Enzymes in organic synthesis

To simplify these reactions, researchers are using a new class of tools to make chiral drugs.

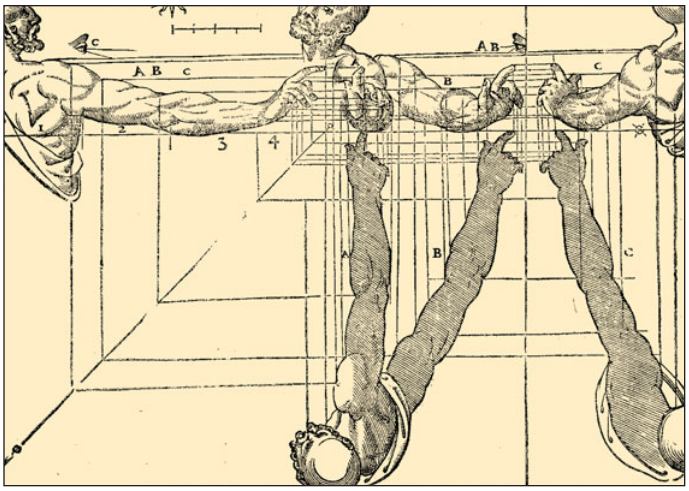
BY GRACE DESANTIS

By exploiting the tools that nature provides, chemists can readily generate chirality where previously there was none. Enzymes offer exquisitely precise chemo-, regio-, and stereocontrol and accelerate chemical transformations that are challenging to perform by conventional chemical synthetic methods. Reactions often proceed at room temperature and under neutral aqueous conditions, in the absence of toxic organic solvents or heavy-metal catalysts. In addition, by virtue of enzyme selectivity, biocatalytic routes can obviate synthetic protecting-group manipulations. Consequently, the application of biocatalysis for the synthesis of chiral as well as achiral pharmaceutical intermediates has received increased attention. The number of commercially available enzymes continues to grow, as do the types of chemical transformations that biocatalysts perform reliably in vitro.

While biocatalysts may prove to be the catalyst of choice for many achiral transformations, their prowess is particularly noteworthy for the synthesis or resolution of chiral molecules. The ability of biocatalysts to impart chirality onto achiral molecules or to perform a chemical transformation on only one stereoisomer of a racemic mixture of a molecule stems from their own chiral nature. Thus, the utility of isolated-enzyme- and whole-cell-catalyzed biotransformations has evolved beyond traditional examples of bioremediation and the industrial processing of pulp, paper, and food.

Chirality

Enantiomers are related to each other like left and right hands, and the biological receptors with which chiral drugs interact are akin to their corresponding gloves. In an achiral milieu, however, enantiomers have the same physical and chemical properties and are thus poorly distinguishable. It is this feature that makes their synthesis challenging. However, preparing drugs in stereochemically pure forms is important because, at best, only one antipode is biologically active while the other "just goes along for the ride", making the formulation of the drug twice as expensive and wasteful. In the worst-case scenario, the opposite antipode fits an altogether different biological receptor or "glove", causing toxicity or a deleterious side effect.



The fact that chemicals can be right-handed or left-handed is one of the most challenging problems facing pharmaceutical companies today: Of the two forms of a drug, one is good, the other ineffective or even dangerous. Illustration from a 17th-century book on drawing and perspective by Jean Cousins.

For example, (*S*)-(–)-penicillamine is a treatment for Wilson's disease, but the *R* isomer is toxic and can cause blindness. Another, infamous, example is thalidomide, for which the *S* isomer is a sedative while the *R* enantiomer is teratogenic. In this case, however, prescribing the single enantiomer may not have been safer because the two enantiomers readily interconvert in vivo. (See also "Redeeming thalidomide" and "How thalidomide was kept out of the U.S. market" in the June 2000 *Modern Drug Discovery*, p 35 and p 69, respectively.)

There has been continuing concern from the U.S. FDA about choices between making drugs available in stereochemically pure form or as mixtures of racemates or diasteriomers. The chiral drug market has soared in recent years, and in 1999 worldwide revenue exceeded $100 billion. Besides the preparation and sale of single-enantiomer drugs from the time of first launch, the introduction of single-enantiomer drug preparations that were previously available only in racemic forms has emerged as a strategy to extend patent life. AstraZeneca's omeprazole, a proton pump inhibitor, is an example of a drug whose patent life was extended by this process of racemic switching. In this context, medicinal and process chemists alike are increasingly being called upon to apply the tools of asymmetric catalysis in general, and biocatalysis in particular, for the preparation of drugs and advanced pharmaceutical intermediates.

Biocatalyst catalog

The number of characterized biocatalysts has been increasing rapidly. In recent years, this production has been spurred by the development of technology to access previously unculturable organisms and thus new enzymes. There is an enzyme-catalyzed counterpart for almost every type of organic reaction, including key synthetic reactions. Biocatalysts can perform classes of reactions that are the staple tools of medicinal chemists, including aldol

condensation, asymmetric epoxidation, Baeyer–Villiger oxidation, and Claisen rearrangement.

The class of enzymes most widely applied to organic synthesis is the hydrolases. Members of the hydrolase family that have been exploited extensively include lipases, esterases, and proteases. The International Union of Biochemistry classifies enzymes by EC number, where each EC family catalyzes a distinct type of chemical transformation, as outlined here:

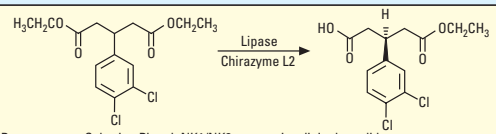
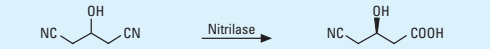
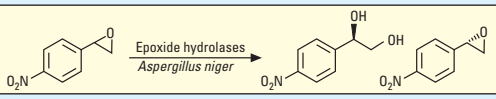
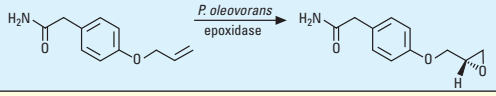
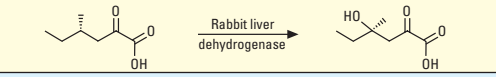
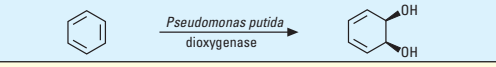
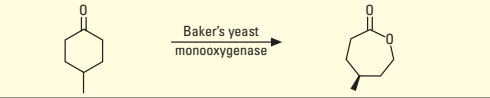
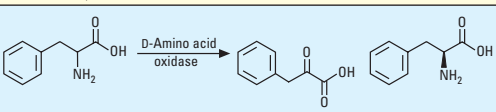
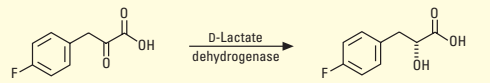
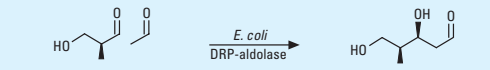
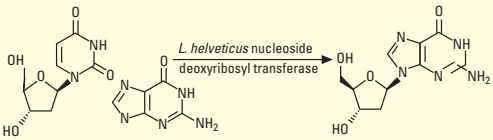
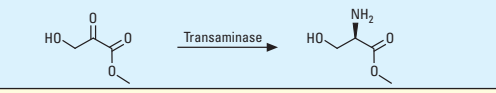
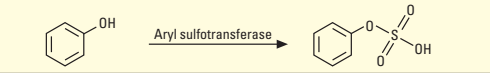
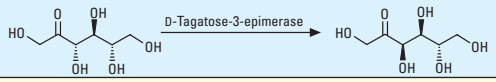
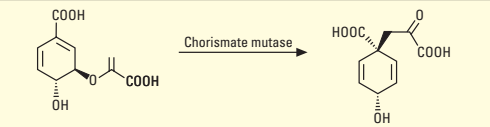
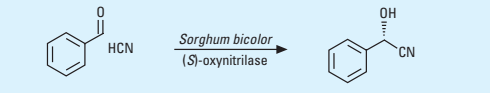
- Oxidoreductases (EC: 1) catalyze oxidation–reduction reactions involving oxygenation, such as C–H → C–OH, or the overall removal or addition of hydrogen atom equivalents, such as CH(OH) ⇄ C=O and CH–CH ↔ C=C.
- Transferases (EC: 2) catalyze the transfer of groups such as acyl, sugar, phosphoryl, and aldehyde or ketone moieties from one molecule to another.
- Hydrolases (EC: 3) catalyze the hydrolytic cleavage of glycosides, anhydrides, esters, amides, peptides, and other C–N moieties.
- Lyases (EC: 4) catalyze additions, usually of HX, to double bonds such as C=C, C=N, and C=O as well as the reverse processes.
- Isomerases (EC: 5) catalyze various rearrangements, including C=C bond migration, cis–trans isomerization, and racemization.
- Ligases (EC: 6) catalyze the formation of C–O, C–S, C–N, C–C, and phosphate ester bonds. These enzymes are also known as synthetases.

Although this classification system is practical and is descriptive from a mechanistic standpoint, it does not do justice to the myriad chemical transformations that may be catalyzed by enzymes and exploited for chiral drug and intermediate synthesis. Many important types of biocatalytic transformations are shown in Table 1, which highlights examples relevant to pharmaceutical intermediates.

Challenges to biocatalysis

Biocatalysts have not been widely applied for large-scale transformations because of their instability, high cost, limited availability, unnatural substrate scope,

Table 1. Biocatalytic reactions and applications

Enzyme	Transformation	Typical application
Hydrolases Esterase Lipase Protease/ amidase	 Precursor to a Schering Plough NK1/NK2 antagonist clinical candidate	Resolution Deprotection Bond formation
Hydrolases Nitrilase Nitrile hydratase	 Diversa route to a precursor to Lipitor (Pfizer's antihypercholesterolemia drug)	Preparation of α- and β-amino acids, hydroxy acids Desymmetrization
Hydrolases Epoxide hydrolase		Resolutions
Oxidases Epoxidases		Asymmetric induction Preparation of epoxides, versatile chiral synthons
Oxidases Monooxygenases		Introduction at oxygen on unactivated carbon
Oxidases Dioxygenases		Bis-hydroxylation of benzene
Oxidases Monooxygenases		Lactonization Desymmetrization
Oxidases Amino acid oxidase		Amino acid resolutions Preparation of α-keto acids
Dehydrogenases	 Ruprinaver, Pfizer's rhinovirus protease inhibitor candidate	Oxidation of alcohols Reduction of carbonyls
C–C bond formation Aldolase	 Precursor to the synthesis of epothilone A, a cytotoxic natural product	Skeletal elaboration concomitant with asymmetric induction/ resolution
Transferases Glycosyl transferase	 Production of dC, dA, and dG, and dideoxy nucleosides (antiviral precursors)	Preparation of nucleoside analogues
Transaminases		Preparation of amino acids
Sulfotransferases		Sulfoxidation reactions
Isomerases Epimerases		Preparation of epimers
Isomerases Chorismate mutase		
Lyases Oxynitrile lyase		Synthesis of chiral hydroxy nitriles, which can be used to make chiral hydroxy acids

product inhibition, need for expensive cofactors, and poor activity in organic solvents. Similarly, biocatalyst development has been hampered by a lack of information about choosing a suitable biocatalyst and by low substrate concentration loading, which leads to low volumetric productivity. Many of these challenges have been addressed, and viable solutions are accessible.

Because of the diversity of possible transformations, the uninitiated biocatalyst user requires guidance to accompany his or her chemical intuition. To facilitate the identification of a suitable enzyme, or at least an enzyme class that may serve as a starting point for a particular application, excellent databases that catalogue biocatalytic transformations are now available. These include a description of the reaction conditions, biocatalyst source, and examples of transformations.

One example is a thematic database compiled by Accelrys (San Diego) and curated by J. Bryan Jones of the University of Toronto (ON) and Bert Holland of Brock University (St. Catharines, ON). This database focuses on chemical synthesis using biocatalysts, including pure enzymes, whole cells, catalytic antibodies, and enzyme analogues. The database is available at *www.accelrys.com/chem_db/biocat.html*.

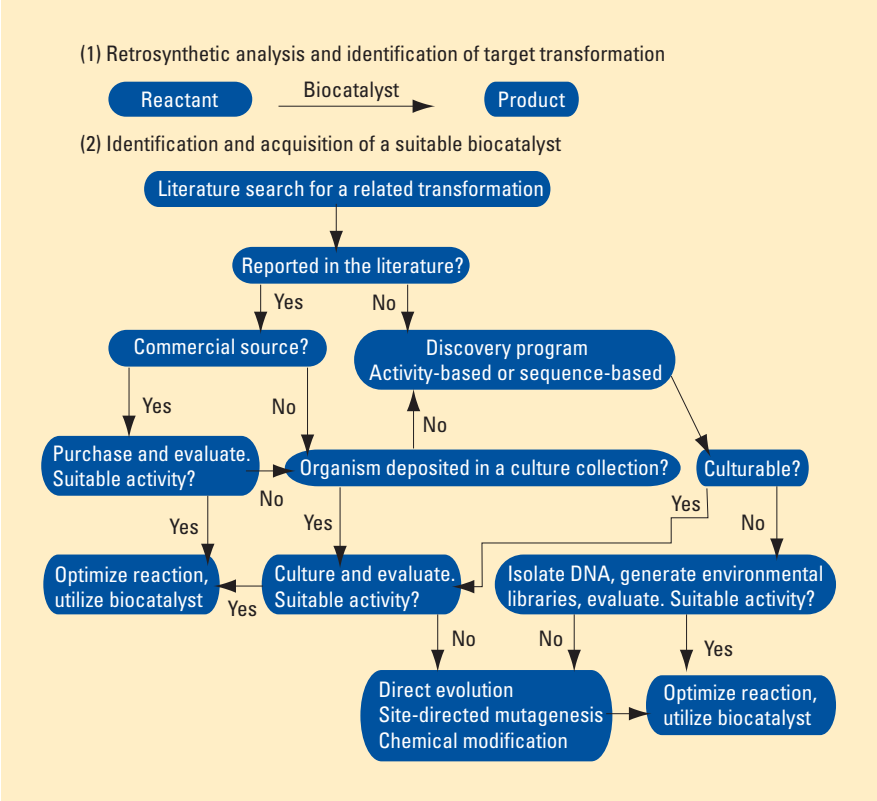


Figure 1. Developing a biocatalyst.

Do-it-yourself biocatalysis

Hundreds of enzymes are available as off-the-shelf preparations from specialty enzyme suppliers and chemical houses, such as Biocatalysts (Pontypridd, Wales, *www.biocatalysts.com*), Diversa Corp. (San Diego, *www.diversa.com*), Roche Molecular Biochemicals (Indianapolis, *http://biochem.roche.com*), and Novozymes (Bagsvaerd, Denmark, *www.novozymes.com*). However, should a suitable biocatalyst for a particular transformation not be commer-

cially available, several approaches are possible for obtaining an appropriate catalyst.

Creating enzymes with new catalytic activities, and tailoring the specificity of existing ones to better accommodate unnatural substrates, is crucial to further increasing the scope of the applications of enzymes in organic synthesis and thus is an area generating considerable research. Insights into the factors that govern enzyme–substrate interactions have been probed by site-directed mutagenesis studies. However, despite recent advances in understanding the intermolecular interactions that determine enzyme structural, regio-, and stereospecificity, truly rational tailoring of enzyme specificity remains an elusive goal.

New, more efficient discovery and directed-evolution programs may now be exploited.

In contrast, directed-evolution programs that use an iterative process of random mutation of enzymes and high-throughput screening have overcome many of the challenges facing practical application of biocatalysis. Companies developing these technologies include Diversa Corp., Prokaria (Reykjavik, Iceland, *www.prokaria.com*), and Evotec OAI (Cologne, Germany, *www.direvo.de*). New, more efficient discovery and directed-evolution programs may now be exploited to access a suitable biocatalyst for virtually any application. A general stepwise procedure that might be used to identify a suitable biocatalyst is illustrated in Figure 1.

The use of biocatalysis in both laboratory-scale and multiton process applications is poised to increase because of the thousands of enzymes available for testing, greater understanding of specificity, availability of databases to guide biocatalyst selection, and advances in protein engineering and high-throughput screening. The diversity of transformations catalyzed by biocatalysts guarantees their applicability to the synthesis of both chiral and achiral drugs in an economic, environmentally responsible, and practical manner.

Suggested reading

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Industrial Biotransformations; Liese, A., Seelbac, K., Wandrey, C., Eds.; Wiley-VCH: Weinheim, Germany, 2000.

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