to vary at will the information content of a macromolecule is to use some sort of backbone and to peg on it various arrays of side chains. Each side chain may carry well-defined information regarding interactions between themselves or with a specific substrate in order to perform specific bond-making or -breaking functions. Nucleic acid–protein interactions should also be mentioned because of their fundamental importance in the evolution of the genetic code.

The backbone just mentioned is a polyamide and the pegs are the amino acid side chains. *Why polyamide*? Because it has the capacity of "freezing" the biopolymer backbone into precise three-dimensional patterns. Flexibility is also achieved and is of considerable importance for conformational

"breathing" effects to occur. A substrate can therefore be transformed in terms of protein conformation imprints and finally, mechanical energy can also be translocated.

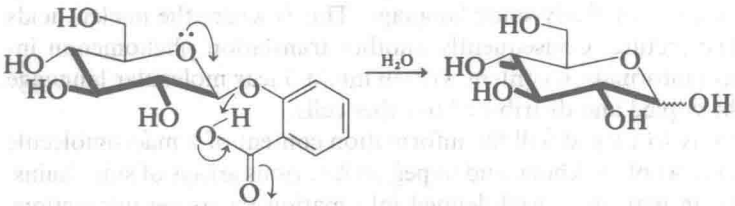
The large variety of organic structures known offer an infinite number of structural and functional properties to a protein. Using water as the translating medium, one can go from nonpolar (structured or nonstructured) to polar (hydrogen bonded) to ionic (solvated) amino acids; from aromatic to aliphatics; from reducible to oxidizable groups. Thus, almost the entire encyclopedia of chemical organic reactions can be coded on a polypeptide backbone and tertiary structure. Finally, since all amino acid present are of L (or *S*) configuration, we realize that *chirality* is essential for order to exist.

1.2 Proximity Effects in Organic Chemistry

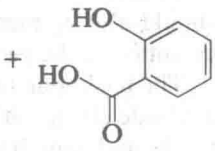
Proximity of reactive groups in a chemical transformation allows bond polarization, resulting generally in an acceleration in the rate of the reaction. In nature this is normally achieved by a well-defined alignment of specific amino acid side chains at the active site of an enzyme.

Study of organic reactions helps to construct proper biomodels of enzymatic reactions and open a field of intensive research: medicinal chemistry through rational drug design. Since a meaningful presentation of all applications of organic reactions would be a prodigious task, we limit the present discussion in this chapter to a few representative examples. These illustrate some of the advantages and problems encountered in conceptualizing bioorganic models for the study of enzyme mechanism. Chapter 4 will give a more complete presentation of the proximity effect in relation to intramolecular catalysis.

The first example is the hydrolysis of a glucoside bond. *o*-Carboxyphenyl *β*-D-glucoside (**1-1**) is hydrolyzed at a rate 10^4 faster than the corresponding



1-1 **1-2**

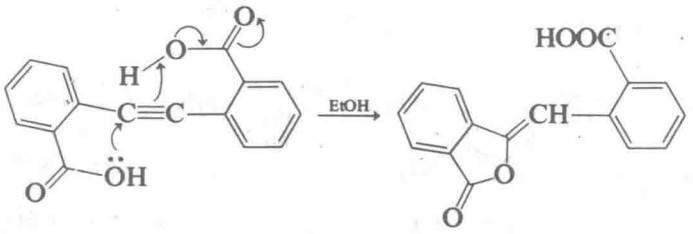


1-3

p-carboxyphenyl analogue. Therefore, the carboxylate group in the *ortho* position must "participate" or be involved in the hydrolysis.

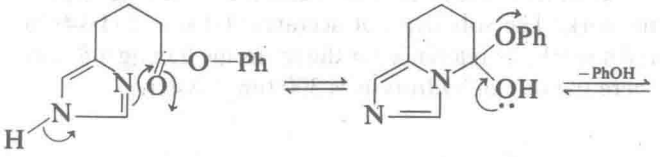
This illustrates the fact that the proper positioning of a group (electrophilic or nucleophilic) may accelerate the rate of a reaction. There is thus an analogy to be made with the active site of an enzyme such as lysozyme. Of course the nature of the leaving group is also important in describing the properties. Furthermore, solvation effects can be of paramount importance for the course of the transformation especially in the transition state. Reactions of this type are called *assisted hydrolysis* and occur by an intramolecular displacement mechanism; steric factors may retard the reactions.

Let us look at another example: 2,2′-tolancarboxylic acid (**1-4**) in ethanol is converted to 3-(2-carboxybenzilidene) phthalide (**1-5**). The rate of the reaction is 10^4 faster than with the corresponding 2-tolancarboxylic or 2,4′-tolancarboxylic acid. Consequently, one carboxyl group acts as a general acid catalyst (see Chapter 4) by a mechanism known as *complementary bifunctional catalysis.*

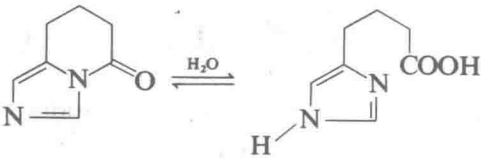


1-4 **1-5**

The ester function of 4-(4′-imidazolyl) butanoic phenyl ester (**1-6**) is hydrolyzed much faster than the corresponding *n*-butanoic phenyl ester. If a *p*-nitro group is present on the aryl residue, the rate of hydrolysis is even faster at neutral pH. As expected, the presence of a better leaving group further accelerates the rate of reaction. This hydrolysis involves the formulation of a tetrahedral intermediate (**1-7**). A detailed discussion of such intermediates



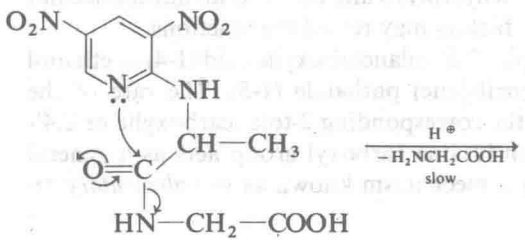
1-6 **1-7**



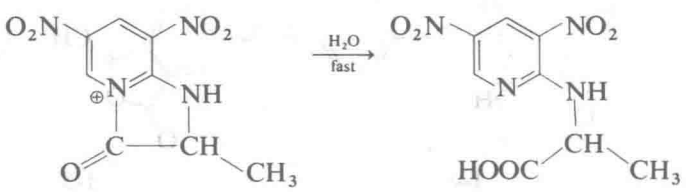
1-8 **1-9**

will be the subject of Chapter 4. The imidazole group acts as a nucleophilic catalyst in this two-step conversion and its proximity to the ester function and the formation of a cyclic intermediate are the factors responsible for the rate enhancement observed. The participation of an imidazole group in the hydrolysis of an ester may represent the simplest model of hydrolytic enzymes.

In a different domain, amide bond hydrolyses can also be accelerated. An example is the following where the reaction is catalyzed by a pyridine ring.



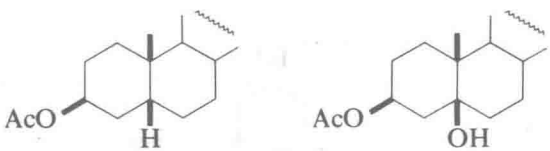
1-10



1-11 **1-12**

The first step is the rate limiting step of the reaction (slow reaction) leading to an acyl pyridinium intermediate (**1-11**), reminiscent of a covalent acyl-enzyme intermediate found in many enzymatic mechanisms. This intermediate is then rapidly trapped by water.

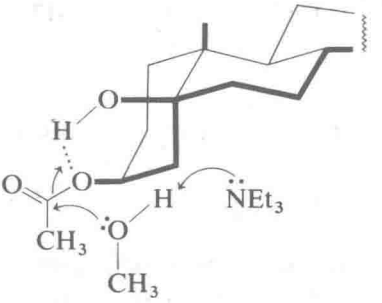
The last example is taken from the steroid field and illustrates the importance of a rigid framework. The solvolysis of acetates (**1-13**) and (**1-14**) in CH_3OH/Et_3N showed a marked preference for the molecule having a β-OH group at carbon 5 where the rate of hydrolysis is 300 times faster.



cis junction

1-13 **1-14**

The reason for such a behavior becomes apparent when the molecule is drawn in three-dimensions (**1-15**). The rigidity of the steroid skeleton thus helps in bringing the two functions in proper orientation where catalysis combining one intramolecular and one intermolecular catalyst takes place.



1-15

The proximal hydroxyl group can cooperate in the hydrolysis by hydrogen bonding and the carbonyl function of the ester becomes a better electrophilic center for the solvent molecules. In this mechanism one can perceive a general acid–base catalysis of ester solvolysis (Chapter 4).

These simple examples illustrate that many of the basic active site chemistry of enzymes can be reproduced with simple organic models in the absence of proteins. The role of the latter is of substrate recognition and orientation and the chemistry is often carried out by cofactors (coenzymes) which also have to be specifically recognized by the protein or enzyme. The last chapter of this book is devoted to the chemistry of coenzyme function and design.

1.3 Molecular Adaptation

Other factors besides proximity effects are important and should be considered in the design of biomodels. For instance in 1950, at the *First Symposium on Chemical-Biological Correlation*, H. L Friedman introduced the concept of *bioisosteric groups* (1). In its broadest sense, the term refers to chemical groups that bear some resemblance in molecular size and shape and as a consequence can compete for the same biological target. This concept has important application in molecular pharmacology, especially in the design of new drugs through the method of variation, or molecular modification (2).

Some pharmacological examples will illustrate the principle. The two neurotransmitters, acetylcholine (**1-16**) and carbachol (**1-17**), have similar muscarinic action.

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