Science of Synthesis

Biocatalysis in Organic Synthesis 2

Volume Editors
K. Faber
W.-D. Fessner
N. J. Turner

Thieme

Science of Synthesis

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- Background information and detailed experimental procedures
- Schemes and tables which illustrate the reaction scope

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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)

[1] Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K, *Nature (London)*, (2012) **485**, 185.

[2] Steinkellner, G.; Gruber, C. C.; Pavkov-Keller, T.; Binter, A.; Steiner, K; Winkler, C.; Łyskowski, A.; Schwamberger, O.; Oberer, M.; Schwab, H; Faber, K.; Macheroux, P.; Gruber. K., *Nature Commun.*, (2014) **5**, 4150; DOI: 10.1038/ncomms5150.

[3] *Cascade Biocatalysis: Integrating Stereoselective and Environmentally Friendly Reactions*, Riva, S.; Fessner, W.-D., Eds.; Wiley-VCH: Weinheim, Germany, (2014).

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

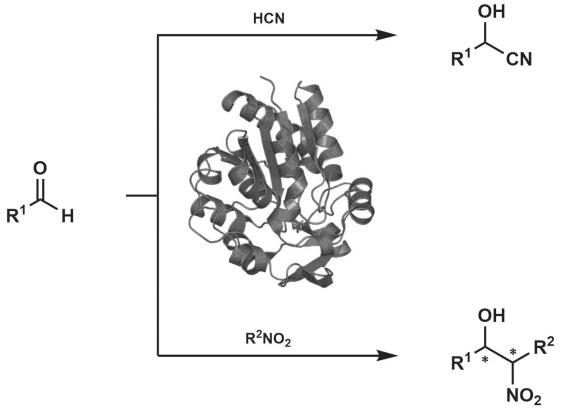
Abstracts

p 1

2.1.1 Cyanohydrin Formation/Henry Reaction

K. Steiner, A. Glieder, and M. Gruber-Khadjawi

Enantiopure cyanohydrins and β-nitro alcohols serve as versatile building blocks for a broad range of chemical and enzymatic reactions, resulting in highly valuable products for many applications. Hydroxynitrile lyases comprise a diverse group of enzymes that catalyze the reversible cleavage of cyanohydrins to carbonyl compounds and hydrogen cyanide. The enzymes have been studied broadly concerning their substrate scope, specificity, structure, and reaction mechanism, and many have been successfully applied and engineered for the synthesis of cyanohydrins from laboratory to industrial scale. Both *R*- and *S*-cyanohydrins are accessible from a broad variety of substrates and, in most cases, high yields and enantiopurities can be obtained after enzyme and reaction engineering. Recent progress in the development and optimization of heterologous expression systems make recombinant hydroxynitrile lyases available in the quantities needed for industrial production. The procedures for safe handling of cyanides are also well-defined and established. In addition, some hydroxynitrile lyases are able to catalyze the nonnatural asymmetric Henry reaction. Although the enzyme activities are rather low, the enzymatic synthesis of enantiopure β-nitro alcohols shows promising results.



Keywords: hydroxynitrile lyase · cyanohydrin · nitroaldol · β-nitro alcohol · Henry reaction · enantioselectivity · enzyme engineering

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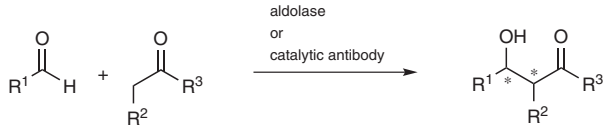
2.1.2 Aldol Reactions

P. Clapés

The asymmetric aldol addition reaction is a cornerstone transformation in organic chemistry and one of the most useful methods for C—C bond formation. Aldolases and catalytic antibodies catalyze aldol and retroaldol reactions with high stereoselectivity and catalytic efficiency. Therefore, they constitute very useful tools in chemical research and the production of complex, multifunctional chiral compounds, such as carbohydrates and amino acids, as well as their derivatives and analogues. In addition, carboligating enzymes and antibodies offer a unique tool to perform asymmetric C—C bond formation in a sustainable, environmentally benign fashion. This review describes the different methodologies

and procedures used for enzymatic C—C bond formation by aldol reaction. These include the asymmetric catalytic aldol additions of dihydroxyacetone phosphate (DHAP), 1-hydroxyalkan-2-ones (i.e., dihydroxyacetone, hydroxyacetone, and 1-hydroxybutan-2-one), pyruvate, glycine, acetaldehyde, and glycolaldehyde as the nucleophilic components to a variety of electrophilic aldehyde structures.



R^1 = diverse; R^2 = H, OH, NH_2, F, Me, Et, SMe; R^3 = H, OH, Me, Et, CO_2^-, CH_2OH, $CH_2OPO_3^{2-}$

Keywords: asymmetric C—C bond formation · aldol additions · DHAP dependent aldolases · D-fructose 6-phosphate aldolase · DHAP mimics · transketolases · pyruvate aldolases · glycine aldolases · catalytic antibodies · self- and cross-aldol additions · 2-deoxy-D-ribose 5-phosphate aldolase · dihydroxyacetone phosphate (DHAP) · dihydroxyacetone · hydroxyacetone · 1-hydroxybutan-2-one · pyruvate · glycine · glycolaldehyde · alkylaldehydes · hydroxyaldehydes · aminoaldehydes

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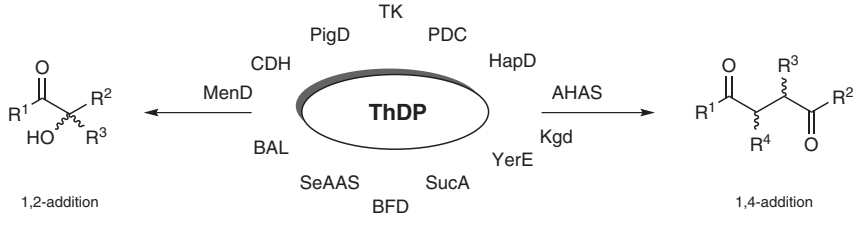
2.1.3 Acyloin, Benzoin, and Related Reactions

M. Pohl, C. Wechsler, and M. Müller

This chapter gives a broad overview of different thiamine diphosphate (ThDP) dependent enzymes and their applicability in organic synthesis as a practical alternative to traditional cross-coupling reactions. Complementary to known nonenzymatic umpolung reactions, enzymatic versions of the benzoin condensation, the asymmetric cross-benzoin condensation, the resolution of racemic 2-hydroxy ketones via C—C bond cleavage, the synthesis of bis(α-hydroxy ketones), the homocoupling of aliphatic aldehydes, the Stetter reaction, and aldehyde–ketone cross-benzoin reactions have been developed. The broad diversity of the products from enzymatic transformations is nicely complemented by the possible subsequent diversity-oriented chemistry. Starting from simple, commercially available aldehydes, many different chiral building blocks can be selectively obtained in a few steps, thus mimicking the diversity-oriented biosynthesis of natural biosynthetic pathways.



Keywords: regioselectivity · enantioselectivity · enzyme catalysis · C—C bond formation · asymmetric Stetter reaction · asymmetric benzoin condensation · asymmetric enzyme catalysis · thiamine diphosphate · stereocontrol · benzoins · acyloins · chiral resolution · kinetic resolution

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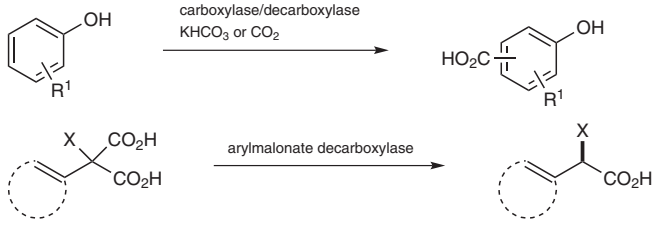
2.1.4 Enzymatic Carboxylation and Decarboxylation

R. Lewin, M. L. Thompson, and J. Micklefield

Carboxylation reactions utilizing whole cells or purified carboxylase/decarboxylase enzymes enable the regioselective formation of new C—C bonds under more benign conditions than are typically used in nonenzymatic transformations such as the Kolbe–Schmitt reaction. A wide variety of substrates have been used in enzymatic carboxylation reactions including phenols, styrenes, pyrroles, and indoles.

Enzymatic decarboxylation can be used to transform simple achiral carboxylic acid substrates into more valuable homochiral building blocks through stereoselective C—H or C—C bond formation. For example, arylmalonate decarboxylases catalyze the enantioselective decarboxylative protonation of α-aryl- and α-alkenylmalonic acids under mild conditions and with excellent enantioselectivity. In addition, thiamine diphosphate dependent decarboxylases catalyze C—C bond formation with a broad range of α-keto acid and aldehyde substrates to produce homochiral α-hydroxy ketones.



Keywords: carboxylation · Kolbe–Schmitt reaction · regioselectivity · whole-cell reaction · C—C bond formation · enantioselectivity · decarboxylation · arylmalonate decarboxylase · malonic acids · stereoselectivity · enantioselective decarboxylative protonation

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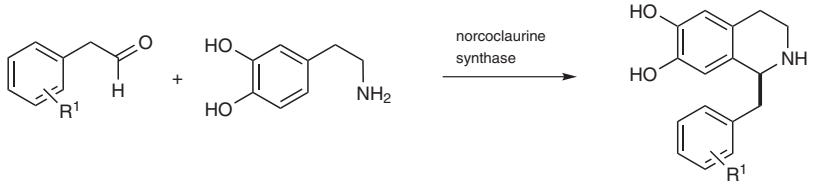
2.1.5 Addition to C=N Bonds

A. Ilari, A. Bonamore, and A. Boffi

The Pictet–Spengler reaction consists of a Mannich-type cyclization in which an electron-rich aromatic carbon attacks a C=N bond, in the form of an electrophilic iminium ion, thus yielding a heterocyclic scaffold and generating a new asymmetric center. In this chapter, the substrate scope and limitations of the best-known Pictet–Spenglerase enzymes are discussed in order to pave the way for development of a general biocatalytic strategy for the stereoselective addition to the C=N bond.



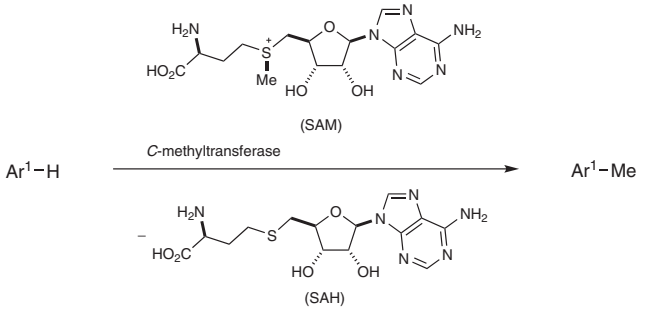
Keywords: Pictet–Spengler reaction · tetrahydroisoquinolines · indole alkaloids · strictosidine synthase · norcoclaurine synthase · β-carbolines · azaindoles

2.2 Enzymatic C-Alkylation of Aromatic Compounds

L. A. Wessjohann, H. F. Schreckenbach, and G. N. Kaluđerović

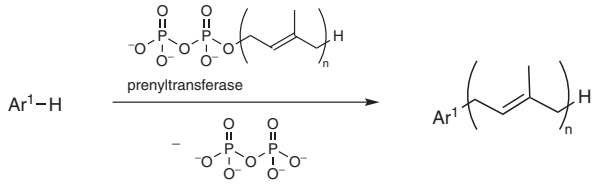
C-Alkylation of aromatic groups, as in Friedel–Crafts chemistry, is an energetically difficult process with significant chemo- and regioselectivity problems, especially if other nucleophiles, such as hydroxy groups or nitrogen atoms, are present in the substrate. Nature provides alkylating enzymes that selectively transfer a methyl, prenyl, or glycosyl group to carbon atoms of aromatic moieties under mild conditions, at room temperature, and mostly with excellent chemo- and regioselectivity. In this review, current enzymatic processes are highlighted and the increasing availability of cosubstrates, cofactors, and suitable enzymes is discussed as a prerequisite for scaling up such processes.

methyltransferases (MTases)

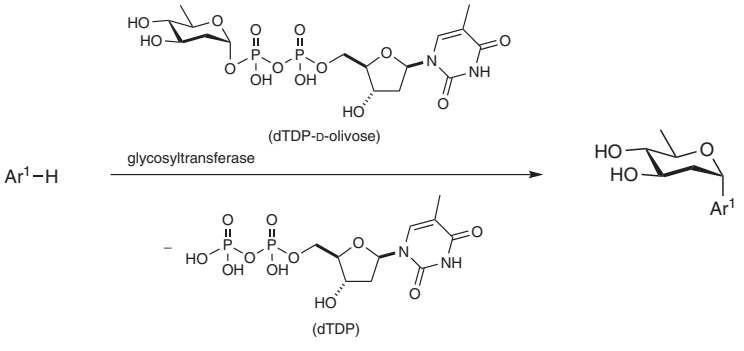


SAM = *S*-adenosyl-L-methionine; SAH = *S*-adenosyl-L-homocysteine

prenyltransferases (PTases)



glycosyltransferases (GTases)



Keywords: transferases · alkylation · methylation · prenylation · glycosylation · phenolic compounds · C—C bond formation · natural products · Friedel–Crafts-like reactions · electrophilic aromatic substitution

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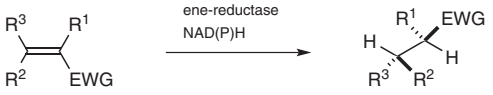
2.3.1 Addition of Hydrogen to C=C Bonds: Alkene Reduction

K. Faber and M. Hall

Ene-reductases are flavoproteins which catalyze the asymmetric reduction of activated alkenes at the expense of a nicotinamide cofactor. The substrate scope is broad and includes α,β-unsaturated carbonyl compounds, carboxylic acid derivatives, and nitro compounds, which upon reduction yield the corresponding saturated products in high enantiopurity.



Keywords: asymmetric catalysis · ene reaction · enzyme catalysis · reduction · stereoselective synthesis

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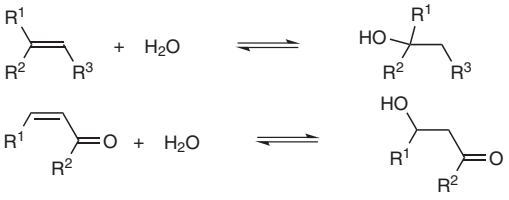
2.3.2 Addition of Water to C=C Bonds

V. Resch and U. Hanefeld

While chemists struggle to find efficient methods to perform the asymmetric addition of water, nature employs countless enzymes (called hydratases or hydro-lyases) to perform this reaction using substrates with both activated and nonactivated double bonds. However, compared to the vast number of hydratases involved in metabolic pathways in nature, only a few are described for their use in organic synthesis. Nevertheless, their potential in asymmetric catalysis has been recognized and some hydratases are used on a large scale in industrial processes. Since hydratases perform the addition of water, water is used as both a solvent and a reagent, opening up a very efficient and green route to both secondary and tertiary alcohols. This chapter focuses on hydratases that catalyze interesting reactions and are tested beyond their biochemical characterization.



Keywords: acetylene hydratase · aconitase · carotenoid hydratases · citraconase · fumarase · hydratase · hydratase–tautomerase bifunctionality · hydro-lyase · kievitone hydratase · limonene hydratase · linalool dehydrogenase–isomerase · malease · oleate hydratase · phaseollidin hydratase · urocanase · water addition

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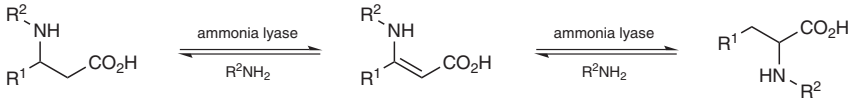
2.3.3 Addition of Ammonia and Amines to C=C Bonds

S. Bartsch and A. Vogel

Ammonia lyases and aminomutases catalyze the reversible, nonreductive, asymmetric amination of α,β-unsaturated carboxylic acids. They utilize ammonia and, to a lesser extent, substituted amines as substrates. The most common acceptors are fumarate and aromatic α,β-unsaturated carboxylic acids. Typical products are optically pure α-amino acids, but production of β-amino acids is also described. No cofactor recycling is required and, by using high concentrations of ammonia, conversion up to 100% can be reached

with excellent enantioselectivity. Ammonia lyases comprise a very heterogeneous group of enzymes from plants and microbes, showing diverse substrate selectivities and reaction mechanisms. The most commonly used members are the aspartate and phenylalanine ammonia lyases.



Keywords: ammonia lyase · aminomutase · nonreductive amination · α,β-unsaturated carboxylic acids · aspartase · 3-methylaspartate ammonia lyase · adenylosuccinate lyase · argininosuccinate lyase · α-amino acids · β-amino acids

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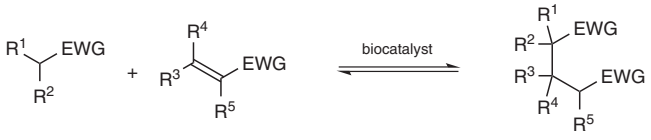
2.3.4 Enzymatic Carbon—Carbon Bond-Forming Michael-Type Additions

E. M. Geertsema and G. J. Poelarends

This chapter gives an overview of practical biocatalytic procedures for C—C bond-forming Michael(-type) additions suitable for organic synthesis purposes. Reported product yields, workup and isolation methods, stereoselectivity, and availability of the applied enzymes are assessed. All methodologies involve promiscuous enzyme activities, since natural enzyme-catalyzed C—C bond-forming Michael additions are extremely rare.



Keywords: enzyme catalysis · Michael addition · C—C bond formation · stereoselective catalysis · enantioselectivity · diastereoselectivity · enzyme promiscuity · lipase · acylase · proteinase · tautomerase

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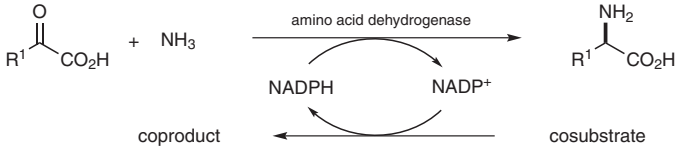
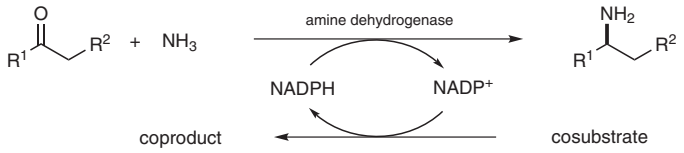
2.4.1 Amino Acid and Amine Dehydrogenases

A. S. Bommarius and S. K. Au

α-Keto acids can be reductively aminated to α-amino acids via amino acid dehydrogenase catalysis, with NAD(P)H as cofactor. Regeneration of the oxidized cofactor $NAD(P)^+$ back to NAD(P)H is required for synthesis and is commonly achieved via formate dehydrogenase catalyzed oxidation of formate to carbon dioxide or glucose dehydrogenase catalyzed oxidation of glucose to gluconic acid. Recently, amine dehydrogenases, which reductively aminate ketones to amines, have been developed via protein engineering. Both amino acid and amine dehydrogenases are exquisitely enantioselective, leading to (*S*)- or (*R*)-amino acids or (*R*)-amines.





Keywords: enantioselectivity · reductive amination · keto acids · α-amino acids · ketones · amines

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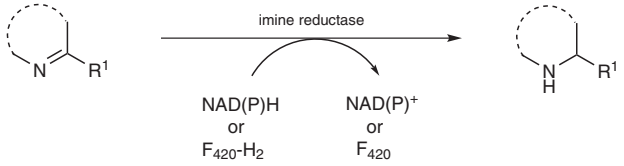
2.4.2 Imine Reductases

F. Leipold, S. Hussain, S. P. France, and N. J. Turner

Imine reductases catalyze the asymmetric reduction of imines to the corresponding chiral amines. The excellent enantioselectivities achieved in these conversions make this biocatalyst an attractive addition to the toolbox for chiral amine synthesis. This chapter details recent developments in the application of different classes of imine reductases in the synthesis of chiral amines as well as amino acids.



Keywords: amines · α-amino acids · imine reduction · reductive amination · tetrahydro-β-carbolines · tetrahydroisoquinolines · piperidines · pyrrolidines

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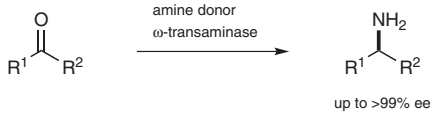
2.4.3 ω-Transaminases

R. C. Simon, E. Busto, E.-M. Fischereder, C. S. Fuchs, D. Pressnitz, N. Richter, and W. Kroutil

Optically pure amines are prepared from the corresponding prochiral ketones via asymmetric amination employing ω-transaminases and selected amine donors.



Keywords: amination · aldehydes · amines · asymmetric catalysis · ketones · ω-transaminases

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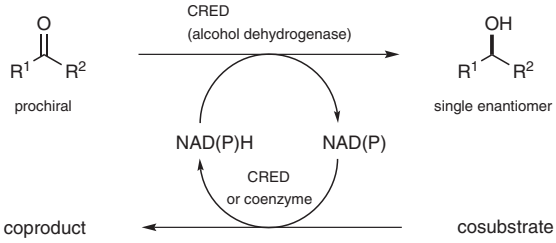
2.5.1 Ketone and Aldehyde Reduction

T. S. Moody, S. Mix, G. Brown, and D. Beecher

The modern organic chemist increasingly uses biotransformations to solve synthetic problems. In particular, stereoselective reduction of prochiral ketones using enzymes has moved from an academic curiosity to a commercial success. Bioreduction using both whole-cell microbial and recombinant systems has proven to be a robust and reliable alternative to other asymmetric chemical methods, resulting in green, economic, and scalable processes for the chemical industry. This review highlights bioreduction applications available to the modern practical chemist.



Keywords: asymmetric catalysis · alcohols · chiral compounds · green chemistry · reduction

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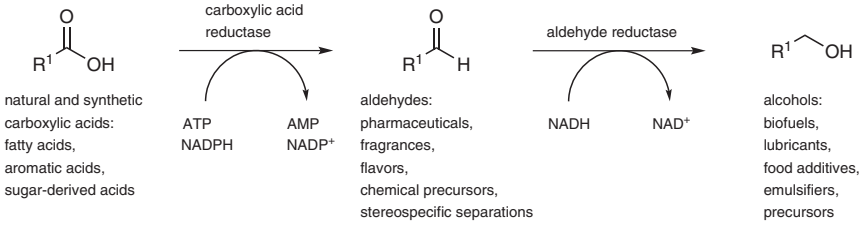
2.5.2 Carboxylic Acid Reductase

A. S. Lamm, P. Venkitasubramanian, and J. P. N. Rosazza

This chapter highlights the versatility of carboxylic acid reductase and its ability to efficiently catalyze the bioconversion of a wide range of natural and synthetic carboxylic acids into their corresponding aldehydes and alcohols.



Keywords: alcohols · arylaldehyde oxidoreductase · biofuels · carboxylate reductase · carboxylic acid reductase · enzymatic reduction · fatty acids · oxidoreductase · racemate resolution

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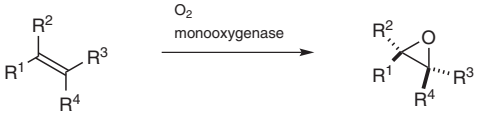
2.6.1 Asymmetric Synthesis of Enantiopure Epoxides Using Monooxygenases

A. T. Li and Z. Li

Monooxygenases catalyze the asymmetric epoxidation of different types of alkenes, providing a green and useful method to synthesize the corresponding epoxides in high enantiomeric excess and good yield. The epoxidations catalyzed by styrene monooxygenase, xylene monooxygenase, alkane monooxygenase, alkene monooxygenase, and cytochrome P450 monooxygenase are reviewed in this chapter.



Keywords: asymmetric epoxidation · asymmetric synthesis · enzyme catalysis · monooxygenase · chiral epoxides · green oxidation

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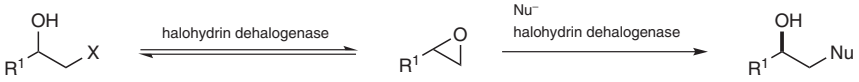
2.6.2 Reactions Catalyzed by Halohydrin Dehalogenases

M. Majerić Elenkov, W. Szymański, and D. B. Janssen

In some bacteria, halohydrin dehalogenases catalyze the conversion of vicinal halo alcohols, such as 1,3-dichloropropane or 3-chloropropane-1,2-diol, into epoxides, and thereby play a role in the biodegradation of halogenated organic compounds. In the reverse reaction, i.e. epoxide ring opening, various small anions can replace the halide, allowing the synthesis of β-substituted alcohols, including β-hydroxynitriles and β-azido alcohols. These remarkable catalytic properties have been modified by structure-based protein engineering, making the enzymes suitable for diverse applications.



Keywords: epoxides · hydroxynitriles · microorganisms · alcohols · cyanides

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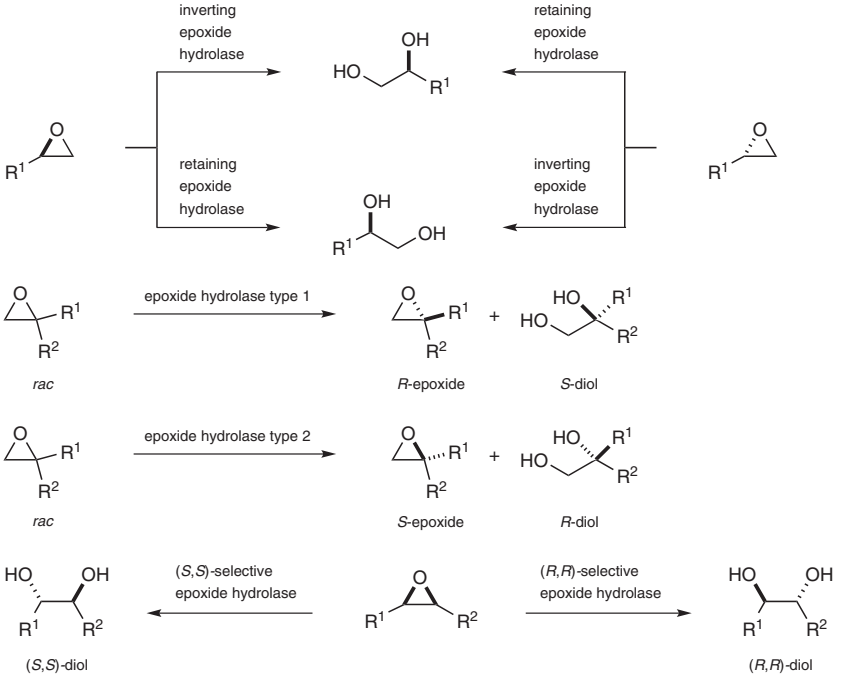
2.6.3 Expoxide Hydrolysis

R. Wohlgemuth

This chapter focuses on the selective biocatalytic ring opening of epoxides by water, leading to vicinal diols or other reaction products. This strategy is also used by nature to prepare a range of important metabolites and natural products by epoxide hydrolase catalyzed ring-opening reactions. The hydrolysis of easily accessible racemic epoxides to enantiomerically pure epoxides or vicinal diols has become of increasing interest as a method for preparing a great variety of chiral intermediates for the synthesis of pharmacologically active compounds, agrochemicals, flavors and fragrances, and metabolites.



Keywords: epoxy compounds · oxiranes · epoxide hydrolase · diols · hydrolysis · resolution · ring opening

Biocatalysis in Organic Synthesis 2

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