1

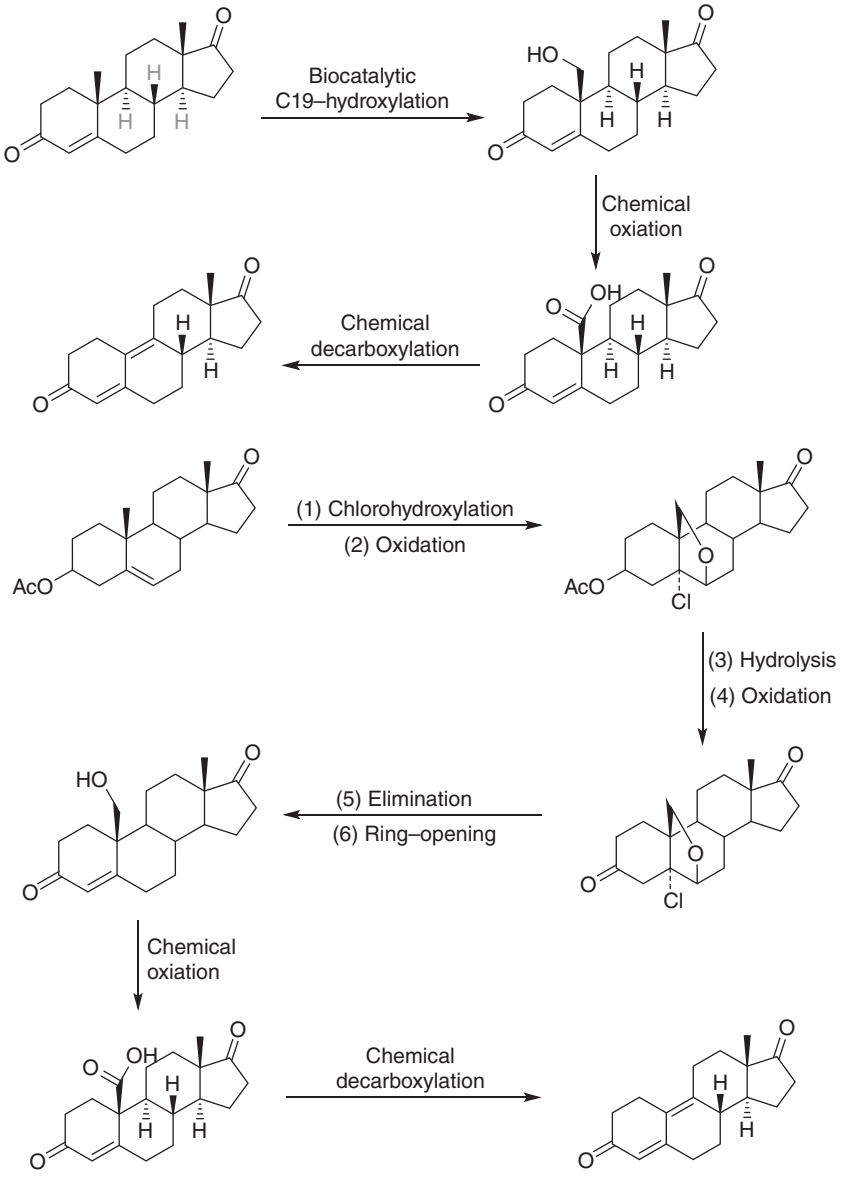
Introduction

Chemical processes are vital for the manufacturing of goods that meet the human's growing needs. Especially since early last century, organic chemical reactions have greatly contributed to the production of fine chemicals as materials, pharmaceuticals, food additives, cosmetics, etc., which are related to our daily lives. In the meantime, fine chemical industry has contributed to increasing air pollution and environmental contamination, which have adverse effects on the earth, our health, and the quality of our daily lives. The *E* (environmental) factor (mass of waste/mass of product, kgs/kg), which is often used to assess the environmental impact of a manufacturing process, for the production of fine chemicals is usually 5–50, or even higher for pharmaceuticals ($25 \geq 100$) [1]. Therefore, it is desirable to develop green organic chemical processes for the manufacturing of the desired chemical products, thus enabling sustainable development of the fine chemical industry [2].

On the other hand, Nature has created and evolved a diversity of enzymes that catalyze numerous kinds of reactions in live organisms and show advantages over traditional chemical reactions, such as high chemo-, regio- and stereoselectivities, and mild reaction conditions. Enzymes can catalyze various reactions that are difficult to be achieved by traditional chemical reactions. When being incorporated into organic synthesis, enzyme catalysis can reduce the number of reaction steps by eliminating the protection and deprotection steps or redesigning the synthetic route with enzymatic reactions to achieve greater efficiency or atom economy [3]. For example, 19-Nor-steroids are key intermediates for the production of contraceptives, such as norethindrone, mifepristone, and tibolone. By employing the biocatalytic hydroxylation at C-19 of steroids, the synthesis of 19-nor-steroids can be achieved in three chemoenzymatic steps [4]. However, the chemical demethylation of steroids usually requires many more steps as shown in Scheme 1.1 [5].

Retrosynthetic analysis involving both chemical and enzyme catalysis enables the design of novel synthetic sequences for the preparation of complex organic molecules such as active pharmaceutical ingredients [6]. Chemoenzymatic cascade reactions thus play an important role in developing green chemical processes by reducing the waste, energy consumption, and production cost. Great advances in this field have been achieved in the last two decades, and industries are paying increasing attention to enzyme application in the green production of chemicals used for pharmaceuticals, food additives, cosmetics, and so on [7].

Chemo-Enzymatic Cascade Reactions, First Edition. Dunming Zhu.



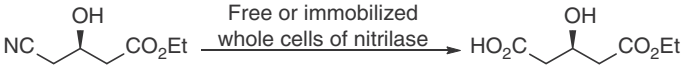
Scheme 1.1 Chemoenzymatic and chemical demethylation of steroids. Source: Based on Wang et al. [5].

In the first chapter of this book, the unique features of enzyme catalysis compared to traditional chemical reactions will be discussed. Next, different modes of chemoenzymatic transformations will be covered with some examples of the operating processes. In the remaining chapters, recent advances in this field, organized according to the modes of chemoenzymatic transformations, will be presented in more detail.

1.1 Advantages of Enzyme Catalysis

1.1.1 Chemoselectivity

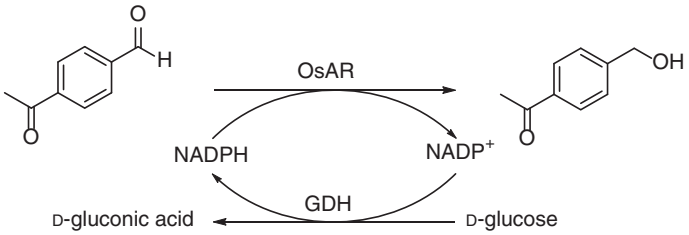
Enzyme catalysis is usually highly chemoselective, and the specific transformation of one functional group can be achieved without affecting the other active functional groups in the same molecule, a feature that otherwise cannot be realized by traditional chemical reactions. For example, chemical hydrolysis of nitrile group to carboxylic acid requires strong basic or acidic conditions at elevated temperature, under which the ester group is also hydrolyzed. Thus, it is impossible to chemo-selectively hydrolyze the nitrile group indiscriminately in the presence of ester group in the same molecule. On the other hand, this challenge can be addressed by using nitrilases, which can catalyze the chemo-specific hydrolysis of nitriles under neutral conditions to give the corresponding carboxylic acids in the presence of other acid- or base-sensitive functional groups [8]. For example, ethyl (*R*)-4-cyano-3-hydroxybutyate was hydrolyzed by a recombinant nitrilase from *Aarabidopsis thaliana* (AtNIT2) to give ethyl (*R*)-3-hydroxyglutarate. The ester group in the molecule remained intact (Scheme 1.2) [9]. This key building block for the synthesis of the cholesterol-lowering drug, rosuvastatin, was produced with excellent biocatalyst productivity (55.6 g/g wet cells weight) and space-time productivity (625.5 g/l d).



Scheme 1.2 Nitrilase-catalyzed chemospecific hydrolysis of ethyl (*R*)-4-cyano-3-hydroxybutyate. Source: From Yao et al. [9]. © 2014, John Wiley & Sons.

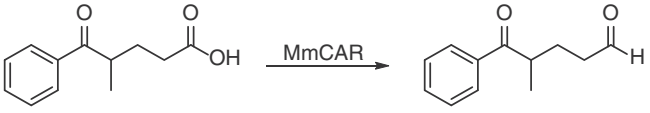
Reduction of aldehyde and ketone to give alcohols is an important transformation in organic synthesis. Traditional chemical carbonyl reduction methods often show low chemo-selectivity toward either aldehyde or ketone. To achieve selective reduction of aldehyde in the presence of keto group, and vice versa, careful selection of the reducing agent and control of the reaction conditions are usually required. On the other hand, Nature has evolved many aldehyde or ketone reductases, which can catalyze the chemo-specific reduction of aldehyde functional group in the presence of keto group, or vice versa, to give the corresponding alcohols. For example, an NADPH-dependent aldehyde reductase from *Oceanospirillum sp*. MED92 (OsAR) catalyzes the selective reduction of the aldehyde group in 4-acetylbenzaldehyde without reducing the keto group, affording 4-acetylbenzyl alcohol as the sole product (Scheme 1.3) [10].

Carboxylic acid functional group is difficult to be reduced and usually requires strong reducing agents, which in turn can often reduce C=O, C=N, and other functional groups. Thus, it is quite challenging to achieve chemo-specific reduction of carboxylic acid group in the presence of other reducible functional groups such as C=O and C=N groups in the same molecule. Furthermore, the reduction of carboxylic acid is difficult to stop at the aldehyde intermediate, since the latter can



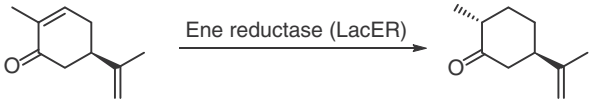
Scheme 1.3 The reduction of 4-acetylbenzaldehyde catalyzed by OsAR. Source: Based on Li et al. [10].

be further reduced to the corresponding alcohol by the reducing agents [11, 12]. However, by using carboxylic acid reductases (CAR, E.C.1.2.1.30) as biocatalyst these problems can be solved, because they catalyze the selective reduction of carboxylic acids into the corresponding aldehydes under mild conditions [13, 14]. This enzyme does not catalyze the reduction of other functional groups such as keto groups and C=N double bonds. Scheme 1.4 shows the enzymatic reduction of a representative keto acid (4-methyl-5-oxo-5-phenylpentanoic acid) to the corresponding keto aldehyde, leaving the keto group intact using a recombinant CAR from *Mycobacterium marinum* (MmCAR) [15].



Scheme 1.4 CAR-catalyzed reduction of keto acid to keto aldehyde.

Ene reductase [16], carbonyl reductase [17], and imine reductase [18] catalyze the specific reductions of C=C, C=O, or C=N functional group, respectively. Ene reductase catalyzes the reduction of C=C bond without affecting the C=O or C=N functional group in the molecule (Scheme 1.5) [19]. This is difficult to be realized by the metal-catalyzed hydrogenation reaction since the C=O or C=N functional group may also be hydrogenated during the reduction of the C=C bond.

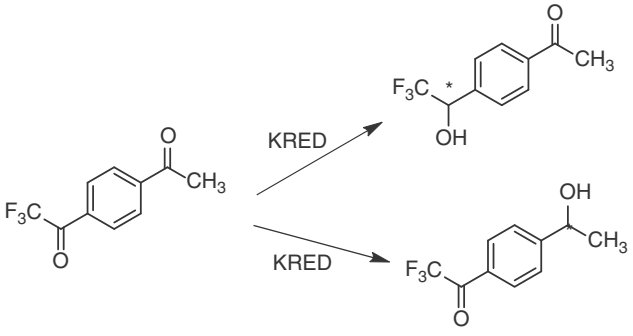


Scheme 1.5 Reduction of (*R*)-carvone by an ene reductase (LacER) from *Lactobacillus casei*. Source: Based on Chen et al. [19].

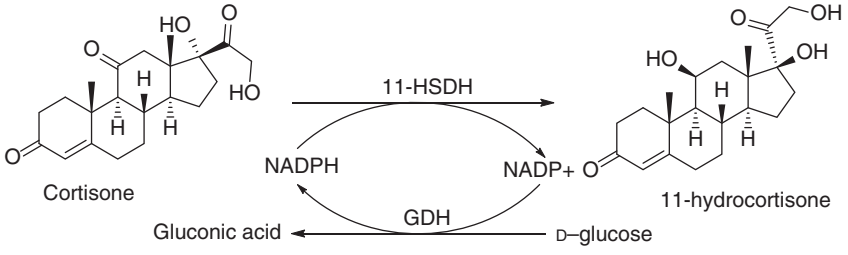
1.1.2 Regioselectivity

Enzymes can differentiate the same functional group at different positions in one molecule; thus, enzymatic reactions are usually highly regioselective. Incorporation of regiospecific enzymatic reaction into organic synthesis often simplifies

the synthetic route of a target compound by eliminating the protecting and de-protecting steps. The regiospecific reduction of either the trifluoromethyl or the methyl keto group in the methyl/trifluoromethyl diketones was achieved by using some commercially available ketoreductases (KREDs) to give either the *R* or the *S* enantiomer with >98% enantiomeric excess (ee), as shown in Scheme 1.6 [20]. Among the three keto groups in the molecule of cortisone, the keto group at 11-position was highly regio- and stereospecifically reduced to give 11β-hydrocortisone by a mutant 11β-hydroxysteroid dehydrogenase (11β-HSDH) from guinea pig (Scheme 1.7) [21].



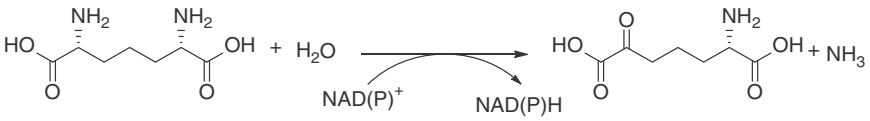
Scheme 1.6 Enzymatic regiospecific reduction of the methyl/trifluoromethyl diketone. Source: Based on Grau et al. [20].



Scheme 1.7 Enzymatic regio- and stereospecific reduction of cortisone. Source: From Zhang et al. [21]. © 2014, Springer Nature.

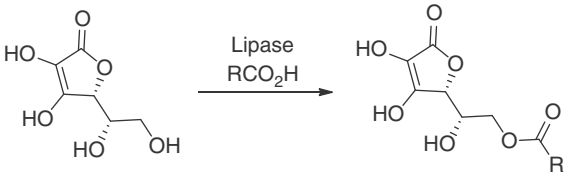
Like alcohol dehydrogenase, other oxidoreductases also show excellent regioselectivity. For example, *meso*-diaminopimelate dehydrogenase (*meso*-DAPDH, EC 1.4.1.16) acts on the D-configuration amino group to generate L-2-amino-6-oxopimelate (Scheme 1.8) [22].

Hydrolases such as lipases and nitrilases often exhibit exquisite regioselectivity. An immobilized lipase from *Staphylococcus xylosus* catalyzed the acylation of one of the hydroxyl groups of ascorbic acid, leading to the lipophilic ascorbyl esters in good yield (Scheme 1.9) [23]. The optically active 3-alkylglutaric acid monoesters bearing various alkyl substituents were prepared by the selective hydrolysis of prochiral 3-alkylglutaric acid diesters using commercially available lipase *Candida antarctica*

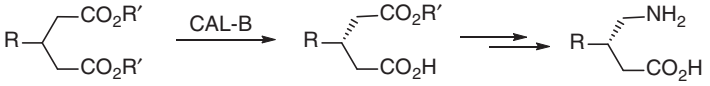


Scheme 1.8 Enzymatic regio- and stereospecific oxidative deamination of *meso*-diaminopimelate. Source: Adapted from Akita et al. [22].

lipase B (CAL-B). The unreacted ester group can then be converted to amino group, affording 3-substituted γ-aminobutyric acids, the important γ-aminobutyric acid (GABA) derivatives (Scheme 1.10) [24].

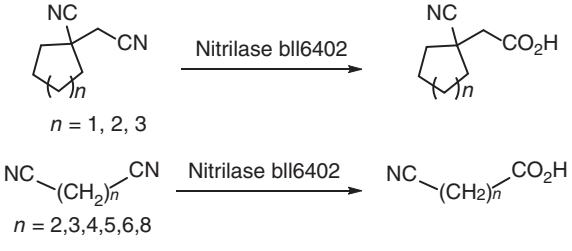


Scheme 1.9 Lipase-catalyzed regioselective acylation of hydroxyl groups. Source: Adapted from Kharrat et al. [23].



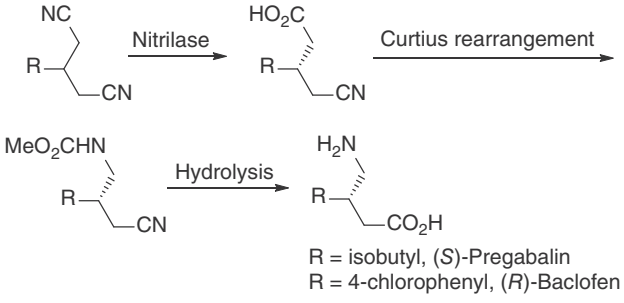
Scheme 1.10 Lipase-catalyzed selective hydrolysis of diesters. Source: Adapted from Jung et al. [24].

A nitrilase (bll6402) from *Bradyrhizobium japonicum* not only catalyzed the regiospecific hydrolysis of cyano group with less steric hindrance in cyclic dinitriles but also acted exclusively on one of the two exactly same CN groups in a molecule to produce the corresponding cyanocarboxylic acids, as shown in Scheme 1.11 [25, 26]. This transformation is not possible by traditional chemical hydrolysis. Furthermore, the desymmetric hydrolysis of prochiral 3-substituted glutaronitriles was achieved using several nitrilases of different origins and their mutant enzymes. The generated optically active 3-substituted-4-cyanobutanoic acids can be further transformed



Scheme 1.11 Nitrilase-catalyzed the regiospecific hydrolysis of dinitriles. Source: Veselá et al. [25]; Zhu et al. [26].

into pharmaceutically important GABA derivatives such as the currently marketed drugs, (*S*)-Pregabalin and (*R*)-Baclofen (Scheme 1.12) [27, 28].

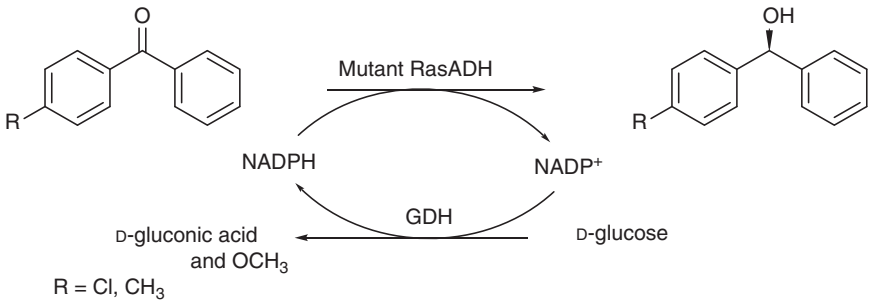


Scheme 1.12 Nitrilase-catalyzed desymmetric hydrolysis of prochiral 3-substituted glutaronitriles. Source: Duan et al. [27]; Yu et al. [28].

1.1.3 Stereoselectivity

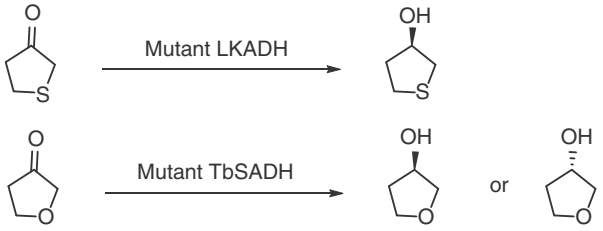
The exquisite stereoselectivity of enzymes often outperforms the traditional chemical catalysis, making enzyme catalysis an alternative or complimentary toolbox for asymmetric transformations [29]. For chemical reduction of ketones, high enantioselectivity is achieved only when the two substituents flanking the carbonyl functional group are sterically and/or electronically very different. For example, the transition-metal-catalyzed hydrogenation of diaryl ketones usually requires an *ortho*-substituent on one of the aryl groups to realize high enantiocontrol in the product formation. The chemical reduction of diaryl ketones with only a *para*- or *meta*-substituent on one of the aryl groups affords the diarylmethanol in low enantiomeric purity (ee often being less than 50%) [30]. In contrast, high enantioselectivity has been achieved by enzymatic reduction. A carbonyl reductase from red yeast *Sporobolomyces salmonicolor* AKU4429 (SSCR) and its mutant enzymes effectively catalyzed the enantioselective reduction of diaryl ketones to give the corresponding chiral alcohols with up to 92% ee [31, 32]. Recently, a mutant alcohol dehydrogenase from *Ralstonia* sp. (RasADH) catalyzed the reduction of these ketones with ee values of 93, 95% for *p*-Cl, *p*-CH_3, respectively, or 97% for *p*-OCH_3 (Scheme 1.13).

(*R*)-Tetrahydrothiophene-3-ol is a key component in Sulopenem, a potent antibacterial prodrug with broad-spectrum antibacterial activity against most gram-positive and gram-negative bacteria. A straightforward synthesis of this alcohol is the reduction of tetrahydrothiophene-3-one; but the near spatial symmetry of the ketone results in low optical purity (23–82% ee) by chemical reduction. However, both high yield (81–88%) and >99% ee of (*R*)-tetrahydrothiophene-3-ol could be obtained by using a mutant Alcohol dehydrogenase from *Lactobacillus kefir* (LKADH) as biocatalyst when tetrahydrothiophene-3-one at a concentration of 100 g/l was used (Scheme 1.14). This biocatalytic process has successfully replaced an original multistep hazardous process starting from an achiral pool of



Scheme 1.13 Mutant RasADH-catalyzed reduction of diarylketones.

substrates [34]. Similarly, highly (*R*)- and (*S*)-selective variants of alcohol dehydrogenase from *Thermoethanolicus brockii* (TbSADH) catalyzed the reduction of tetrahydrofuran-3-one and other difficult-to-reduce ketones to both enantiomers of the corresponding chiral alcohols with high ee values (Scheme 1.14) [33].

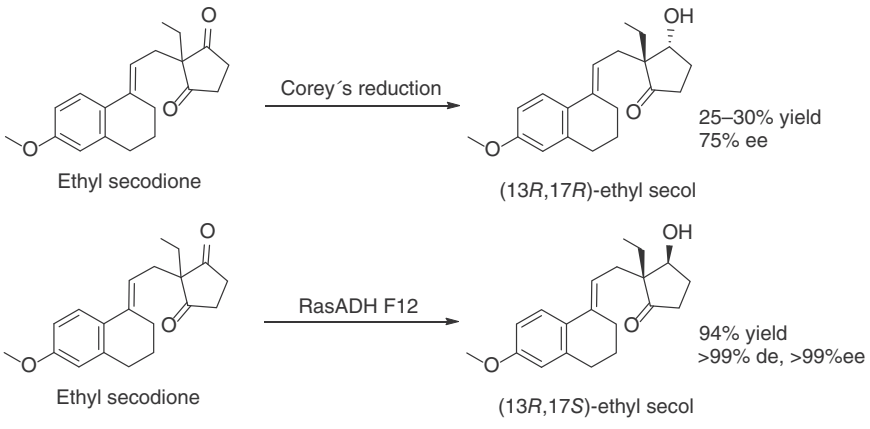


Scheme 1.14 Ketoreductase-catalyzed reduction of tetrahydrothiophene-3-one and tetrahydrofuran-3-one. Source: Based on Sun et al. [33].

(13*R*, 17*S*)-Ethyl secol is a key intermediate for the synthesis of steroidal medicines, such as gestodene and levonorgestrel. A straightforward approach to access (13*R*, 17*S*)-ethyl secol is the desymmetric reduction of ethyl secodione, which creates two chiral centers in one reaction step. The chemical reduction of ethyl secodione at low temperature led to the isolation of (13*R*, 17*R*)-ethyl secol in 25–30% yield and 75% ee [35]. Recently, by using an engineered carbonyl reductase (RasADH F12) from *Ralstonia* sp. as the biocatalyst, reductive desymmetrization of ethyl secodione and other 2,2-disubstituted cyclopentadiones led to the production of essentially one single stereoisomer, (13*R*, 17*S*)-ethyl secol, in up to 94% isolated yield (Scheme 1.15) [36].

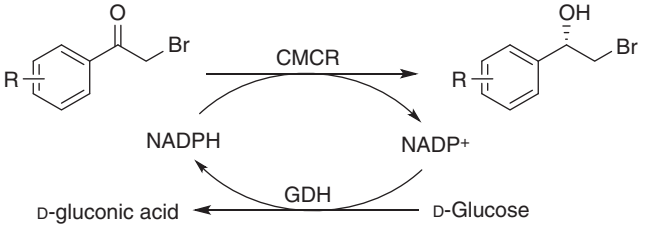
1.1.4 Mild Reaction Conditions

In addition to high chemo-, regio-, and stereoselectivity, it is well known that enzymatic reactions are usually carried out in aqueous buffer under neutral or close to neutral pH and at room temperature. These mild conditions can keep the potentially labile functional groups in the molecule intact in the synthesis of a target compound without involving the protection/deprotection steps of a



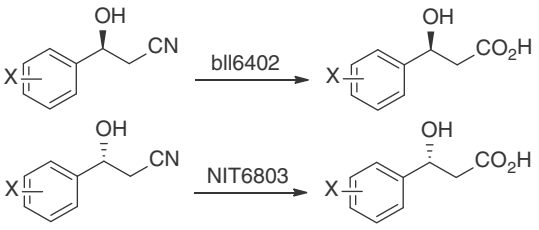
Scheme 1.15 Chemical and biocatalytic reduction of ethyl secodione. Source: Adapted from Chen et al. [36].

traditional chemical transformation. Additionally, the enzymatic reaction may proceed efficiently and cleanly, and the formation of unwanted by-products can be minimized. One example is the reduction of α-bromoacetophenones, an important transformation for the preparation of optically active β-bromo alcohols, epoxides, or diols. The reduction, using metal hydrides as the reducing agent, often causes loss of the bromo group, leading to low yield of α-bromohydrins and difficulty in product purification. In order to address this challenge [37], the isolated carbonyl reductase from *Candida magnolia* (CMCR) was employed in combination with a D-glucose dehydrogenase/D-glucose cofactor regeneration system for the reduction of α-bromoacetophenones. The reaction was performed in a two-phasic reaction medium and the concomitant loss of bromine was effectively prevented. The optically pure α-bromohydrins were prepared in 79–92% yields and >99% ee (Scheme 1.16) [38].



Scheme 1.16 Enzymatic reduction of α-bromoacetophenones. Source: From Ren et al. [38]. © 2012, Elsevier.

Chemical hydrolysis of nitriles requires strong basic or acidic conditions and elevated reaction temperature. Therefore, for the chemical hydrolysis of β-hydroxy nitriles to the β-hydroxy acid, it is difficult to avoid the undesirable elimination of OH group that results in the formation of the unsaturated by-products, because it



Scheme 1.17 Nitrilase-catalyzed hydrolysis of β-hydroxy nitriles. Source: From Ankati et al. [40]. © 2009, American Chemical Society.

cannot tolerate such harsh reaction conditions [39]. A couple of nitrilases (bll6402, NIT6803) from different sources have been shown to effectively catalyze the hydrolysis of β-hydroxy nitriles without affecting the β-hydroxy group. The corresponding β-hydroxy carboxylic acids were obtained in excellent yields (Scheme 1.17) [40].

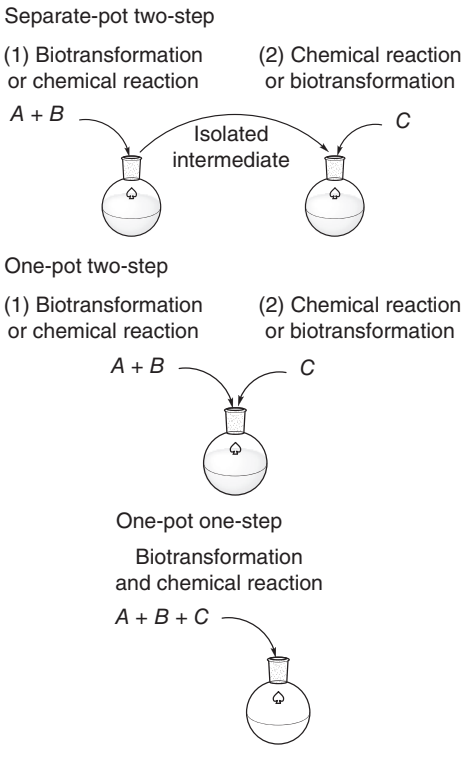
1.2 Modes of Chemoenzymatic Transformations

Because of the unparalleled selectivity and mild reaction conditions, enzymes are expected to play an exciting role in "green chemistry." Chemoenzymatic cascade strategies offer unprecedented opportunities for developing efficient and sustainable synthetic technologies to address the issues of health, environment, energy, and security that we face today.

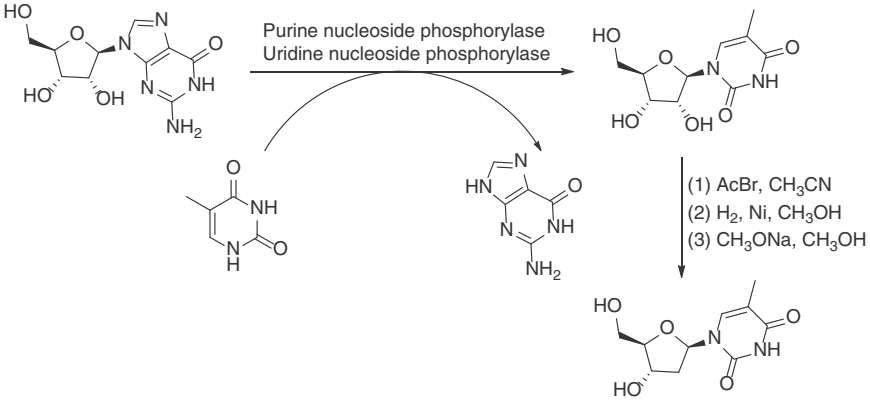
Chemoenzymatic transformations can be carried out in one of the three modes: separate-pot two-step, one-pot two-step, and one-pot one-step (Scheme 1.18). This is a simplified presentation because more than one enzymatic reaction can be combined with multiple chemical transformations to achieve a synthetic goal. Especially for the "separate-pot two-step" mode, infinite number of either enzymatic or chemical reactions can be incorporated into a synthetic route of a target compound as the case may be. For the other two modes, the number of reactions may be limited by the compatibility of chemical reactions and biotransformations.

1.2.1 "Separate-Pot Two-Step" Mode

For the "separate-pot two-step" mode, the sequential steps of chemical reaction and biotransformation are carried out in separate reactors, with the intermediate being isolated or partially purified. The chemical reaction and biotransformation can be performed under quite different reaction conditions, and thus have been widely used to achieve complex synthetic goals. An example is the chemoenzymatic process for the preparation of β-thymidine. β-Thymidine is a precursor for the production of anti-AIDS drugs stavudine (d4T) and zidovudine (AZT). The enzymatic transglycosylation of guanosine and thymine yielded 5-methyluridine (5-MU) and guanine. The resulting 5-MU was then converted into β-thymidine by bromination and hydrogenation, as shown in Scheme 1.19 [41].



Scheme 1.18 Three simplified modes of chemoenzymatic cascade transformations.

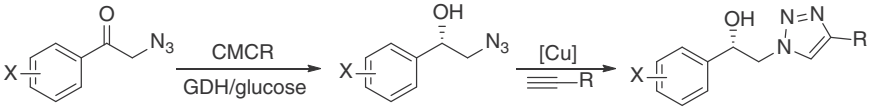


Scheme 1.19 Chemoenzymatic synthesis of β-thymidine by a separate-pot two-step process. Source: Adapted from Gordon et al. [41].

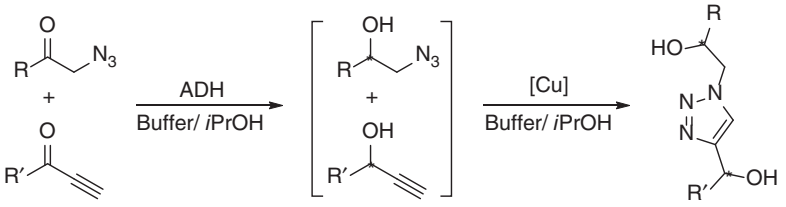
1.2.2 "One-Pot Two-Step" Mode

In the case of "one-pot two-step" mode, the chemical reaction and biotransformation are carried out sequentially in one pot without the isolation of the intermediates. This helps the simplification of process operation and reduction in

solvent usage, waste generation, and operational cost. The enzymatic reduction of α-azidoacetophenone derivatives generates 2-azido-1-arylethanols with excellent optical purity, which can react with alkynes employing click chemistry to afford optically pure triazole-containing β-adrenergic receptor blocker analogs. The bioreduction of ketones and Cu-catalyzed "click" reaction was first carried out in the "separate-pot two-step" mode, in which the 2-azido-1-arylethanol intermediates were isolated (Scheme 1.20) [42]. This process was later performed in a "one-pot two-step" mode. The corresponding chiral 1,2,3-triazole-derived diols were prepared in high yields and excellent enantio- and diastereoselectivities under very mild conditions in aqueous medium (Scheme 1.21) [43].



Scheme 1.20 Chemoenzymatic synthesis of 1,2,3-triazole-derived diols by a separate-pot two-step process. Source: Adapted from Ankati et al. [42].

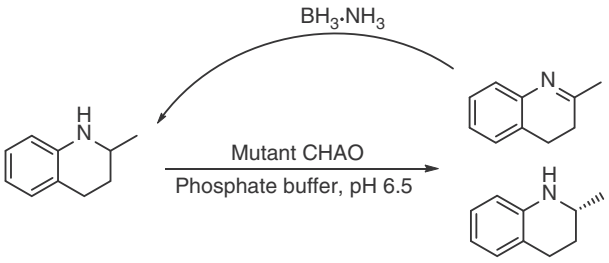


Scheme 1.21 Chemoenzymatic synthesis of 1,2,3-triazole-derived diols by a one-pot two-step process. Source: Adapted from Cuetos et al. [43].

1.2.3 "One-Pot One-Step" Mode

The third mode of chemoenzymatic transformation is "one-pot one-step," in which the chemical reaction and biotransformation proceed concurrently without high concentration accumulation of the intermediates. An example is our recent report that deracemization of 2-methyl-1,2,3,4-tetrahydroquinoline was achieved by using a mutant cyclohexylamine oxidase (CHAO) with the reducing agent $BH_3{\cdot}NH_3$. The bio-oxidation of (*S*)-2-methyl-1,2,3,4-tetrahydroquinoline led to the imine intermediate, which was immediately reduced by the chemical reducing agent, resulting in the production of (*R*)-2-methyl-1,2,3,4-tetrahydroquinoline with 76% isolated yield and 98% ee after cycles of enantioselective oxidation and nonselective reduction (Scheme 1.22) [44]. Tetrahydroquinoline is a "privileged" scaffold or substructure in many biologically active natural products and therapeutic agents used in cancer drug development.

Both chemical reaction and biotransformation possess advantageous features and shortcomings in terms of their capability for molecular construction and functional transformation. Combination of chemical reaction and biotransformation

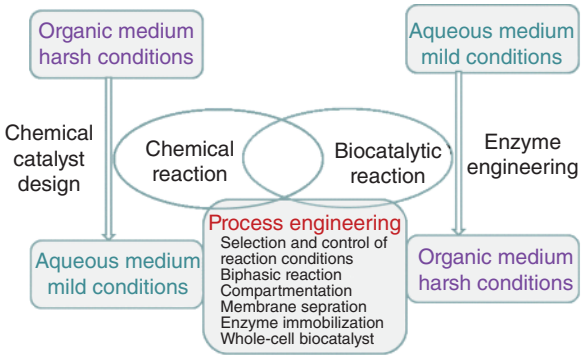


Scheme 1.22 Chemoenzymatic deracemization of 2-methyl-1,2,3,4-tetrahydroquinoline by a one-pot one-step process. Source: From Li et al. [44]. © 2014, American Chemical Society.

may give full play to their advantages, and thus offer tremendous opportunity for developing efficient and sustainable processes by designing novel synthetic routes from "starting materials to products" with retrosynthesis strategy [3, 6]. This can be relatively easily realized by the "separate-pot two-step" mode, because both biocatalytic and chemical transformations are individually treated as a synthetic step without considering the compatibility of the reaction conditions that are often quite different for chemical reactions and biotransformations. However, this "separate-pot" mode is less efficient than the two "one-pot" modes. The "one-pot two-step" and "one-pot one-step" modes are actually cascade reactions defined in organic chemistry [45]. A cascade reaction is a chemical process that comprises at least two consecutive reactions such that each subsequent reaction occurs by virtue of the chemical functionality formed in the previous step. In the strictest definition of cascade reaction, the reaction conditions do not change among the consecutive steps of a cascade and no new reagents are added after the initial step. This kind of sequential reactions is also called domino reaction. But a rather inclusive definition, which does not preclude the addition of new reagents or the change of conditions after the first reaction, is often adopted in the literature. In this book, the inclusive definition of cascade reaction is adopted, and includes one-pot sequential and concurrent transformations.

The undeniable benefits of cascade reactions include higher atom economy and production efficiency, and less resource consumption and waste generation. Especially, in the "one-pot one-step" mode, if one carries out several transformations in one synthetic operation, the involved savings can be considerable. However, for this module, there are many challenges, such as the reaction condition compatibility of the chemical reaction with biotransformation, the inhibition or inactivation of (bio)catalysts, and so on, which must be addressed before industrial application [46, 47]. In this context, scientists and engineers are designing chemical catalysts for use in aqueous reaction medium and evolving enzyme catalysts with tolerance of organic solvents, so that these catalysts can work under the same reaction conditions (Scheme 1.23). Process engineering involving various strategies such as reaction medium engineering [48] and reaction site separation [46] has also been carried out for developing efficient and economical chemoenzymatic cascade processes. When further advances in (bio)catalyst design and process engineering have been achieved in the future, we can expect that chemoenzymatic cascade

reactions will definitely play an ever-increasing role in the transition of the currently non-sustainable fine chemical industry to a green and sustainable one.



Scheme 1.23 Scientists and engineers are working together to develop efficient and economical chemoenzymatic cascade.

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