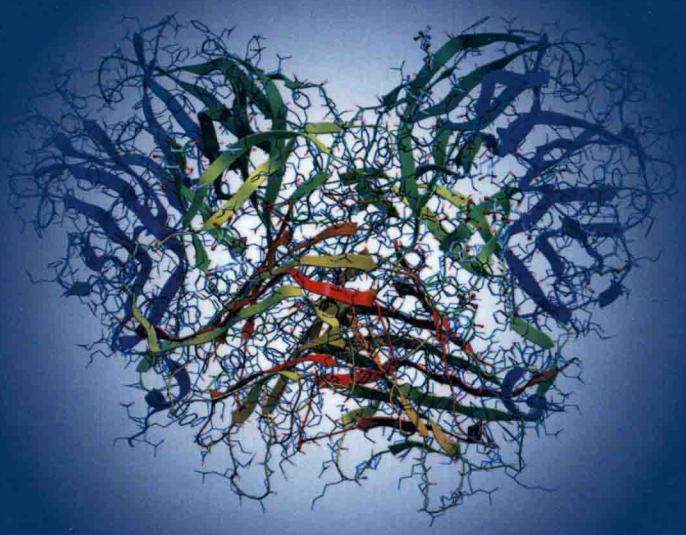
ELSEVIER

ORGANIC SYNTHESIS USING BIOCATALYSIS



Edited by

Animesh Goswami
Jon D. Stewart

Organic Synthesis Using Biocatalysis

Edited by

Animesh Goswami
Chemical Development, Bristol-Myers Squibb,
New Brunswick, NJ, USA

Jon D. Stewart
Department of Chemistry, University of Florida,
Gainesville, FL, USA

ELSEVIER

Amsterdam • Boston • Heidelberg • London • New York • Oxford
Paris • San Diego • San Francisco • Singapore • Sydney • Tokyo

Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK
225 Wyman Street, Waltham, MA 02451, USA

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

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

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-411518-7

Organic Synthesis Using Biocatalysis

List of Contributors

Samantha K. Au
School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Parker H. Petit Institute of Bioengineering and Bioscience, Atlanta, GA, USA

Andreas S. Bommarius
School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Parker H. Petit Institute of Bioengineering and Bioscience; School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA

Chen Cao
Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta-cho, Midori-ku, Yokohama, Japan

Pere Clapés
Department Química Biológica y Modelización Molecular, Instituto de Química Avanzada de Cataluña, IQAC-CSIC, Barcelona, Spain

Rodrigo O.M.A. de Souza
Biocatalysts and Organic Synthesis Lab, Organic Chemistry Department, Chemistry Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Brent D. Feske
Chemistry and Physics Department, Armstrong State University, Savannah, GA, USA

Michael J. Fink
Vienna University of Technology, Institute of Applied Synthetic Chemistry, Vienna, Austria

Animesh Goswami
Chemical Development, Bristol-Myers Squibb, New Brunswick, NJ, USA

Gideon Grogan
Department of Chemistry, University of York, Heslington, York, UK

Harald Gröger
Faculty of Chemistry, Bielefeld University, Universitätsstr, Bielefeld, Germany

Jonathan Groover
Chemistry and Physics Department, Armstrong State University, Savannah, GA, USA

Melissa L.E. Gutarra
Escola de Química, Federal University of Rio de Janeiro, Pólo Xerém, Estrada de Xerém, Xerém, Duque de Caxias, Rio de Janeiro, Brazil

Romas Kazlauskas
Department of Biochemistry, Molecular Biology & Biophysics and The Biotechnology Institute, University of Minnesota, Saint Paul, MN, USA

Tomoko Matsuda
Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta-cho, Midori-ku, Yokohama, Japan

Marko D. Mihovilovic
Vienna University of Technology, Institute of Applied Synthetic Chemistry, Vienna, Austria

Leandro S.M. Miranda
Biocatalysts and Organic Synthesis Lab, Organic Chemistry Department, Chemistry Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Thomas S. Moody
Department of Biocatalysis and Isotope Chemistry, Almac, Craigavon, Northern Ireland, UK

Ramesh N. Patel
SLRP Associates, Consultation in Biotechnology, Bridgewater, NJ, USA

Laila Roper
Department of Chemistry, University of York, Heslington, York, UK

J. David Rozzell
Provivi, Santa Monica, CA, USA

Florian Rudroff
Vienna University of Technology, Institute of Applied Synthetic Chemistry, Vienna, Austria

Jon D. Stewart
Department of Chemistry, University of Florida, Gainesville, FL, USA

Table of Contents

CHAPTER 1
Introduction, Types of Reactions, and Sources of Biocatalysts

Animesh Goswami*, Jon D. Stewart†
*Chemical Development, Bristol-Myers Squibb, New Brunswick, NJ, USA
†Department of Chemistry, University of Florida, Gainesville, FL, USA

1 INTRODUCTION

1.1 Enzymes and Their Roles in Nature

Enzymes are nature's catalysts, facilitating the creation, functioning, maintenance, and ultimately the demise of all living cells. Enzymes are proteins composed of 20 natural amino acids joined together by peptide bonds, in some cases augmented with additional organic or inorganic species known as cofactors.[1] In addition to their primary molecular structures dictated by the amino acid sequence, enzyme catalytic function also depends upon subsequent folding into specific three-dimensional shapes that contain a variety of secondary and tertiary structural elements. These architectures determine not only enzyme function but also how they interact with the external solvent medium in which they are dissolved or suspended. This can have important ramifications when enzymes are employed under partially or completely nonaqueous conditions.

Like all catalysts, enzymes increase reaction rates by lowering their activation energies. The most important difference between enzymes and simple catalysts such as a proton or hydroxide is that the former are much more restrictive in the range of acceptable substrates. The three dimensional structure of the enzyme allows binding of only those starting materials (usually referred to as substrates) whose structures are congruent with the size, shape and polarity of the catalytic portion of the enzyme (the "active site"). *Formation of this noncovalent complex prior to chemical conversion is the key to the high selectivity of enzyme-catalyzed reactions since it places the substrate into a specific location where its functional groups are oriented precisely with those on the enzyme.*

[1] Some RNA molecules also possess catalytic abilities; however, their substrate and range of chemical conversions seems rather limited and for this reason, catalytic RNA molecules lie outside the scope of this book.

Organic Synthesis Using Biocatalysis. http://dx.doi.org/10.1016/B978-0-12-411518-7.00001-9

Noncovalent complex formation allows chemical reactions between specific amino acids on the enzyme and functional groups on the substrate to occur in an environment that is kinetically equivalent to a unimolecular process. Transforming what would otherwise be bimolecular reactions into effectively intramolecular conversions is a major reason that enzymes can accelerate reactions by up to 23 orders of magnitude over the background (uncatalyzed) reaction [1].

Selectivity is the second benefit from forming a noncovalent enzyme–substrate complex prior to chemical conversion. By focusing the catalytic attention of the enzyme onto a specific area of the substrate, reactions can be restricted to a single portion of the molecule that may or may not be the most reactive portion of the overall substrate structure. This allows enzyme-catalyzed reactions to be selective in many respects: chemoselective (carrying out only one specific transformation while others are possible), regioselective (transforming only one among several possible sites), and stereoselective (producing and/or consuming one stereoisomer in preference to others).

2 DEFINITION OF BIOCATALYSIS

In nature, enzymes catalyze transformations of metabolites that occur within and/or outside of living cells. Although some enzymes accept only a limited variety of substrates, a large fraction is more tolerant and allows conversions of nonnatural starting materials. The field of biocatalysis rests upon this partial promiscuity. If enzymes were truly selective for only a single substrate, it would be impossible to utilize them for synthesizing new molecules from nonnatural substrates. The goal is to identify or engineer enzymes that are sufficiently *general* to accept a variety of related substrates, but *selective* enough to yield single products or stereoisomers. We use the term "biocatalysis" to describe the use of enzymes (either native or modified) for synthetic transformations of nonnatural starting materials. Enzymes used for *in vitro* synthetic transformations are called biocatalysts, and the processes are called biocatalytic transformations.

3 SCOPE OF THIS BOOK

Some enzymes catalyze the reactions that build up large molecules from simple building blocks, for example, complex carbohydrates from carbon dioxide and water or the synthesis of steroids and terpenoids from acetate. Others are involved in the degradation of large assemblies to small molecules, for example, hydrolysis of proteins to amino acids and the oxidative degradation of lignin. Although some of these native conversions are industrially important and practiced on large scales, the use of enzymes to produce their normal primary and secondary products of cells lies outside the scope of this book. Here, our focus is on preparing *nonnatural* compounds using enzymes since this addresses the need commonly encountered in organic synthesis. However, it should be noted that the native reactions of an enzyme can often be used as starting points for their applications to nonnative reactions.

The field of metabolic engineering also lies outside the scope of this book. These efforts use two or more enzymes to catalyze sequential steps in a pathway that

links a simpler starting material such as glucose with a final intracellular target product such as butanol or lysine. In some cases, the complete pathway already exists within a single organism; in others, enzymes from different sources are assembled into an artificial metabolic pathway in a suitable host cell. The key difference between biocatalysis and metabolic engineering is that the molecular skeletons are provided *in vitro* in the former case and *in vivo* in the latter. Although it is economically attractive to produce a target molecule by metabolic engineering, this benefit must be balanced against the (usually) lengthy optimization phase required for efficient production and the restriction that intermediates and the final product should be nontoxic to the host cells.

4 KEY BENEFITS OF EMPLOYING ENZYMES IN SYNTHESIS

Enzymes offer several attractive features as catalysts for organic synthesis. They often show high selectivities, they can operate under mild conditions and they are completely biodegradable catalysts constructed solely from renewable resources. They are thus ideal strategies as chemistry embraces sustainability.

4.1 Selectivity: Chemo-, Regio- and Stereo-

Biocatalytic reactions can show very high selectivities in all respects (Figure 1.1). When similarly-reactive functional groups are present in a molecule, the enzymes often catalyze only the reaction of one while leaving the others intact. For example, nitrile hydratases catalyze the partial hydrolysis of a nitrile group to yield a primary amide without cleaving an ester moiety present in the same molecule or further hydrolyzing the amide product, a property referred to as "chemoselectivity."

"Regioselectivity" is another useful property displayed by many enzymes. This refers to the transformation of one functional group while leaving other identical (or nearly identical) moieties at different locations within the molecule untouched. For example, among three esters in a triacylglyeride, many lipases hydrolyze only one position, and do not catalyze further hydrolysis of the diester product.

All but one of the amino-acid building blocks have at least one chiral center,[2] and for this reason, enzymes are intrinsically asymmetric catalysts. The asymmetric nature of enzymes results in enantioselectivity when biocatalysts convert a prochiral starting material into a single product enantiomer, for example, in ketone or imine reductions. The same asymmetric nature also causes the biocatalysts to preferentially transform only one stereoisomer of a starting material that contains a mixture of diastereomers or enantiomers. Such a process is referred to as a kinetic resolution and the ratio of rate constants for the fast- and slow reacting enantiomers is termed the enantioselectivity (E) ratio. E values higher than 100 are commonly observed for biocatalysts, and values in this range allow both the residual starting material (the slow-reacting enantiomer) and the product

[2] Glycine is the only achiral amino acid normally found in proteins.

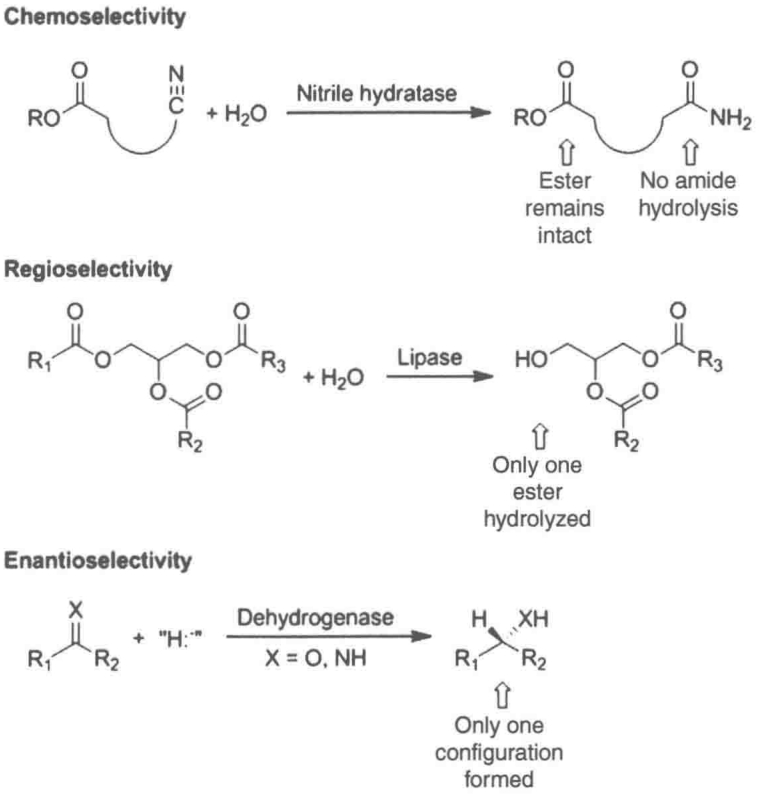


FIGURE 1.1
Types of selectivity exhibited by enzymes. Biocatalytic processes can provide chemo-, regio-, and stereoselective conversions. Representative examples can be observed in reactions catalyzed by nitrile hydratases, lipases and dehydrogenases.

(from the faster-reacting enantiomer) to be obtained with high optical purities from a single reaction run to 50% conversion.

4.2 Biocatalyst Reaction Conditions

Enzymes have generally evolved to function best under the conditions that exist within their respective cellular environments. This usually means ambient temperatures (20–40°C), near-neutral pH values, and with water as the solvent. Such conditions are particularly appropriate for sensitive starting materials and/or products, and this constitutes an important reason to employ enzymes in organic synthesis. It should be noted, however, that some enzymes have evolved to function under extreme conditions. For example, thermophilic bacteria that thrive at temperatures more than 100°C have been valuable sources of enzymes with much higher-than-usual thermal stabilities. In addition, enzymes normally found outside cells (extracellular enzymes) are also generally more stable since their operating environment is less predictable and largely uncontrolled. This diversity in thermal stabilities often allows one to choose a reaction temperature that balances good reaction rates with enzyme stability and also maximizes space–time yields.

In nature, most enzyme-catalyzed reactions occur in an aqueous environment and many synthetic applications, therefore, also utilize water as solvent. This is often advantageous with regard to maximizing the sustainability of a chemical process. Moreover, enzymes are usually most stable in water. These benefits, however, must be balanced against two disadvantages of using water as a solvent for biocatalytic processes. Water is a reactant in hydrolytic processes and its concentration must be minimized when such reactions are run in reverse in order to shift the equilibrium, for example, when using enzymes to synthesize esters or amides from carboxylic acids and alcohols or amines, respectively. In such cases, water-organic biphasic systems or completely organic solvents can be used. The second complication associated with aqueous conditions is that many of the starting materials and products of synthetic interest have very limited solubilities in water. This either requires the use of dilute solutions, which lowers space–time yields and also generates large volumes of wastewater that must be treated, or the use of organic solvents as additives or replacements for water.

Biocatalysts are proteins and composed of natural amino acids. In some cases, additional natural ligands (cofactors) are present. This means that biocatalysts are inherently nonhazardous materials, although it should be noted that, because they are proteins, some enzymes may cause allergenic reactions in susceptible individuals. Although such reactions are rare, normal care should be taken when handling solid enzyme powders.

5 MECHANISM AND KINETICS OF ENZYME-CATALYZED REACTIONS

Although it is not necessary to determine kinetic parameters in order to use enzymes for chemical synthesis, this knowledge can often be useful in deciding which avenues offer the best opportunities for process improvements. Likewise, it is not essential – but often highly useful – to understand the mechanism of the enzyme-catalyzed reaction for the same reasons that one often benefits from knowing the mechanisms of any reaction employed in a synthetic route. Although every individual enzymatic reaction has a unique combination of kinetic properties and reaction mechanism, several useful generalizations are summarized in the subsequent section.

5.1 Features of Enzyme Catalyzed Reactions

As noted previously, noncovalent association between the enzyme and its substrate(s) is the essential first step in biocatalytic reactions. Although early theories of enzyme catalysis focused primarily on interactions between the enzyme and substrate, it was later appreciated that maximizing selective, noncovalent interactions between the enzyme and the high-energy transition state(s) that linked enzyme-bound complexes of substrates and products was the key to efficient rate enhancements. A somewhat oversimplified view is that ground-state interactions determine substrate specificity and transition-state interactions yield rate enhancements.

In addition to noncovalent associations (by van der Waals forces, hydrogen bonds, electrostatic and hydrophobic interactions), some enzymes also form covalent bonds between the enzyme and portions of the substrate. Lipases are a well-known example of this phenomenon. These enzymes utilize a specific protein hydroxyl group (most commonly a serine side chain) to form an ester intermediate with the substrate that is subsequently cleaved by an exogenous nucleophile to form the final reaction product and regenerate the free protein hydroxyl group, making the active site suitable for the next catalytic cycle.

5.2 Coenzymes

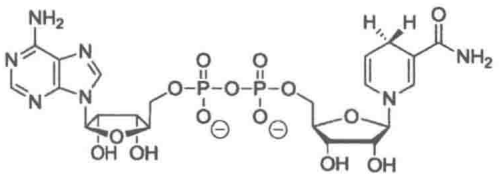
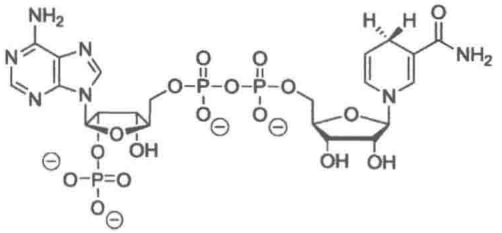
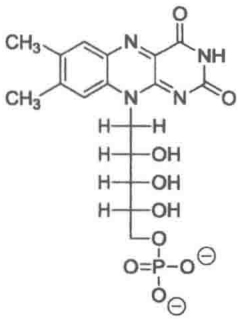
Although some enzyme-catalyzed reactions utilize only functional groups found in the protein, the limited number and variety of amino-acid side-chain moieties severely limits the range of accessible reactions. For example, there are neither common amino acids with electrophilic side chains nor amino-acid functional groups suitable for redox catalysis.[3] Nature has circumvented this problem by evolving a suite of coenzymes (also known as cofactors) that specialize in particular types of chemical conversions. Table 1.1 lists some common cofactors for biocatalytic processes. In some cases, for example, biotin and some flavins, these cofactors are covalently coupled to the enzyme within the active site. Other cofactors such as nicotinamides are bound reversibly by noncovalent forces during the entire catalytic cycle. Finally, a few cofactors such as pyridoxal phosphate form reversible covalent linkages with the resting form of the enzyme that are cleaved during the catalytic cycle, and then re-formed at the end. When needed by specific enzymes, provision for cofactor supply must also be made. Because of their expense, cofactors are normally supplied in substoichiometric quantities (usually $\ll 0.1$ mole %). This means that they must be regenerated prior to the start of the next catalytic cycle, and strategies for cofactor regeneration have been developed as an essential adjunct for biocatalytic reactions, particularly for reductions and oxidations.

5.3 Kinetics and Reaction Mechanisms

Reaction mechanisms describe the sequence of bond breaking and bond making steps, whereas kinetics are concerned with the nature and timing of the noncovalent complexes that form and break down during the catalytic cycle. Depending on the number of substrates and products, kinetics can be simple or complex. Single-substrate/single-product reactions are the most straightforward schemes, although there are relatively few examples of such reactions in biocatalysis. More commonly, two or more molecules are bound and/or released. This introduces the question of timing with respect to formation and breakdown of enzyme – ligand complexes. Three common reaction mechanisms for a two-substrate/two-product reaction are illustrated schematically in Figure 1.2. In an ordered mechanism, the enzyme cannot productively bind the second substrate

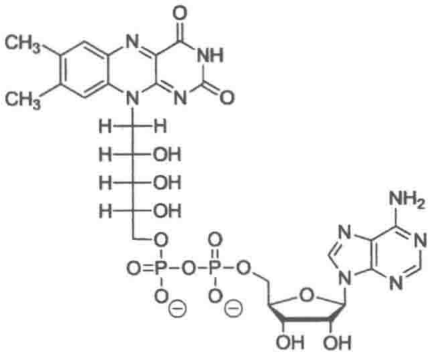
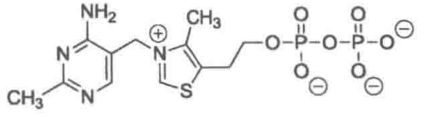
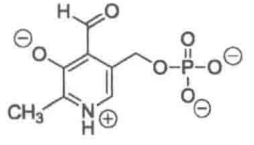
[3] The only exception is disulfide bond formation between a pair of suitably positioned cysteine side-chains.

Table 1.1 Cofactors Commonly Encountered in Biocatalytic Reactions Applied to Chemical Synthesis

Name	Cofactor Structure	Chemical Function	Enzyme
Nicotinamide adenine dinucleotide, reduced form (NADH)[a]		Donates a hydride for polar reductions of functional groups such as C = O and C = N; acts as an electron source for monooxygenases	Alcohol dehydrogenase/ketoreductase; alkene reductase/enoate reductase; amino acid dehydrogenase; monooxygenase; dioxygenase
Nicotinamide adenine dinucleotide phosphate, reduced form (NADPH)[b]		Donates a hydride for polar reductions of functional groups such as C = O and C = N; acts as an electron source for monooxygenases	Alcohol dehydrogenase/ketoreductase; alkene reductase/enoate reductase; amino acid dehydrogenase; monooxygenase; dioxygenase
Flavin mononucleotide (FMN)		Forms a hydroperoxy intermediate from O_2 that is used by monooxygenases; following 2 electron reduction, donates a hydride for reductions of electron-deficient C = C bonds	Alkene reductase/enoate reductase; amino acid oxidase; Baeyer–Villiger monooxygenase

(Continued)

Table 1.1 Cofactors Commonly Encountered in Biocatalytic Reactions Applied to Chemical Synthesis *(cont.)*

Name	Cofactor Structure	Chemical Function	Enzyme
Flavin adenine dinucleotide (FAD)		Forms a hydroperoxy intermediate from O_2 that is used by monooxygenases; following 2 electron reduction, donates a hydride for reductions of electron-deficient C = C bonds	Alkene reductase/enoate reductase; amino acid oxidase; Baeyer–Villiger monooxygenase
Thiamine pyrophosphate		Allows umpolung anion formation by aldehyde carbonyl groups; facilitates α-keto acid decarboxylations	Pyruvate decarboxylase; benzoylformate decarboxylase; phenylpyruvate decarboxylase
Pyridoxal phosphate		Following reversible Schiff's base formation, the cofactor stabilizes an anion on the carbon adjacent to the amine	Transaminase; threonine aldolase

[a]*Known as DPNH in the very old scientific literature*
[b]*Known as TPNH in the very old scientific literature*