



Science of  
Synthesis

# Biocatalysis in Organic Synthesis 3

## Volume Editors

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W.-D. Fessner  
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## Science of Synthesis

**Science of Synthesis** is the authoritative and comprehensive reference work for the entire field of organic and organometallic synthesis.

**Science of Synthesis** presents the important synthetic methods for all classes of compounds and includes:

- Methods critically evaluated by leading scientists
- Background information and detailed experimental procedures
- Schemes and tables which illustrate the reaction scope



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# Science of Synthesis

## Biocatalysis in Organic Synthesis 3

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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.

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## **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:<sup>[1]</sup>

- 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.<sup>[2]</sup>
- The compatibility of enzymes with each other has enabled the design of highly efficient synthetic cascades, thereby avoiding the separation of sensitive intermediates.<sup>[3]</sup> 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”.<sup>[4]</sup> 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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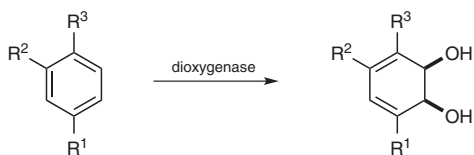
## Abstracts

p 1

### 3.1 Dihydroxylation of Aromatics and Alkenes

C. C. R. Allen

The use of ring-hydroxylating dioxygenase enzymes for the biotransformation of aromatic hydrocarbons, alkenes, and phenols to give chiral *cis*-dihydrodiol metabolites is of significant potential for the preparation of chiral precursors for organic synthesis. Many products are produced with high enantiomeric excess, and a wide number of biotransformations have been studied. This type of biotransformation is typically used to convert readily available starting materials into single enantiomer bioproducts in a single step. The enzymes are multicomponent systems comprising two or more protein subunits. Furthermore, there is a requirement for reducing equivalents (e.g., NADH) and therefore whole-cell biocatalysts are used, either as wild-type strains, mutants, or clones. Recently, there have been significant developments in the use of molecular biology methods to improve these biocatalysts. This review covers the approaches employed to perform specific types of biotransformation, namely arene, alkene, and phenol hydroxylation.



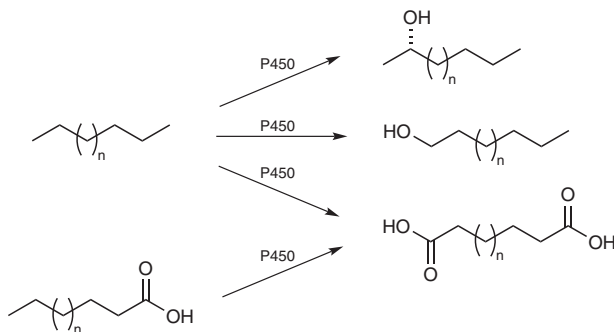
**Keywords:** biocatalysis • chiral pool • dihydroxylation • enzyme catalysis • diols • phenols

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### 3.2.1 Cytochrome P450 in the Oxidation of Alkanes

J. C. Nolte and V. B. Urlacher

Selective direct oxidation of relatively cheap alkanes leads to valuable synthons that can be used as building blocks for the chemical and pharmaceutical industry. This chapter describes the hydroxylation of alkanes and fatty acids catalyzed by cytochrome P450 monooxygenases (CYP). It summarizes early and more-recent methods for the selective production of terminal and subterminal alcohols, dicarboxylic acids, and other oxidation products of alkanes using isolated enzymes and whole-cell biocatalysts.

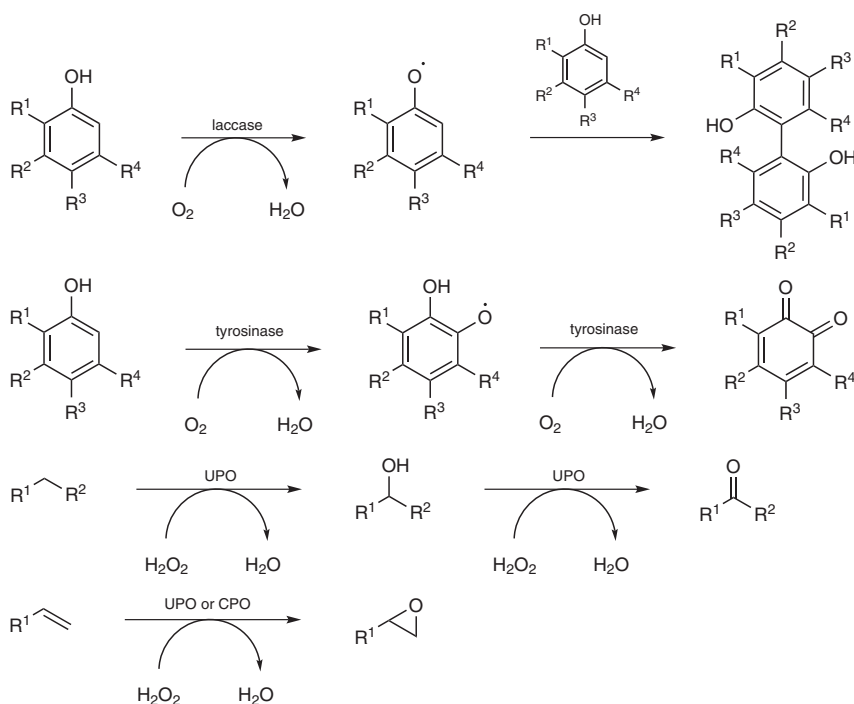


**Keywords:** alkanes • fatty acids • microbial oxidation • biocatalysts • enzyme catalysis

## 3.2.2 Oxidation Other Than with Cytochrome P450s

S. Herter and N. J. Turner

The laccase- or tyrosinase-catalyzed oxidation of phenolic compounds leads to the generation of radical cations which subsequently produce quinoid derivatives. Acting as electrophilic Michael acceptors, quinones can undergo successive reactions amongst themselves yielding homomolecular products. In the presence of nucleophiles acting as coupling partners, enzymatically generated quinones undergo hetero-cross-coupling reactions to give novel hybrid molecules. In contrast to laccases, tyrosinase enzymes also catalyze the *ortho*-hydroxylation of monophenols, giving rise to catechols and benzo-1,2-quinones. Unspecific peroxygenases (UPOs) catalyze the hydroxylation of a broad range of C–H containing substrates, including small aromatic compounds, larger polycyclic aromatic hydrocarbons, heteroaromatics, alkanes, and cycloalkanes. A common feature of UPOs and chloroperoxidases (CPOs) is found in the asymmetric epoxidation of alkenes to yield the corresponding epoxides, often with high enantiomeric excess.



**Keywords:** 1,4-addition reactions • C–C coupling • C–H activation • chloroperoxidases • C–O coupling • Diels–Alder cyclization • domino reactions • epoxidation • homomolecular coupling • hetero-cross-coupling reactions • hydroxylation • laccases • oxidation • quinones • tyrosinases • unspecific peroxygenases

## 3.3.1 Oxidations Using Dehydrogenases

F. Hollmann

The use of alcohol dehydrogenases has a number of advantages over traditional chemical methods for the oxidation of alcohols. These include the mild reaction conditions, the avoidance of tedious protecting-group strategies, and the high regio- and chemoselectivities. This review highlights the most important alcohol dehydrogenases used for bioca-