6

ENZYME-CATALYZED ASYMMETRIC SYNTHESIS

Harald Gröger
Department of Chemistry and Pharmacy, University of Erlangen-Nuremberg, Erlangen, Germany

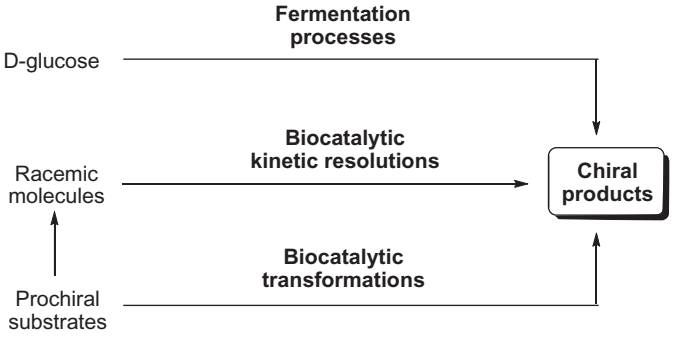
6.1. INTRODUCTION

Biocatalysis has been recognized over the past decades as a highly valuable tool for organic chemists to prepare enantiomerically pure molecules, so-called chiral building blocks, in a highly efficient way. Besides a multitude of academic work, it is noteworthy that enzyme catalysis belongs to the standard repertoire in industry when facing challenging enantioselective synthetic routes. A broad range of biocatalytic methods is already in use in particular for large-scale manufacture of drug intermediates [1].

The research in the field of enzyme catalysis has already been comprehensively reviewed some years ago [2]. Thus, the focus of the current review is on a selection of (particularly recently developed) enantioselective enzymatic reactions, which turned out to be highly useful and applicable in organic synthesis, fulfilling criteria such as high productivity, substrate concentrations, conversions, and enantioselectivities. The presented methods are an interesting complementary tool to existing "classic organic" or "chemocatalytic asymmetric" methodologies. Among biocatalytic reactions, both resolution of racemates and asymmetric synthesis starting from prochiral substrates are attractive routes already applied, in part, in industry. A third type of biotechnological approach, which is not a subject of this review, are fermentation processes. A graphical summary of these three types of so-called "white biotechnology" methodologies is given in Scheme 6.1.

Hydrolases are the enzyme class most commonly applied as biocatalysts in organic chemistry. This is mainly due to the accessibility of these enzymes (used, e.g., in the textile and detergents industry), their suitability for transformations in organic media

Catalytic Asymmetric Synthesis, Third Edition, Edited by Iwao Ojima



Scheme 6.1.

(in particular when using lipases), and the lack of a need for cofactors. However, in recent years, we have also seen an increasing tendency to apply redox enzymes in organic syntheses as well as lyases in C–C bond formations and transferases. Isomerases also turned out to be very useful in particular in combination with hydrolases for dynamic kinetic resolutions. Thus, a broad range of biotransformations is available for organic chemists. A challenge, however, is the use of ATP cofactor-dependent enzymes, which contribute to only a negligible number of typical organic biotransformations (e.g., due to the high price of ATP).

The field of biocatalysis benefited significantly from the tremendous progress in molecular biology. Highly efficient methods for screening and optimization of biocatalysts (by, e.g., directed evolution in the latter case) have been developed, which allows access to tailor-made enzymes. Furthermore, the design of recombinant microorganisms gives access to highly productive whole-cell catalysts, which contain only the desired enzymes in large amount, thus significantly reducing the required biomass for biotransformations compared with the use of wild-type enzymes. Such designer cells are economically attractive (bio-)catalysts and can be easily prepared in high cell-density fermentations.

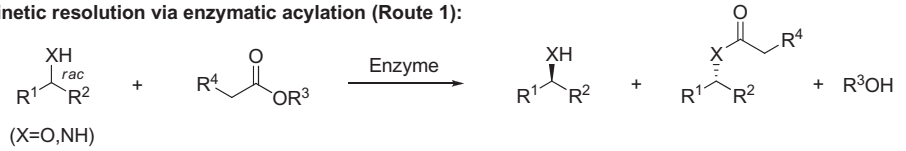
The majority of applications of biotransformations in organic synthesis are related to asymmetric synthesis of chiral molecules with an emphasis on pharmaceutically relevant molecules or intermediates thereof. Examples for application of enzymes as biocatalysis in drugs synthesis will be given in the sections below. It should be noted that biotransformations are not only attractive methods for lab-scale synthesis, but also are very effective and technically feasible process technologies on industrial scale.

6.2. ENANTIOSELECTIVE ACYLATION OF ALCOHOLS AND AMINES WITH CARBOXYLATES

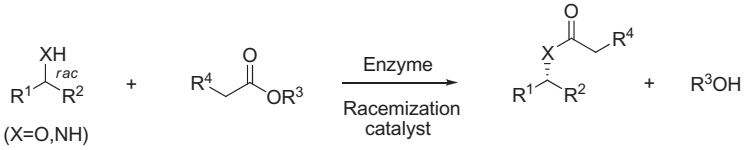
6.2.1. Overview

Enantioselective acylation is an excellent method for resolution of alcohol and amines. Although recently a range of chemocatalysts has been developed for enantioselective acylation reactions, the most commonly applied catalysts are enzymes, in particular lipases. Lipases [3] are one of the rare types of enzymes that tolerate a broad range of

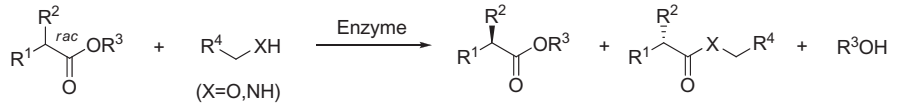
Kinetic resolution via enzymatic acylation (Route 1):



Dynamic kinetic resolution via enzymatic acylation and chemocatalytic racemization (Route 2):



Kinetic resolution via transesterification or aminolysis (Route 3):



Scheme 6.2.

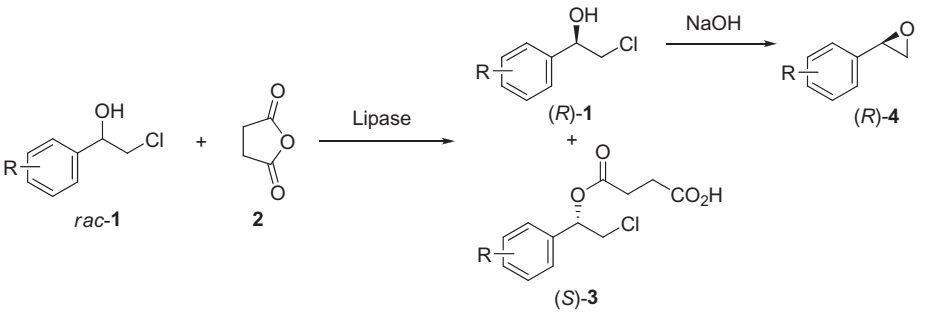
solvents and can be used in organic media. Besides two-phase media, lipases can be used even in pure organic media, which makes them easily compatible with "typical" organic transformations. Further reasons for lipases' attractiveness are their easy and cheap access since these enzymes often originate from applications in other (bulk product) sectors such as detergents and food enzymes. In synthetic applications, lipases turned out to be highly enantioselective and productive catalysts for enantioselective acylation of both alcohols and amines. When using a racemic alcohol, lipases generate enantioselectively the acyl form of the preferred enantiomer while leaving the undesired enantiomer untouched (Scheme 6.2, Route 1, X=O). While this reaction represents a "classic" enzymatic resolution, combination of a lipase with a chemocatalyst for racemization of the alcohols enables a dynamic kinetic resolution under (theoretically) quantitative formation of acylated alcohol (Route 2, X=O) [4]. Replacing the alcohol substrate with a racemic amine component (Route 1 and 2: X=NH) allows the analogous syntheses of enantiomerically pure amines. An alternative option is the use of achiral alcohols and racemic acid components (e.g., esters). The resulting lipase resolution is a so-called transesterification reaction, which can be useful for the resolution of acid or derivatives thereof (Route 3, X=O). When carrying out the same reactions with achiral amines instead of alcohols, the resulting synthesis is a so-called aminolysis reaction (Route 3, X=NH). This reaction can be useful for the resolution of chiral acid or derivatives thereof as well (see Section 6.2.4).

6.2.2. Acylation Using Racemic Alcohols

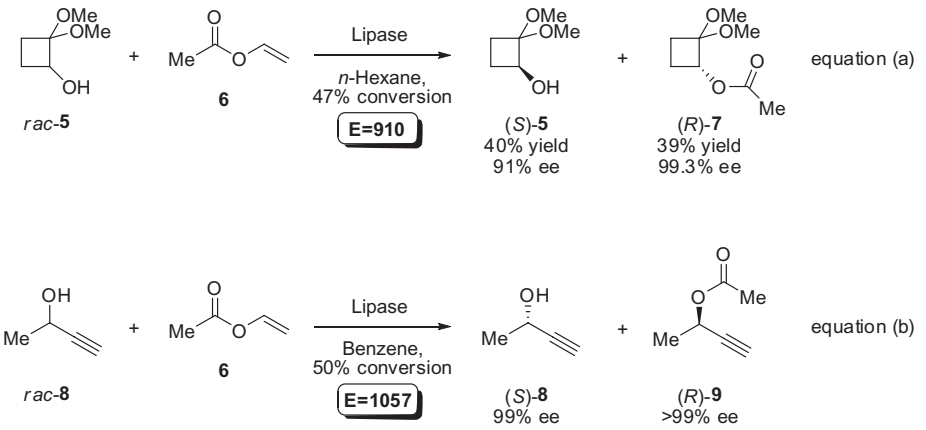
The resolution of racemic alcohols in the presence of lipases is a widely known technology. To review this type of reaction comprehensively would be beyond the scope of this review [5]. Therefore, in the following, selected examples are given.

Notably, at BASF AG, enzymatic acylation of racemic alcohols has already been established as an industrial platform [6]. For example, using a racemic aromatic halohydrin *rac*-**1** in combination with succinic acid anhydride **2** as a donor molecule and a lipase as biocatalyst furnished the (*R*)-halohydrin (*R*)-**1**, which has been subsequently transformed into the corresponding epoxide (*R*)-**4** (Scheme 6.3). The simple separation of the unconverted (*R*)-enantiomer (*R*)-**1** from the acylated halohydrin (*S*)-**3** by acid–base extraction is advantageous.

Recently, efforts have been made to acylate enantioselectively secondary alcohols that bear additional functional groups such as a cyano, carbonyl, or an alkine moiety. In a (nondynamic) kinetic resolution, cyanohydrins are acylated in the presence of a lipase very efficiently, leading to enantiomerically enriched cyanohydrin acetates [7]. Furthermore, protected cyclobutanones *rac*-**5** bearing an α-hydroxy keto moiety are suitable substrates for lipase-catalyzed acylation (Scheme 6.4, equation (a)) [8]. Using these types of substrates, excellent enantioselectivities were obtained with E values of up to 910 when using a lipase from *Candida antarctica* B. This enzyme (the so-called lipase CAL-B) is regarded as the most versatile lipase in organic chemistry. In contrast,



Scheme 6.3.

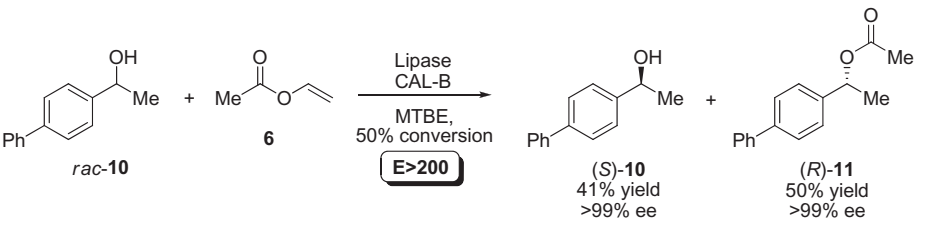


Scheme 6.4.

only low enantioselectivities were found when using α-hydroxy cyclobutanone in non-protected form.

Propargylic alcohols have been resolved as well very successfully in the presence of lipase CAL-B by Porto and coworkers [9]. In the presence of this enzyme, acylation of racemic phenyl propargylic alcohol *rac*-**8** proceeded highly enantioselectively, reaching an excellent E value of 1057 (Scheme 6.4, equation (b)). Further propargylic alcohols with a different substitution pattern also undergo CAL-B-catalyzed acylation in a highly enantioselective manner. In addition, Bornscheuer et al. reported the development of new lipases highly suitable for the resolution of propargylic alcohols [10].

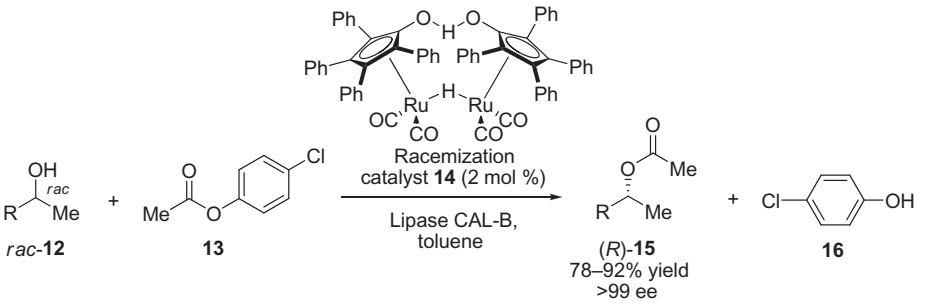
Another very interesting structural motif is the biaryl-substituted secondary alcohol moiety. Enantioselective enzymatic acylation of such molecules has been reported by Rebolledo and coworkers [11]. Notably, excellent enantioselectivities with E values of >200 were obtained in the acylation of a range of biaryl-substituted secondary alcohols of type *rac*-**10** when using lipase from *C. antarctica* A (CAL-B) as a biocatalyst (Scheme 6.5). Excellent enantiomeric excess of >99% ee has been obtained for both the formed ester (*R*)-**11** and remaining alcohol substrate (*S*)-**10**.



Scheme 6.5.

A very interesting one-pot process to improve overall process efficiency has been reported by Kamal and coworkers. Starting from ketone reduction with sodium in the presence of neutral alumina in hexane, racemic alcohols are yielded after the initial step. Without isolation of these intermediates, subsequent enzymatic acylation in the presence of a lipase from *Pseudomonas cepacia* proceeded with a conversion of 49–50%, and gave both the formed ester as well as the remaining alcohol with excellent >99% ee in most cases [12].

A disadvantage of lipase-catalyzed resolutions, however, is the limitation of a maximum yield of 50%. This limitation can be overcome when coupling the lipase-catalyzed enantioselective resolution with a racemization of the alcohol substrate, thus obtaining a dynamic kinetic resolution process. The latter step can be carried out in a highly efficient manner by a (nonchiral) metal complex as a chemocatalyst as has been demonstrated independently in pioneering work by the Williams [13] and Bäckvall groups [14,15]. Such a type of dynamic kinetic resolution process has been further developed by the Bäckvall group into a highly efficient approach toward enantiomerically pure acylated alcohols (Scheme 6.6) [16]. Ru complexes, for example, of type **14**, were used as racemization catalysts, and CAL-B was used as a lipase. The acylated alcohols (*R*)-**15** were obtained in good to high yields of 78–92% and with excellent enantioselectivities of >99% ee. The reaction is shown for the use of *p*-chlorophenylacetate as a



Scheme 6.6.

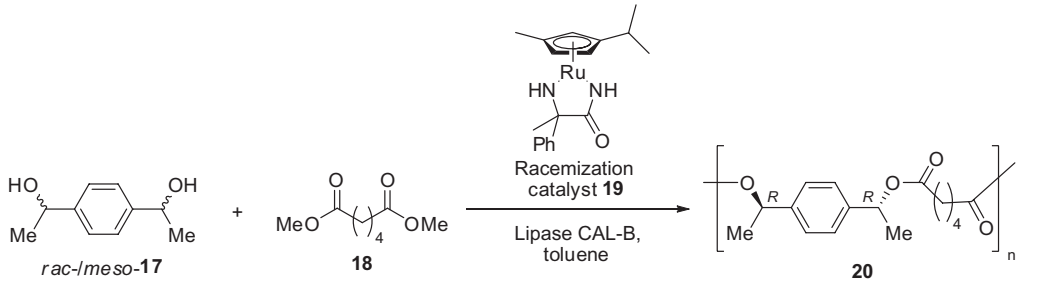
donor in Scheme 6.6. Commercially available isopropenyl acetate can be used as an alternative donor but requires the additional use of hydrogen to prevent decreased yields due to ketone formation.

Modified versions of this chemoenzymatic dynamic kinetic resolution based on the use of other racemization catalytic systems were reported by the Kim and Park group [17,18] as well as the Sheldon group [19]. Notably, a large-scale industrial process at DSM has been developed recently. Therein, a modified Ru-Noyori-type catalyst was used in combination with immobilized CAL-B [16,20].

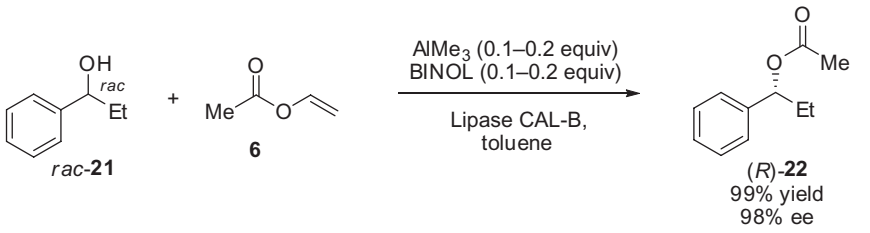
Besides methodology development, this dynamic kinetic resolution process has been applied for the highly enantioselective synthesis of a broad range of compounds bearing (at least one) alcohol functionality. For example, racemic/*meso*-diols [21–23], allylic alcohols [24,25], α- and β-hydroxy esters [26,27], halohydrins [28], and hydroxyl phosphonates [29] served as substrates. The reactions typically proceed with high conversion and excellent enantioselectivities, leading to the corresponding desired esters in high enantiomeric excess. Thus, this type of dynamic kinetic resolution based on the use of metal racemization catalysts in combination with a lipase for enzymatic resolution in organic media is already considered a highly efficient and mature technology for the highly efficient preparation of chiral esters with a broad substrate tolerance with respect to the alcohol moiety.

A very specific, recent application of this type of dynamic kinetic resolution is the use of this technology by DSM researchers for the enantio- and diastereoselective synthesis of chiral polymers [30]. Dimethyl adipate (**18**) was used as acceptor in combination with a mixture of racemic and *meso*-alcohols of type **17**, leading to the corresponding chiral polyester **20** in an enantioselective manner. The ruthenium complex **19** was used as racemization catalyst in combination with lipase CAL-B as biocatalyst for the resolution (Scheme 6.7).

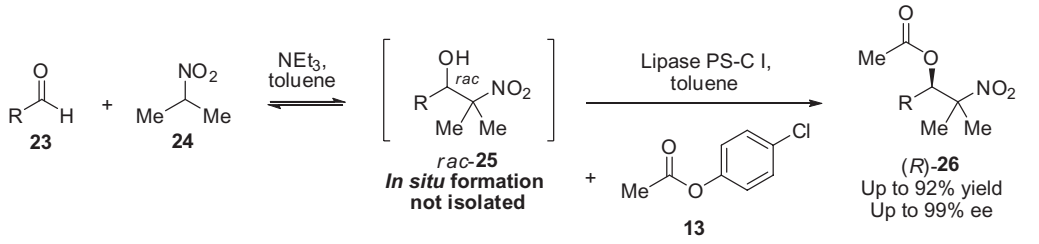
One limitation is the dependency on relatively expensive heavy metal racemization catalysts. In the search for a cheap source, the Berkessel group developed a highly efficient approach based on a cheap and readily available aluminum racemization catalyst (Scheme 6.8) [31]. The aluminum racemization catalyst was prepared from $AlMe_3$ and binaphthol. CAL-B was applied as an enzyme component. The resulting chemoenzymatic dynamic kinetic resolution gave the desired products in excellent conversion and enantioselectivity. For example, racemic 1-phenyl-1-propanol (*rac*-**21**) was acylated enantioselectively under formation of the resulting ester (*R*)-**22** with 99% yield and 98% ee.



Scheme 6.7.



Scheme 6.8.



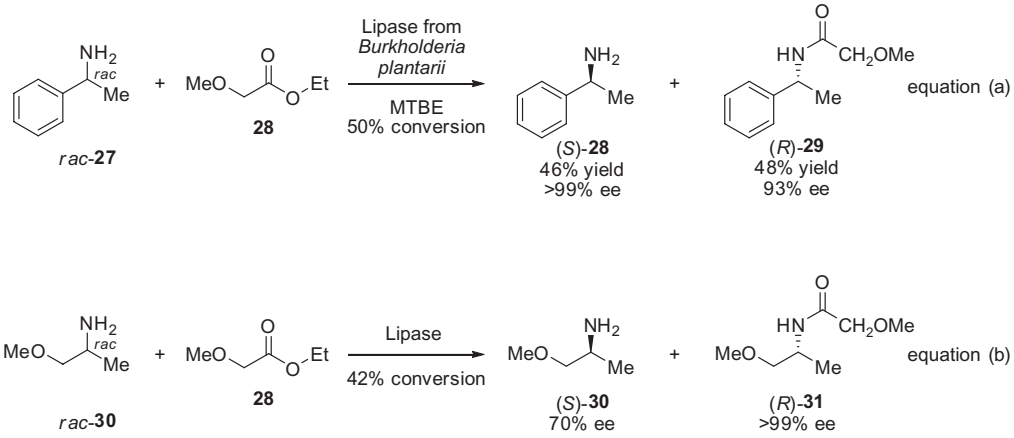
Scheme 6.9.

Lipase-catalyzed enantioselective acylation has also been combined with a reversible nitroaldol reaction (Henry reaction) for the preparation of *O*-acylated β-nitroalkanols (Scheme 6.9) [32]. In this type of dynamic kinetic resolution, the products (*R*)-**26** were obtained in yields of up to 92% and with enantioselectivities of up to 99% ee. Immobilized lipase from *P. cepacia* (PS-C I) turned out to be particularly useful, and 2-nitropropane was used as the nitroaldol donor component.

Since cyanohydrins are intermediates for the synthesis of a variety of valuable chiral building blocks, much effort has been made toward dynamic kinetic resolution of racemic cyanohydrins. *In situ* racemization has been achieved via reversible hydrocyanation reaction, leading to an easy cleavage of the cyanohydrin. In contrast, the acylated cyanohydrin, which is formed in the lipase-catalyzed resolution in a highly enantioselective fashion, is stable with regard to racemization [33–35].

6.2.3. Acylation Using Racemic Amines

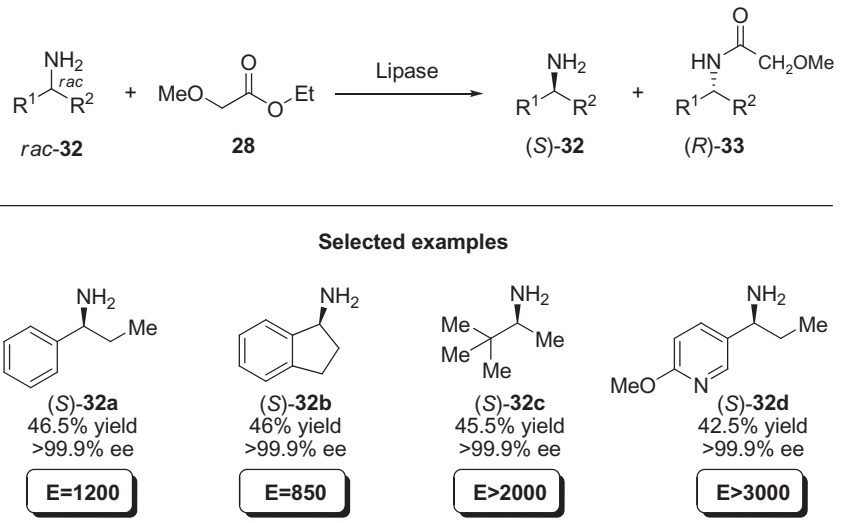
Further chiral building blocks of broad industrial interest, in particular for the life sciences industry, are enantiomerically pure amines that are widely used as an intermediate in the manufacture of pharmaceuticals and agrochemicals [36]. Also, for these target molecules, enzymatic resolution via lipase-catalyzed aclation turned out to be an excellent synthetic methodology. This process shows in particular an impressive efficiency when using methoxy acetate as an acylation agent, as has been demonstrated by BASF researchers [37]. Compared with "standard" acceptors (such as ethyl acetate), a rapid increase of the reactivity was observed when using methoxy acetate. Starting from racemic amines, lipased-catalyzed acylation proceeds highly enantioselectively, thus forming the amide with excellent enantioselectivities. Accordingly, also the remaining amine enantiomer can be obtained easily in enantiomerically enriched form. The enantioselectivities are impressive, with E values for this process exceeding 2000. In addition, the process runs in pure organic media with, for example, MTBE as solvent, with a high volumetric productivity. Different types of lipases can be applied, such as lipase from *C. antarctica* B and lipase from *Burkholderia plantarii*. The substrate range of this process technology is very broad and excellent results have been obtained for a diverse set of amines. Selected examples are given in Scheme 6.10 showing that the desired products were obtained in excellent enantiomeric excess.



Scheme 6.10.

The power of this method is underlined by the fact that this methodology is applied at BASF AG on an annual 2.500-t scale for the production of (*S*)-methoxyisopropylamine, which is an intermediate in the production of the herbicide "Outlook" [38]. Furthermore, resolution of a range of other racemic amines is carried out at an annual scale of 1.500 t via this methodology at BASF AG. This method impressively demonstrates how efficient resolution can be (even on large scale) in spite of the general limitation of a maximum yield of 50%.

Very recently, a broad investigation of the substrate scope has been reported by Ditrich using this resolution methodology [39]. Notably, a broad range of aliphatic, as



Scheme 6.11.

well as aromatic, amines *rac*-**32** are suitable substrates, and the obtained enantioselectivities are very impressive with E values of up to >3000. In Scheme 6.11, yields, enantiomeric excesses, and E values are given for selected substrates in this kinetic resolution.

Functionalized amines can be resolved as well. This has been shown, for example, for the enantioselective acylation of β-amino acid esters [40]. For example, when using ethyl acetate as aclyation reagent and CAL-B as a suitable lipase, the resolution furnished the acetylated ethyl 3-amino butyrate with a high enantioselectivity (E = 80) [41].

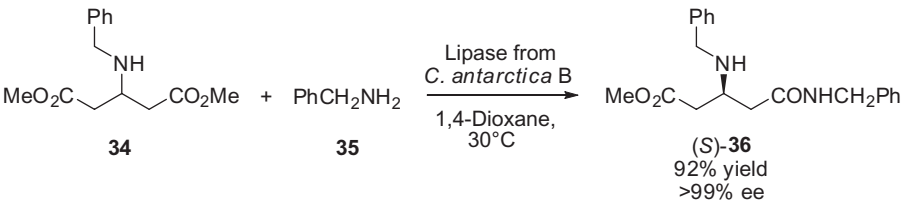
Very recently, Bertrand, Gastaldi and Gil et al. found that pure long-chain fatty acids (e.g., lauric acid) are suitable acylation reagents and lead to high enantioselectivities with E values exceeding 500 for 2-amino-4-phenylbutane even when carrying out the resolution at the high temperature of 80°C [42]. A further acylation reagent is (*R*)-phenylglycine amide, which reacts with racemic amines under the formation of the (*R*,*R*)-amide while leaving the remaining (*S*)-enantiomer of the amine unchanged [43]. Notably, this acylation reported by Svedas et al. is carried out in aqueous solution. As a catalyst, penicillin acylase from *Alcaligenes faecalis* was used.

A major challenge in the last few years has been the development of efficient dynamic kinetic resolution of racemic amines by combination of lipase-catalyzed resolution and a synthetic racemization catalyst in organic media (according to the concept discussed above for the dynamic kinetic resolution of racemic alcohols). Since amines are much more difficult to racemize in comparison with alcohols, the key step was the development of a suitable racemization protocol enabling amine racemization at (relatively) smooth reaction conditions. In a very recent work, De Vos et al. studied the suitability of Raney metals as heterogeneous catalysts for racemization of amines [44]. After identifying Raney nickel and cobalt as selective racemization catalysts for amines, subsequent dynamic kinetic resolution of aliphatic amines using CAL-B gave, for example, a yield of 95% ee and 97% ee for the corresponding *N*-acetyl 2-hexylamide.

6.2.4. Other Acylations

In principle, one could also carry out a kinetic resolution with an achiral alcohol or amine and a racemic acid moiety (e.g., an ester). When using an achiral alcohol and a racemic ester, this type of reaction is called transesterification. Although numerous transesterifications are known for different types of esters [45], a drawback from a practical point of view is the difficulty of product separation. Since the reaction mixture contains substrate and products in similar amounts and since both substrate and products are (related) esters, workup can be tedious in many cases. The analogous enzyme catalytic transformation of achiral amines and racemic acid components is also known as aminolysis [46]. In this case, the resulting reaction mixture can be separated more easily by converting the ester in a subsequent hydrolytic step in its acid while the formed amide remains unchanged. Thus, enantioselective aminolysis can be an elegant approach toward enantiomerically enriched acids. Aminolysis reactions have also been reported [47]. Recent examples of aminolysis reactions used in desymmetrization as well as resolution reactions have been reported by various groups for the synthesis of, for example, optically active β-amino acids and derivatives thereof.

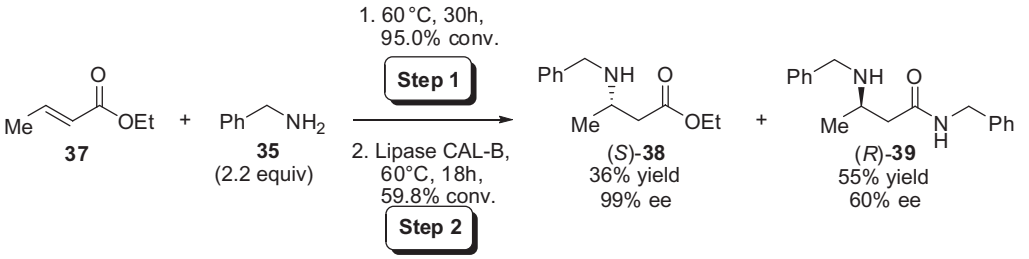
An interesting example for an enzymatic aminolysis and ammonolysis in an enantioselective desymmetrization reaction has been reported by the Gotor group [47]. Using a lipase from *C. antarctica* B, enzymatic aminolysis and ammonolysis of dimethyl 3-(benzylamino)glutarate, **34**, gave the corresponding monoamides in very good yields of up to 92% and in enantiomerically pure form. A selected example is shown in Scheme 6.12. The resulting monoamides are interesting intermediates for the synthesis of non-natural β-amino acids, as has been demonstrated for the preparation of (*R*)-3,4-diaminobutanoic acid.



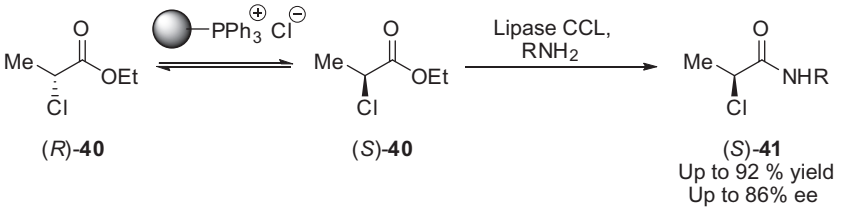
Scheme 6.12.

An example for an enzymatic resolution via aminolysis is the chemoenzymatic synthesis of short-chain aliphatic β-amino acid esters by the Gröger group [48]. This reaction is carried out as a one-pot two-step synthesis starting from easily available enoates and benzylamine. The initial step is a thermal Michael addition leading to the corresponding racemic β-amino esters. Subsequent resolution via aminolysis with benzylamine as donor and lipase from *C. antarctica* (CAL-B) yields the desired optically active β-amino ester of type **38** with good conversion and excellent enantioselectivities of up to 99% ee. An example is shown in Scheme 6.13.

A dynamic kinetic resolution with enzymatic aminolysis as the key step has been reported by the Kostic group [49]. In the presence of an immobilized phosphonium chloride for racemization of ethyl 2-chloropropionate **40** and lipase from *Candida*



Scheme 6.13.



Scheme 6.14.

cylindracea (CCL), aminolysis led to the formation of the desired amides (*S*)-**41** in yields of up to 92% and with enantiomeric excess of up to 86% ee (Scheme 6.14).

6.3. HYDROLYTIC REACTIONS

6.3.1. Overview

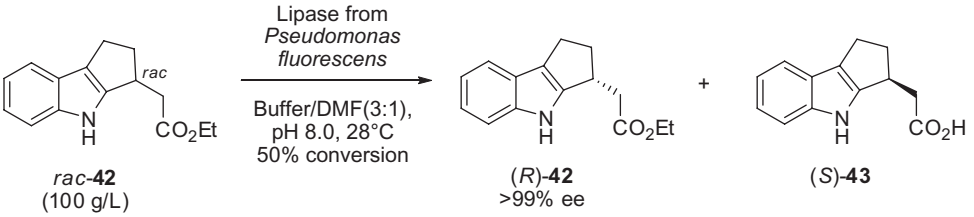
Besides their use in acylation reactions (see Section 6.2), hydrolases are very useful catalysts for the hydrolysis of carboxylic acid derivatives, in particular esters and amides. The hydrolysis of esters has been applied not only for the resolution of racemic carboxylic esters but also for the desymmetrization of prochiral diesters such as malonates or *meso*-compounds bearing two ester moieties. Although ester hydrolysis is probably the most frequently applied hydrolytic transformation with hydrolases, hydrolysis of other functional groups plays a role as well. The hydrolysis of nitriles to carboxylic acids turned out to be a versatile approach toward α-hydroxy acids and α-amino acids. Notably, this process can be carried out as a dynamic kinetic resolution since racemization of the substrate proceeds easily under the applied reaction conditions. Furthermore, hydrolysis of amide bonds plays a very important role in the field of enantioselective amino acid synthesis. Notably, hydrolytic reaction can proceed at the acid amide moiety (with amidases) as well as at the *N*-acylated amino functionality (by using aminoacylases). Both routes are highly efficient and are widely applied already on technical scale in the fine chemicals industry for the synthesis of chiral amino acids. Racemases are available for both of these processes, which make dynamic kinetic resolutions possible. Another dynamic kinetic resolution for amino acid production by means of hydrolytic enzymes

is the transformation of racemic hydantoins into enantiomerically pure D- or L-amino acids. In this process, hydantoinases and carbamoylases are the enzymes of choice for the hydrolytic steps, whereas racemization is dependent on pH or occurs by addition of a hydantoin racemase.

6.3.2. Ester Hydrolysis

A "classic" biocatalytic approach toward chiral carboxylic acids is hydrolysis of racemic or prochiral esters in enzymatic hydrolysis reactions. Lipases, esterases, and proteases were used as enzyme components. For resolution based on the use of these types of hydrolases, a broad range of racemic substrates can be used. Alternatively, prochiral substrates or *meso*-substrates can be used, which give the opportunity for a desymmetrization reaction with a (theoretically) quantitative yield of the resulting enantiomer. The field of enzymatic hydrolytic reactions for the synthesis of chiral carboxylic acids has been comprehensively reviewed [46]. Thus, in the following, the focus is on selected recent examples, which additionally show a high degree of synthetic efficiency, for example, high volumetric productivity, substrate concentration, and selectivity.

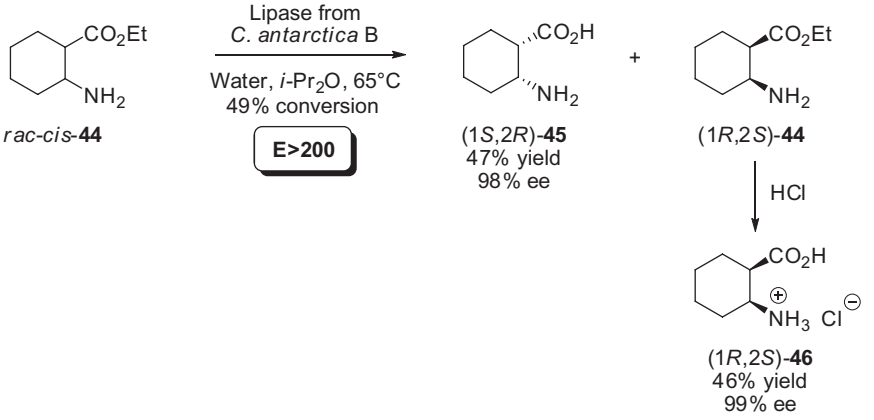
A highly efficient synthesis of an indole ethyl ester (*R*)-**42** as an intermediate for a prostaglandin D2 receptor antagonist via lipase-catalyzed hydrolysis of the corresponding racemate has been reported by Merck researchers [50,51]. The desired (*R*)-ester (*R*)-**42** was obtained with an excellent enantiomeric excess of >99% ee as remaining ester after hydrolysis of *rac*-**42** with a conversion of 50% (Scheme 6.15). As a catalyst, a lipase from *Pseudomonas fluorescens* was used. Notably, the reaction runs at a high substrate concentration of 100 g/L. Furthermore, the process turned out to be technically feasible and was applied successfully on a 40-kg scale [51].



rac-**42** (100 g/L) → (*R*)-**42** >99% ee + (*S*)-**43**

Scheme 6.15.

Highly enantioselective hydrolysis of racemic alicyclic *cis*- and *trans*-β-amino esters *rac*-**44** in the presence of lipase from *C. antarctica* B has been reported by the Fülöp group [52]. Notably, hydrolysis was performed in diisopropylether with only 0.5 equivalents of water. For example, the resulting *cis*-β-amino acids, for example, (1*S*,2*R*)-**45**, were obtained in high yields of 42–46% and with excellent enantiomeric excess of 96–99% ee. The opposite enantiomeric forms have been isolated as hydrochloride salts of the resulting acids (e.g., (1*R*,2*S*)-**46**) after hydrolysis also in high enantiomeric excess. In Scheme 6.16, a representative example is given. Enzymatic hydrolysis also turned out to be very suitable for the lipase-catalyzed resolution of a broad range of other types of β-amino acids [53–55].

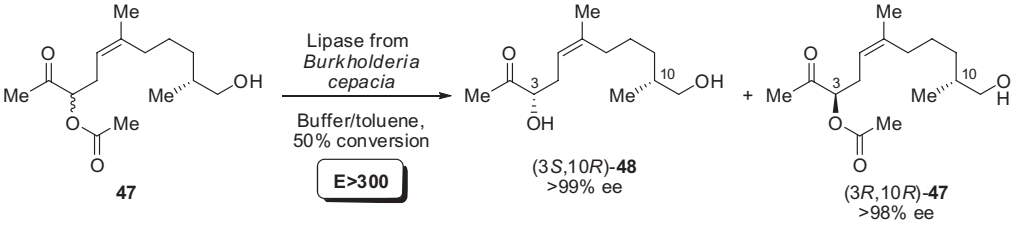


Scheme 6.16.

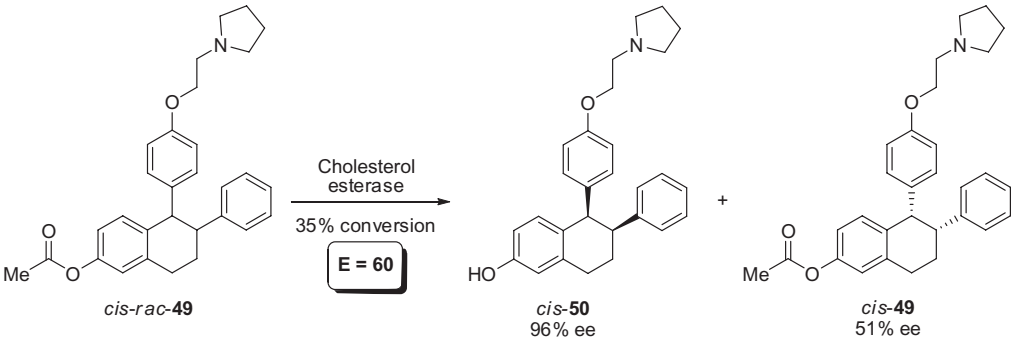
A further widely applied lipase-catalyzed resolution is the enantioselective hydrolysis of racemic α-amino acids esters [56]. As a representative example, an efficient kinetic resolution of racemic octyl pipecolate has been achieved by Kazlauskas et al. using a lipase from *Aspergillus niger* [57]. The desired (*S*)-2-piperidinecarboxylic acid was obtained with an enantioselectivity of E > 100. The engineering of the reaction medium for lipase-catalyzed resolution via ester hydrolysis has been reported jointly by Landfester, Gröger, and coworkers. In the presence of porcine pancreas lipase as enzyme, the hydrolytic resolution of racemic phenylalanine *n*-propyl ester reaction proceeds at high substrate concentrations of up to 827 g/L of solvent [55]. Furthermore, a dynamic kinetic resolution, which is based on a hydrolase-catalyzed ester hydrolysis in combination with amino ester racemization using an aromatic aldehyde, has been developed by the Beller group. For this type of reaction, a protease was used as hydrolase. For example, L-tyrosine is formed in 92% yield and with an enantioselectivity of 97% ee from the corresponding benzyl ester when using alcalase in combination with 3,5-dichlorosalicylaldehyde [58]. Alternatively, pyridoxal 5-phosphate was successfully used in combination with α-chymotrypsin and alcalase, respectively [59,60].

Lipases also turned out to be suitable for the resolution of complex molecules bearing more than one additional functional group. This is exemplified by the enzymatic hydrolysis of **47**, which represents a key building block of epothilones. The Wessjohann and Bornscheuer group found that in the presence of a lipase from *Burkholderia cepacia* (Amano PS), the acyloin acetate **47** was hydrolyzed highly enantioselectively with an E value of >300, leading to the corresponding diol (3*S*,10*R*)-**48** in >99% ee (Scheme 6.17) [61].

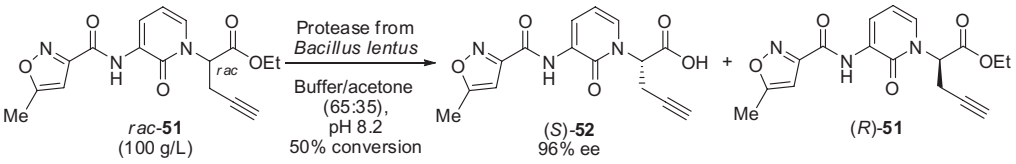
The power of hydrolases to recognize also "remote chiral centers" has been demonstrated by Liu et al. in the synthesis of Lasofoxifene, a potent and selective estrogen receptor modulator [62]. The Pfizer researchers found that, in particular, a cholesterol esterase from porcine pancreas is capable for this type of resolution. Although in substrate *rac-cis*-**49** the functional group for enzymatic hydrolysis (ester group) is separated from the stereogenic center by an aromatic group, enzymatic resolution proceeds with a high enantioselectivity as the E value of 60 indicates. The desired product Lasofoxifene (*cis*-**50**) is obtained at 35% conversion with an enantiomeric excess of 96% ee (Scheme 6.18).



Scheme 6.17.



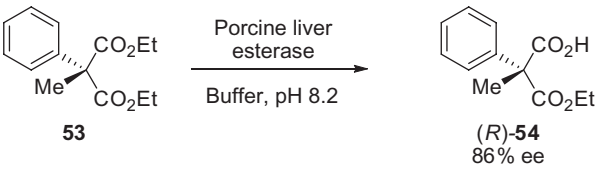
Scheme 6.18.



Scheme 6.19.

A further impressive resolution for the synthesis of a drug intermediate has been reported by Tao and coworkers at Pfizer with the hydrolytic synthesis of the desired acid (*S*)-**52** as an intermediate for a rhinovirus protease inhibitor [63,64]. The resolution proceeds at a high substrate input of 100 g/L of *rac*-**51**, and delivered the required acid (*S*)-**52** with 50% conversion and a high enantiomeric excess of 96% ee (Scheme 6.19). The unwanted enantiomer (*R*)-**51** can be separated and subsequently recycled. The biocatalyst, a protease from *Bacillus lentus*, which has not been reported before to be used as a catalyst, has been identified by screening of a comprehensive library of hydrolases. Other efficient resolutions of pharma intermediates via enzymatic hydrolysis have been also reported by the same group [65,66].

The desymmetrization of 2,2′-disubstituted malonates (e.g., **53**) is a versatile approach for enantiomerically enriched carboxylic acids of type (*R*)-**54**. Pioneer work by



Scheme 6.20.

Schneider et al. showed the suitability of porcine liver esterase (PLE) for many different types of malonate substrates [67]. High enantioselectivities of up to 86% ee were obtained. A representative example is shown in Scheme 6.20. It should be added that, in the meantime, the PLE is available also in recombinant form [68]. Reaction medium engineering for the desymmetrization of malonates with PLE has been done as well [69,70]. By means of an optimized reaction medium consisting of aqueous buffer, 2-propanol, and *tert*-butanol (with a ratio of 8:1:1), the enantioselectivity has been increased from 81% without alcohol additives to 96% ee for the PLE-catalyzed hydrolysis of diethyl 2-methyl-2-phenylmalonate [70].

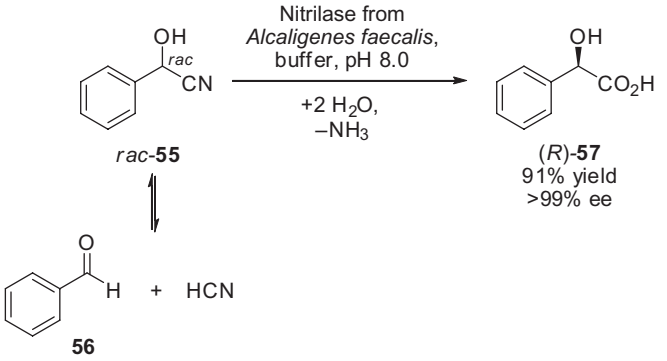
The application of PLE in asymmetric synthesis has been reviewed comprehensively very recently [71].

6.3.3. Nitrile Hydrolysis

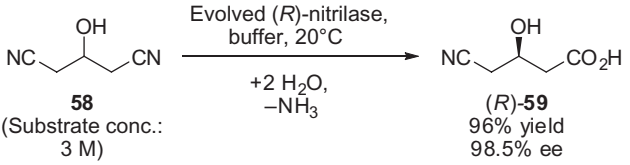
The transformation of racemic or prochiral nitriles into carboxylic acids can be done enantioselectively in the presence of nitrilases. For example, racemic α-hydroxy nitriles or α-amino nitriles have been used in particular, since these substrates racemize under the reaction conditions.

The transformation of α-hydroxy nitriles into its corresponding acids has been reported by Yamamoto et al. already in 1991 for the synthesis of (*R*)-mandelic acid ((*R*)-**57**) with excellent enantiomeric excess of >99% ee. As a biocatalyst, resting cells of *A. faecalis* were used bearing an (*R*)-selective nitrilase. The desired (*R*)-mandelic acid was obtained in 91% yield in a dynamic kinetic resolution with spontaneous racemization of mandelonitrile (caused by the equilibrium with benzaldehyde and hydrogen cyanide) under the chosen reaction conditions (Scheme 6.21) [72,73]. Benzaldehyde and hydrogen cyanide can be used as substrates as well as an alternative. This type of process already found commercial application for the technical production of (*R*)-mandelic acid at Mitsubishi Rayon and BASF AG [6].

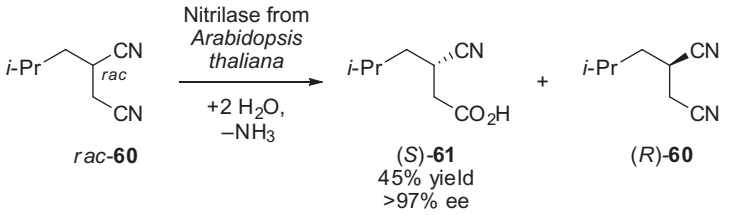
In addition, nitrilases suitable for a broad range of substituted mandelonitriles, 3-aryl-2-hydroxypropionitiles, and 3-hydroxyglutaronitrile were found by DeSantis and Burk et al. by means of a screening of genomic libraries [74]. The desymmetrization of prochiral 3-hydroxyglutaronitrile **58** via nitrilase-catalyzed hydrolysis affords the corresponding hydroxy acid (*R*)-**59**, which represents an intermediate used in the production of the drug Lipitor. By means of the identified nitrilases, high conversion of >95% and high enantioselectivities of >90% ee were obtained [74]. Furthermore, enzyme engineering delivered an optimized nitrilase (mutant), which gave the Lipitor intermediate (*R*)-**59** in 96% yield and 98.5% ee when operating at a high 3 M substrate concentration (Scheme 6.22) [75]. Thus, both excellent volumetric productivity and high enantiomeric excess were realized by means of this optimized evolved nitrilase.



Scheme 6.21.



Scheme 6.22.



Scheme 6.23.

Furthermore, nitrilases can also catalyze enantioselectively the hydrolysis of α-amino nitriles under formation of optically active amino acids. Using a nitrilase of *Rhodococcus rhodochrous*, Furuhashi et al. reported the formation of L-leucine with an enantiomeric excess of about 97% ee with racemic aminoisocapronitrile as a starting material [76].

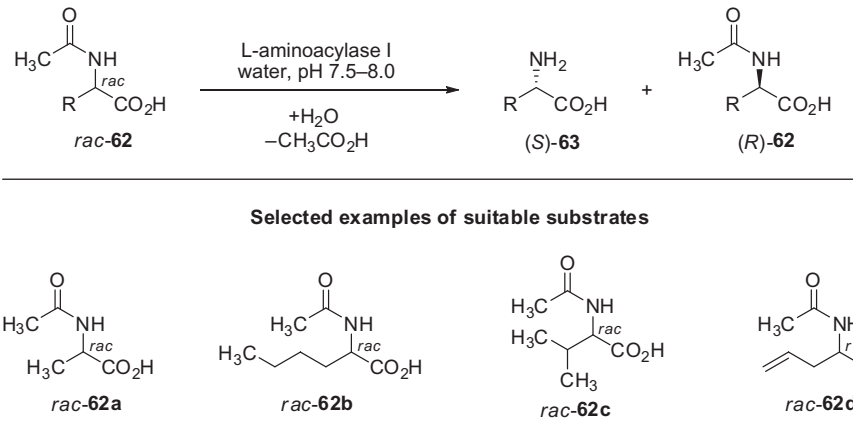
A further highlight in nitrilase-based asymmetric synthesis is the preparation of a key intermediate for a second generation process for pregabalin (Scheme 6.23) [64]. Starting from a racemic dinitrile *rac*-**60** regio- and enantioselective hydrolysis delivered the corresponding acid (*S*)-**61** in 45% yield and with an excellent enantiomeric excess of >98% ee. Notably, the remaining undesired enantiomer (*R*)-**61** can be easily recycled. Thus, the overall yield of pregabalin is increased from 18% to 21% in the original process [77] up to 40% (after one recycling) in this biocatalytic second generation process [64]. Notably, a further efficient biocatalytic synthetis route toward pregabalin based

on a lipase-catalyzed resolution via hydrolysis has also been developed by Tao et al. recently [78].

6.3.4. Amide Hydrolysis

Enantioselective resolution of racemic amides belongs to the most important biotransformations in the field of enantioselective amino acid synthesis. In general, two types of amides can be used as substrates, namely *N*-acylated amino acids or amino acid amides. Both substrates can be easily prepared on large scale as well. For hydrolysis of *N*-acylated amino acids, aminoacylases or penicillin acylases are the enzymes of choice, whereas amidases are used in case of amino acid amides. Both resolution techniques are applied on large scale and represent key industrial technologies for the technical production of L- and D-amino acids.

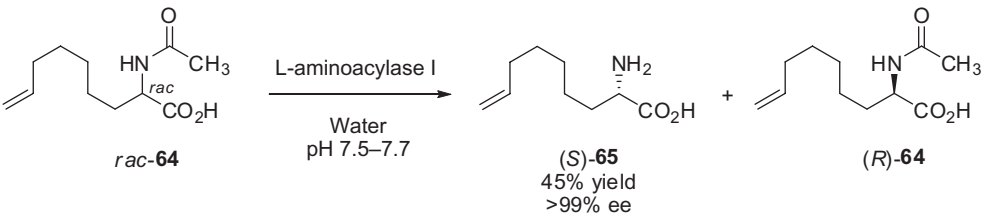
To start with the aminoacylase-catalyzed resolution of racemic *N*-acetyl α-amino acids, *rac*-**62**, this methodology is robust and highly efficient. An impressive study on the substrate range by Whitesides et al. revealed that amino acylases I from porcine kidney and *Aspergillus oryzae* tolerate a broad range of aliphatic, aromatic, and heteroaromatic substrates, leading to the desired L-amino acids of type L-**63** in good yields and with excellent enantiomeric excess of >99% ee in many cases [79]. Selected examples of this type of resolution for the synthesis of L-amino acids (*S*)-**63** are shown in Scheme 6.24.



Scheme 6.24.

Due to the high process efficiency, the amino acylase technology has been industrially applied for decades on a several hundred ton scale [80]. An elegant technical concept developed by Wandrey, Kula and Leuchtenberger et al. represents the enzyme membrane reactor (EMR), which is applied at Degussa AG (now Evonik Degussa GmbH) for the manufacture of L-methionine [81,82]. In a continuous resolution process in homogeneous solution, the enzyme cleaves enantioselectively the L-*N*-acetyl methionine under formation of the desired L-amino acid L-methionine. Due to its high efficiency, amino acylases-type resolution has become a widely applied

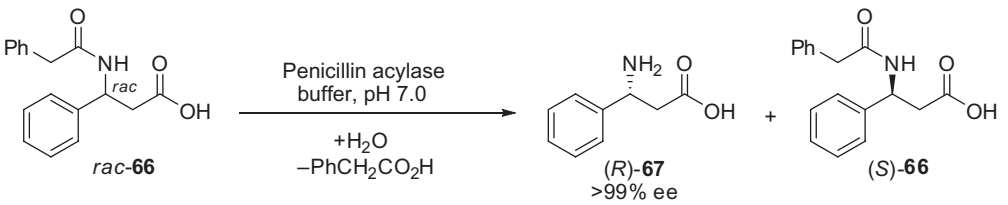
"standard" method in α-amino acid synthesis. Besides natural substrates, this method turned out to be very suitable also for (non-natural) α-amino acid pharma intermediates. For example, recently, researchers at Boehringer Ingelheim Pharmaceuticals, Inc. reported the synthesis of the non-natural α-amino acid (*S*)-**65**, a key building block in the synthesis of a protease inhibitor [83]. In the presence of an amino acylase I, the resolution proceeds in aqueous buffer at pH 7.0 under the formation of (*S*)-2-amino-8-nonenoic acid, (*S*)-**65**, in ≥45% yield and with >99% ee (Scheme 6.25).



Scheme 6.25.

D-enantioselective amino acylases are available for the analogous synthesis of the corresponding D-amino acids [84,85]. Furthermore, dynamic kinetic resolution based on the combination of an aminoacylase and an *N*-acetyl amino acid racemase has been successfully developed by the Tokuyama group and Kula group [86,87]. This type of dynamic kinetic resolution has been applied in a continuous synthesis of optically active methionine using a recombinant *N*-acylamino acid racemase from *Amycolatopsis* sp. and an L- and D-aminoacylase, respectively [88]. The dynamic kinetic resolution in the presence of an L-amino acylase gave enantiomerically pure L-methionine in a continuous production process in >99% yield.

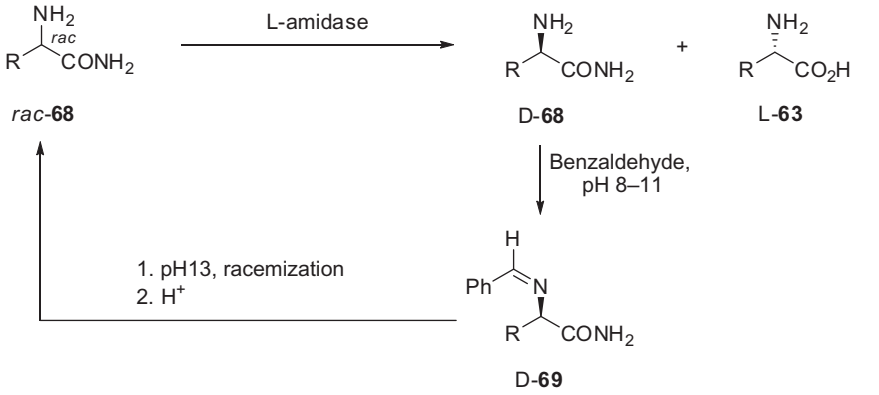
Starting with *N*-phenylacetyl amino acids as substrates, a penicillin acylase, which is a highly efficient enzyme used in the large-scale manufacture of 6-amino penicillanic acid (6-APA) for side-chain cleavage, is a suitable enzyme [89]. The corresponding L-α-amino acids are obtained with excellent enantioselectivities. Although the phenylacetyl group is the preferred acyl-substituent, the synthetically useful Z-protecting group (Z = benzyloxycarbonyl) is also tolerated by penicillin acylase [90]. An impressive application of penicillin acylase for the highly enantioselective synthesis of both aliphatic and aromatic β-amino acids has been reported by Soloshonok and coworkers [91]. Besides the broad substrate range, high conversion and excellent enantioselectivities make this route particularly attractive. Accordingly, most β-amino acids are obtained in enantiomerically pure form (>99% ee). A selected example is shown in Scheme 6.26. The use



Scheme 6.26.

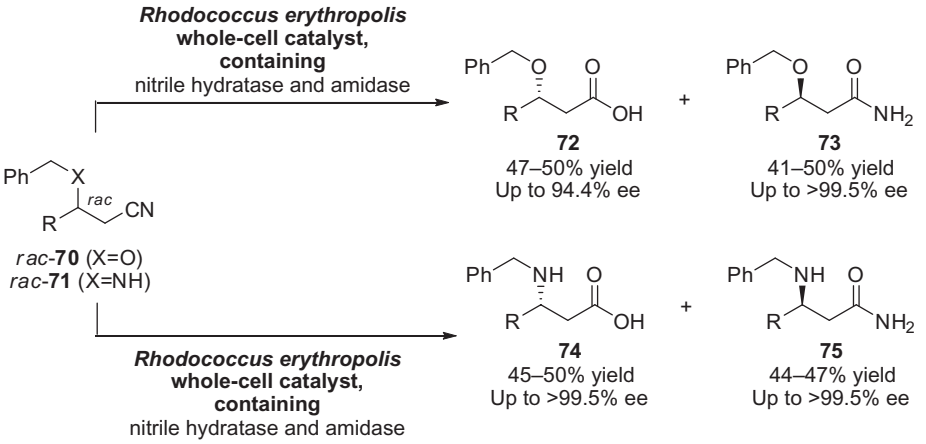
of a penicillin acylase immobilized on Eupergit for this type of reaction has been reported by the Tomasini group, and the resulting (*R*)-3-amino-3-phenylpropionic acid ((*R*)-**67**) was also obtained in enantiomerically pure form [92].

Another broadly applicable α-amino acid technology already established on industrial scale are resolutions based on the use of amidases and racemic carboxylic acid amides *rac*-**68** as easily available starting materials. The biotransformation proceeds highly enantioselectively under formation of the desired α-amino acids with high conversion. Notably, L- and D-enantioselective amidases are known and available in recombinant form, and are widely used in industrial production processes for manufacture of chiral α-amino acids, for example, at DSM [93,94]. The synthetic concept is shown below in Scheme 6.27. Starting from racemic amide (*rac*-**68**), an L-amidase furnishes the desired L-amino acid, L-**63**, in a resolution process with high enantioselectivities of typically >99% ee. The undesired D-enantiomer, D-**68**, is separated and subsequently racemized in a chemical process using benzaldehyde and basic conditions. The amidase from *Pseudomonas putida* turned out to be a highly useful enzyme showing both a broad substrate range and high enzymatic activities [95]. Aliphatic, aromatic, and heteroaromatic α-amino acids are accessible in enantiomerically pure form by means of this technology.



Scheme 6.27.

For example, at Lonza, the amidase technology has been used for the synthesis of (*S*)-piperazine-2-carboxylic acid [96,97]. The reaction is carried out in the presence of whole cells of *Klebsiella terrigena*, which contain a suitable amidase. The enzymatic resolution of the corresponding racemic amide runs at a substrate input of 20 g/L and gave the desired α-amino acid in 41% yield and with 99.4% ee. The recently reported development of an efficient amidase racemase by the Asano group represents a further impressive milestone in this field [98]. An α-amino-ε-caprolactam racemase turned out to be suitable for the racemization of a broad range of acyclic amides besides caprolactam. In a representative example, the complete conversion of L-alanine amide into D-alanine by combination of the racemase with a D-amidase in a one-pot process has been demonstrated. This concept appears to be highly suitable for dynamic kinetic resolution of, in particular, aliphatic racemic α-amino acid amides. Another key feature of the amidase technology is the potential to accept racemic α,α-disubstituted amides



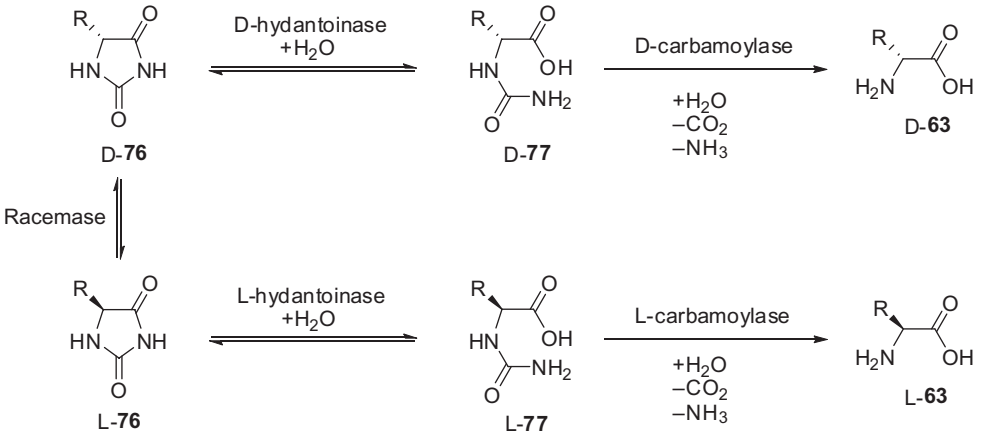
Scheme 6.28.

[99,100]. A representative example is the successful synthesis of L-α-methyl-3,4-dihydroxyphenylalanine. Other types of racemic α-methylated amides have been successfully resolved as well. Furthermore, biocatalytic resolution of α-amino acids bearing two stereogenic centers has been done by means of amidases, and was applied, for example, for the synthesis of an intermediate of the pharmaceutically important antibiotics florfenicol and thiamphenicol [101].

An amidase-catalyzed resolution was also the key step in the synthesis of highly enantiomerically enriched β-hydroxy and β-amino acids **72** and **74**, and amides **73** and **75** reported by the Wang group [102]. Therein, a wild-type microorganism, namely *Rhodococcus erythropolis* AJ270, containing a nitrile hydratase and an amidase was used as biocatalyst. The initial step is a transformation of the racemic nitrile *rac*-**70** and *rac*-**71** into the amide catalyzed by the nitrile hydratase followed by amidase-catalyzed amide hydrolysis. The enantioselectivity was caused predominantly by the amidase. Notably, *O*- and *N*-benzyl protection of the substrates turned out to be a key prerequisite for high enantioselection. The corresponding products **72**, **73**, **74**, and **75** are obtained in high yield and with enantioselectivities of up to 94.4% ee and >99.5% ee, respectively (Scheme 6.28).

6.3.5. Hydantoin Hydrolysis

A further versatile category of substrate for enzymatic resolution is racemic hydantoins: In the presence of hydantoinases and carbamoylases, racemic hydantoins *rac*-**76** are enantioselectively converted into enantiomerically pure α-amino acids [80,103]. In the initial step, the hydantoinase catalyzes the hydrolytic ring opening of the hydantoin under (reversible) formation of an *N*-carbamoyl amino acid. Subsequent cleavage of the *N*-carbamoyl amino acid of type **77** furnishes the desired α-amino acid, L- or D-**63**. This step is irreversible and is known to proceed with excellent enantioselectivity. Racemization of the hydantoin allows a dynamic kinetic resolution process. For racemization, a hydantoin racemase can be used. Alternatively, some hydantoins also racemize *in situ*



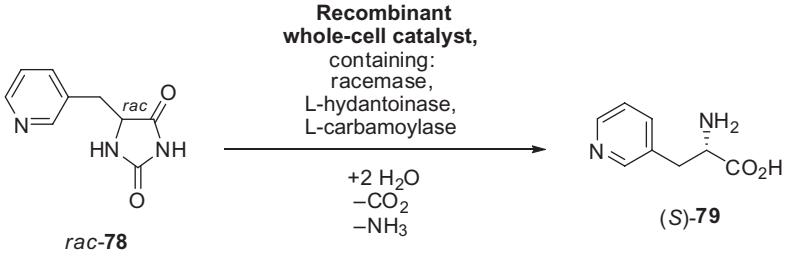
Scheme 6.29.

under conditions of enzymatic hydrolysis. The synthetic concept of such a dynamic kinetic resolution is shown in Scheme 6.29. Since D- as well as L-selective carbamoylases and hydantoinases are known, this synthetic concept can be used for the production of both L- and D-amino acids, (*S*)- or (*R*)-**63**, respectively.

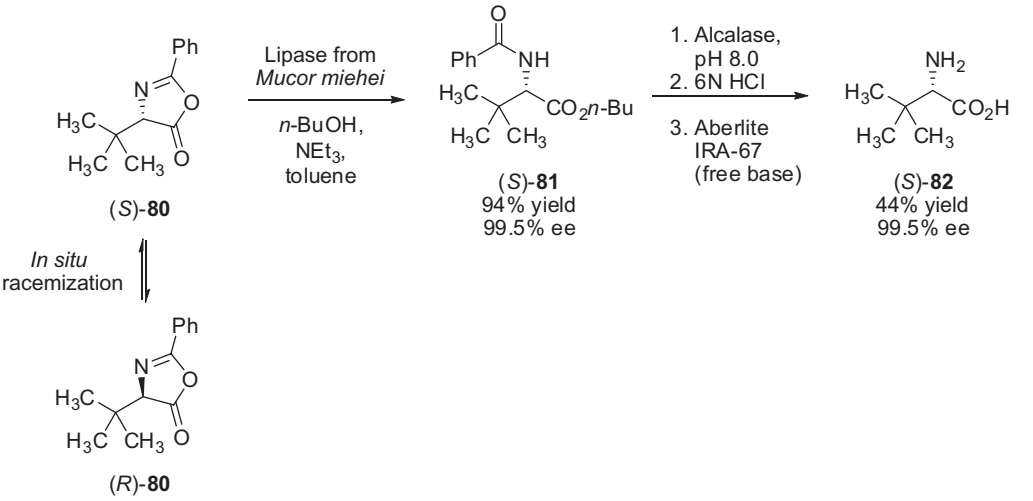
A selected application of the D-selective hydantoin resolution is the production of D-phenylglycine and D-4-hydroxyphenylglycine [103–106]. Required as side chains for β-lactam antibiotics, their annual production volume exceeds 1.000 t. Such a process, which is based on the use of a *Bacillus brevis* strain, has been developed by Kanegafuchi Chemical Industries Co., Ltd. The L-selective approach has been limited for a long time by low productivities due to the lack of suitable L-hydantoinases. This limitation has been overcome by converting a D-hydantoinase into an L-hydantoinase, applying the concept of "directed evolution" as a modern molecular biologic tool for the improvement of enzymes [107]. The combination of an L-hydantoinase with the construction of whole-cell biocatalysts then led to a highly efficient L-selective hydantoinase technology platform, which has been established at Degussa AG (now Evonik Degussa GmbH) on industrial scale [108]. In the recombinant whole-cell biocatalyst, all three required enzymes, namely racemase, hydantoinase, and carbamoylase, are overexpressed in a host organism. In the presence of an L-enantioselective recombinant whole-cell catalyst, the racemic hydantoin is converted into its corresponding L-amino acid with high conversion and excellent enantioselectivity in a dynamic kinetic resolution process. It is further noteworthy that excellent substrate concentrations can be achieved as well. In Scheme 6.30, the synthesis of (*S*)-**79** as a selected example is shown. This technology platform is also applicable toward the synthesis of D-amino acids when using D-enantioselective recombinant whole-cell catalysts.

6.3.6. Hydrolysis of Other Carboxylic Acid Derivatives and Other Functional Groups

Besides racemic α-amino acids esters, racemic azlactones of type **80** also turned out to be suitable substrates. Notably, a dynamic kinetic resolution is achieved with this type



Scheme 6.30.



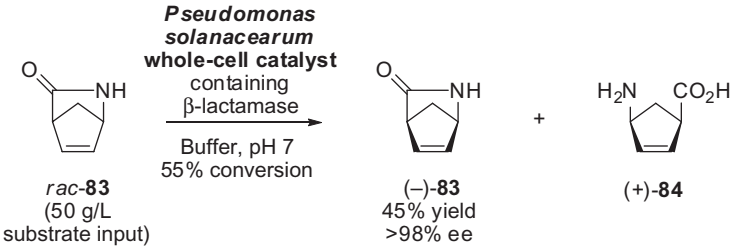
Scheme 6.31.

of substrate **80** due to easy racemization of azlactones under conditions of lipase-catalyzed resolution via azlactone ring opening with an alcohol (Scheme 6.31) [109,110]. Using this resolution concept, *N*-benzoyl L-*tert*-leucine butyl ester, (*S*)-**81**, has been synthesized with 94% yield and with 99.5% ee in the presence of a lipase from *Mucor miehei*. The ring-opening reaction has to be carried out in a nonaqueous system due to a competing nonenzymatic ring opening of the azlactone with water. The product (*S*)-**81** was converted into L-*tert*-leucine, (*S*)-**82**, via chemoenzymatic two-step hydrolysis (alcalase, pH 8.0, followed by 6N HCl, reflux) and subsequent neutralization of the hydrochloride of (*S*)-**82**.

A further class of hydrolases used in the resolution of amino acid precursors are lactamases. In the presence of an α-amino-ε-caprolactam hydrolase (ACL-hydrolase), racemic 3-α-amino-ε-caprolactam was enantioselectively hydrolyzed under the formation of enantiomerically pure L-lysine [111]. Notably, this enzymatic resolution step was coupled with an *in situ* racemization of the substrate by means of an ACL-racemase. The resulting dynamic kinetic resolution proceeds with a high substrate input of 100 g/L

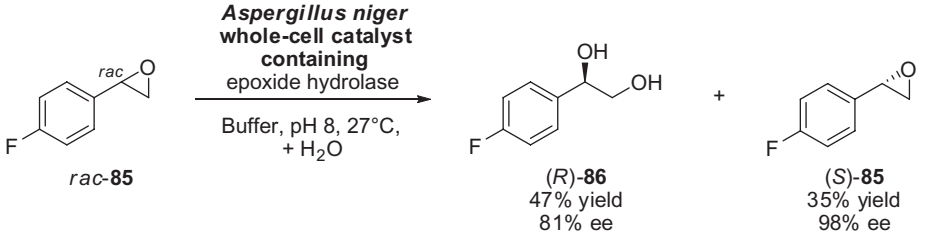
and has been applied at technical scale for the production of L-lysine at Toray Industries on a 4.000-t scale for some time [111d]. Since large-scale fermentation of L-lysine, however, turned out to be a superior manufacturing method, this dynamic kinetic resolution process is not industrially applied anymore.

A further lactam used in a (technical) hydrolytic resolution process is the racemic γ-lactam *rac*-**83** as precursor for the synthesis of Carbovir, which is a potent inhibitor of HIV-1 [112,113]. In the presence of suspended whole cells of *Pseudomonas solanacearum* bearing a β-lactamase, the resolution proceeds at a substrate input of 50 g/L with 55% conversion, thus delivering the desired lactam enantiomer (–)-**83** (as remaining substrate) in 45% yield and with >98% ee (Scheme 6.32). The substrate input has been increased up to >100 g/L when using a recombinant whole-cell catalyst overexpressing the β-lactamase. This process has been scaled up and runs on a scale of tons.

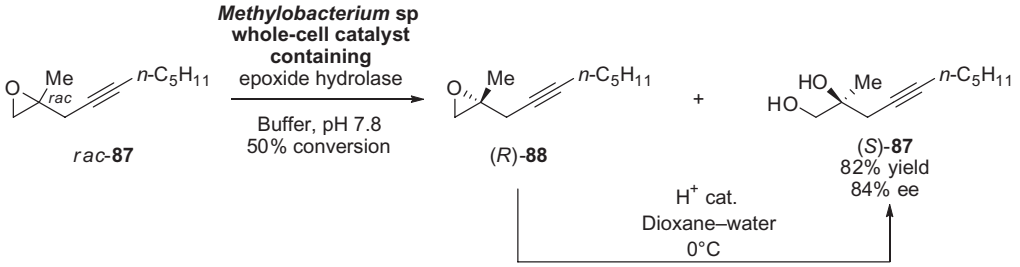


Scheme 6.32.

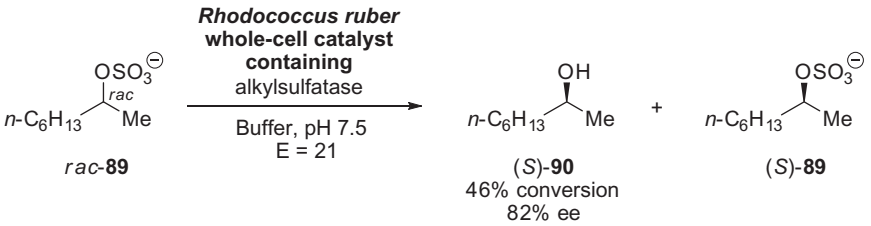
Other compounds than carboxylic acid derivatives can also be enantioselectively hydrolyzed. In the presence of epoxide hydrolases, ring opening of racemic epoxides occurs highly enantioselectively with a broad range of substrates. Notably, enzymatic ring opening might occur in an opposite manner compared to the chemical ring opening in basic media, thus offering an approach to (theoretically) a 100% yield. An efficient hydrolytic ring opening of racemic styrene epoxide and substituted derivatives thereof, for example, *rac*-**85**, has been reported by the Furstoss group [114,115]. Using *A. niger*, whole-cell catalysts bearing an epoxide hydrolase furnished the corresponding diol (*R*)-**86** in 47% yield and with 81% ee. The remaining epoxide (*S*)-**85** was obtained in 35% yield and with a high enantiomeric excess of 98% ee (Scheme 6.33). Notably, it was found by the same group that whole cells from *Beauveria sulfurescens* contain an enantiocomplementary epoxide hydrolase, showing the opposite stereopreference.



Scheme 6.33.



Scheme 6.34.



Scheme 6.35.

Suitable epoxide hydrolases for the hydrolytic ring opening of aliphatic racemic epoxides have been reported by the Faber group [116,117]. Notably, this methodology also tolerates highly functionalized epoxides. A selected example is shown in Scheme 6.34. In the presence of whole-cell catalysts of *Methylobacterium* sp. bearing an epoxide hydrolase, the resolution of epoxide *rac*-**87** proceeds with a high enantioselectivity, which is indicated by an E value of 66. Subsequent acid-catalyzed ring opening under inversion of configuration delivers the diol (*S*)-**88** as sole product in 82% yield and with an enantiomeric excess of 84% ee. The diol (*S*)-**88** has been used by the Faber group as an intermediate in the total synthesis of the antibiotic (*R*)-fridamycin E.

Another interesting functional group for enzymatic enantioselective hydrolysis are sulfate esters. When using racemic sulfatases, those racemic esters can be enzymatically hydrolyzed under inversion of the absolute configuration, whereas chemical hydrolysis of sulfate esters proceeds under retention. The Faber group developed such an enzymatic resolution of racemic sulfates using whole cells of *Rhodococcus ruber* bearing an alkylsulfatase as a catalyst [118,119]. For example, hydrolysis of *rac*-**89** gave the (*S*)-alcohol (*S*)-**90** at 46% conversion with an enantiomeric excess of 82% ee, corresponding to an E value of 21 (Scheme 6.35).

6.4. CARBON–CARBON BOND-FORMING REACTIONS

6.4.1. Overview

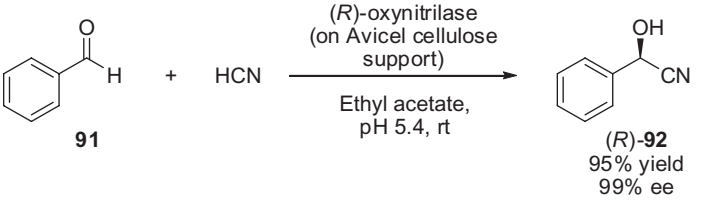
Formation of C–C bonds belongs to the most important asymmetric transformations. Besides many highly efficient developed chemocatalysts, biocatalysis also turned out to

be suitable catalysts for asymmetric C–C bond formations. Among the broad range of lyases available in nature for the formation of C–C bonds, several of them are used (mainly in recombinant form) as catalysts in enantioselective organic synthesis [120]. In the presence of oxynitrilases, cyanohydrins are formed, which can be subsequently converted into valuable chiral building blocks such as amino alcohols and α-hydroxy acids, in particular mandelic acid and substituted derivatives thereof. A further group of lyases, which are of interest for applications in organic chemistry, are benzaldehyde lyases or decarboxylases. These enzymes are capable of catalyzing the benzoin condensation and related reactions thereof with aliphatic aldehydes. The biocatalytic aldol reactions represent another interesting reaction class of C–C bond formation. Notably, aldolases tolerate in general a broad range of acceptors, whereas specificity is high for the corresponding donor molecules. Of particular interest are easily accessible nonphosphorylated donor molecules. For example, using glycine as a donor results in the direct formation of α-amino β-hydroxy acids without a need of protecting groups. Recently, enzyme promiscuity has become a popular field of research. Among discovered asymmetric biocatalytic "non-natural" reaction types are, for example, nitroaldol reaction (Henry reaction) using oxynitrilases.

6.4.2. Hydrocyanation of Aldehydes

The hydrocyanation of aldehydes is one of the oldest biocatalytic transformations in organic chemistry. Already in 1907, Rosenthaler reported the addition of HCN to aldehydes in aqueous reaction medium when using almond meal as a biocatalyst [121]. However, in spite of further improvement—in particular by Becker and Pfeil [122,123] in the 1960s—it took until 1987 before the first highly enantioselective hydrocyanation with excellent enantiomeric excess of the resulting cyanohydrins was reported by the Effenberger group [124]. The key step of this methodology was suppression of the undesired (non-enantioselective) hydrocyanation by using an organic phase as reaction medium. In the presence of an immobilized (*R*)-oxynitrilase from bitter almond (*Prunus amygdalus*), Effenberger et al. achieved high conversions and excellent enantioselectivities for a broad range of substrates. For example, (*R*)-mandelonitrile ((*R*)-**92**) was obtained in 95% yield and with 99% ee (Scheme 6.36). The Effenberger group also achieved an elegant access to (*S*)-cyanohydrins when using an immobilized (*S*)-oxynitrilase from *Mannihot esculenta* as biocatalyst [125]. Furthermore, the substrate spectrum was studied in detail [126].

Since immobilized enzymes turned out to be valuable biocatalysts, the development of novel immobilization methodologies gained interest. A particular goal was the

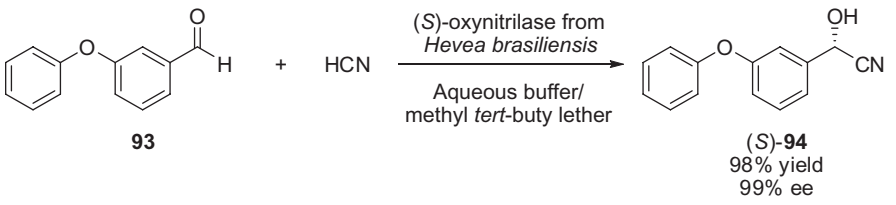


Scheme 6.36.

development of immobilisates, which allow easy separation from the reaction mixture and which are more stable regarding abrasion. Such a technology has been developed jointly by Vorlop and Gröger et al. using oxynitrilase entrapped in highly flexible polyvinyl alcohol lenses [127]. A prerequisite is the extension of the molecular weight by cross-linking the oxynitrilase by means of glutaraldehyde and chitosane. The resulting immobilized oxynitrilase turned out to be suitable for recycling within at least 20 recycling cycles. The preparation of cross-linked enzyme aggregates represents a further efficient immobilization methodology for oxynitrilases as well as sol-gel immobilization [128,129]. This has been demonstrated by Hanefeld and Sheldon et al. for various oxynitrilases. For example, in the presence of immobilized oxynitrilase from *Linum usitatissimum* as cross-linked enzyme aggregate, the desired product (*R*)-2-butanone cyanohydrin was formed enantioselectively, and subsequent hydrolysis gave (*R*)-2-hydroxy-2-methylbutyric acid in 85% yield (from 2-butanone) and with an enantiomeric excess of 87% ee.

An alternative process technology for the suppression of the unwanted formation of racemic cyanohydrins was reported by the Kula group [130]. To carry out the oxynitrilase-catalyzed reaction in pure aqueous media, however, the pH has to be kept below pH 3.25. A broad range of substrates was converted enantioselectively into the desired cyanohydrins under these reaction conditions.

A further milestone in this field was achieved by the Griengl group developing an asymmetric hydrocyanation in an aqueous–organic two-phase solvent system in the presence of a recombinant oxynitrilase from *Hevea brasiliensis* [131–133]. Excellent experimental data were obtained for the expression of this enzyme, which ensures availability for large-scale applications. The process concept of the Griengl group is currently applied at DSM for the production of (*S*)-3-phenoxybenzaldehyde cyanohydrin ((*S*)-**94**), which is a valuable intermediate in industrial pyrethroid manufacture. Impressive space-time yield of 2.1 mol/(L·h) has been reported for the synthesis of (*S*)-**94** as well as both excellent yields of 98% and enantioselectivity of 99% ee (Scheme 6.37) [134]. In addition, numerous aldehydes turned out to be suitable substrates [135–137]. Since recombinant (*R*)-oxynitrilases are also available, the Griengl process has been extended to the synthesis of (*R*)-cyanohydrins products as well [138–140]. In summary, this technology is already regarded as a well-established and mature technology for large-scale applications.



93

(*S*)-**94**
98% yield
99% ee

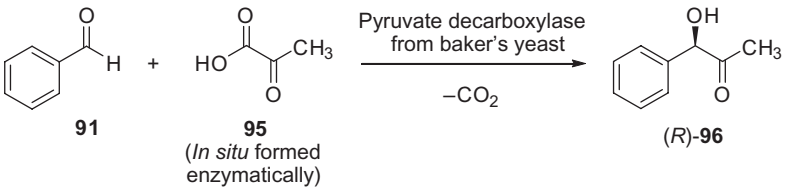
Scheme 6.37.

Besides the use of isolated oxynitrilases, in particular in recombinant form, much work has been done with defatted almond meal powder as biocatalyst. This type of biocatalyst is easily accessible and can be used directly in the corresponding hydrocyana-

tion biotransformation. An interesting approach is the use of defatted almond meal in microaqueous media. The resulting products were obtained in yields of up to 100% and with excellent enantioselectivities of up to 99% ee [141].

6.4.3. Benzoin Condensation and Related Reactions

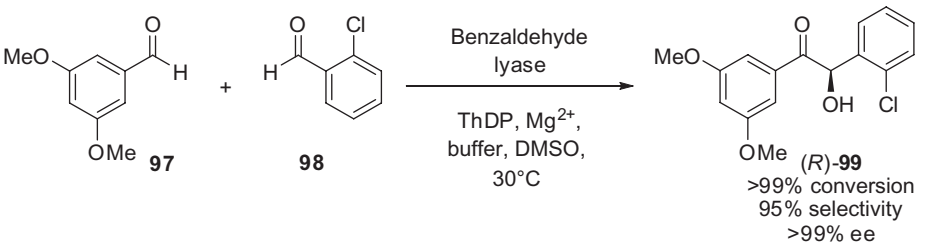
This condensation and related reactions thereof belong to the "classic" chemical transformations, known to proceed with cyanide as an achiral catalyst. The development of enantioselective versions of this type of reaction and related reactions using organocatalysts [142] and biocatalysts [143] has gained a lot of interest in the last decade. Notably, however, an enantioselective version of the synthesis of (*R*)-acetyl phenylcarbinol ((*R*)-**96**) as a related reaction had already been developed in the 1920s by Neumann and has been applied since the 1930s by Knoll AG as a key step for the production of ephedrine (Scheme 6.38) [144]. As a catalyst, a pyruvate decarboxylase from baker's yeast is used in combination with molasses as a starting material. The key reaction is the condensation of *in situ*-formed pyruvate (**95**) with added benzaldehyde (**91**) under the formation of α-hydroxyketone (*R*)-**96** with high enantioselectivity. This intermediate is then chemically transformed into ephedrine via imine formation and subsequent diastereoselective metal-catalyzed reductive amination.



Scheme 6.38.

Several recent breakthroughs in the field of benzoin condensation have been achieved jointly by the groups of Müller and Pohl [145,146]. A key prerequisite was the development of efficient enzymes available in recombinant form. For example, an impressively broad substrate spectrum leading to the desired carboligation products with excellent enantioselectivities has been found for the (recombinant) benzoyl formate decarboxylase from *P. putida* [145]. A synthetically valuable application is the development of an asymmetric cross-benzoin condensation via enzymatic cross-coupling reactions of different aldehydes [146]. By means of this methodology from Müller et al., highly enantiomerically enriched mixed benzoins are obtained when using two different types of substituted benzaldehydes in the presence of thiamine diphosphate-dependent enzymes. A selected example is shown in Scheme 6.39. The Müller and Pohl group achieved the preferred synthesis of one of these products when using benzaldehyde lyase or a mutant of a pyruvate decarboxylase as catalysts in such cross-benzoin condensations. For the formation of these products, typically one of the two aldehydes is preferred as donor, whereas the other one acts as the preferred acceptor molecule.

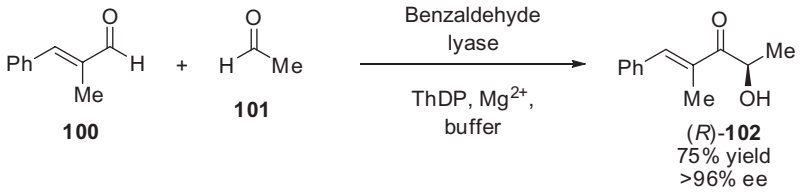
Very recently, Degussa researchers in cooperation with Liese and Pohl demonstrated that whole-cell catalysts containing benzaldehyde lyase in overexpressed form are highly



Scheme 6.39.

attractive catalysts for the benzoin condensation [147]. Notably, due to the use of whole cells with cofactor therein, the addition of external amount of cofactor is not required. In addition, high substrate concentrations were reported, which represents a further prerequisite for a technical process with favorable economical data.

Recent achievements have been made in the condensation of aliphatic aldehydes. This reaction has also been known for a long time for acetoin condensation. However, extension to other types of aldehydes has been a challenge for a long time. Enzymatic carboligation of linear aldehydes has been reported recently by Trauthwein and Müller et al. resulting in high conversions and enantioselectivities of up to 80% ee [148]. When using branched aliphatic aldehydes in the presence of a benzaldehyde lyase, high conversion and an enantioselectivity of up to 89% ee were obtained. Very recently, the Müller group also reported the suitability of unsaturated aldehydes for such types of umpolung reactions [149]. Depending on the type of enzyme, regioselectivity can be varied, and the desired α-hydroxy ketones, for example, (*R*)-**102**, are formed regioselectively and with enantiomeric excess of up to >99%. An example is shown in Scheme 6.40.



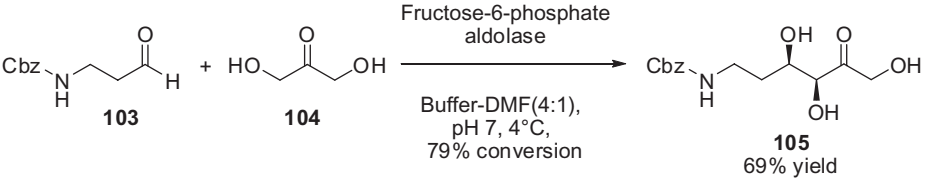
Scheme 6.40.

6.4.4. Aldol Reactions

Aldol reactions are one of the most important transformations in organic chemistry, and play a key role in biochemical processes for the construction of sugars. A key feature of biocatalytic aldol reactions [150,151] is the high specificity with respect to the donor component, whereas a broad substrate range is usually observed for the acceptor molecules. Accordingly, the aldolases are typically divided according to the type of required donor. Some examples of donors are dihydroxyacetone phosphate (DHAP), pyruvate, acetaldehyde, and glycine. Aldolases have found many synthetic applications, which

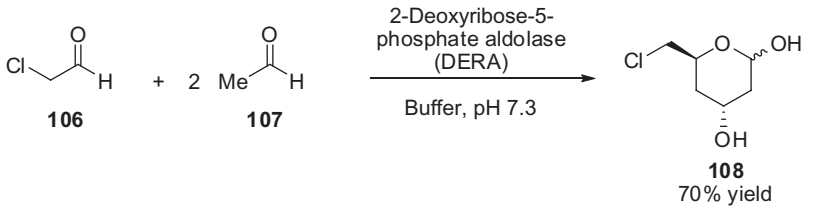
recently have been reviewed comprehensively [150,151]. In the following, some selected recent contributions will be described.

Many aldolases require DHAP as a donor. Since phosphorylated dihydroxyacetone (DHA) is difficult to prepare and handle under *in vitro* conditions, preparative type biotransformations with expensive phosphorylated donors and isolated enzymes are rare. Recently, however, aldolases that tolerate DHA directly have been found, and the chemistry with these enzymes opens up a new perspective for biocatalytic aldol reactions. For example, recombinant D-fructose-6-phosphate aldolase was used by Joglar and Clapés et al. in a chemoenzymatic two-step synthesis of D-fagomine and *N*-alkylated derivatives thereof [152]. This enzyme, overexpressed in *Escherichia coli*, catalyzes the aldol reaction of DHA and *N*-Cbz-3-aminopropanal under the formation of the aldol adduct **105** in 69% yield (Scheme 6.41). DHA (**104**) can be used directly instead of phosphorylated DHA, which simplifies the process tremendously. After purification and diastereomer enrichment by cation-exchange chromatography, D-fagomine was obtained in an overall yield of 51% and with a diastereomeric ratio of ≥99:1. The Sprenger group also reported the application of this recombinant enzyme for the aldol reaction of DHA and hydroxyacetone with α-hydroxyaldehydes for the synthesis of sugar derivatives [153].



Scheme 6.41.

A synthetic highlight in the enzymatic aldol chemistry is the use of 2-deoxy-D-ribose 5-phosphate aldolase (called DERA) as enzyme in *in situ* aldol condensations of two molecules of acetaldehyde (**107**) and one molecule of chloroacetaldehyde (**106**) under the formation of 6-chloro-2,4,6-deoxyhexapyranoside (**108**) with 70% yield in a tandem aldol reaction (Scheme 6.42) [154]. This reaction had been developed in the 1990s by the Wong group, who also applied a DERA mutant in the synthesis of a variety of other sugar analogues such as deoxyriboses, dideoxyhexoses, trideoxyhexoses, and deoxythiosugars. The compound **108** is a valuable intermediate in the construction of statine side

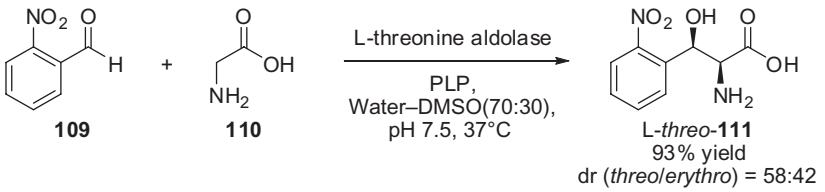


Scheme 6.42.

chain. Since statines are blockbuster drugs of high industrial interest, it is not surprising that this process has gained attention. Notably, access to a highly efficient recombinant form of the enzyme that tolerates a high concentration of chloroacetaldehyde has been achieved by DSM researchers, thus fulfilling a key criterion for industrial applicability of this process [155].

The use of aldolases in diastereoselective aldolization reactions as key steps in stereospecific biocatalytic synthesis of novel pancratistatin analogues has been reported by Fessner and coworkers [156]. Notably, such structures with a high molecular complexity and several stereogenic centers were prepared without the need for protective group strategies. As enzymes, a fructose-1,6-bisphosphataldolase and the stereocomplementary rhamnulose-1-phosphataldolase were used.

When using glycine as a donor, corresponding aldol reaction gives enantio- and diastereoselectively α-amino β-hydroxy acids such as threonine and β-phenylserine. Suitable enzymes for these reactions are in particular threonine aldolases. The Wong group reported an elegant approach for β-phenylserine and substituted derivatives thereof, obtaining the desired products, for example, L-*threo*-**111**, in yields of up to 93% and with excellent enantioselectivity (for both diastereomers) in general (Scheme 6.43) [157]. Diastereoselectivity, however, turned out to be modest for most of these reactions. It is noteworthy that the L-threonine aldolase (from *E. coli*) gave *erythro*-α-hydroxy-β-L-amino acids with aliphatic aldehydes whereas the *threo*-diastereomers were obtained as preferred (kinetically controlled) products when using aromatic aldehydes as substrates. When using a D-threonine aldolase (from *Xanthomonus oryzae*), however, *threo*-α-hydroxy-β-D-amino acids were obtained as kinetically controlled products with aliphatic as well as aromatic aldehydes. Diastereoselectivity, however, in general varies broadly with threonine aldolases, and dependent on substrate and enzyme, low or medium to high diastereomeric ratios were obtained [158–161].

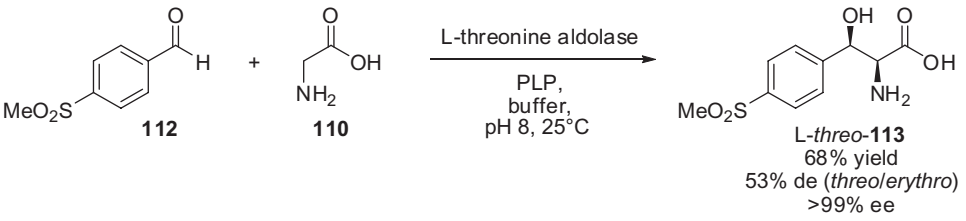


109 **110** L-*threo*-**111**
93% yield
dr (*threo*/*erythro*) = 58:42

Scheme 6.43.

This reaction also gained interest for the synthesis of a key intermediate of the drug thiamphenicol. The Griengl group reported the threonine aldolase-catalyzed aldol reaction of glycine (**110**) with 4-(methylsulfonyl)benzaldehyde (**112**) under the formation of the corresponding α-amino β-hydroxy acid L-*threo*-**113** as thiamphenicol intermediate with 68% analytical yield, a diastereoselectivity of 53% de, and excellent enantiomeric excess of >99% ee (Scheme 6.44) [162].

Since an excess of glycine is required for sufficient conversion and because of low diastereoselectivity but high stereochemical preference for formation of the α-stereogenic center, often the reverse *retro*-aldol reaction is carried out as an alternative. In such enzymatic resolution processes, diastereomerically pure *threo*-racemates are used as



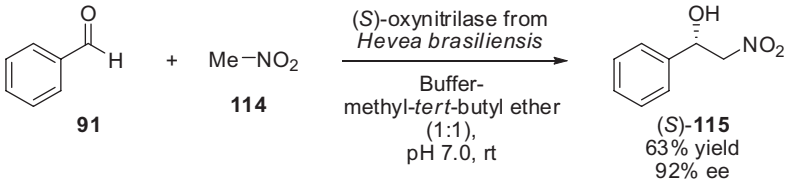
Scheme 6.44.

substrates and lead to the formation of diastereo- and enantiomerically pure α-amino β-hydroxy acids [163,164].

6.4.5. "Non-Natural Reaction Types" in C–C Bond Formation

A very exciting issue in enzyme chemistry is their use for so-called non-natural reactions. These phenomena, also known as enzyme promiscuity [165], allow interesting organic synthesis with enzymes, which are known to catalyze other types of reactions in nature. Notably, a broad range of such "non-natural reaction types" with enzymes as catalysts are known in the field of C–C bond formations.

Very recently, the Griengl group reported the first enzymatic nitroaldol reaction, also widely known as Henry reaction [166,167]. An (*S*)-oxynitrilase from *H. brasiliensis*, which is a highly efficient biocatalyst for asymmetric hydrocyanation, served as biocatalyst, and nitromethane and nitroethane were used as aldol donors. With nitromethane as donor, a broad range of nitroaldol adducts were obtained with yields up to 77%, and with enantioselectivities of up to 92% ee. In Scheme 6.45, an example is shown. When using nitroethane as donor and aldehydes as acceptors, two stereogenic centers are formed in a diastereo- and enantioselective biotransformation (with a diastereomeric ratio of d.r. = 90:10 and an enantioselectivity of up to 95% ee).



Scheme 6.45.

Interestingly, lipases also turned out to be suitable catalysts for Michael reactions. In the presence of lipases from *C. antarctica* B (CAL-B), several heteroatom nucleophile donors (such as amines and thiols) were tolerated as well as malonates [168]. However, the reaction does not proceed enantioselectively (which is in contrast to an early contribution from Kitazume et al. with several amine donors and other hydrolases; see Reference 169).

A further non-natural reaction catalyzed by enzymes is the Morita–Baylis–Hillman reaction. The Reetz group reported that this reaction is catalyzed by carrier proteins such as serum albumins or certain lipases [170]. In the presence of these enzymes, the Morita–Baylis–Hillman reaction of cyclohexenone with 4-nitrobenzaldehyde gives the corresponding Morita–Baylis–Hillman adduct with conversions of up to 35% and enantioselectivities of up to 19% ee.

6.5. ENANTIOSELECTIVE REDUCTIONS

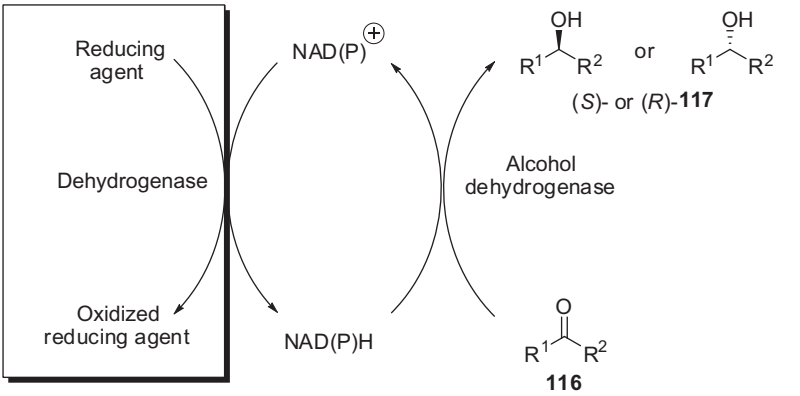
6.5.1. Overview

The enantioselective transformation of C=X double bonds (with X=O, N, C) into corresponding reduced CH–XH single bonds (with X=O, N, C) plays a major role in asymmetric synthesis. Notably, a range of redox enzymes (namely dehydrogenases) are available, which catalyze the reduction of C=O double bonds under the formation of the corresponding alcohol moieties. The reaction range comprises reduction of, for example, ketones, α- and β-keto esters, and α-keto acids. Furthermore, reductive amination of C=O double bonds (of α-keto acids) using amino acid dehydrogenases turned out to represent a highly efficient approach toward the synthesis of enantiomerically pure amino acids. A further class of redox enzymes (oxidoreductases) of common interest in organic synthesis are enoate reductases. These enzymes catalyze the reduction of activated C=C double bonds bearing at least one electron-withdrawing group as substituent. Although not belonging to the group of redox enzymes, transaminases also catalyze "reductive processes" with both α-keto acids and ketones, thus leading to corresponding amines and amino acids in an asymmetric fashion. In the following, such types of organic synthetic reactions using oxidoreductases and transaminases will be discussed.

6.5.2. Reduction of Ketones

The asymmetric reduction of ketones represents a straightforward and an atom-economical approach toward the synthesis of optically active alcohols, and numerous efficient catalytic routes thereof have been developed up to date. Outstanding chemocatalytic technologies are metal-catalyzed asymmetric hydrogenation of ketones [171] and borane reduction [172], which are applied on technical scale and represent landmarks in industrial asymmetric catalysis. In addition, biocatalytic reduction [173] turned out to be a highly efficient alternative and competitive technology for asymmetric ketone reduction. This is underlined by an increasing number of industrial applications of biocatalytic asymmetric reductions of ketones.

The principle of enantioselective biocatalytic reduction of ketones **116** is based on the use of an alcohol dehydrogenase (ADH) as a catalyst, and a cofactor as a reducing agent. An ADH is an enzyme capable of reducing carbonyl moieties under formation of (chiral) alcohols (*R*)- or (*S*)-**117** and requires a specific "cofactor" as reducing agent. The most preferred cofactors are either NADH or NADPH. Since the cofactors are expensive reducing agents, and too costly to be applied in stoichiometric amount, a common key feature of all preparative (and technical) biocatalytic reductions is the use of cofactors in catalytic amount and their recycling *in situ* by coupling the ketone reduction process with a second process, in which the cofactor is regenerated (Scheme 6.46).

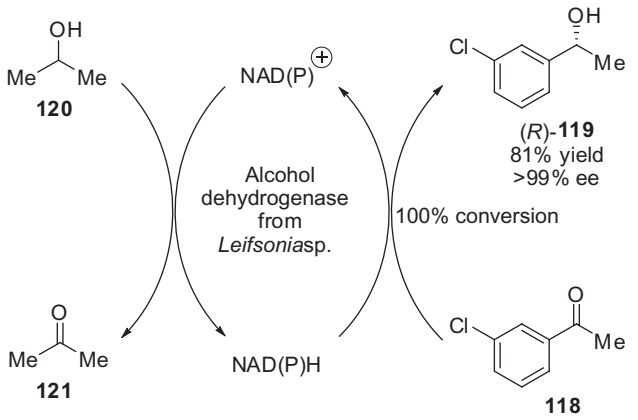


Scheme 6.46.

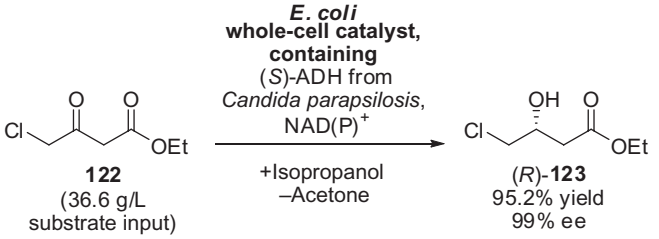
Toward this end, two approaches have been developed with the so-called substrate-coupled or enzyme-coupled cofactor regeneration. In the substrate-coupled cofactor recycling, the same ADH, which reduces the ketone substrate, is used for the dehydrogenation of isopropanol (as reducing agent) under the formation of acetone. An alternative approach is the enzyme-coupled cofactor regeneration: In the presence of a second enzyme, a (preferably) cheap compound (e.g., formate, glucose) is used as reducing agent and oxidized, thus regenerating the required reduced form of the cofactor, NAD(P)H. Thus, both types of cofactor regenerations are based on the use of a cheap and easily available source for the reduction of the oxidized form of the cofactor, namely, NAD^+ or $NADP^+$.

The ADHs can be used as isolated enzymes (in purified form or as crude extract) or incorporated in whole cells. With respect to the latter approach, the use of wild-type cells or recombinant whole-cell organisms is conceivable. Recently, tailor-made whole-cell catalysts, bearing the ADH and (in case of the enzyme-coupled cofactor regeneration) an additional enzyme, gained tremendous interest due to their beneficial properties. Due to overexpression, the desired enzymes are available within the cells in large amounts, thus avoiding undesired side reactions by other dehydrogenases and allowing an economically attractive access—in particular, when using high cell-density fermentation for biocatalyst production. In the following, selected examples are given with a particular focus on recently reported contributions.

To start with an example in the field of substrate-coupled cofactor regeneration for asymmetric ketone reduction, Wong et al. reported such a process with an isolated NADH-dependent ADH from a *Pseudomonas* sp. strain [174]. Notably, a broad substrate spectrum has been observed when carrying out the reactions in a two-phase solvent system with *n*-hexane as an organic phase. Another ADH with a broad substrate spectrum is the recombinant ADH from *Leifsonia* sp., which was developed by Itoh and coworkers [175,176]. A selected example of reductions with this purified enzyme using isopropanol for cofactor regeneration is given in Scheme 6.47. The potential of ionic liquids for the biocatalytic reduction of ketones under cosubstrate cofactor recycling has been demonstrated by Kragl, Liese, and coworkers [177].



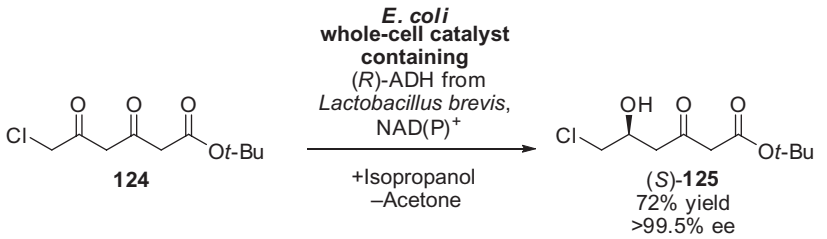
Scheme 6.47.



Scheme 6.48.

The use of whole-cell catalysts in reductions under substrate-coupled cofactor recycling was demonstrated by Matsumura and coworkers using wild-type cells of *Candida boidinii* [178], and by Itoh et al. [179] using recombinant *E. coli* whole-cell catalyst overexpressing an ADH from a *Corynebacterium* strain. Furthermore, an efficient recombinant whole-cell *E. coli* biocatalyst overexpressing an ADH from *Candida parapsilosis* was reported by Daicel researchers [180]. This biocatalyst is suitable for the asymmetric reduction of ethyl 4-chloroacetate using isopropanol as reducing agent and without the addition of external cofactor. At a substrate input of 36.6 g/L, the desired ethyl (*R*)-4-chloro-3-hydroxybutanoate ((*R*)-**123**) was obtained in a yield of 95.2% and with an excellent enantioselectivity of 99% ee (Scheme 6.48). Weuster-Botz et al. [181] developed an analogous synthesis of the corresponding (*S*)-enantiomer using *Lactobacillus kefir* wild-type cells. When using 5% (v/v) of isopropanol as cosubstrate, a final product concentration of 1.2 M was achieved in combination with 97% yield and an enantioselectivity of 99.5%.

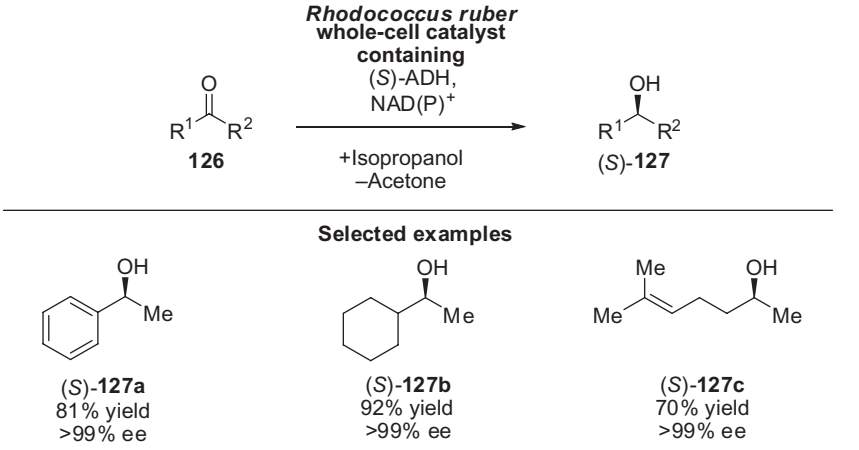
A regio- and enantioselective reduction of 3,5-dioxocarboxylates has been developed by the Müller group by means of recombinant *E. coli* cells with an overexpressed ADH from *Lactobacillus brevis* [182]. With isopropanol as reducing agent, the 3,5-diketo ester **124** was transformed into (*S*)-6-chloro-5-hydroxy-3-oxohexanoate (*R*)-**125** in 72% yield and with an excellent enantiomeric excess of >99.5% ee (Scheme 6.49). Notably, the 3-oxo-group remained untouched.



Scheme 6.49.

An interesting process development has been reported by Liese et al. [183] demonstrating that by pervaporation or stripping off the acetone the conversion can be increased significantly. This is due to shifting the equilibrium in the favored direction by removing the side-product acetone from the reaction mixture.

A further efficient whole-cell biocatalyst has been reported by the Faber group with an *R. ruber* wild-type strain converting a broad range of ketones very selectively and efficiently to the corresponding (*S*)-alcohol at high isopropanol concentrations of up to 50% (v/v) [184,185]. The utility of high isopropanol concentrations is particularly attractive for commercial applications. Therefore, the high isopropanol tolerance of this biocatalyst represented a major breakthrough. Selected synthetic applications are given in Scheme 6.50 demonstrating that a broad range of aliphatic and aromatic ketones are



Scheme 6.50.

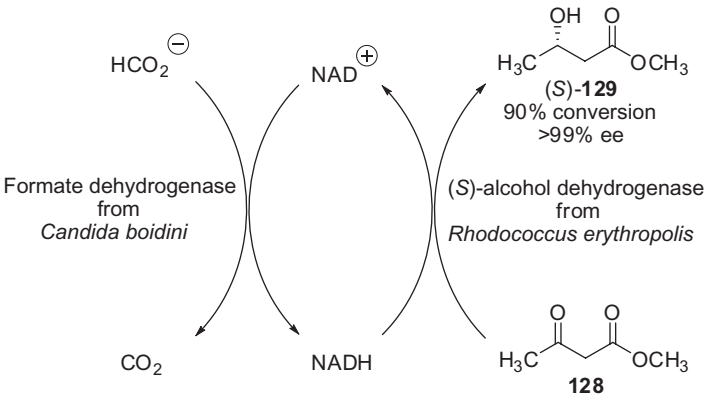
reduced with high enantioselectivities of >99% ee in most cases. The recombinant expression of the *R. ruber* ADH has also been reported, thus even further expanding the scope of this versatile enzyme [186]. Besides mono- and biphasic aqueous–organic solvent media, the substrate-coupled regeneration with isopropanol in the presence of ADH from *R. ruber* has been successfully applied in microaqueous organic systems with 99% (v/v) of an organic solvent. Notably, high substrate concentrations of up to ~2 M were realized [187]. Starting from α-chloro ketones, the corresponding halohydrins were obtained with enantioselectivities of up to >99% ee when using *R. ruber* as a lyophilized catalyst [188]. For example, (*R*)-octanol was formed with >99% conversion and 99% ee. Furthermore, highly enantio- and diastereoselective reduction of diketones under formation of the corresponding diols with >99% ee and >99% de has been reported by the Kroutil group [189].

The high efficiency of enzymatic asymmetric ketone reduction with substrate-coupled cofactor regeneration is also underlined by commercial applications thereof, as has been reported, for example, by Wacker.

Very recently, the Gröger group reported the combination of an ADH-catalyzed reduction of ketones under substrate-coupled cofactor regeneration with a palladium-catalyzed Suzuki cross-coupling reaction in a one-pot synthesis in aqueous media [190]. When carrying out the Suzuki cross-coupling reaction in the initial step starting from aromatic boronic acids and a halogenated acetophenone, subsequent biocatalytic reduction gave enantiomerically pure biaryl alcohols with conversions of up to 91%.

When applying an enzyme-coupled cofactor regeneration for asymmetric biocatalytic reduction processes, the use of a formate dehydrogenase (FDH) represented a popular approach. The FDH catalyzes the oxidation of formate into carbon dioxide, while reducing the oxidized form of the cofactor into its reduced form, NAD(P)H. The most widely applied FDH is probably the FDH from *C. boidinii* and optimized mutants thereof [191] developed in the Kula group who are—jointly with the Hummel and Wandrey groups—pioneers in the field of FDH-based applications [192,193] in addition to the Whitesides group [194]. A key advantage when using FDH for cofactor regeneration certainly is the irreversible step of carbon dioxide formation and removal, thus shifting the equilibrium toward (complete) product formation. In addition, downstream processing is simplified since (ideally) no organic by-product remains in the reaction mixture. The initial work on enzymatic reduction of ketones has been carried out based on the use of isolated enzymes in homogeneous aqueous media. Due to the low solubility of the hydrophobic ketones in water, the reactions were carried out at low substrate concentrations for a long time, typically in the range of 5–20 mM or below. In the 1990s, Hummel et al. as well as the Kula group studied in detail the suitability of different types of ADHs in combination with an FDH for asymmetric reduction of a broad range of ketones comprising keto esters, aromatic ketones, and aliphatic 2-alkanones [195–197]. The Kula group also carried out preparative transformations based on these enzymes by coupling the ADH reduction reactions with FDH regeneration [198,199]. As enzymes, ADHs from *R. erythopolis* and *C. parapsilosis* were used in combination with the FDH from *C. boidinii*. Carrying out reductions of several keto esters and a keto dialkyl acetal at a substrate concentration of 100 mM furnished the desired alcohols in most cases with high conversion (up to 100%) and high enantioselectivities of >99%. A selected example is given in Scheme 6.51.

The issue of high space-time yields in spite of the limitation of low ketone solubility has been successfully addressed by the Wandrey group, who developed elegant

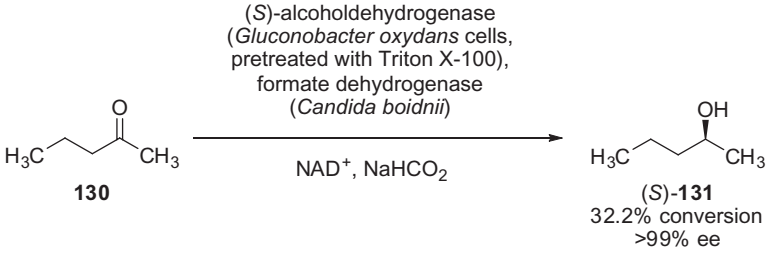


Scheme 6.51.

engineering solutions by means of continuously operating processes with an EMR. An efficient "three-loop" concept is based on an enzymatic reaction in pure aqueous medium, a separation of the aqueous phase from the enzyme via ultrafiltration, and a subsequent continuous extraction of the aqueous phase with an organic solvent. Organic and aqueous phases are separated by a hydrophobic membrane [200–202]. Although the reaction in this EMR is limited by the low solubility of the ketone in water (9–12 mM), good space-time yields in the range of 60–104 g/(L·d) have been obtained as has been demonstrated for the synthesis of, for example, (*S*)-1-phenylpropan-2-ol and (*S*)-4-phenylbutan-2-ol in enantiomerically pure form. An extended, newly designed emulsion membrane reactor concept has also been applied by Wandrey et al. for the asymmetric reduction of 2-octanone [203]. A conversion of 97% has been achieved at a residence time of 1 h, corresponding to a space-time yield of 21.1 g/(L·d). Notably, this emulsion membrane reactor has been operated over a period of >4 months.

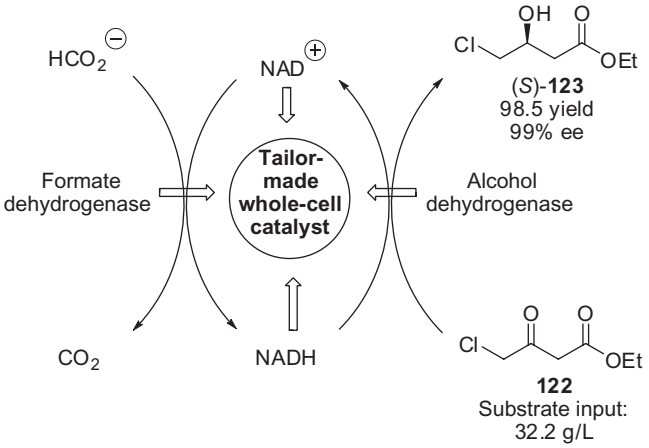
Although the presence of an organic solvent could improve the solubility of poorly water-soluble ketones, the known instability of the FDH from *C. boidinii* toward many organic solvents remained a challenge. Addressing the issue, Gröger and Hummel et al. developed a suitable aqueous–organic two-phase solvent reaction medium based on the use of *n*-heptane and *n*-hexane as organic phases [204,205]. In this reaction medium, a recombinant (*S*)-ADH and FDH from *C. boidinii* (mutant C235, C262A) remain stable, and preparative reductions therein gave good conversions and high enantioselectivities with a variety of aromatic ketone substrates. Although reactions proceed at substrate concentrations of up to 200 mM sufficiently, at higher substrate concentrations conversions are decreasing and prolonged reaction times are required. A further improvement of the substrate concentrations up to 500 mM has been realized when using an "emulsion system" for the synthesis of the corresponding alcohols [206,207]. For example, the reduction of 4-chloroacetophenone as a model substrate on a 6-L scale gave the desired (*S*)-alcohol with >98% conversion and >99.4% ee. As enzymes, the ADH from *R. erythropolis* and the FDH from *C. boidinii* have been used.

Reductions based on the use of ADH and FDH already proved their technical feasibility. This has been successfully demonstrated by the Patel group in the production of (*S*)-2-pentanol ((*S*)-**131**) on pilot scale using an ADH from *Gluconobacter oxydans* (SC 13851) [208]. *G. oxydans* cells, pretreated with Tritone X-100, were used as biocatalyst in combination with the FDH from *C. boidinii*. This reduction was carried out at a 1.500-L scale with a substrate input of 3.2 kg (~2.13 g/L). The desired (*S*)-2-pentanol ((*S*)-**131**) has been formed with a conversion of 32.2% and an enantioselectivity of >99% ee (Scheme 6.52).



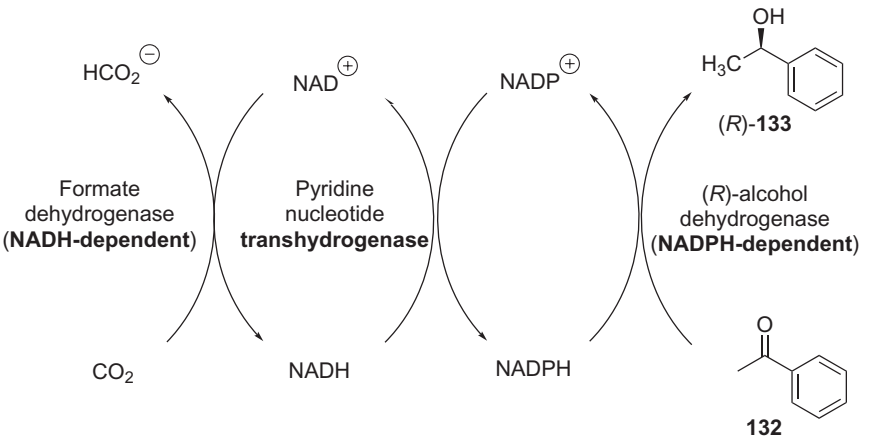
Scheme 6.52.

The potential of an FDH-based whole-cell catalyst for synthetic applications has been recognized by Matsuyama et al., constructing a recombinant *E. coli* W3110 strain, which coexpresses an ADH from *Pichia finlandica* and an FDH from *Mycobacterium* [209]. The tailor-made whole-cell catalyst has been successfully applied, for example, in the enantioselective reduction of ethyl 4-chloro-3-oxobutanoate (**122**) under the formation of the corresponding (*S*)-alcohol (*S*)-**123** at 32.2 g/L substrate input with 98.5% yield and 99% ee (Scheme 6.53).



Scheme 6.53.

For a long time, a major limitation for applications using the FDH from *C. boidinii* was its inability to regenerate $NADP^+$, thus being limited to the regeneration of NAD^+ only. An elegant solution of this problem has been recently found by the Hummel group, thus expanding the application range of FDH-based cofactor regeneration also to $NADP^+$-dependent ADHs [210]. As such an ADH, the highly efficient ADH from *L. kefir* [211,212] was chosen. The key step is the integration of an additional enzymatic step within the cofactor-regeneration cycle, namely the pyridine nucleotide transhydrogenase (PNT)-catalyzed regeneration of NADPH from $NADP^+$ under consumption of NADH forming NAD^+ [210]. The concept is graphically shown in Scheme 6.54, exemplified for the synthesis of (*R*)-phenylethanol ((*R*)-**133**).

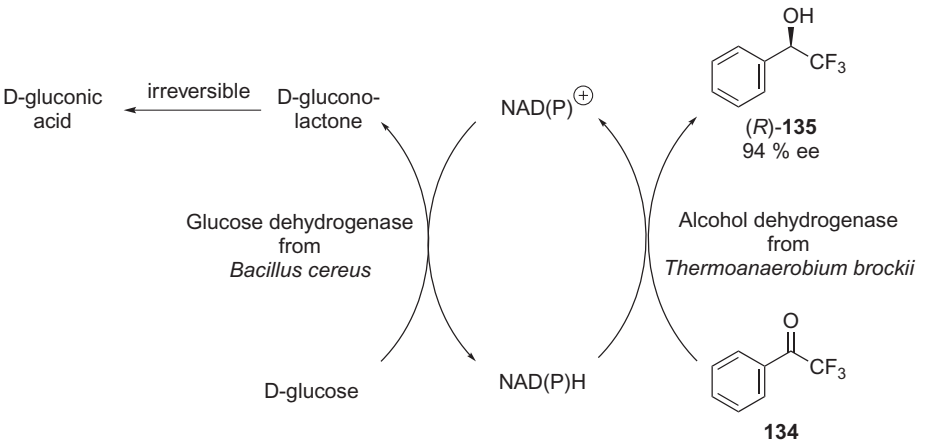


Scheme 6.54.

A further efficient option for recycling the cofactor NAD(P)H, which is oxidized during the reduction process, is based on the use of a glucose dehydrogenase (GDH). Therein, D-glucose is oxidized to D-gluconolactone, while the oxidized cofactor $NAD(P)^+$ is reduced to NAD(P)H (which is the required reducing agent for the reduction process). Since D-gluconolactone is subsequently hydrolyzed under the formation of D-gluconic acid (as its sodium salt at neutral pH), this reaction can also be regarded as an irreversible step, thus shifting the whole reaction into the direction of the desired alcohol product. Although some preparative synthetic applications by means of isolated enzymes are known [213–215], most of the reported applications of GDH-coupled cofactor regeneration in asymmetric reduction are based on the use of recombinant whole-cell systems. Notably, industrial applications of this recombinant whole-cell technology based on an ADH and a GDH have already been reported in particular by Kaneka Corporation and Degussa AG (now Evonik-Degussa GmbH). Some selected examples of this technology are given in the following.

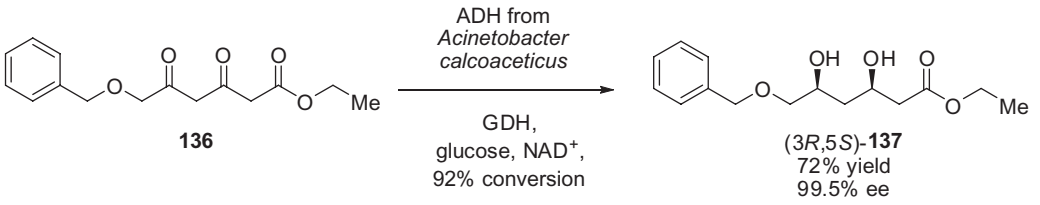
The proof of principle and pioneering work for a biocatalytic reduction using a GDH-coupled cofactor-regeneration process has been done by Wong and coworkers [213,214]. The corresponding enzymatic reduction of ketones in the presence of different types of ADHs such as ADHs from horse liver, yeast, and *Thermoanaerobium brockii* gave the

desired alcohols, for example, (*R*)-**135**, with good to high enantioselectivities. Both ADH and GDH were used in an immobilized form. The conversions of these enzymatic biotransformations were in the range of 72–90%. Although enantioselectivities varied, they exceeded 90% ee in many cases. A selected example is shown in Scheme 6.55.



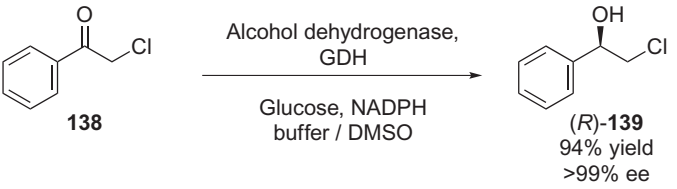
Scheme 6.55.

A recent contribution to this field has been made by the Hua group focusing on the asymmetric reduction of α-chlorinated ketones in the presence of isolated ADHs and under regeneration of the cofactor with a GDH [216]. A range of α-chlorinated alcohols were formed in high yields of 72–99%, and with excellent enantioselectivities of typically >99% ee. The joint use of ADH and GDH has also been successfully applied for the enantioselective reduction of substituted benzophenones by Merck researchers [217]. The feasibility of this methodology for an enantio- and diastereoselective reduction of ethyl 6-benzyloxy-3,5-dioxohexanoate, has been demonstrated by the Patel group [218,219]. When using cell extracts of *Acinetobacter calcoaceticus* in combination with a GDH and glucose, the desired product ethyl (3*R*,5*S*)-6-benzyloxy-3,5-dihydroxyhexanoate ((3*R*,5*S*)-**137**) was formed with 92% conversion and an enantioselectivity of 99% ee (Scheme 6.56). After product isolation, (3*R*,5*S*)-**137** was obtained in 72% yield and with an enantiomeric excess of 99.5% ee.



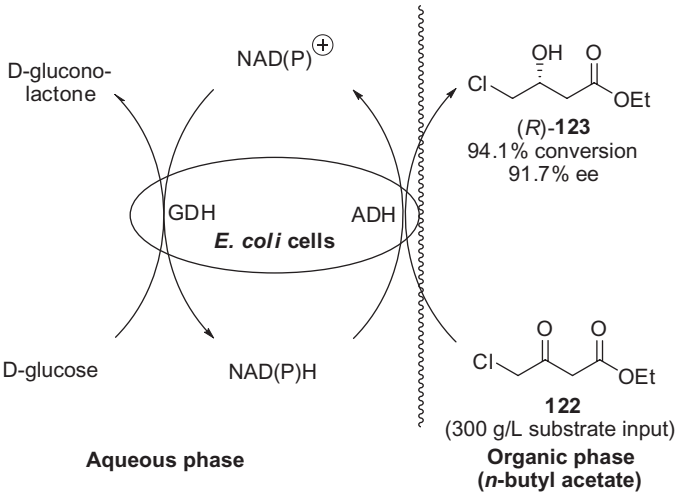
Scheme 6.56.

Besides a screening for various ketones, the preparative asymmetric reduction of benzoyl hydroxyacetone and α-tetralone in the presence of isolated ADH and GDH enzymes has been described by BioCatalytics researchers [220]. Using the isolated ADH enzymes in an amount of 1–7% (w/w) compared with the amount of substrate and a catalytic amount of cofactor led to the synthesis of the optically active alcohols, for example, (*R*)-**139**, in high yields. The reductions have been carried out at high substrate concentrations of up to 0.75–1.4 M. A selected example is shown in Scheme 6.57.



Scheme 6.57.

The design of recombinant whole cells is an elegant approach toward tailor-made (bio-)catalysts, which contain not only the cofactor "for free" but also both of the desired enzymes, ADH and GDH, in overexpressed form. The corresponding reduction of ketones proceeds within the cell according to the concept shown in Scheme 6.58. Advantages of such a recombinant whole-cell system over the wild-type ones are the higher amount of the desired enzymes within the cell (due to overexpression), their cost-effective access, and excellent performance in synthetic applications. With respect to this recombinant whole-cell concept, the pioneers in the design and application of highly



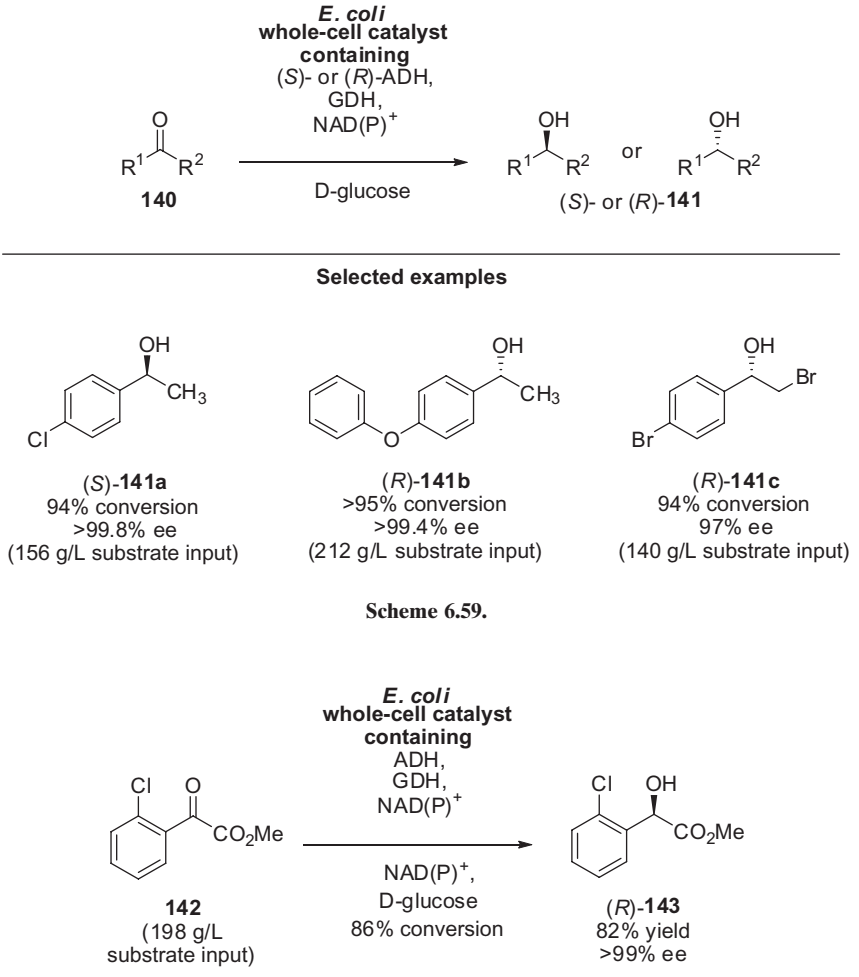
Scheme 6.58.

efficient recombinant whole-cell biocatalysts, consisting of an ADH and GDH, are Shimizu and coworkers [221]. As a GDH, the GDH from *Bacillus megaterium*, which accepts both NADH and NADPH as a cofactor, was used. Already in the 1990s, Shimizu et al. developed an effective *E. coli* catalyst, as well as a highly efficient reaction system for the reduction of 4-chloro-3-oxobutanoate [222–226]. The use of these efficient recombinant whole-cell catalysts in the asymmetric reduction of 4-chloro-3-oxobutanoate (**122**) forming the corresponding pharmaceutically important alcohol (*R*)-**123** has been intensively investigated and optimized by the Shimizu group. As a reaction media, an *n*-butyl acetate/water two-phase solvent system turned out to be suitable [227]. When using the *E. coli* host organism overexpressing an $NADP^+$-dependent ADH from *Sporobolomyces salmonicolor*, and an isolated GDH enzyme or GDH-expressing cells as biocatalysts, the desired optically active (*R*)-alcohol (*R*)-**123** was formed with up to 255 g/L in the organic phase under optimized conditions [228,229]. The conversion reached 91% and an enantioselectivity of 91% ee was found. Besides glucose as a cosubstrate, a low amount of NADP+ is required. A further improvement has been achieved when using *E. coli*, co-expressing both the ADH from *S. salmonicolor* and the GDH from *B. megaterium*, resulting in the formation of the desired optically active (*R*)-alcohol with 94.1% conversion and an enantioselectivity of 91.7% ee when operating at a substrate concentration of 300 g/L and adding a catalytic amount of the $NADP^+$-cofactor [230]. Scheme 6.58 illustrates the concept of this application of a tailor-made whole-cell biocatalyst in a two-phase reaction media, as well as experimental results.

It is noteworthy that the Shimizu group also designed a whole-cell catalyst for the synthesis of the analogue (*S*)-enantiomeric form of ethyl 4-chloro-3-hydroxybutanoate [231]. In addition, Kaneka researchers jointly with the Shimizu group reported the extension of this reduction technology for the reduction of other type of functionalized β-keto ester substrates, for example, 4-bromo-3-oxobutanoate [232], and a range of other substrates [231]. This impressive biocatalytic reduction technology developed by the Shimizu group has already been commercialized. Since 2000, Kaneka Corporation applies this methodology for the manufacture of ethyl (*S*)-4-chloro-3-hydroxybutanoate on industrial scale [231].

A recombinant whole-cell catalyst, containing an ADH and GDH, has also been developed by Patel et al., and successfully applied for the reduction of an acetophenone substituted with a keto ester-containing moiety [233]. The reaction proceeded with a reaction yield of 95% and gave an excellent enantioselectivity of 99.9% ee. Notably, this biotransformation has been scaled up to a 500-L scale. The construction of an *E. coli* whole-cell catalyst, harboring the widely used (*R*)-selective ADH from *L. kefir* and a GDH from *Bacillus subtilis*, has also been successfully accomplished by the Hummel group [234].

In addition, Degussa researchers jointly with the Hummel group reported the application of recombinant whole-cell biocatalysts in asymmetric reductions of a range of ketones at high substrate input, exceeding 150 g/L, in pure aqueous media, and in general without the need of addition of external amount of cofactor [235]. Both types of enantiomers are available due to the use of (*S*)- and (*R*)-selective whole-cell biocatalysts. This methodology, which is both economical and simple to be carried out, has been used for the preparation of a wide range of optically active alcohols (*S*)- and (*R*)-**141**. Typically, the substrate concentrations are in the range of 1 M, thus exceeding 100 g/L. The reduction proceeds with high conversions of up to >95%, and with high enantioselectivities of up to >99.4% ee. An overview about selected examples is given in Scheme 6.59. The synthesis of a fluorinated 4-phenylethan-1-ol as well as aliphatic halohydrins also



Scheme 6.59.

Scheme 6.60.

turned out to proceed efficiently and with high enantioselectivity of >99% ee when using these types of recombinant whole-cell catalysts [236,237]. After further optimization, this recombinant whole-cell reduction technology platform has already been applied on industrial scale at Degussa AG.

Recently, a further highly efficient application of recombinant *E. coli* cells overexpressing ADH and GDH was demonstrated by Ema and Sakai et al. with the efficient biocatalytic synthesis of methyl (*R*)-*o*-chloromandelate, which is an intermediate for the drug clopidogrel [238]. When starting from the corresponding α-keto ester, the biocatalytic reduction proceeds at an impressive substrate input of 198 g/L with 86% conversion, leading to the desired product (*R*)-**143** with 82% yield and an excellent enantioselectivity of >99% ee (Scheme 6.60). Furthermore, this type of biocatalyst also turned out to be suitable for the efficient asymmetric reduction of a broad range of ketones [239].

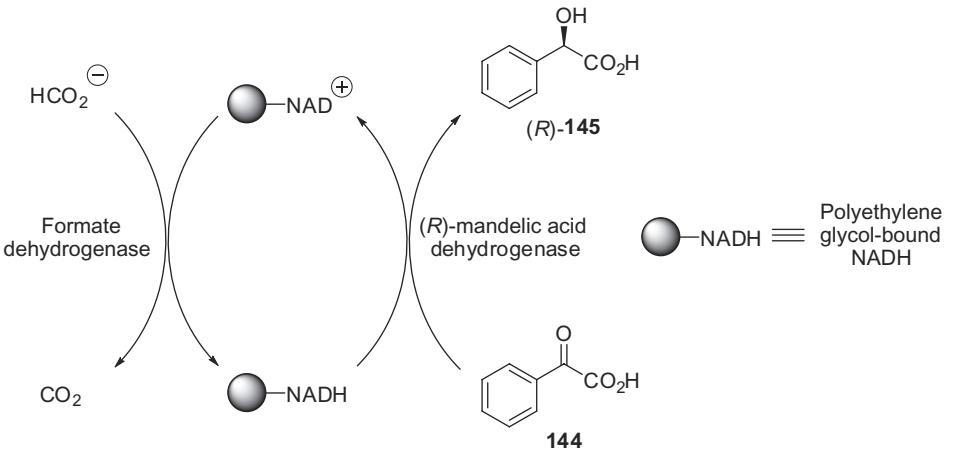
A further type of cofactor regeneration related to the one with a GDH is based on the use of a glucose-6-phosphate dehydrogenase (G-6-PDH). The synthesis of chiral alcohols by means of this methodology has already been reported in 1981 by Wong and Whitesides [240,241]. The combination of the G-6-PDH with the ADH from *L. kefir* for the synthesis of optically active (*R*)-phenylethan-1-ol has been additionally reported by Hummel [242], and the Stewart group applied their impressive set of 19 recombinant ADHs from *Saccharomyces cerevisiae* in a screening of numerous α- and β-keto ester substrates by means of G-6-PDH-based reductions [243,244]. As a substrate, glucose-6-phosphate is needed, which can be formed by the cells starting from glucose (in case of a whole-cell approach) or used directly (in case of isolated enzymes). The latter option, however, is less attractive since glucose-6-phosphate itself is an expensive compound. Accordingly, syntheses based on this type of cofactor regeneration with isolated enzymes have only been reported for small-scale applications. In contrast, the whole cell-based approach has potential for large-scale applications, and promising process development has already been reported by Hanson and Patel et al. [245] The applied whole-cell biocatalyst turned out to reduce 2,3′-dichloro-4′-fluoroacetophenone enantioselectively, thus leading to the desired product (*S*)-2-chloro-1-(3′-chloro-4′-fluorophenyl)-ethanol in 89% yield and with >99% ee. The substrate input of this reduction was ~20 g/L, and the intact *E. coli* cells have been provided with glucose directly.

In summary, numerous methodologies for asymmetric biocatalytic reductions of ketones have been developed based on the use of isolated enzymes as well as whole-cell catalysts. High efficiency in organic synthetic transformations of ketones into optically active alcohols has been demonstrated, which is underlined by applications on industrial scale.

6.5.3. Reduction of α-Keto Acids

In contrast to α-keto ester, the analogous α-keto acids are not substrates for ADHs. However, a range of hydroxy acid dehydrogenases suitable for the reduction of α-keto acids under formation of enantiomerically pure α-hydroxy acids are available. An efficient enantioselective synthesis of (*R*)-mandelic acid via enzymatic reduction of phenylglyoxylic acid has been developed by Hummel and Kula et al. [246]. As a biocatalyst, an (*R*)-mandelic acid dehydrogenase from *Lactobacillus curvatus* was found. Based on this enzyme, a highly efficient asymmetric reduction process by means of such a dehydrogenase route has been disclosed by Wandrey, Hummel, Kula, and coworkers [247]. A continuous conversion of phenylglyoxylic acid (**144**) to (*R*)-mandelic acid ((*R*)-**145**) was performed in an EMR with simultaneous cofactor regeneration. A mandelate dehydrogenase and an FDH have been used as biocatalysts. This two-enzyme process allowed the production of (*R*)-mandelic acid ((*R*)-**145**) with high space-time yields of 700 g/(L*d) at a low enzyme consumption. The concept of this continuous (*R*)-mandelic acid production is shown in Scheme 6.61. Thus, this efficient type of biotransformation complements other biocatalytic routes to enantiomerically pure aromatic α-hydroxy acids such as oxynitrilase-catalyzed asymmetric cyanohydrin synthesis and subsequent hydrolysis or nitrilase-catalzyed hydrolysis of racemic cyanohydrins [248].

Aliphatic α-keto acids also turned out to represent suitable substrates. The most prominent example is the transformation of pyruvate into D- or L-lactic acid in the presence of D- and L-lactate dehydrogenases. Besides lactate dehydrogenases, however, other dehydrogenases have also been developed as recombinant biocatalysts for the



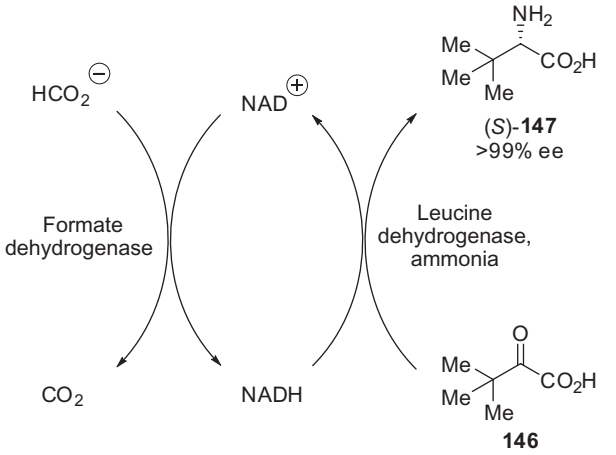
Scheme 6.61.

reduction of aliphatic α-keto acids. For example, Hummel and Kula et al. applied an L-2-hydroxyisocaproate dehydrogenase from *Lactobacillus confusus* for a highly enantioselective reduction of a range of aliphatic 2-keto acids [249].

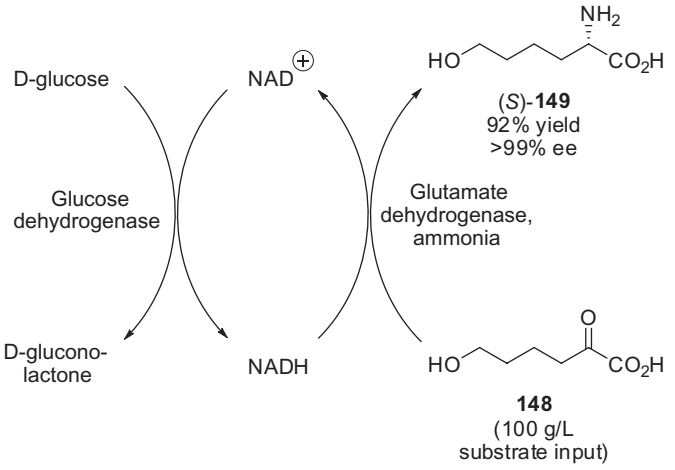
6.5.4. Reductive Amination of α-Keto Acids

The asymmetric reductive amination of α-keto acids represents a straightforward approach to (in particular nonproteinogenic) α-amino acids in enantiomerically pure form [250]. The most prominent representative amino acid obtained by this route is the bulky amino acid L-*tert*-leucine, which serves as an important building block for the pharmaceutical industry [251]. For the synthesis of amino acids via reductive amination of keto acids, one can use chemocatalysts [252] as well as enzymes, namely amino acid dehydrogenases, as catalyst components. An enzymatic route is based on the combined use of two isolated and purified dehydrogenases, namely, a leucine dehydrogenase (LeuDH) for reductive amination and an FDH from *C. boidinii* [253,254]. The FDH is needed for an *in situ* recycling of the cofactor NADH. By means of such a biocatalytic reductive amination, L-*tert*-leucine ((*S*)-**147**) is obtained with high conversion and excellent enantioselectivity of >99% ee (Scheme 6.62). This process is already applied on a scale of tons at Degussa AG [255] and additionally turned out to be applicable to a range of other types of α-amino acids [251,254].

A further example for the value of enzymatic reductive amination is the synthesis of the nonproteinogenic amino acid L-6-hydroxynorleucine ((*S*)-**149**), required as intermediate for the synthesis of an antihypertensive drug [256]. This process, which was developed by Patel and coworkers, gave the desired L-amino acid with complete conversion, a yield of 92%, and an enantioselectivity of >99% ee (Scheme 6.63). In this synthesis, which runs at a high substrate input of 100 g/L, a beef liver glutamate dehydrogenase was used as L-amino acid dehydrogenase and a GDH from *B. megaterium* was used for cofactor regeneration.



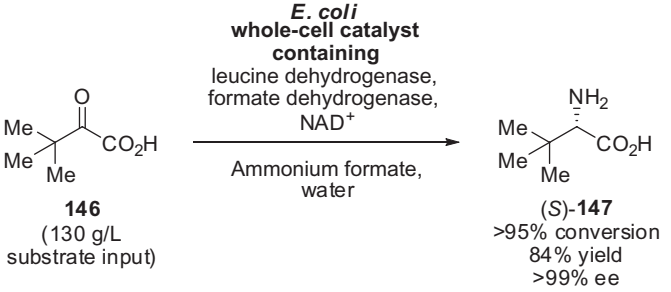
Scheme 6.62.



Scheme 6.63.

However, in spite of high efficiency, the need for isolated, costly enzymes as well as the need for the addition of expensive cofactor NAD^+ (although used in catalytic amounts) is disadvantageous. Thus, efforts have been made to address these issues. The direct use of a whole-cell catalyst, containing both an amino acid dehydrogenase and FDH in overexpressed form, has been reported by Esaki et al. in their pioneer work for several amino acids [257]. The desired amino acids were obtained with high conversion and excellent enantioselectivity. For example, L-leucine, L-valine, and L-norvaline were synthesized with a recombinant whole-cell catalyst overexpressing a LeuDH with conversions of 95–97% and enantioselectivities of >99% ee. When using a whole-cell catalyst bearing a phenylalanine dehydrogenase as amino acid dehydrogenase, L-tyrosine (92% conversion) and L-phenylalanine (95% conversion) were formed in enantiomerically pure form.

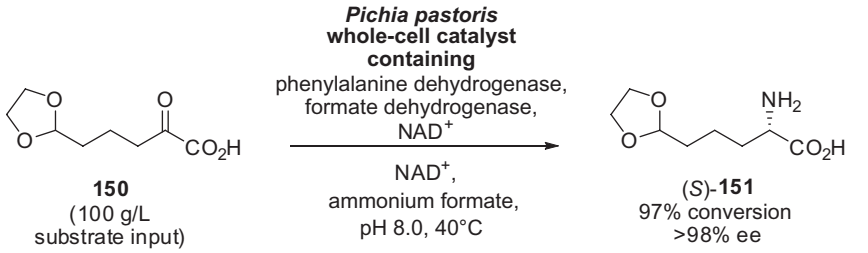
Recently, a highly efficient process for the synthesis of L-*tert*-leucine, which is based on the use of tailor-made recombinant whole-cell catalysts, has been reported by Gröger, Altenbuchner, and coworkers (Scheme 6.64) [258]. Notably, the process runs at an overall substrate input of 130 g/L and leads to the desired L-*tert*-leucine ((*S*)-**147**) with >95% conversion. After isolation, L-*tert*-leucine ((*S*)-**147**) was obtained in 84% yield with an enantiomeric excess of >99% ee.



Scheme 6.64.

A whole cell-catalyzed process has also been developed by Gröger and Altenbuchner et al. for the enantioselective synthesis of L-neopentylglycine as a further bulky α-amino acid [259]. The synthesis of this non-natural amino acid proceeds highly efficiently, leading to the desired L-neopentylglycine with >95% conversion and a high enantioselectivity of >99% ee at substrate concentrations of up to 88 g/L.

Patel reported a whole cell-catalyzed reductive amination toward the synthesis of L-allysine ethylene acetal ((*S*)-**151**) as a further L-amino acid intermediate for an antihypertensive drug (Scheme 6.65) [260]. As a whole-cell catalyst, *Pichia pastoris* cells containing a phenylalanine dehydrogenase from *Thermoactinomyces intermedius* were used in combination with an FDH from *P. pastoris*. The desired L-amino acid (*S*)-**151** was obtained with 97% conversion and an enantioselectivity of >98% ee when using a substrate input of 100 g/L.



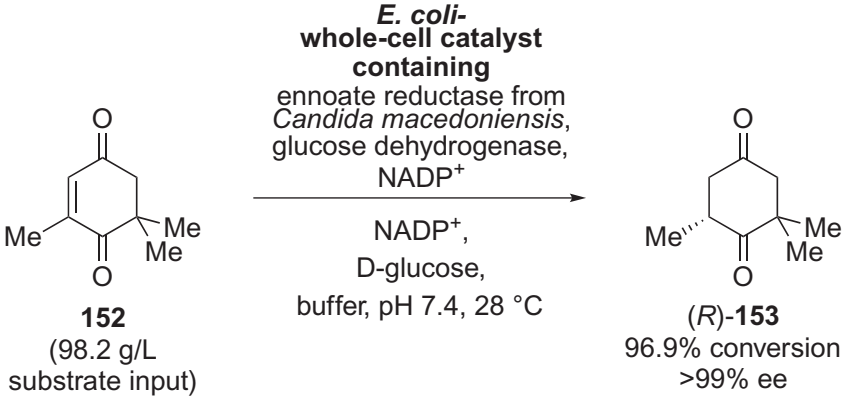
Scheme 6.65.

6.5.5. Reduction of Activated C=C Double Bonds

The biocatalytic reduction of activated C=C double bonds represents an enzymatic reaction with high application potential in organic synthesis [261]. By means of such a

reaction type, one can obtain access to, for example, chiral ketones, aldehydes, carboxylates and derivatives thereof, and nitroalkanes. However, in contrast to reduction of C=O double bonds of ketones and keto esters (see Section 6.5.2) with ADHs, the field of biocatalytic C=C reduction is still rarely explored. This is, in part, due to the limited number of readily available and sufficiently stable recombinant enoate reductases. The "workhorses" in this field are enoate reductases from lower fungi and bacteria, belonging to the family of so-called old yellow enzymes. These enzymes show a broad substrate range, and many of them are available in recombinant form.

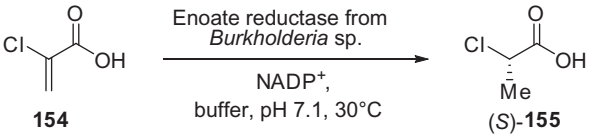
In the field of chiral ketone synthesis, an impressive contribution has been reported by Shimizu et al. in the asymmetric reduction of the C=C double bond in ketoisophorone (**152**) [262,263]. In the presence of a recombinant whole-cell catalyst overexpressing an enoate reductase from *Candida macedoniensis* and a GDH, the desired reduction of the C=C double bond proceeds under the formation of (*R*)-levodione ((*R*)-**153**) with both excellent conversion and enantioselectivity. Furthermore, the process runs at a high substrate concentration leading to (*R*)-levodione with 96.9% conversion at a substrate input of 98.2 g/L (Scheme 6.66). Thus, this process also fulfils the criteria for a technically feasible process and can be regarded as one of the major pioneering works in the field of asymmetric enzymatic C=C bond reduction.



Scheme 6.66.

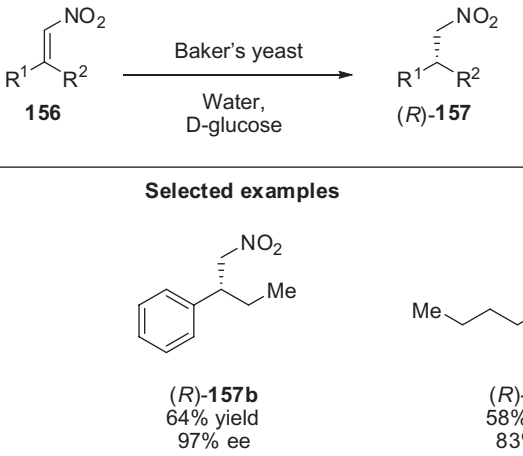
Other cyclic α,β-unsaturated enones serve as substrates as well, and reduction of these types of substrates can be carried out in a highly enantioselective manner as has been demonstrated by the Stewart group using recombinant old yellow enzymes [264].

Notably, also α,β-unsaturated carboxylic acids and their esters with very different substitution pattern can be used as substrates. For example, the C=C double bond in α-chloroacrylic acid (**154**) has been reduced in the presence of an enoate reductase from *Burkholderia* sp., leading to the desired α-chloropropionate (*S*)-**155**, which is an important pharmaceutical building block, in high enantioselectivity (Scheme 6.67) [265]. Enoate reductases were also applied successfully in asymmetric synthesis of γ-lactones, which were obtained in high enantiomeric excess [266]. In addition, enzymatic reduction of 2-decen-5-olide for the synthesis of δ-decalactone has found commercial interest due to its use as a constituent of natural flavorings [267].



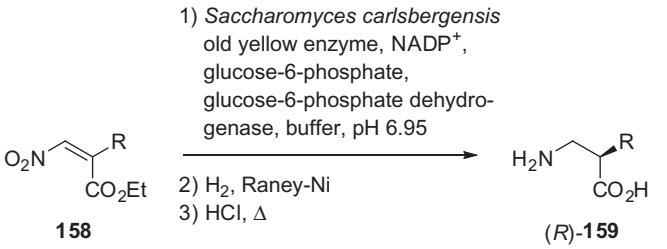
Scheme 6.67.

Besides enones, enals, and α,β-unsaturated esters, nitroalkenes are also suitable substrates for enoate reductases, which has been impressively demonstrated by the Otha group in their pioneering work (Scheme 6.68) [268]. A broad range of 1-nitro-1-alkenes of type **156** were reduced enantioselectively in the presence of baker's yeast, which contains enoate reductases. The synthesized 1-nitroalkanes (*R*)-**157** were formed with enantiomeric excess of up to 98% ee. These products represent interesting intermediates for the formation of enantiomerically enriched primary amines through subsequent reduction of the nitro group.

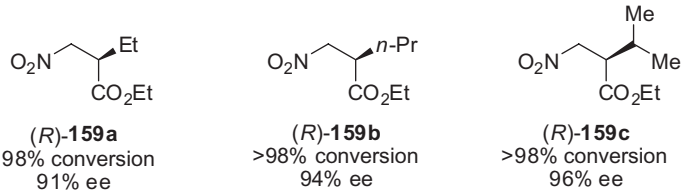


Scheme 6.68.

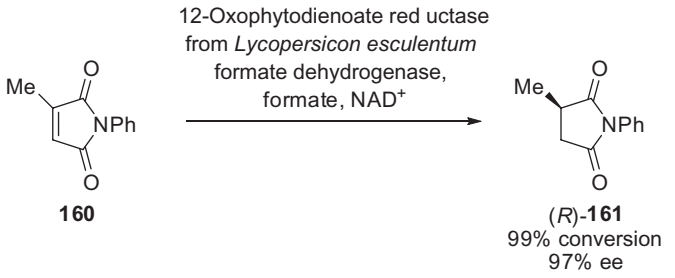
A further impressive application of enoate reductases has recently been reported by the Stewart group [269]. Nitroalkenes of type **158**, which were prepared starting from an α-keto ester and nitromethane in a Henry reaction and subsequent dehydration, turned out to be suitable substrates. The reduction of the C=C double bond in (Z)-nitroalkenes of type **158** proceeds very efficiently, leading to the corresponding 2-substituted 3-nitropropanoates, which were subsequently transformed as crude products into the corresponding chiral β^2-amino acid esters (*R*)-**159**. These products (*R*)-**159** were obtained with a high overall conversion of up to >98% and in most cases high enantiomeric excess of up to 96% ee. Selected examples are given in Scheme 6.69.



Selected examples



Scheme 6.69.



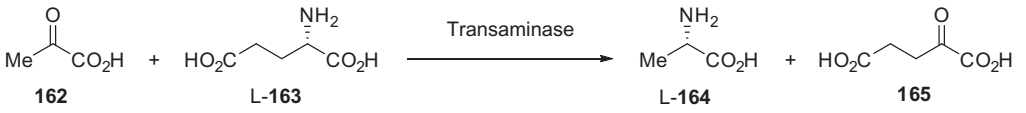
Scheme 6.70.

In spite of high suitability of these enoate reductases, there is an increasing demand for further enzymes capable of reducing C=C double bonds, thus expanding the diversity of biocatalysts for this important reaction. Recently, various microbial enzymes have been studied with respect to their application in organic synthesis, and recombinant forms thereof are now available [270,271]. A further elegant step in this direction has been recently reported by Faber et al., demonstrating the suitability of an 12-oxophytodienoate reductase from *Lycopersicon esculentum* (tomato) as an air-stable enoate reductase in organic syntheses [271,272]. This example also shows that enzymes of plant origin can be used as efficient biocatalysts in organic synthesis. A broad range of activated alkenes have been successfully reduced, leading to high enantioselectivities in most cases. For example, reduction of an α-substituted maleimide **160** proceeds under the formation of the desired product (*R*)-**161** with excellent 99% conversion and 97% ee when using an FDH for cofactor regeneration (Scheme 6.70).

6.5.6. Transamination

Besides amino acid dehydrogenases, further catalysts suitable for the transformation of carbonyl functionality into an amine moiety are transaminases. Notably, depending on the type of transaminase, both α-keto acids and ketones are tolerated as substrates, thus leading to α-amino acids and amines with a stereogenic center in α-position, respectively.

The synthesis of chiral α-amino acid starting from keto acids by means of a transamination has been developed by NSC Technologies [273,274]. This process can be used for the synthesis of both L- and D-amino acids and is based on the transfer of an amino group from an inexpensive amino donor, for example, L-glutamic acid or L-aspartic acid, to the carbonyl moiety of the keto acid substrate. This reaction is catalyzed by a transaminase (aminotransferase) and requires pyridoxal phosphate as a cofactor (which is bound to the transaminase). A broad substrate range has been observed and enantioselectivities are excellent in general, thus leading to the desired D- or L-amino acids in enantiomerically pure form [275]. For example, starting from pyruvic acid (**162**) the desired product L-alanine (L-**164**) is formed in an efficient transamination process with an impressive space-time yield of 4.8 kg/(L·d) when using L-glutamic acid (L-**164**) as an amino donor (Scheme 6.71). Furthermore, several nonproteinogenic α-amino acids such as L-phosphinothricine, L-homophenylalanine, and L-*tert*-leucine have been produced as well using transamination.

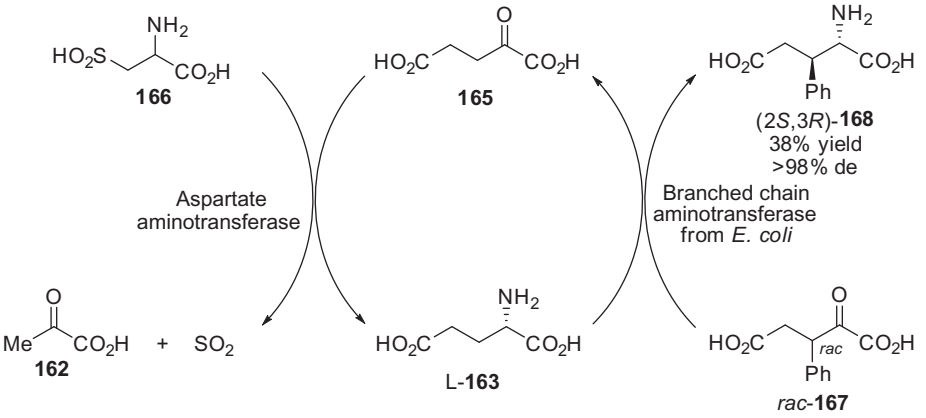


162 L-**163** L-**164** **165**

Scheme 6.71.

A drawback of transaminations is incomplete reactions (with yields typically around 50% in "standard" processes) due to thermodynamic reasons [275,276]. This problem has been overcome by coupling the transamination reaction with a subsequent reaction, which consumes the synthesized α-keto acid (as an undesired side product) in an irreversible step. For example, decarboxylation of oxaloacetate, which is the keto acid side product when using L-aspartate as amino donor, turned out to be such a suitable subsequent irreversible step [277].

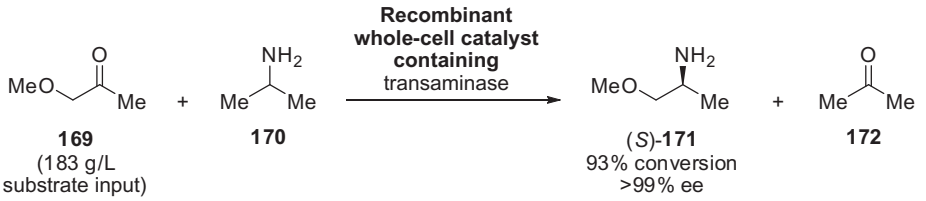
Other efficient approaches to shift the equilibrium in the desired direction have been developed by the Gefflaut group [278].These methods are based on a coupling of the transaminase process with either an irreversible aspartate aminotransferase-catalyzed transamination process using cysteine sulfinic acid (**166**) as an amino donor or an amino dehydrogenase-catalyzed reaction under *in situ* cofactor recycling. In the latter methodology, the applied cofactor recycling is based on the use of formate in combination with an FDH. In the presence of a branched chain aminotransferase from *E. coli*, these types of transaminations turned out to be suitable for the synthesis of various types of nonnatural, 3- or 4-substituted glutamic acid analogues, for example, (2*S*,3*R*)-**168**. A selected example is shown in Scheme 6.72.



Scheme 6.72.

Furthermore, ω-transaminases turned out to be capable for the transformation of a range of prochiral ketones into the corresponding enantiomerically pure amines (bearing a primary amino moiety).

A highlight in this field is the highly efficient synthesis of (*S*)-methoxyisoproylamine ((*S*)-**171**), which is an intermediate for the production of the herbicide metolachlor [279]. Based on a recombinant whole-cell catalyst overexpressing a transaminase, which was optimized through directed evolution, an impressive synthesis of this molecule was developed by Celgene researchers. A key feature is the high substrate concentration of 2.08 M, corresponding to 183 g/L. The desired (*S*)-enantiomer (*S*)-**171** was obtained with excellent >99% ee (Scheme 6.73). Isopropylamine (**170**) was used as an amine donor. The high conversion of 93% was achieved through removal of acetone in vacuo. Besides this process, Celgene also applied this transaminase technology for numerous other types of chiral (*S*)- and (*R*)-amines. Furthermore, this transamination technology has been scaled up to the production of chiral amines on a >500-kg scale [280].



Scheme 6.73.

Besides the use of isopropylamine as an amine source, L-amino acids also represent suitable cosubstrates. The use of L-alanine as an alternative amino donor has been investigated by the Kim group [281]. To make this process synthetically useful, biocatalytic removal of the pyruvic acid formed as a result of the oxidation of the cosubstrate L-alanine was required. Thus, the equilibrium is shifted in the desired direction, leading

to a high yield of the transamination product. This was achieved in particular by means of transaminase-containing recombinant whole cells, which are able to consume the formed pyruvic acid. When using such types of recombinant whole cells, (*S*)-phenylethyl-1-amine was formed with 90% conversion and an enantioselectivity of >99% ee.

Other efficient methodologies to shift the equilibrium by means of enzymatic derivatization of the formed α-keto acids have been reported recently by the Bornscheuer group and Kroutil group. The Bornscheuer group applied a pyruvate decarboxylase for the removal of pyruvate through decarboxylation and formation of carbon dioxide in an irreversible process. In the presence of such a combination of transaminase and pyruvate decarboxylase, the transamination of 1-*N*-Boc-3-oxopyrrolidine gave the corresponding (*S*)-amine with 80% conversion and 99% ee [282]. The Kroutil group used an alanine dehydrogenase and an FDH to regenerate L-alanine from pyruvate under the formation of carbon dioxide from formate. By means of this methodology, the transamination reactions proceed with both high conversion (of up to >99%) and enantioselectivity (of up to >99% ee) [283].

For a long time, enzymatic transamination of ketones has been limited to the synthesis of (*S*)-amines. The extension of this technology toward the (*R*)-enantiomeric forms has been recently reported by Hasegawa et al. by means of a transaminase, which requires in particular α-methylbenzylamine as an amine donor [284].

6.6. ASYMMETRIC OXIDATIONS

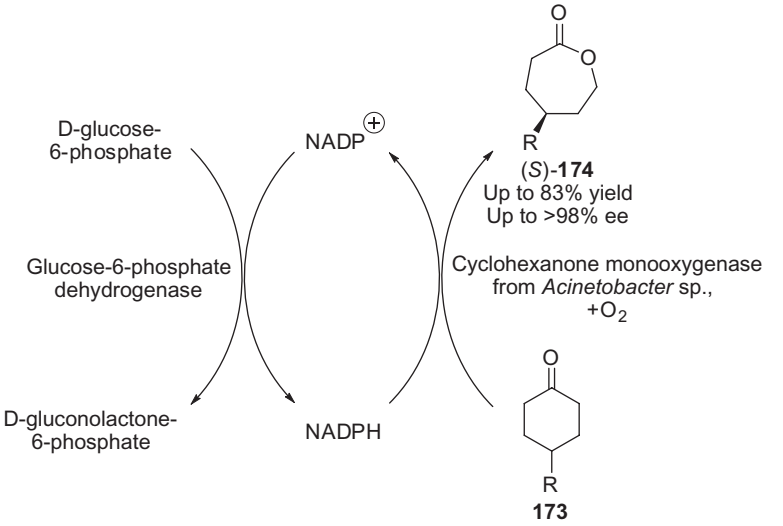
6.6.1. Overview

Asymmetric oxidations play an important role in organic synthesis. Many valuable, highly efficient chemocatalytic methods have been developed. Besides chemocatalysts, enzymes turned out to represent useful (bio-)catalysts. Notably, a broad range of different types of oxidation reactions can be catalyzed by biocatalysts. To start with the oxidation of a carbon atom, one can convert a ketone moiety into an ester in an asymmetric fashion by means of a so-called Baeyer–Villiger monooxygenase. When starting from alkenes, a range of monooxygenases turned out to be suitable for enantioselective epoxidation. A further challenging reaction that can be catalyzed by monooxygenases is the enantioselective hydroxylation of alkanes. The asymmetric synthesis of chiral amino moieties can be achieved by resolutions based on enzymatic amine oxidation or amino acid dehydrogenation. Heteroatoms can be oxidized as well, which has been demonstrated for, for example, sulfoxidation. In the following, these enzymatic asymmetric oxidations are described in more detail.

6.6.2. Baeyer–Villiger Oxidation

The Baeyer–Villiger reaction is a key reaction in organic chemistry. Although known for more than 100 years, asymmetric catalytic Baeyer–Villiger reactions remained a challenge. Depending on the type of ketone substrate, enantioselective Baeyer–Villiger oxidation can be carried out as a resolution of racemic ketones as well as as an asymmetric desymmetrization reaction when starting from a prochiral ketone. Since a quantitative yield can theoretically be obtained in the latter version, asymmetric desymmetrizations are particularly attractive. As chiral catalysts, both chemocatalysts and enzymes have been developed and successfully used. Suitable enzymes for this type

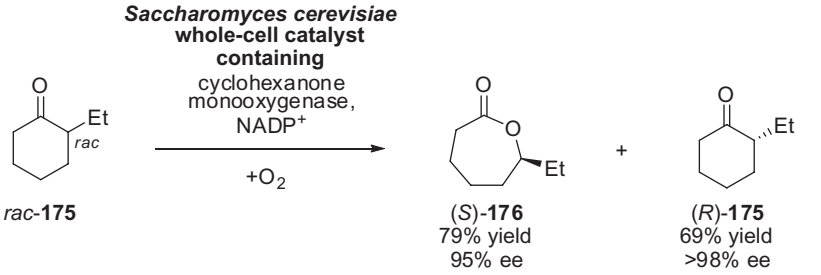
of reaction are known as Baeyer–Villiger monooxygenases [285,286]. Notably, these enzymes are also capable of oxidizing heteroatoms such as sulfur in sulfoxidation reactions (see Section 6.6.6). Baeyer–Villiger monooxygenases are cofactor-dependent enzymes and are usually obtained from microbial sources. The probably most widely used Baeyer–Villiger monooxygenase for synthetic purposes so far is a cyclohexanone monooxygenase from *Acinetobacter* sp. [286,287]. The enzymatic Baeyer–Villiger oxidation can be carried out with a broad range of substrates. To start with prochiral 4-substituted monocyclic cyclohexanones **173**, corresponding (*S*)-lactones (*S*)-**174** are formed in good yield and with high enantioselectivity of up to >98% ee in an enzymatic desymmetrization (Scheme 6.74) [288,289]. Notably, for this oxidation process, the reduced form of the cofactor (NADPH) is required under the formation of $NADP^+$, which is *in situ* recycled using an enzymatic coupled cofactor regeneration.



Scheme 6.74.

Further applications of monooxygenases have been desymmetrizations starting from *meso*-compounds. For example, 2,6- or 3,5-disubstituted (*meso*-)cyclohexanones have been converted successfully by Stewart et al. into the corresponding lactones with high enantioselectivity of up to >98% ee [290]. Furthermore, a series of prochiral 3-substituted cyclobutanones was converted by recombinant whole cells, overexpressing a Baeyer–Villiger monooxygenase, into the corresponding (*S*)-lactones with moderate to good enantioselectivities [291,292]. An application of the enzymatic Baeyer–Villiger oxidation for the synthesis of an intermediate of (*R*)-baclofen based on the use of 3-(*p*-chlorophenyl)-substituted cyclobutanone as prochiral substrate has been reported by the Furstoss group [293].

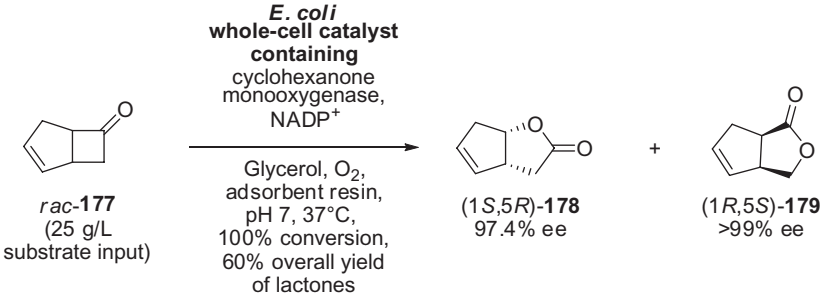
Besides desymmetrization, resolutions can also be carried out with Baeyer–Villiger monooxygenases when starting from, for example, racemic 2-substituted cyclohexanones [294,295]. Excellent enantioselectivities with E values of up to E>200 have been achieved by Stewart and Kayser et al. in a range of resolution processes. A selected example is



Scheme 6.75.

shown in Scheme 6.75. Further examples of successful applications of Baeyer–Villiger monooxygenases in resolution processes have been described by several groups for the resolution of racemic bicyclic ketones [296–298].

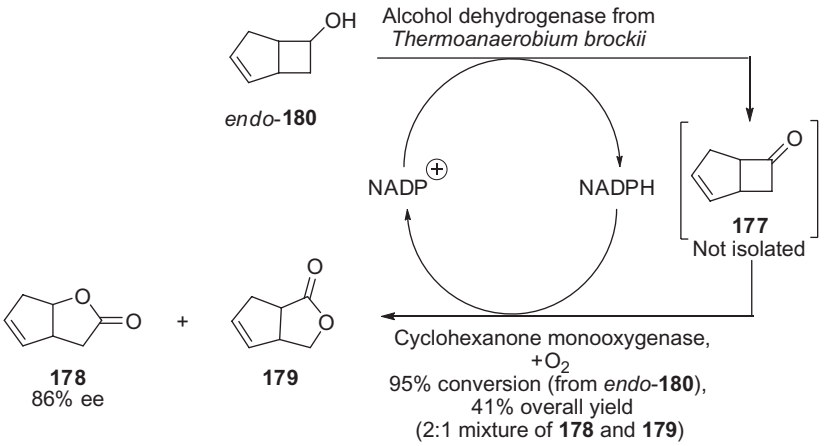
Due to the high efficiency of enzymatic Baeyer–Villiger reactions and availability of a recombinant production strain of the biocatalyst, scale-up of this methodology has been reported as well [299]. For example, a biotransformation at kilogram scale has been reported by Furstoss and coworkers in collaboration with Sigma-Aldrich for the enzymatic Baeyer–Villiger oxidation of racemic bicyclo[3.2.0]hept-2-en-one (*rac*-**177**) in a 50-L bioreactor. Besides a high productivity, high enantioselectivities were obtained for both regioisomeric lactones (1*S*,5*R*)-**178** and (1*R*,5*S*)-**179** when operating at a substrate input of 25 g/L (Scheme 6.76) [300].



Scheme 6.76.

As a very nice extension of the enzymatic Baeyer–Villiger oxidation, Alphand et al. reported a combination of this reaction (as a resolution) with an anion-exchange resin-catalyzed racemization of the substrate toward a dynamic kinetic resolution process [301]. High yields of up to 85% accompanied by excellent enantioselectivities of up to 97% ee were achieved when using racemic benzyloxycyclopentanone as a substrate.

A further process improvement with respect to the type of cofactor recycling was reported by the Willetts group. By coupling a cyclohexanone monooxygenase with an ADH from *T. brockii*, a cosubstrate-free "double oxidation" of an alcohol *endo*-**180** into lactones **178** and **179** is achieved (Scheme 6.77) [302]. The oxidized form of the cofactor



Scheme 6.77.

is consumed in the initial (ADH-catalyzed) step, while the resulting reduced form of the cofactor is then required for the second, monooxygenase-catalyzed oxidation step. In this second step, the oxidized form of the cofactor, which is then required for the first step, is generated again. This process technology has been successfully used for the regio- and enantioselective synthesis of **178**, which was obtained in 41% yield (as a mixture with regioisomer **179**) and with 86% ee.

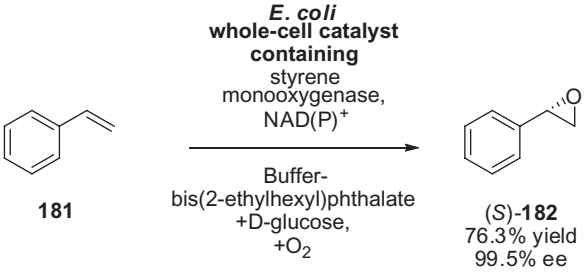
Very recently, the Bornscheuer group and Reetz group independently reported access to improved Baeyer–Villiger monooxygenase mutants by means of directed evolution technology [303], and its combination with site-directed mutagenesis [304].

6.6.3. Epoxidation of Alkenes

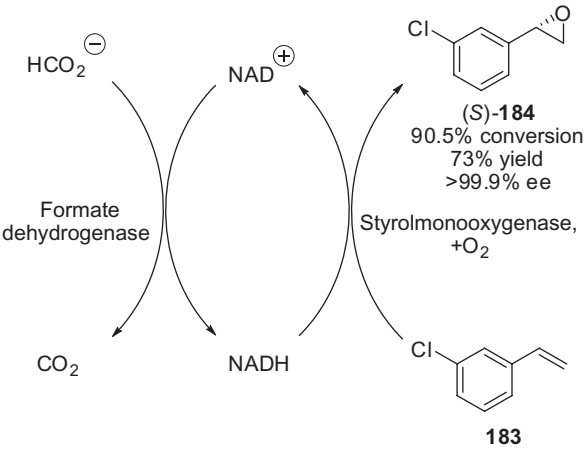
Asymmetric catalytic epoxidation of alkenes represents a straightforward approach to enantiomerically enriched epoxides. Besides metal catalysts and organocatalysts, cofactor-dependent monooxygenases turned out to be valuable catalyst for this type of reaction [305].

Pinoneer work on asymmetric biocatalytic epoxidation of (substituted) styrene has been done by the Schmid and Witholt group, who developed an efficient epoxidation methodology including an initial scale-up to a gram-scale synthesis of chiral oxiranes [306,307]. As a biocatalyst, recombinant *E. coli* whole cells overexpressing a styrene monooxygenase have been applied. Using glucose as the carbon source in a two-phase fed-batch process with bis(2-ethylhexyl)phthalate as organic solvent gave (*S*)-styrene oxide ((*S*)-**182**) with 99.5% ee (Scheme 6.78). Scale-up of this type of process up to a 30-L scale has been reported by the same group [308].

Furthermore, enzymatic asymmetric epoxidation has been carried out with isolated enzymes by coupling with an FDH-catalyzed regeneration of the cofactor NADH in organic–aqueous emulsions (Scheme 6.79) [309]. Using a stable recombinant FAD/NADH-dependent styrene monooxygenase StyAB as well as dodecane as an organic phase (which led to advantageously low substrate concentration in the aqueous phase,



Scheme 6.78.

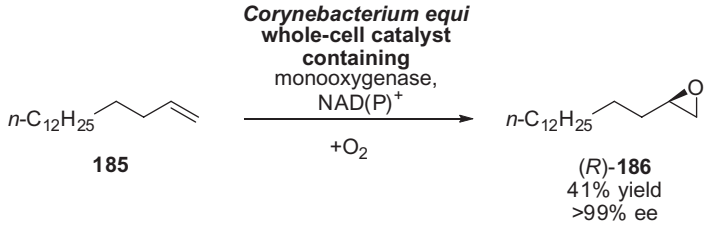


Scheme 6.79.

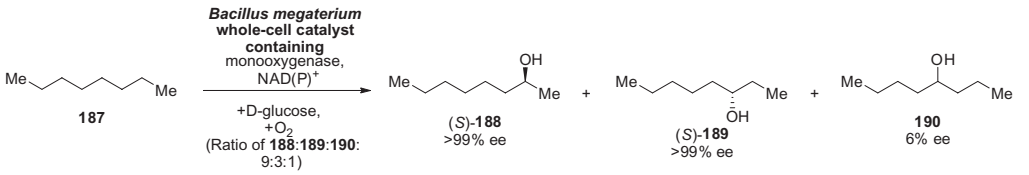
thus keeping deactivation of the monooxygenase to a minimum) gave (*R*)-3-chlorostyrene epoxide ((*S*)-**184**) with 90.5% conversion, in 73% yield and with >99.9% ee (Scheme 6.79). Other styrene derivatives have been epoxidized with both high conversion and enantioselectivity as well.

The Schmid group also reported the first direct regeneration of an FAD-dependent monooxygenase for the epoxidation of styrene leading to (*S*)-epoxides with >98% ee [310]. An organometallic Rh complex catalyzes the transhydrogenation between formate and the cofactor FAD, thus delivering FADH2 required as cofactor for the applied of styrene monooxygenase StyA. Furthermore, the Schmid group reported a direct electrochemical regeneration of FAD within a process for the asymmetric monooxygenase-catalyzed epoxidation [311].

The Ohta group reported the epoxidation of an aliphatic linear *n*-alkane in a pioneer work [312,313]. Notably, a high enantioselectivity was observed in the microbial epoxidation of hexadec-1-ene (**185**). The resulting epoxide (*R*)-**186** was obtained in 41% yield and with an excellent enantioselectivity of >99% ee (Scheme 6.80).



Scheme 6.80.



Scheme 6.81.

The enantioselectivity of monooxygenases has been improved by the Arnold group [314]. Applying an engineered cytochrome P450 BM-3 enzyme to the epoxidation of several terminal aliphatic alkenes led to enantioselectivities between 55% ee and 83% ee. Notably, inversion of enantioselectivity in the epoxidation of styrene has been achieved by the Schwaneberg group by means of a single mutation in the P450 BM3 monooxygenase [315]. This mutant was found via directed evolution

Furthermore, a regio- and enantioselective epoxidation of linolenic has been developed by the Turner group using a P450-monooxygenase from *B. megaterium* [316]. In the presence of this biocatalyst, an enantioselectivity of 60% ee was obtained.

6.6.4. Hydroxylation of Alkanes

Selective functionalization of alkanes is still among the most current challenges in the field of organic chemistry. Enzymes, namely monooxygenases, turned out to be versatile and selective catalysts [317–319]. In some fields, in particular related to steroid hydroxylation, technical applications have already been reported [320]. In the following, a brief overview of main achievements in asymmetric enzymatic hydroxylation in recent years will be given. It is noteworthy that in spite of excellent selectivities for many oxidoreductase-catalyzed reactions (e.g., reductions with ADHs; see Section 6.5.2), highly asymmetric hydroxylation protocols are still rare.

Pioneer work in the field of asymmetric hydroxylation has been done by the Adam group [321]. When using a *B. megaterium* strain as a whole-cell biocatalyst, asymmetric hydroxylation proceeds with a range of linear *n*-alkanes such as *n*-heptane and *n*-octane (**187**) in a highly enantioselective fashion. However, regioselectivity still needs improvement since different types of regioisomers are formed in significant amount. Furthermore, undesired "overoxidation" under formation of the corresponding ketones from the alcohols plays a role. An example for the formation of regiosiomers with up to >99% ee is shown in Scheme 6.81.

A recombinant P450-monooxygenase from *B. megaterium* was engineered by Arnold et al. and turned out to be suitable for stereoselective hydroxylation of a cyclopentanecarboxylate derivative [322]. In the presence of various mutants, the desired hydroxylation proceeds with both high diastereoselectivity (of up to 96% de) and enantioselectivity (of up to 89% ee).

The hydroxylation of *N*-benzyl pyrrolidine with a recombinant *E. coli* strain bearing an alkane hydroxylase was reported by the Witholt group [323]. The desired *N*-benzyl (*R*)-3-hydroxypyrrolidine was formed with 70% ee. When replacing the *N*-benzyl group with other *N*-protecting groups, the corresponding substrates were hydroxylated enzymatically with enantioselectivities of up to 75% ee [324]. An enantioselective hydroxylation of 2- and 3-alkylpyridines based on the use of whole cells of *P. putida* was described by the Sheldrake group [325].

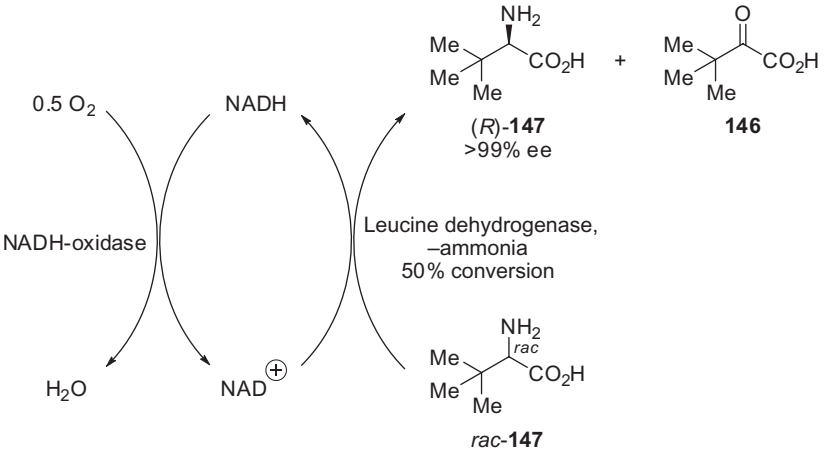
So far, a main application area of monooxygenases on large scale is the regio- and diastereoselective hydroxylation of steroid molecules. Although not starting from a prochiral substrate, this technology is briefly described in the following. An example for a technical application of monooxygenases in this field represents the diastereo- and regioselective 11β-hydroxylation of the steroid Reichstein S to hydrocortisone using *Curvularia* sp. whole cells. This process runs at about 100 t per year at Schering [326,327]. The industrial applications of monooxygenases at Schering have been summarized in a recent review [320]. A further application of monooxygenases for steroid functionalization, which has been developed by Pharmacia and Upjohn, is the conversion of progesterone to cortisone by *Rhizopus* sp. whole cells [328].

6.6.5. Oxidation of Amines, Amino Acids, and Alcohols

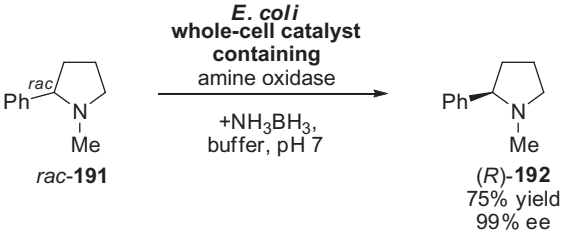
The enantioselective oxidation of (racemic) amines can be used for the preparation of enantiomerically pure amines, which play an important role as intermediates in drugs synthesis. Although this route still represents a resolution process with the limitation of a maximum of 50% yield, it turned out to be very useful for, in particular, the preparation of unnatural D-amino acids and chiral amines. In the latter case, dynamic resolution processes have been developed as well. Either amine oxidases (in case of amines) or amino acid dehydrogenases in combination with an NADH-oxidase (for D-amino acid synthesis) have been used as enzymes. Both concepts are described in the following.

In the presence of amino acid dehydrogenases, L-amino acids can be oxidized under the formation of keto acids [329]. The required oxidized form of the cofactor, NAD^+, is reduced to NADH. *In situ* recycling of the cofactor is then carried out using an NADH-oxidase, which makes use of the (expensive) cofactor in catalytic amounts possible. This reaction is particularly attractive for the synthesis of D-amino acids when starting from easily available racemic amino acids as substrates. A prerequisite is a (nearly) quantitative conversion of 50% to obtain high enantioselectivities. The Hummel group applied such a concept successfully for the synthesis of D-*tert*-leucine ((*R*)-**147**) starting from racemic *tert*-leucine (*rac*-**147**) as a starting material [329]. When using a leucine amino dehydrogenase and an NADH-oxidase from *E. coli*, the desired D-*tert*-leucine ((*R*)-**147**) is formed with excellent enantioselectivity of >99% ee, which also indicates quantitative oxidation of the L-enantiomer (Scheme 6.82).

A highly efficient approach toward enantiomerically pure primary, secondary, and tertiary amines by means of an amine oxidase has been reported by the Turner group [330–332]. Starting from the corresponding racemic amines (or their imine and iminium



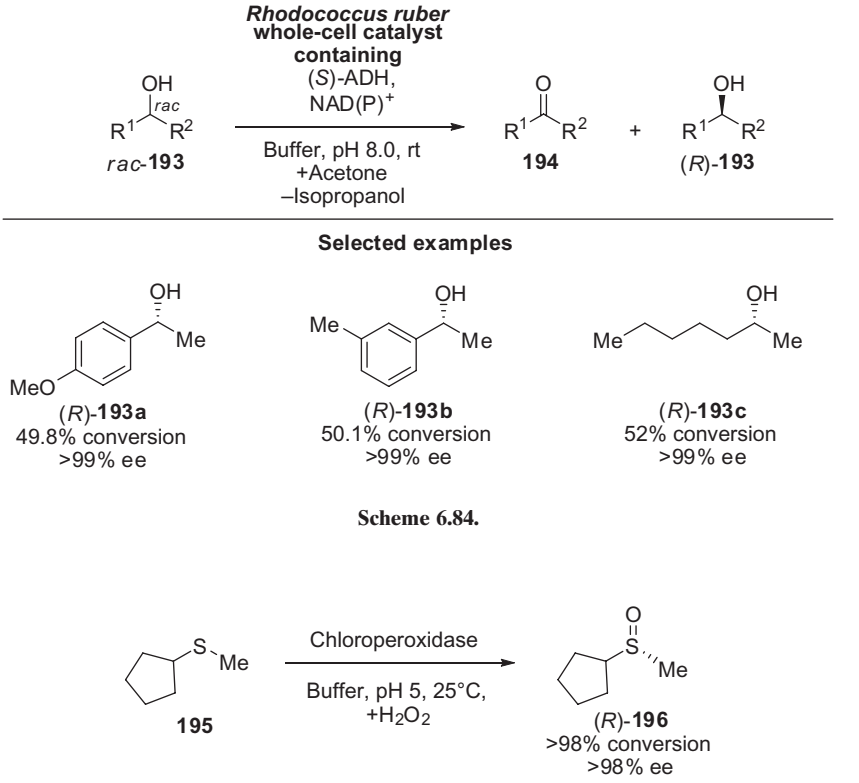
Scheme 6.82.



Scheme 6.83.

precursors in case of cyclic secondary and tertiary amines), an amine oxidase-catalyzed enantioselective oxidation of one enantiomer under imine formation and nonasymmetric *in situ* reduction of the imine with a borohydride were combined in a one-pot synthesis. By means of such an enantioselective chemoenzymatic process, amines were obtained in good yield and excellent enantioselectivity of 99% ee. A selected example for such an impressive deracemization process starting from a racemic cyclic tertiary amine is given in Scheme 6.83 [332]. This chemoenzymatic one-pot methodology complements the highly efficient acylation technology with lipases for the enantioselective synthesis of amines.

The (bio-)transformation of secondary alcohols into ketones by means of ADHs has been investigated as well. For this reaction, the oxidized cofactor form, $NAD(P)^+$, is required. The Kroutil and Faber group reported a highly efficient resolution of racemic secondary alcohols by means of an enantioselective oxidation in the presence of an ADH from *R. ruber* [185]. *In situ* cofactor recycling of formed NADPH was carried out using acetone, which is converted into isopropanol under the formation of $NADP^+$. Selected examples are shown in Scheme 6.84.



Scheme 6.84.

Scheme 6.85.

Alternatively, cofactor regeneration can also be carried out using an NADH-oxidase. This has been demonstrated by the Hummel group for the resolution of racemic phenylethan-1-ol [333]. In this process, the (*R*)-enantiomer is completely oxidized, thus resulting in an enantiomerically pure remaining (*S*)-enantiomer of this chiral alcohol.

6.6.6. Sulfoxidation

A further interesting oxidation reaction is the sulfoxidation for the enantioselective preparation of chiral sulfoxides. Dialkyl sulfides serve as suitable substrates for enzymatic sulfoxidation. For example, the Colonna group reported the enantioselective sulfoxidation of a range of dialkyl sulfides in the presence of a chloroperoxidase or cyclohexanone monooxygenase [334]. For example, using a chloroperoxidase as a biocatalyst gave both an excellent conversion (>98%) and enantioselectivity (>98% ee) when using cyclopentyl methyl sulfide (**194**) as a starting material in the sulfoxidation reaction (Scheme 6.85).

In addition, the enantioselective biocatalytic sulfoxidation has been reported for a range of aromatic sulfides [335,336] and for 1,3-dithioacetals [337].

6.7. SUMMARY

In summary, a broad range of enzymes turned out to be efficient catalysts in asymmetric synthesis. Often, excellent enantioselectivities are achieved, thus making biocatalysis an attractive tool for the enantioselective preparation of chiral molecules. Enzymes can be used in different forms, such as isolated and immobilized enzymes or whole-cell catalysts. The use of recombinant whole cells, containing the desired enzymes in overexpressed form, is gaining more and more attention. In particular, recombinant whole-cell catalysts are attractive in the field of redox biocatalysis due to the need of (often) more than one enzyme for the biotransformation. Besides excellent enantioselectivities (as a key feature of many enzymatic processes), high conversions at high substrate concentrations have also been realized for a broad range of biotransformations. Thus, it is no surprise that there is also an increasing tendency to apply biocatalysis on industrial scale, and numerous biocatalytic manufacturing processes are already running successfully on industrial scale. Without any doubt, in the future, we can expect further efficient biocatalytic syntheses on lab and technical scales.

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