

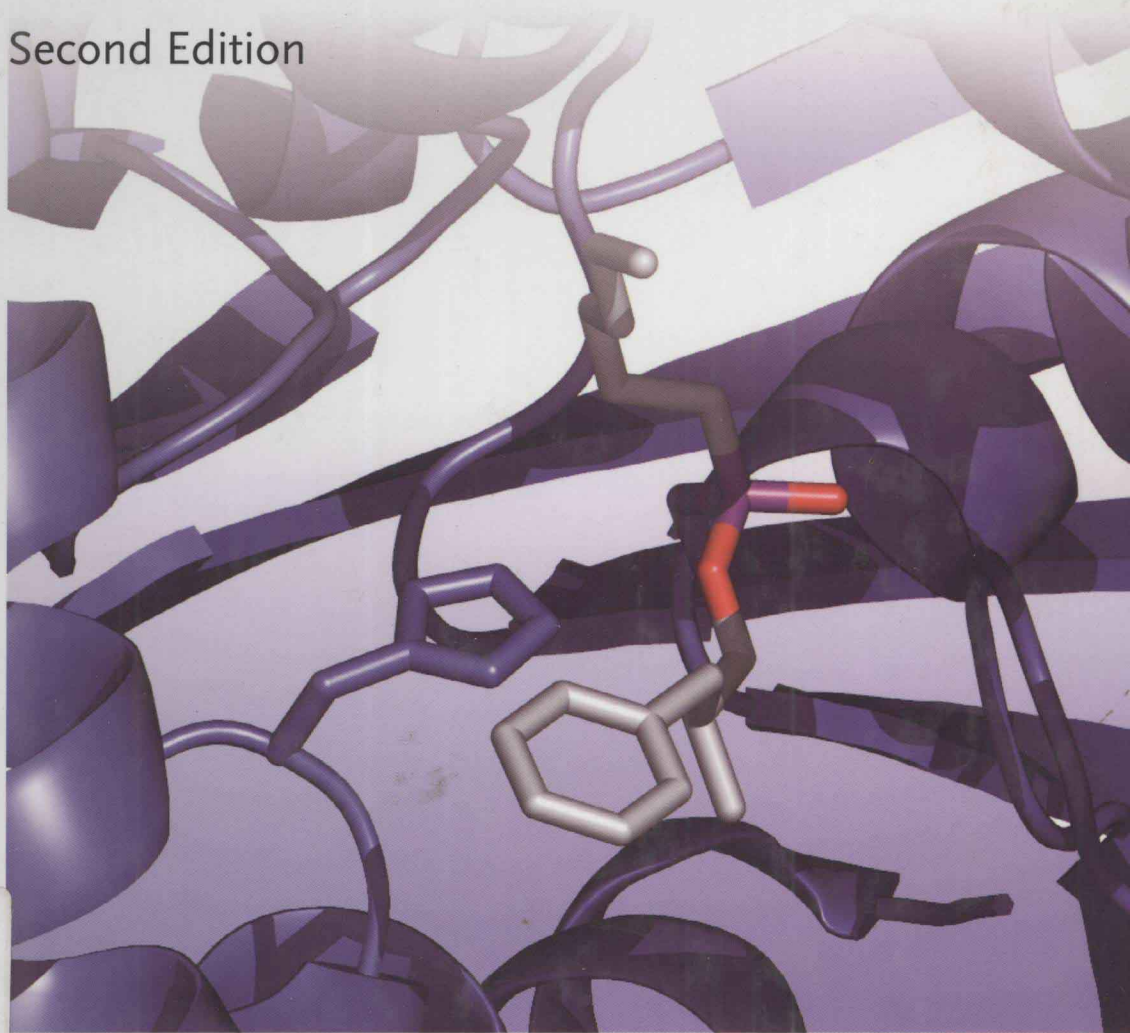
Uwe T. Bornscheuer and
Romas J. Kazlauskas

 WILEY-VCH

Hydrolases in Organic Synthesis

Regio- and Stereoselective Biotransformations

Second Edition



Uwe T. Bornscheuer and Romas J. Kazlauskas

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Regio- and Stereoselective Biotransformations

2nd edition



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Xray structure showing a phosphonate transition-state analog bound in the active site of Pseudomonas cepacia lipase (PDB code: 1ys2)

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*Uwe T. Bornscheuer and
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To

Aleli and Tanja

and to Jonas and Stasè

and also to

Lottie, Anna, Jonas and Annika

Preface for the 1st edition

Each traveller to a city seeks something different. One wants to see that special painting in the museum, another wants to drink the local beer, a third wants to meet a soulmate.

Each organic chemist also seeks something different from the field of biocatalysis. One wants high enantioselectivity, another wants reaction under mild conditions, a third wants to scale up to an industrial scale. We hope this book can be a guide to organic chemists exploring the field of biocatalysis. Enzyme-catalyzed reactions, especially hydrolase-catalyzed reactions, have already solved hundreds of synthetic problems usually because of their high stereoselectivity.

The organization is aimed at the chemist – by reaction type and by different functional groups. This information should help organic chemists identify the best hydrolase for their synthetic problem. In addition, we suggest how to choose an appropriate solvent, acyl donor, immobilization technique and other practical details. We hope that learning how others solved synthetic problems will generate ideas that solve the next generation of problems.

Although this book has more than 1 700 references, we might have missed important hydrolase-catalyzed reactions. The choices on what to include usually reflect our own research interests, but were sometimes arbitrary or even inadvertent. We will post corrections and additions on a web site: <http://pasteur.chem.mcgill.ca/hydrolases.html>.

Montreal/Stuttgart, February 1999

Romas J. Kazlauskas, Uwe T. Bornscheuer

Preface for the 2nd edition

Over the decade, hydrolases have become key parts of the growing area of industrial biotechnology (Schmid et al., 2001; Shoemaker et al., 2003). We hope that the first edition of *Hydrolases in Organic Syntheses* contributed to this success. It was a key reference in our laboratories and an excellent starting point for new researchers in the field. For this success to continue, we felt that we had to keep it current with new developments in the area – directed evolution and protein design, dynamic kinetic resolutions, and the use of structures to explain selectivity.

Planning the 2nd edition presented a challenge – more than 900 publications in the area since 1999. We chose to focus on new areas, new insights, and new frontiers and not to focus on comprehensive lists of substrates. For this information, databases are more efficient than a book. We've added a new chapter on protein sources and optimization of biocatalyst performance for organic synthesis, an expanded chapter about directed evolution and a new chapter about catalytic promiscuity. In addition, we expanded sections dynamic kinetic resolution and immobilization and added new classes of hydrolases, such as haloalkane dehalogenases and organophosphorus hydrolases. In turn, we shortened some sections including a major shortening of the sections on lipid modification, which is of limited interest to organic chemists. These changes required a rearrangement of the book chapters. In addition, many sections contain minor updates.

We sincerely hope that these changes will extend and enhance the usefulness of this book.

Minneapolis/Greifswald, August 2005

Romas J. Kazlauskas, Uwe T. Bornscheuer

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1 Introduction

Hydrolases are the group of enzymes that catalyze bond cleavage by reaction with water. The natural function of most hydrolases is digestive – to break down nutrients into smaller units for digestion. For example, proteases hydrolyze proteins to smaller peptides and then to amino acids and lipases hydrolyze lipids (triglycerides) to glycerol and fatty acids (Fig. 1). Because of the need to break down a wide range of nutrients, hydrolases usually have a broad substrate specificity.

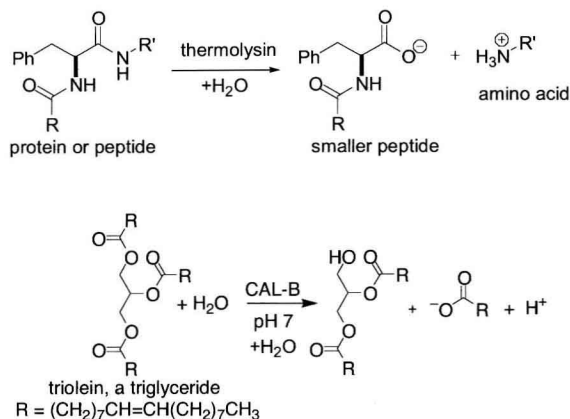


Fig. 1. The natural role of most hydrolases is digestive – to break down nutrients into smaller units. Thermolysin, a protease secreted by thermophilic bacteria, catalyzes the hydrolysis of proteins to peptides and then further to amino acids. Lipase B from *Candida antarctica* (CAL-B) catalyzes the stepwise hydrolysis of triglycerides (e.g., triolein) to fatty acids and glycerol. The reaction shows only the first step from a triglyceride to a diglyceride.

Several characteristics make hydrolases useful to the organic chemist. First, because of their broad substrate specificity, hydrolases often accept as substrates various synthetic intermediates. Second, hydrolases often show high stereoselectivity, even toward unnatural substrates. Third, besides hydrolysis, hydrolases also catalyze several related reactions – condensations (reversal of hydrolysis) and alcoholysis (a cleavage using an alcohol in place of water). Two examples from industry are shown in Fig. 2. Thermolysin catalyzes the condensation of two amino acid derivatives to make an aspartame derivative (Isowa et al., 1979). The reaction proceeds in the condensation direction because the product precipitates from solution. The high enantioselectivity permits using racemic starting materials and the high regioselectivity of thermolysin eliminates the need to protect the β -carboxyl group of the aspartic acid derivative. The second example is an alcoholysis reaction (Morgan et al., 1997a). The ester, vinyl acetate, is cleaved not by water, but by the substrate alcohol. The liberated vinyl alcohol (not shown) tautomerizes to acetaldehyde. These alcoholysis reactions are also called transesterification reactions.

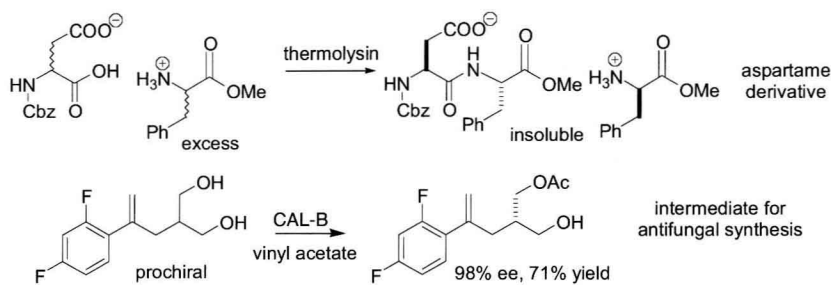


Fig. 2. Several unnatural, synthetically useful reactions catalyzed by hydrolases. Thermolysin catalyzes the regio- and enantioselective coupling of *N*-benzyloxycarbonyl-L-aspartate with L-phenylalanine methyl ester. Precipitation of the product drives this reaction in the condensation direction instead of the normal hydrolysis direction. This condensation is a key step in the manufacture of aspartame, a low-calorie sweetener. Because of the high enantioselectivity of thermolysin, racemic substrates may be used. Because of the high regioselectivity of thermolysin for the α -carboxyl group, the β -carboxyl group in the aspartic acid derivative needs no protection. Lipase B from *Candida antarctica* (CAL-B) catalyzes the enantioselective acetylation of a prochiral diol yielding an intermediate for the synthesis of antifungal agents. This example is an alcoholysis where the ester, vinyl acetate, is cleaved not by water, but by the substrate alcohol. This reaction is run in an organic solvent to avoid the competing hydrolysis.

Several other features make hydrolases convenient to use as synthetic reagents. Many hydrolases (approximately several hundred) are commercially available. They do not require cofactors and they tolerate the addition of water-miscible solvents (e.g., DMSO, DMF). Lipases, esterases and some proteases are also stable and active in neat organic solvents.

Enzymes are often classified according to the reaction catalyzed using an Enzyme Commission (EC) number. According to this classification, hydrolases form group 3 and are further classified according to the type of bond hydrolyzed. For example enzymes in the group 3.1 hydrolyze ester bonds (Tab. 1). Further classification into subcategories yields a four digit EC number. For example, lipases have the number EC 3.1.1.3. Classification of the more useful enzymes for organic synthesis is given in Tab. 1. A convenient web site to look up numbers and classification is at <http://www.expasy.ch/enzyme>. One disadvantage of this classification is that all enzymes catalyzing the same reaction have the same number, even though they may have very different structures, properties and other characteristics. For example, all lipases have the same number even though there are more than one hundred different lipases.

Tab. 1. Selected Hydrolases Useful in Organic Synthesis.

EC Number	Type of bond hydrolyzed	Examples
3.1	Ester	
3.1.1	in carboxylic acid esters	triacylglycerol lipase, acetylcholine esterase, phospholipase A ₁ , phospholipase A ₂ , gluconolactonase, lipoprotein lipase
3.1.3–4	in phosphoric acid mono- or diesters	phospholipase C, phospholipase D
3.2	Glycosidic	
3.2.1	in <i>O</i> -glycosides	α -amylase, oligo-1,6-glucosidase, lysozyme, neuraminidase, α -glucosidase, β -galactosidase, α -mannosidase, N-acetyl- β -glucosaminidase, sucrose α -glucosidase, nucleosidases
3.3	Ether	
3.3.2	in epoxides	epoxide hydrolase
3.4	Peptide	
3.4.11	aminopeptidase	leucine aminopeptidase
3.4.16, 21	serine proteinase	subtilisin, chymotrypsin, thermitase
3.4.18, 22	cysteine proteinase	papain
3.4.17, 24	metalloproteinase	thermolysin
3.5	Other amides	
3.5.1	in linear amides	penicillin amidase (penicillin G acylase)
3.5.2	in cyclic amides	hydantoinase
3.5.5	in nitriles	nitrilase ^a
3.8	Halide bonds	
3.8.1	carbon-halide bonds	haloalkane dehalogenase

^aNitrile hydratase (EC 4.2.1.84), which catalyzes addition of water to a nitrile yielding an amide, is not a hydrolase, but a lyase.

This book describes the application of lipases and proteases in organic syntheses, but also surveys esterases, epoxide hydrolases, nitrile hydrolyzing enzymes and glycosidases. The emphasis is on examples that are synthetically useful, especially those that exploit the regio- and stereoselectivity of hydrolases.

