Science of Synthesis

Biocatalysis in Organic Synthesis 1

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Preface

As the pace and breadth of research intensifies, organic synthesis is playing an increasingly central role in the discovery process within all imaginable areas of science: from pharmaceuticals, agrochemicals, and materials science to areas of biology and physics, the most impactful investigations are becoming more and more molecular. As an enabling science, synthetic organic chemistry is uniquely poised to provide access to compounds with exciting and valuable new properties. Organic molecules of extreme complexity can, given expert knowledge, be prepared with exquisite efficiency and selectivity, allowing virtually any phenomenon to be probed at levels never before imagined. With ready access to materials of remarkable structural diversity, critical studies can be conducted that reveal the intimate workings of chemical, biological, or physical processes with stunning detail.

The sheer variety of chemical structural space required for these investigations and the design elements necessary to assemble molecular targets of increasing intricacy place extraordinary demands on the individual synthetic methods used. They must be robust and provide reliably high yields on both small and large scales, have broad applicability, and exhibit high selectivity. Increasingly, synthetic approaches to organic molecules must take into account environmental sustainability. Thus, atom economy and the overall environmental impact of the transformations are taking on increased importance.

The need to provide a dependable source of information on evaluated synthetic methods in organic chemistry embracing these characteristics was first acknowledged over 100 years ago, when the highly regarded reference source **Houben–Weyl Methoden der Organischen Chemie** was first introduced. Recognizing the necessity to provide a modernized, comprehensive, and critical assessment of synthetic organic chemistry, in 2000 Thieme launched **Science of Synthesis, Houben–Weyl Methods of Molecular Transformations**. This effort, assembled by almost 1000 leading experts from both industry and academia, provides a balanced and critical analysis of the entire literature from the early 1800s until the year of publication. The accompanying online version of **Science of Synthesis** provides text, structure, substructure, and reaction searching capabilities by a powerful, yet easy-to-use, intuitive interface.

From 2010 onward, **Science of Synthesis** is being updated quarterly with high-quality content via **Science of Synthesis Knowledge Updates**. The goal of the **Science of Synthesis Knowledge Updates** is to provide a continuous review of the field of synthetic organic chemistry, with an eye toward evaluating and analyzing significant new developments in synthetic methods. A list of stringent criteria for inclusion of each synthetic transformation ensures that only the best and most reliable synthetic methods are incorporated. These efforts guarantee that **Science of Synthesis** will continue to be the most up-to-date electronic database available for the documentation of validated synthetic methods.

Also from 2010, **Science of Synthesis** includes the **Science of Synthesis Reference Library**, comprising volumes covering special topics of organic chemistry in a modular fashion, with six main classifications: (1) Classical, (2) Advances, (3) Transformations, (4) Applications, (5) Structures, and (6) Techniques. Titles will include *Stereoselective Synthesis*, *Water in Organic Synthesis*, and *Asymmetric Organocatalysis*, among others. With expert-evaluated content focusing on subjects of particular current interest, the **Science of Synthesis Reference Library** complements the **Science of Synthesis Knowledge Updates**, to make **Science of Synthesis** the complete information source for the modern synthetic chemist.

The overarching goal of the **Science of Synthesis** Editorial Board is to make the suite of **Science of Synthesis** resources the first and foremost focal point for critically evaluated information on chemical transformations for those individuals involved in the design and construction of organic molecules.

Throughout the years, the chemical community has benefited tremendously from the outstanding contribution of hundreds of highly dedicated expert authors who have devoted their energies and intellectual capital to these projects. We thank all of these individuals for the heroic efforts they have made throughout the entire publication process to make **Science of Synthesis** a reference work of the highest integrity and quality.

The Editorial Board July 2010

Science of Synthesis Reference Library

Biocatalysis in Organic Synthesis (3 Vols.)
C-1 Building Blocks in Organic Synthesis (2 Vols.)
Multicomponent Reactions (2 Vols.)
Cross Coupling and Heck-Type Reactions (3 Vols.)
Water in Organic Synthesis
Asymmetric Organocatalysis (2 Vols.)
Stereoselective Synthesis (3 Vols.)

Volume Editors' Preface

The field of biocatalysis, defined as the use of enzymes for the transformation of unnatural compounds, dates back almost a century and in its infancy it was driven by curiosity about biochemical pathways and enzyme mechanisms. It was mainly during the 1980s that the enormous catalytic potential of enzymes was recognized for the asymmetric synthesis of unnatural, high-value targets. Subsequently, the increasing demand for environmentally compatible procedures paved the way for the application of biocatalysts for low-cost bulk chemicals. The ability to develop the next generation of biocatalysts was enabled by major technology advances in the biosciences, which triggered several distinct innovation waves:[1]

- In the 1980s, only crude commercial enzyme preparations from the food, detergent, and tanning industries were available, and their use for stereoselective synthesis had much of a black-box approach. Aiming to broaden the arsenal of enzymatic reactions, chemists began to screen whole microbial cells in the search for novel activities in the 1990s, but enzyme isolation was still a cumbersome task.
- Rapid advances in molecular biology widened the quantitative understanding of biocatalytic systems by means of genomics, proteomics, and metabolomics. These advances facilitated the sequence-based search and subsequent production of suitably tagged enzymes via cloning and overexpression into a reliable host, which has become simple and affordable enough to be carried out by chemists.
- The exponential growth in the availability of crystal structures of proteins has significantly contributed to the understanding of enzyme mechanisms, which allows biocatalysts to be tuned for improved selectivity and stability under process conditions by site-directed mutagenesis. Exploitation of the "catalytic promiscuity" of proteins has often led to unprecedented catalytic activities.
- New methods for activity testing enable high-throughput screening of large libraries of mutant enzymes generated through selective pressure by directed evolution.
- In the near future, the search for a desired catalytic activity, which is generally guided by sequence analogy today, will include the third dimension of a desired catalytic site derived from crystal structures to accommodate the transition state of almost any organic transformation.[2]
- The compatibility of enzymes with each other has enabled the design of highly efficient synthetic cascades, thereby avoiding the separation of sensitive intermediates.[3] It is expected that the ever-increasing complexity of cascade design will merge with the field of metabolic engineering, which allows the use of renewable carbon sources more efficiently as alternatives to petroleum-based platform chemicals.

As a result of these developments, it is now possible to obtain biocatalysts that catalyze a much more diverse range of synthetic transformations, including asymmetric amination of ketones (transaminases), C—C bond formation (aldolases, oxynitrilases), oxidation (amine/alcohol oxidases, P450 monooxygenases, Baeyer–Villiger monooxygenases), and reduction (ene reductases, amino acid dehydrogenases), as well as new enzymes for hydrolysis (nitrilases, nitrile hydratases, epoxide hydrolases). The increased availability of new biocatalysts will become even more prominent in the next five years as new biocatalyst platforms (e.g., imine reductases, alkyltransferases, halogenases) move from academic laboratories into practical application.

One impact of this rapidly changing landscape will be that process and medicinal chemists will have additional options for replacing expensive or toxic chemical reagents with more selective and sustainable biocatalysts. Although replacing a chemical reagent

with a biocatalyst represents a significant step forward for biocatalysis, more transformative opportunities are presented when the use of a biocatalyst enables a new synthetic route to the target molecule to be developed. Such routes can be more efficient and cost effective, since they cut out steps in the synthesis and hence reduce costs and waste. Thus, the synthetic chemists of the future will be able to redesign their routes to target molecules using biocatalysts that can catalyze reaction steps not achievable by alternative chemical approaches. Increasingly, chemo- and biocatalysts will be used in concert to develop efficient and telescoped reaction processes including dynamic kinetic resolution and deracemization reactions.

The conversion of an unnatural substrate in a laboratory or industrial process is often limited by the low performance of commercial "off-the-shelf" biocatalysts, which not long ago required an extensive search from biodiversity for an enzyme variant that is sufficiently effective and stable for an economical operation. In this respect, directed in vitro evolution has emerged as a powerful technology enabling us to improve essentially any desired property of an enzyme, including its substrate scope, stereoselectivity, catalytic efficiency, robustness to organic solvents, high substrate concentration, pH extremes, and elevated temperatures, or other external factors frequently dictated by optimum process conditions. Since the proof-of-principle stage two decades ago, significant developments with respect to advanced mutagenesis technologies, smart library design, high-throughput-screening methodology, and the introduction of powerful computer algorithms for the prediction of new enzyme function have revolutionized our abilities to rapidly create tailor-made enzymes with optimized properties. The exponential growth in the field of enzyme engineering by evolutive techniques and semi-rational design, drawing from a rapidly increasing wealth of (genome) sequences, protein X-ray structures, and biochemical data, is currently lifting the traditional limitations of enzymes as practical catalysts for synthetic organic chemistry and for the development of sustainable biocatalytic processes of the future.

As a consequence, it is now routinely possible to adapt enzymes to a specific reaction of interest with predefined process conditions rather than vice versa, as proven by the many success stories including the introduction of various new industrial processes on large scale that are based on specifically designed biocatalysts. Successful reports of enzymes being designed in silico ("theozymes") to catalyze unnatural reactions are already emerging. Although computational enzyme design is in its infancy and its impact on biocatalysis still limited, such methods point the way for the future and promise deeper insights into the origins of efficient enzymatic catalysis.

One way to promote the use of biocatalysis when designing synthetic routes to chemical targets is to embrace the concept of "biocatalytic retrosynthesis".[4] The fundamental premise of biocatalytic retrosynthesis is that target molecules are disconnected into smaller fragments based upon the increased availability of engineered biocatalysts to catalyze the forward synthetic reactions. Retrosynthesis is a standard tool used by organic chemists when designing novel synthetic routes, but biocatalysts are rarely considered during this design process; this is not surprising, since only recently has a diverse toolbox of biocatalysts become generally available. The now routine application of protein engineering and directed evolution for the creation of novel, robust biocatalysts has radically changed the landscape. With the current rate of progress, it is clear that during the next few years the number of biocatalysts available for use will greatly increase. One area where biocatalysis is having a major impact is in the synthesis of chiral amines. In the future, the synthesis of enantiomerically pure chiral amines will develop along similar lines to asymmetric ketone reduction, i.e. biocatalysts will become the preferred method of choice rather than a replacement for traditional chemical approaches in second-generation processes.

We believe that this broad contemporary overview on the state-of-the-art in enzymatic methods for asymmetric synthesis will be a useful portal for anyone interested in applying biocatalysis as a highly potent, selective, and sustainable technology complementary to metal catalysis and organocatalysis, and that this three-volume set will be a valuable addition to the acclaimed suite of *Science of Synthesis* resources as part of the *Reference Library*, which has an approach orthogonal to the original concept of focusing on product types rather than methodology. We as editors have benefited enormously from the excellent scientific expertise of the many authors from all over the world, and we are grateful for their outstanding efforts and their precious time dedicated to the successful completion of this unique project. Finally, we also would like to express our sincere appreciation to the entire editorial team at Thieme for their extraordinary efforts made toward a seamless handling of manuscripts throughout the entire publication process, but in particular for the excellent collaboration with volume coordinators Alex Russell, Toby Reeve, Matthew Weston, and Mark Smith, and not least to our colleague Joe Richmond for his initiative.

Volume Editors October 2014

K. Faber (Graz, Austria)
W.-D. Fessner (Darmstadt, Germany)
N. J. Turner (Manchester, UK)

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[4] Turner, N. J.; O'Reilly, E., *Nature Chem. Biol.*, (2013) **9**, 285.

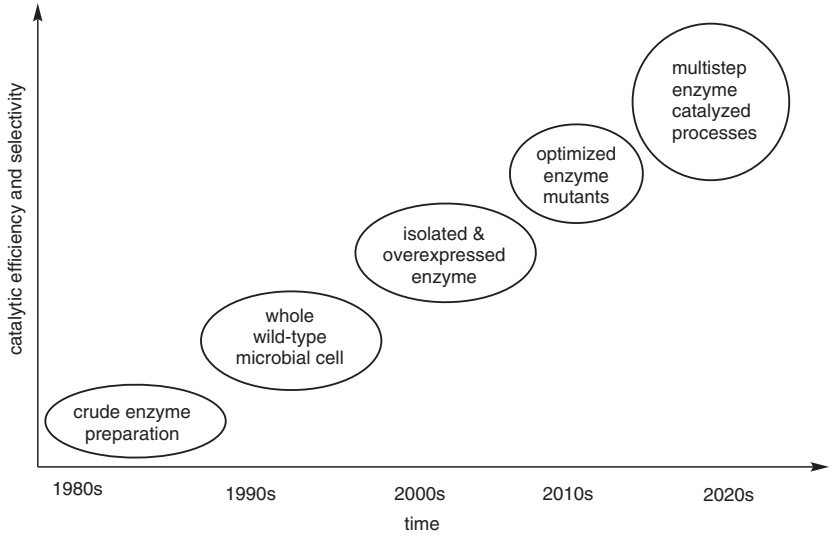
Abstracts

p 1

1.1.1 Historical Perspectives: Paving the Way for the Future

S. Servi, D. Tessaro, and F. Hollmann

This chapter describes the evolution of modern biocatalysis, focusing on the application of both whole-cell biocatalysts and isolated enzymes in organic synthesis. Milestones in this process are the application to β-lactam and amino acid chemistry, the preparation of chiral synthons as single enantiomers for the synthesis of pharmaceutical intermediates, the modification of carbohydrates and the synthesis of value-added products from lipids. The application of hydrolytic enzymes (lipases, proteases, esterases, and nitrile hydratases) has evolved in time toward more complex enzymatic systems such as oxidoreductases involving cofactor recycling or aminotransferases (transaminases) leading to the formation of chiral amines. The recently developed techniques of molecular biology and directed evolution toward the preparation of better enzymatic catalysts are dramatically improving the availability and efficiency of the enzymes and thus significantly increasing the role of biocatalysis in organic synthesis.



Keywords: chiral synthons · whole-cell biocatalysis · hydrolytic enzymes · oxidoreductases · cofactor recycling · directed evolution · cascade biocatalysis

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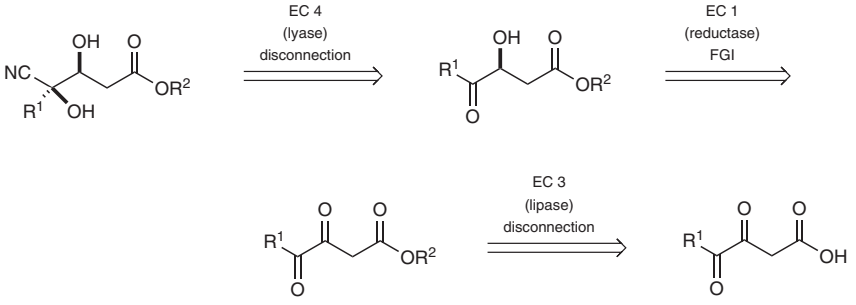
1.1.2 Enzyme Classification and Nomenclature and Biocatalytic Retrosynthesis

A. Liese and L. Pesci

The enzyme nomenclature system is based on six different enzyme classes, defined by the type of chemical reaction catalyzed; hence, for a given synthetic step, it is possible to plan an enzymatic transformation (even thinking in a retrosynthetic manner) for the synthesis and/or modification of a certain compound. With this premise, the possibility of combining the methods of traditional chemical retrosynthesis with biocatalytic transformations provides an enormous potential benefit for organic chemists, including the use of mod-

ern feedstocks and "sustainable chemistry" criteria. In this chapter, enzyme nomenclature is discussed, and the related information is used as a basis for applying biocatalytic retrosynthetic analysis to several classes of organic molecules. Some key examples are provided in order to appreciate the real potential of biocatalytic retrosynthesis, especially when used in combination with more traditional chemical strategies.



Keywords: enzyme nomenclature · reaction types · organic synthesis · retrosynthesis · green chemistry

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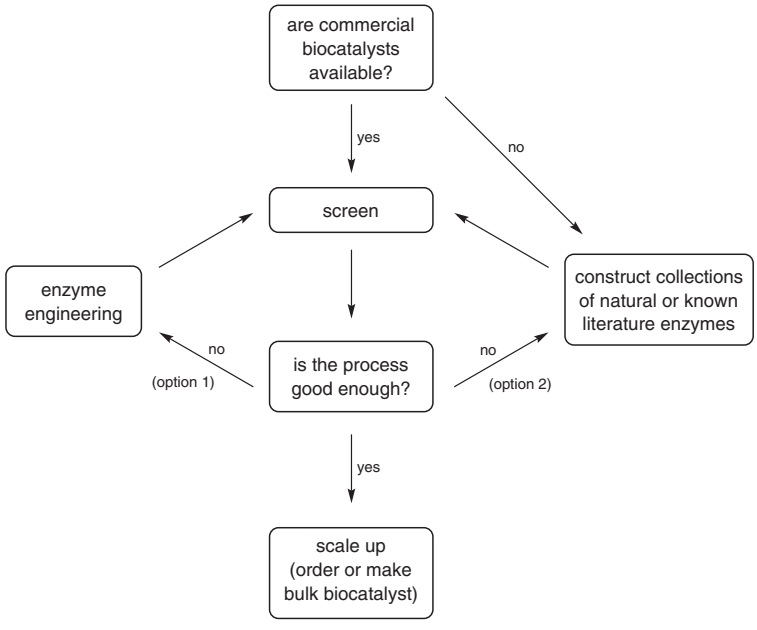
1.1.3 Enzyme Sources and Selection of Biocatalysts

R. Lauchli and D. Rozzell

Biocatalysts can be obtained from commercial suppliers, natural organisms, or from enzyme engineering efforts. This chapter discusses the sources from which one can obtain biocatalysts, and presents strategies for efficiently obtaining enzymes that meet the demands of medium- to large-scale chemical processes.



Keywords: enzyme catalysis · catalysts · genomics · green chemistry

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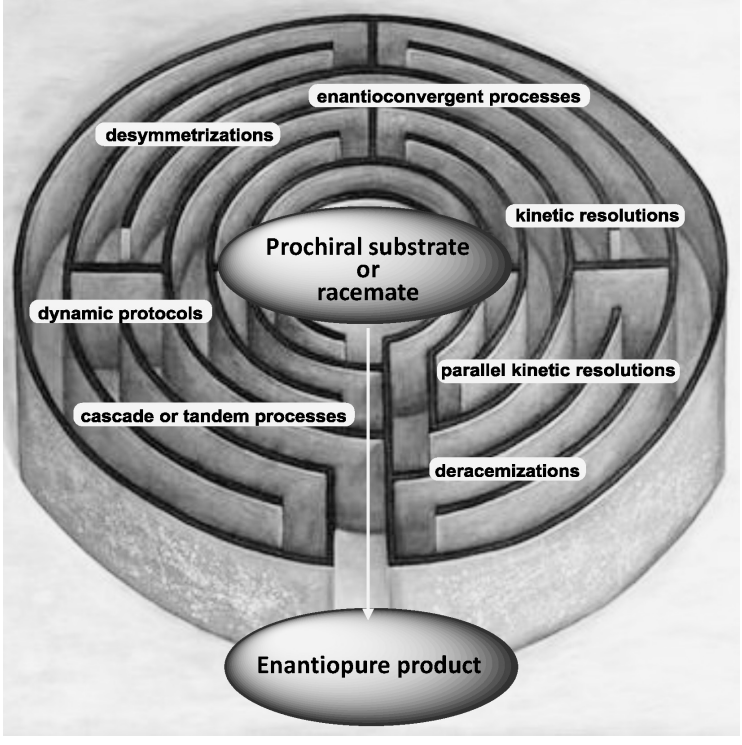
1.2 Strategies and Methods in Biocatalysis

A. Díaz-Rodríguez and I. Lavandera

The use of biocatalysts in organic synthesis and, particularly, in the preparation of optically pure chemicals offers major advantages in terms of selectivity, efficiency, safety, and sustainability. Thus, research groups are becoming more interested in biocatalysis as a tool for challenging synthetic routes. Herein we focus on the different strategies and methods that chemists have designed in order to obtain enantioenriched compounds starting from prochiral or racemic derivatives using enzymes or whole cells as catalysts. In the first part of the chapter, enzymatic desymmetrizations are presented, followed by other established systems dealing with racemates to attain a single or two enantiopure derivatives in the same reaction vessel. Then, the preparation of optically pure compounds in excellent yields and enantiomeric excesses by means of deracemization techniques is discussed. Finally, some recent examples where the combination of enzymes with other (bio)catalysts has provided high-added-value targets are shown.



Keywords: desymmetrizations · kinetic resolutions · parallel kinetic resolutions · (cyclic) deracemizations · enantioconvergent processes · dynamic kinetic resolutions · dynamic kinetic asymmetric transformations · concurrent catalysis · cascade reactions · tandem reactions · one-pot reactions · multistep catalysis

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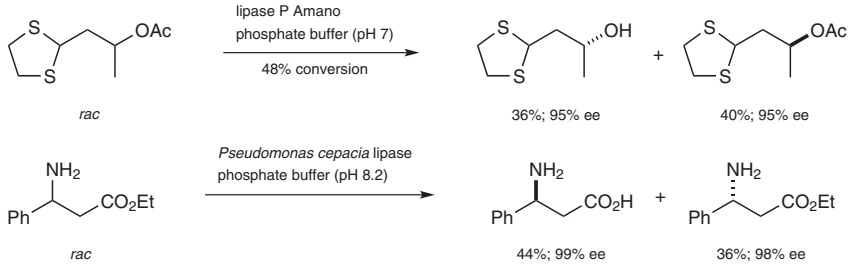
1.3.1 Resolution of Alcohols, Acids, and Esters by Hydrolysis

M. Bertau and G. E. Jeromin

This chapter reviews the use of enzymes, principally esterases and lipases, as catalysts for the resolution of racemic carboxylic acid derivatives via hydrolysis. The resolution of esters of chiral primary, secondary, and tertiary alcohols, as well as diols, are examined. Bio-

catalytic hydrolytic methods for the desymmetrization of prochiral substrates and *meso*-compounds are also considered.



Keywords: carboxylic acids · carboxylic esters · alcohols · diols · resolution · esterase · lipase · hydrolysis · *meso*-trick · desymmetrization · dynamic kinetic resolution · substrate engineering · substrate design

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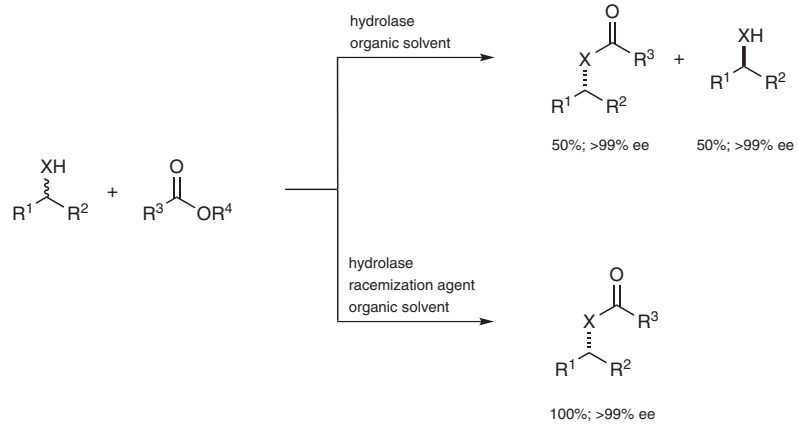
1.3.2 Resolution of Alcohols, Amines, Acids, and Esters by Nonhydrolytic Processes

M. Rodríguez-Mata and V. Gotor-Fernández

The use of hydrolases has become a conventional process in organic synthesis, not only for the preparation of optically pure compounds, but also for regio- and chemoselective processes. Their utility for selective transformations under mild reaction conditions make hydrolases attractive catalysts for performing certain transformations that are difficult to achieve by nonenzymatic strategies. Nowadays, many companies use lipases for the preparation of high-added-value compounds and pharmaceuticals because of the advantages of hydrolase-catalyzed processes, which include cost and environmental benefits. Their commercial availability, lack of cofactor dependency, and activity in both aqueous and organic media has allowed the development of asymmetric transformations which are summarized in this chapter. After a brief general introduction discussing the potential of hydrolases in organic synthesis, asymmetric reverse hydrolytic processes are analyzed, substituting the conventional hydrolase nucleophile, water, for other species such as alcohols, amines, esters, or ammonia. The kinetic resolution and dynamic kinetic resolution reactions of alcohols and amines are presented, using esters or carbonates for the production of esters, amides, and carbamates in optically active form. Finally, the resolution of carboxylic acids or esters is described via less-employed interesterification, aminolysis, and ammonolysis processes.



Keywords: acylation · alcohols · alkoxycarbonylation · amines · aminolysis · ammonolysis · asymmetric synthesis · carbonates · esters · hydrolases · interesterification · kinetic resolution · lipases · transesterification

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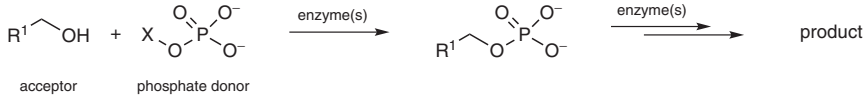
1.3.3 Transphosphorylation

R. Wever, L. Babich, and A. F. Hartog

The transfer of phosphoryl groups from one compound to another is one of the most important mechanisms by which cell function is controlled and orchestrated. Phosphorylated compounds find several applications such as in prodrugs or drugs, flavor enhancers, and key intermediates in the synthesis of pharmaceuticals. Regiospecific introduction of a phosphate group into a biomolecule via chemical methods is a challenge, particularly when the molecule has several potential phosphorylation sites or is labile. Protection and deprotection steps have to be introduced in the synthetic procedure, leading to waste and poor yields. Enzymes are able to catalyze reactions in a regio- or stereoselective manner and to date many synthetic methods and routes using enzymes have been developed. In particular, enzymatic cascade reactions in one pot are being used either in one step or multiple steps. These cascades make use of (parts of) naturally occurring biochemical pathways in which high-energy phosphorylated compounds drive the reaction to the desired product. This chapter describes the more classical enzymatic methods as well as the more recently developed cascade reactions to synthesize (phosphorylated) compounds.



Keywords: transphosphorylation · kinases · adenosine triphosphate · phosphoenol pyruvate · acetyl phosphate · glucose 6-phosphate · fructose 1,6-bisphosphate · glycerol phosphate · dihydroxyacetone phosphate · pyrophosphate · polyphosphate · glyceraldehyde 3-phosphate · phosphorylated nucleosides · inosine monophosphate · phosphorylated carbohydrates · alkaline phosphatase · aldolases · acid phosphatases · phosphorylases · enzymatic cascade reactions

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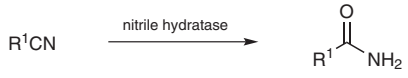
1.4.1 Hydrolysis of Nitriles to Amides

Y. Asano

Nitrile hydratase (NHase; EC 4.2.1.84) catalyzes the hydration of nitriles to form amides. The reaction catalyzed by nitrile hydratase is strikingly fast and versatile and a wide range of nitriles, including aromatic and arylalkyl nitriles, α- and β-substituted nitriles, and aminonitriles can be hydrated to the corresponding amides. Although nitrile hydratase generally has low stereoselectivity, its use in conjunction with highly stereospecific amidases provides a valuable route for the stereoselective synthesis of carboxylic acids. The powerful nature of nitrile hydratase has had a huge impact on the progress of applied microbiology, enzyme engineering, and enzyme-catalyzed organic synthesis. The best-known applications of nitrile hydratase on an industrial scale are the production of acrylamide and nicotinamide from acrylonitrile and pyridine-3-carbonitrile, respectively.

This chapter provides an overview of the current scope of nitrile hydratase mediated reactions and focuses on whole-cell biotransformations.



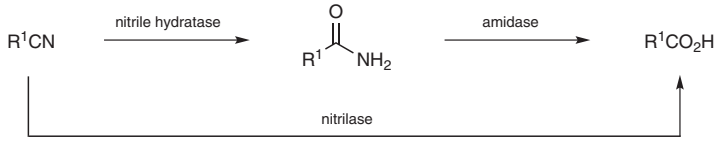
Keywords: nitriles · amides · nitrile hydratase · hydrolysis · hydration · chemoselectivity · carboxylic acids · kinetic resolution · desymmetrization · whole-cell biotransformations · aldoxime–nitrile pathway

p 277

1.4.2 Hydrolysis of Nitriles to Carboxylic Acids

L. Martínková and A. B. Veselá

The synthesis of carboxylic acids from nitriles utilizes two pathways of nitrile biotransformations: direct hydrolysis by nitrilase and bienzymatic hydrolysis by nitrile hydratase and amidase. General procedures consist of using whole cells or isolated enzymes as catalysts in aqueous media with a small fraction of organic cosolvent. These methods afford a number of products that are often difficult to prepare by chemical means such as 3-oxoamides, cyano carboxamides and cyano carboxylic acids, enantiopure 2- and 3-substituted carboxylic acids and carboxamides, and enantiopure (hetero)cyclic carboxylic acids and carboxamides. Stereochemistry is mainly recognized by amidase, but in some cases also by nitrilase and nitrile hydratase. Nitrile hydrolysis has also been employed in chemoenzymatic and multienzymatic methods such as the synthesis of aromatic and heterocyclic amides from aldehydes, the synthesis of enantiopure 2-hydroxy acids from aldehydes, the synthesis of enantiopure 3-hydroxy acids from 3-oxonitriles, and the synthesis of cyclophellitols from benzo-1,4-quinone.



Keywords: nitriles · carboxylic acids · carboxamides · aldehydes · nitrilase · nitrile hydratase · amidase · stereochemistry · chemoenzymatic methods · multienzymatic methods

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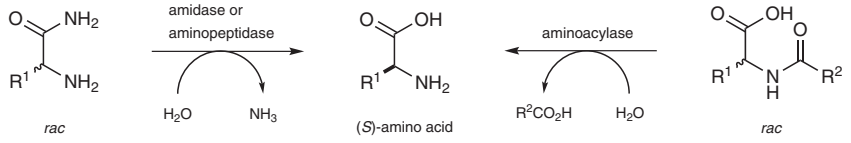
1.4.3 Hydrolysis of Amides

M. Hall, K. Faber, and G. Tasnádi

This chapter describes the enzymatic hydrolysis of amide substrates. The main target compounds are amino acids, obtained via the kinetic resolution of amino acid amides and N-acylated amino acids using aminopeptidases, amidases, and aminoacylases. In addition, methods leading to enantiopure carboxylic acids and amines as well as lactamase-catalyzed processes are presented.



Keywords: amide hydrolysis · amino acids · amines · carboxamides · lactams · Vince lactam · amidases · aminopeptidases · aminoacylases · lactamases

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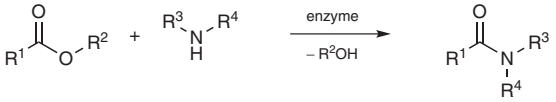
1.4.4 Enzymatic Synthesis of Amides

J. W. Schmidberger, L. J. Hepworth, A. P. Green, and S. L. Flitsch

The synthesis of amides is one of the most common reactions performed in organic chemistry. Biocatalysis is an attractive alternative to chemical methodologies because of the mild reaction conditions and excellent atom economy, combined with the potential for stereoselectivity. Here, we provide an overview of the literature on enzyme-catalyzed amide-bond formation on a preparative scale, with a focus on nonnatural substrates.



Keywords: amines · amides · esters · aminolysis · enzymes · regioselectivity · chemoselectivity · stereoselectivity · kinetic resolution · lipases · esterases · penicillin acylases · proteases · nitrile hydratases

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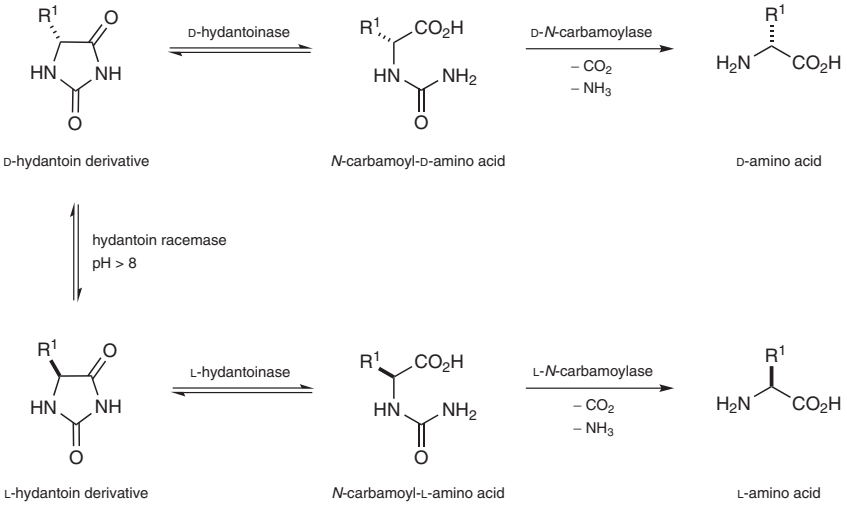
1.4.5 Hydrolysis of Hydantoins, Dihydropyrimidines, and Related Compounds

C. Slomka, U. Engel, C. Syldatk, and J. Rudat

Providing advantages including high chemo-, regio-, and enantioselectivity as well as mild reaction conditions, biocatalytic reaction systems are becoming increasingly important for the synthesis of chiral fine chemicals. This chapter focuses on hydantoins and related compounds as promising substrates for the synthesis of optically pure amino acids and on the enzymes involved in these processes. In particular, the production of D-amino acids, such as D-4-hydroxyphenylglycine, via the so-called "hydantoinase process" is now well established. Many investigations regarding the synthesis of L-amino acids with the help of this process have also been carried out. A further interesting application is the synthesis of β-amino acids, which are gaining importance in the pharmaceutical industry due to their special structure. Different possibilities for the application of modified hydantoinase processes are discussed, in which dihydropyrimidines serve as substrates for β-amino acid synthesis. Moreover, various methods to improve the synthesis of amino acids are described.



Keywords: hydantoins · dihydropyrimidines · hydrolysis · racemization · enantioselectivity · *N*-carbamoylamino acids · hydantoinase process · amino acids · D-4-hydroxyphenylglycine · β-amino acids · directed evolution · whole-cell biocatalysis · immobilization · recombinant expression

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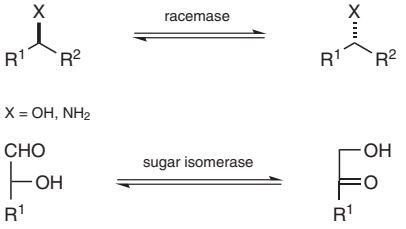
1.5 Isomerizations: Racemization, Epimerization, and *E*/*Z*-Isomerization

K. Faber and S. M. Glueck

Biocatalytic racemization represents the reversible interconversion of an enantiomer to its mirror image and is catalyzed by racemases. In the context of organic synthesis, it represents the key step to turn a kinetic resolution into a dynamic process. In contrast, sugar isomerases, acting as intramolecular oxidoreductases, are a subclass of isomerases and catalyze the interconversion of aldoses into ketoses, which finds application in the biotechnological production of (unnatural) rare sugars. The field of enzymatic isomerization is complemented by (carbohydrate) epimerization, alkene *E*/*Z*-isomerization, and mutase-catalyzed rearrangement reactions.



Keywords: isomerization · racemization · epimerization · *E*/*Z*-isomerization · mutase · aldose · ketose

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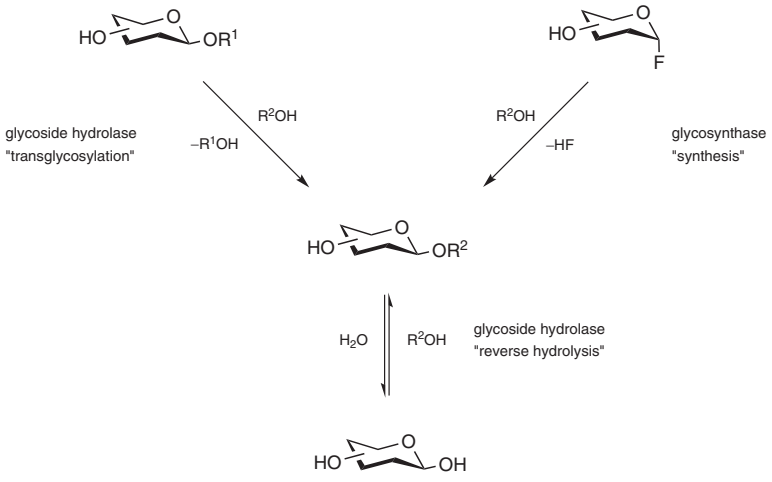
1.6.1 Glycosidases and Glycosynthases

B. Cobucci-Ponzano and M. Moracci

Enzymatic synthesis of glycans, as an alternative to classical chemical synthesis, is of great interest due to the exquisite stereospecificity and improved processivity and regioselectivity of the biological catalysts, and for the possibility of using reagents less toxic to the environment. Nonetheless, the limitations intrinsic to the natural enzymes promoting sugar synthesis, namely glycoside hydrolases and glycosyltransferases, have prompted efforts to engineer the former catalysts, obtaining glycosynthases that promote the synthesis of oligosaccharides, polysaccharides, and glycoconjugates in quantitative yields from inexpensive substrates. In this chapter we survey methods that exploit glycosidases and glycosynthases to allow the efficient and reliable preparation of glycans of synthetic relevance.



R^1 = leaving group; R^2 = glycoside/sugar

Keywords: carbohydrate active enzymes · carbohydrate synthesis · enzyme engineering · glycobiology · glycochemistry · glycoconjugates · glycosidase reaction mechanism · glycosyltransferase · oligosaccharides · polysaccharides · protein glycosylation

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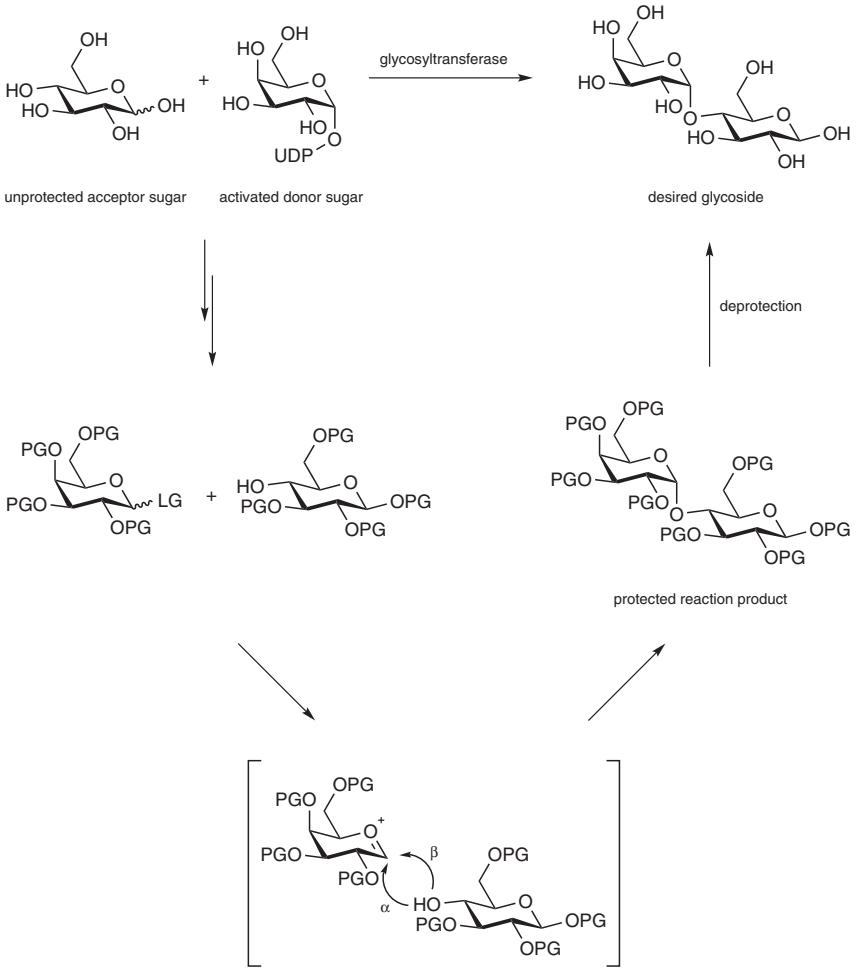
1.6.2 **Glycosyltransferases**

J. Voglmeir and S. L. Flitsch

The stereo- and regioselective properties and the high selectivity of glycosyltransferases toward donor and acceptor substrates make these enzymes highly attractive for synthetic applications. Various examples of recombinantly expressed glycosyltransferases demonstrate the versatility of both in vivo and in vitro syntheses of oligosaccharides from milligram to kilogram scale. However, due to the enormous variety of carbohydrate structures in living organisms, to date only a small proportion of carbohydrate epitopes have been synthesized in a routine manner. This chapter summarizes recent approaches to the application of glycosyltransferases in both preparative sugar synthesis and biotransformation.



Keywords: glycosyltransferases · enzymatic synthesis · nucleotide sugars · glycoconjugates · green chemistry

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