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Key Building Blocks via Enzyme-Mediated Synthesis

Thomas Fischer and Jörg Pietruszka

Abstract Biocatalytic approaches to valuable building blocks in organic synthesis have emerged as an important tool in the last few years. While first applications were mainly based on hydrolases, other enzyme classes such as oxidoreductases or lyases moved into the focus of research. Nowadays, a vast number of biotransformations can be found in the chemical and pharmaceutical industries delivering fine chemicals or drugs. The mild reaction conditions, high stereo-, regio-, and chemoselectivities, and the often shortened reaction pathways lead to economical and ecological advantages of enzymatic conversions. Due to the enormous number of enzyme-mediated syntheses, the present chapter is not meant to be a complete review, but to deliver comprehensive insights into well established enzymatic systems and recent advances in the application of enzymes in natural product synthesis. Furthermore, it is focused on the most frequently used enzymes or enzyme classes not covered elsewhere in the present volume.

Keywords Asymmetric catalysis, Biocatalysis, Chiral building blocks, Enzymes, Natural products

Contents

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Abbreviations

2,5-/3,6-DKCMO	2,5-/3,6-Diketocamphane 1,2-monooxygenase
Ac	Acetyl
ADH	Alcohol dehydrogenase
BAL	Benzaldehyde lyase
BVMO	Baeyer–Villiger monooxygenase
CAL-B	*Candida antarctica* lipase B
CHD	Cyclohexadienediol
CHMO	Cyclohexanone monooxygenase
CPMO	Cyclopentanone monooxygenase
CYP450	Cytochrome P450 monooxygenase
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DERA	2-Deoxy-D-ribose 5-phosphate aldolase
DHAP	Dihydroxyacetone phosphate
DKR	Dynamic kinetic resolution
DMAP	4-*N*,*N*-Dimethylaminopyridin
EDC	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide
ee	Enantiomeric excess
Et	Ethyl
FDH	Formate dehydrogenase
GDH	Glucose dehydrogenase
GluDH	Glutamate dehydrogenase
HHDH	Halohydrin dehydrogenase
HNL	Hydroxynitrile lyase
KRED	Ketoreductase
Me	Methyl
NCR	NAD(P)H-dependent 2-cyclohexen-1-one reductase
n-Pe	*n*-Pentyl
OYE	Old yellow enzyme
PAL	*Pseudomonas aeruginosa* lipase
PCC	Pyridinium chlorochromate
PDC	Pyruvate decarboxylase

Ph	Phenyl
PheDH	Phenylalanine dehydrogenase
PMB	*p*-Methoxybenzyl
PMP	*p*-Methoxyphenyl
PPL	Porcine pancreatic lipase
Pr	Propyl
rac	Racemic
TBS	*tert*-Butyldimethylsilyl

1 Introduction

Nowadays, an increasing number of new pharmaceuticals have a natural lead structure or are directly derived from nature [1–4]. Their often remarkable physiological activities are frequently linked to structures bearing a number of stereogenic centers. Consequently, asymmetric synthesis is becoming even more important both in industry and in academic research [5, 6].

For providing chiral intermediates, different possibilities of asymmetric catalysis have been established. Various examples of transition metal-catalyzed or organocatalytic reactions can be found in the literature [7–9]. Biocatalysis – the application of isolated enzymes or whole cells in biotransformations – has a long historical background, being already known in ancient Egypt and the Far East for the preparation of food and alcoholic beverages [8, 10]. Plants, fruit, and extracts thereof were used for these purposes in the past, but they still find applications in recent research [11–13]. For example, coconut water was investigated as a new biocatalytical system for organic synthesis with some very promising results (Fig. 1) [14].

Major advances in the knowledge of biochemical pathways and the establishment of computer-based predictions of three-dimensional structures of proteins led to the development of new microbiological methods, such as rational protein design or directed evolution, giving scientists the possibility to provide tailor-made biocatalysts [15–19]. These methodological works on the disclosure of new efficient biocatalysts are not explicitly mentioned in this review unless they were applied in natural product synthesis. Biotransformations do not always compete with known chemical syntheses, but rather complement the portfolio of catalytic methods in organic chemistry.

Enzymatic processes are basically considered to be environmentally benign due to the mild reaction conditions needed and the high enantio-, regio-, and chemoselectivity of the enzymes. The ecological effect of using water as a solvent is quite often counterbalanced by low solubility of the substrates, making organic solvent resistant enzymes necessary [20], or by problems in downstream-processing, in most cases extracting the product with huge amounts of organic solvents [21]. Nevertheless, a biotransformation can sometimes substitute heavy metal catalysts

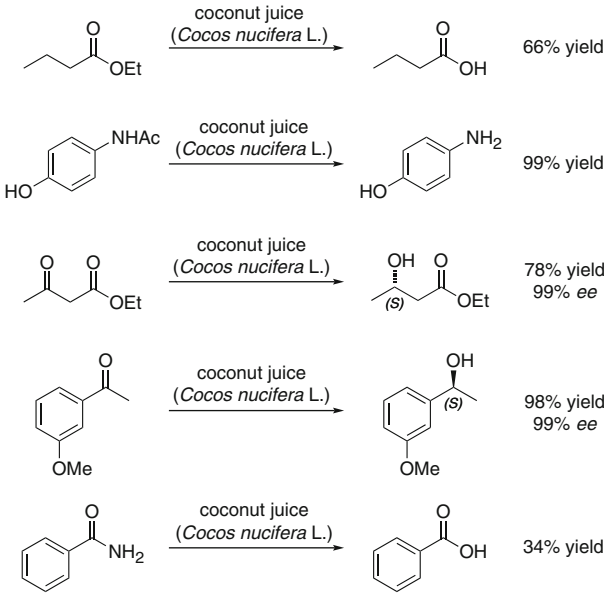

Fig. 1 Enzymatic reactions catalyzed by coconut juice (*Cocos nucifera*)

or raise immensely the efficiency of a process and thus deliver both ecological and economical advantages.

In general there are two principle possibilities using a biocatalyst in organic synthesis, namely as whole cells or as isolated enzymes – free or immobilized. The advantages and disadvantages of each can be intensively discussed, but the outcome of this consideration always depends on the whole system and the kind of application. There are numerous examples of both and thus there is no partitioning between whole cell biotransformation and isolated enzymes in this review.

Biocatalytic approaches to key building blocks in the synthesis of natural products and pharmaceuticals have been reviewed and summarized in various books in the last few years [22–48]. The variety of enzymes that were used is rising day by day, so this chapter is only meant to be instructive, not complete. The compilation is also restricted to the most frequently used enzyme classes, trying to give brief insights by selected examples of some older, but mainly recent, applications.

2 Lipases and Esterases in Organic Synthesis

Hydrolases are widely used enzymes in organic synthesis, with most applications concentrating on lipases and esterases. This chapter discloses the possibilities of asymmetric accesses to chiral building blocks for the synthesis of natural products

using lipases and esterases. Other hydrolases, such as amidases or peptidases, are not dealt with explicitly.

Asymmetric synthesis with lipases and esterases can basically be performed by two different approaches – the desymmetrization of prochiral or meso compounds and the enzymatic kinetic resolution of racemic mixtures. The main bottleneck of kinetic resolutions, product yields of maximum 50%, can be overcome if an in situ racemization of the starting material is possible. In this case all starting material can theoretically be converted to the desired product [34].

2.1 Lipase-Catalyzed Desymmetrizations

Desymmetrization of prochiral or meso compounds is a powerful tool in organic synthesis, yielding key building blocks with high enantiopurity [49]. Therefore, chiral reagents or catalysts can be used to achieve the differentiation between enantiotopic groups. Some of the enantioselective enzymatic desymmetrizations using lipases that have been described in the literature represent interesting opportunities in natural product synthesis.

Enantiomerically pure cyclopropanes are a frequent motif in the structure of natural products. Their synthesis is often demanding and many approaches have been made [50, 51]. Porcine pancreatic lipase (PPL) was used for the stereoselective desymmetrization of a cyclopropane dibutanoate (Fig. 2). The asymmetric hydrolysis of the meso compound yielded the corresponding enantiopure alcohol almost quantitatively. The intermediate obtained was successfully applied in the total synthesis of dictyopterenes A and C, sexual pheromones of brown algae [52], and constanolactones (see below) [53].

Due to its structural complexity and interesting physiological activities as an antifungal agent and especially as an immunosuppressive drug, the macrolide rapamycin has been a target of many total syntheses [54–58]. In their approach, Ley and co-workers used a lipase-catalyzed desymmetrization of a meso diol (Fig. 3). In early studies, the selective acetylation of *meso*-2,4-dimethylpentane-1,5-diol was achieved by a PPL immobilized on celite with moderate yields and 92%

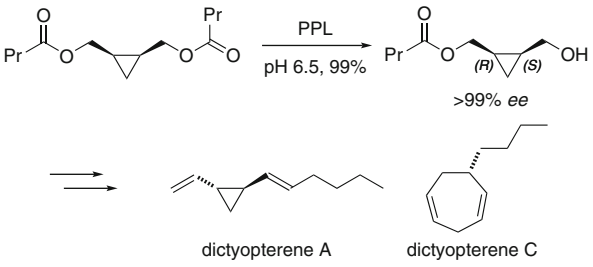

Fig. 2 Chemoenzymatic synthesis of dictyopterenes A and C

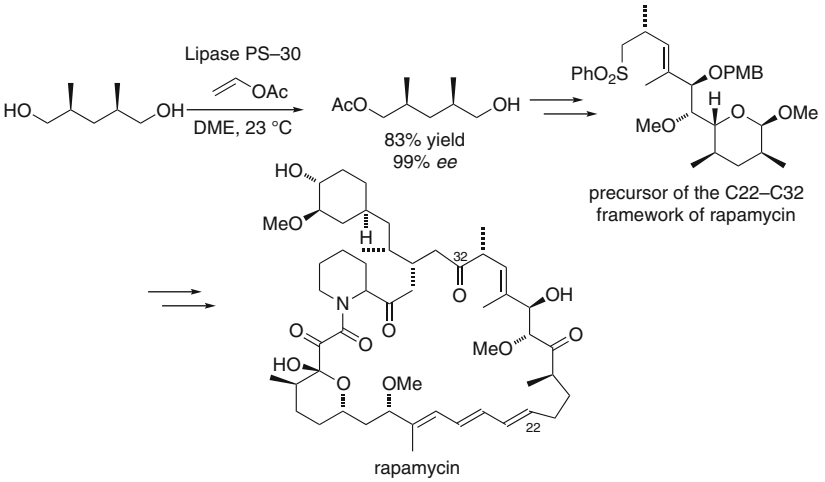

Fig. 3 Enantioselective acylation catalyzed by porcine pancreatic lipase (PPL)

enantiomeric excess (*ee*) [59]. Later, advances in the enzymatic desymmetrization led to the substitution of the catalyst with the also commercially available and inexpensive Lipase PS-30, providing the monoacetate in 83% yield and 99% *ee* [60, 61].

2.2 Kinetic Resolutions with Lipases and Esterases

Kinetic resolutions in general are regularly applied in organic synthesis. Since enzymes are highly attractive for asymmetric synthesis, various types of biocatalysts have been used in enzymatic (dynamic) kinetic resolutions, but the focus will remain on lipase- and esterase-mediated resolutions as the most common tools in early steps of natural product syntheses.

The enantiomeric excess of substrate and product shifts during the course of a kinetic resolution, making the determination of the efficiency of the reaction dependent on the reaction time. Hence, two kinetic resolutions can only be compared at the same extent of conversion [45, 62]. For a better comparison of two reactions, equations for the calculation of the enantioselectivity were established [63, 64]. In most cases, the enantiomeric excess of both substrate and product is determined for greater accuracy, but the determination of one and the conversion can also be used for the calculation of the *E* value (enantioselectivity):

$$E = \frac{ln[1 - c(1 + ee_p)]}{ln[1 - c(1 - ee_p)]}; \quad E = \frac{ln[(1 - c)(1 - ee_s)]}{ln[(1 - c)(1 + ee_s)]}; \quad E = \frac{ln\left[\dfrac{1 - ee_s}{1 + (ee_s/ee_p)}\right]}{ln\left[\dfrac{1 + ee_s}{1 + (ee_s/ee_p)}\right]} \quad (1)$$

E values above 100 are very inaccurate due to the logarithmic function, so that usually such high enantioselectivities are stated as $E \geq 100$. In these calculations the *E* value increases with increasing enantioselectivity. For the enzymatic kinetic resolutions, only *E* values greater than 20 indicate a potentially useful application in organic synthesis.

However, this theoretical approach does not cover all side effects of applied biocatalysis. The calculation of *E* values for enzyme mixtures or in systems with inherent inhibitory effects must be handled with care. Nevertheless, calculating *E* from (1) usually provides a very useful tool for estimating the efficiency of a resolution system.

An example for the application of enzymatic kinetic resolutions with high *E* values in natural product synthesis is the chemoenzymatic synthesis of the northern half of epothilones (also see Sect. 4.1). Various lipases and esterases could be found with outstanding enantioselectivity (up to >100); among these were lipase B from *Candida antarctica*, a lipase from *Burkholderia cepacia*, a lipase from *Pseudomonas* sp., and a lipase from *Streptomyces diastochromogenes*, all affording the desired (*S*)-configurated alcohol with >99% enantiomeric excess (Fig. 4) [65].

Allenes are versatile intermediates in organic synthesis and a variety of useful applications has been established [66]. A very interesting feature of allenes is their axial chirality along the cumulated diene system, so that optically active allenes were frequently used in asymmetric synthesis, in most cases being prepared from enantiomerically enriched precursors.

Since allenic systems are rather rare in nature, enzymatic approaches were hardly investigated due to a lack of known organisms metabolizing these structures. Recently, a mutant of a lipase from *Pseudomonas aeruginosa* (PAL) was generated by directed evolution, which was applied in the kinetic resolution of an allenic *p*-nitrophenolester [67]. Further investigations on the possibility of lipase-catalyzed kinetic resolutions of allenes with different lipases led to the application of PPL in the total synthesis of (−)-striatisporolide A, a fungal metabolite isolated from an

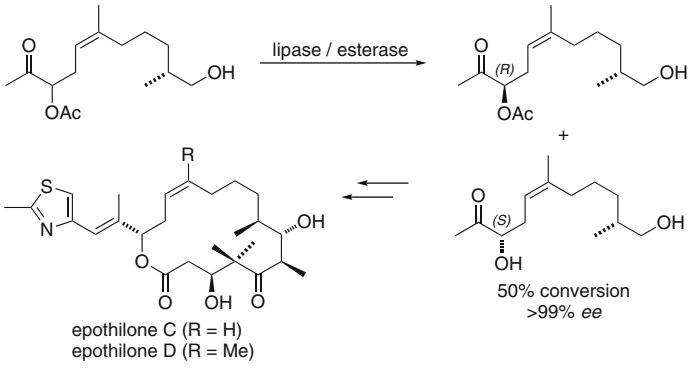

Fig. 4 Enzymatic kinetic resolution of epothilone precursors

Australian *Penicillium striatisporum* strain (Fig. 5) [68]. Before, neither a synthetic approach, nor the absolute configuration of this butenolide had been reported. Combining the enzymatic kinetic resolution with a palladium(II)-mediated racemization [69] could lead to a highly efficient dynamic kinetic resolution (DKR) of allenes.

The complex tetramic acid lactam cylindramide was isolated from the marine sponge *Halichondria cylindrata* and is known to possess a significant cytotoxic activity against human B16 melanoma cells [70]. A chemoenzymatic access to the characteristical substituted bicyclo[3.3.0]octane moiety was recently established, using lipase PS (Amano) for an enzymatic kinetic resolution (Fig. 6) [71]. The racemic diol was generated by a transannular Pd-catalyzed ring-closure of cycloocta-1,5-diene and removal of the acetyl groups. The resolution step provided the (1*S*,3a*R*,4*S*,6a*R*)-configured diol in 44% yield with 98% *ee*. Side products, the monoacetate (8%) and the (1*R*,3a*S*,4*R*,6a*S*)-configured diacetate (38%), could easily be separated by chromatography.

Psymberin (irciniastatin A), a cytotoxin of the pederin family, has been isolated from the marine sponges *Psammocinia* sp. and *Ircinia ramosa*. Its highly cytostatic

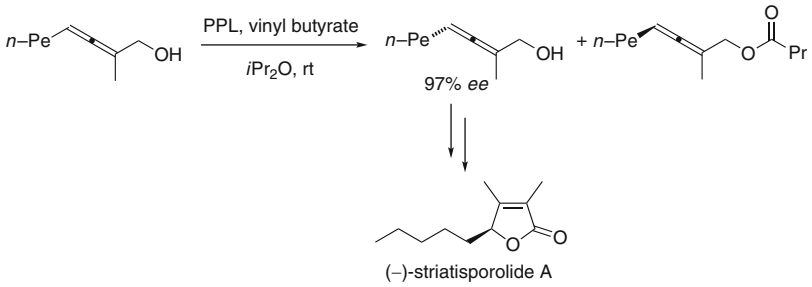

Fig. 5 Enzymatic kinetic resolution of primary allenic alcohols

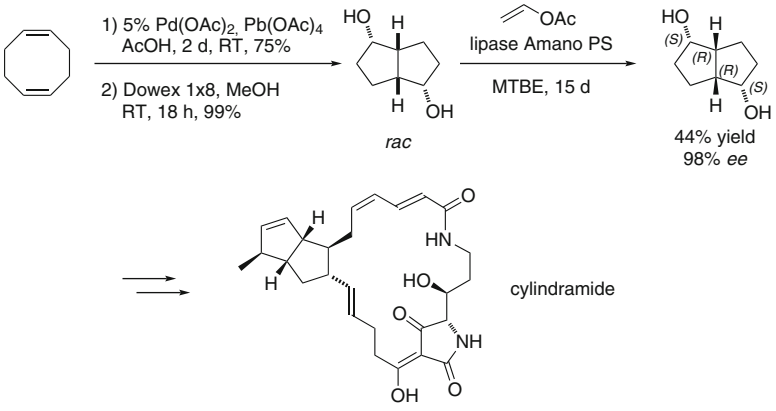

Fig. 6 Enzymatic kinetic resolution in the total synthesis of cyclindramide

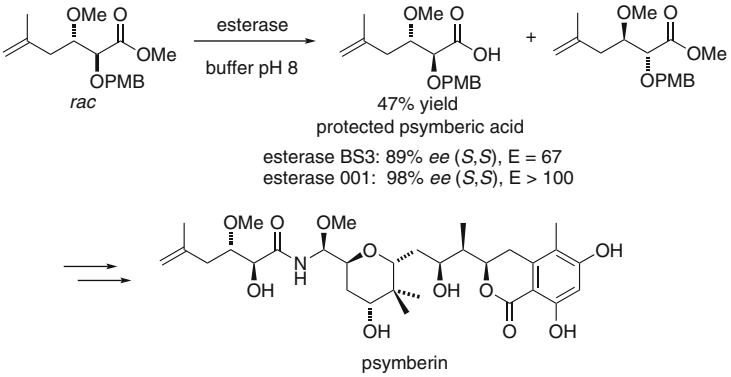

Fig. 7 Chemoenzymatic synthesis of PMB-protected psymberic acid

activity against various human cancer cell lines made it an attractive target for total syntheses [72–74]. Lately, a chemoenzymatic approach by an esterase-catalyzed kinetic resolution towards psymberic acid, one of the main structural elements of psymberin, was disclosed [75]. A number of lipases and esterases was screened affording esterase BS3 from *Bacillus subtilis* and commercially available esterase 001 (Codexis) as suitable biocatalysts for enantioselective hydrolysis. Especially esterase 001 showed superior performance, yielding the protected psymberic acid with 98% *ee* (Fig. 7).

The importance of enantiomerically pure cyclopropane derivatives has already been pointed out (see Sect. 2.1) and many examples of lipase- or esterase-catalyzed kinetic resolutions of cyclopropanes can be found in the literature, but, unfortunately, high *E* values are scarce [76–84]. For instance, several lipases were screened for their ability to perform a kinetic resolution of cyclopropylmethanols [85].

Among the enzymes tested, *Candida antarctica* lipase B (CAL-B) (commercially available as Novozyme 435 or Chirazyme L-2) was the most efficient. For the kinetic resolution of the corresponding acetate in tetrahydrofuran at 60°C, a comparably high *E* value was achieved ($E = 44$). Hence, this enzyme was applied in the total synthesis of constanolactones A and B, marine oxylipins isolated from the red algae *Constantinea simplex* [86]. An enantiomerically enriched cyclopropylmethanol was submitted to enzymatic kinetic resolution after acetylation, giving the enantiopure (>98% *ee*) in good yield (76%) over the two steps (Fig. 8). Application of enzymatic kinetic resolution to other cyclopropylmethanol derivatives delivered the series with opposite cyclopropane configuration, yielding building blocks for the total synthesis of solandelactones [87], halicholactone, and neohalicholactone [88].

The kinetic resolution of 5-hydroxymethyl-2-cyclohexenone towards the total synthesis of penienone, a fungal metabolite of *Penicillium* sp. no. 13 [89], has been performed by using Lipase PS from *B. cepacia* for the acetylation of the (*S*)-enantiomer, leaving the desired (*R*)-alcohol. As a main problem inherent with enzymatic kinetic resolutions, the reaction yielded both products in a 1:1 mixture

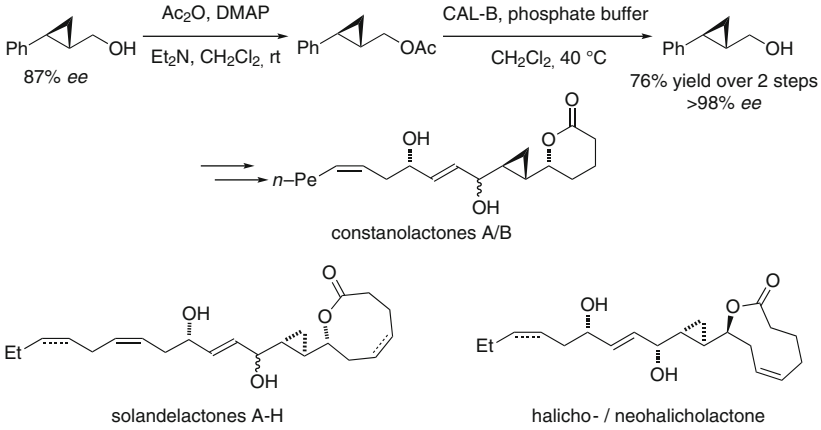

Fig. 8 Enzymatic kinetic resolution of cyclopropylmethanols

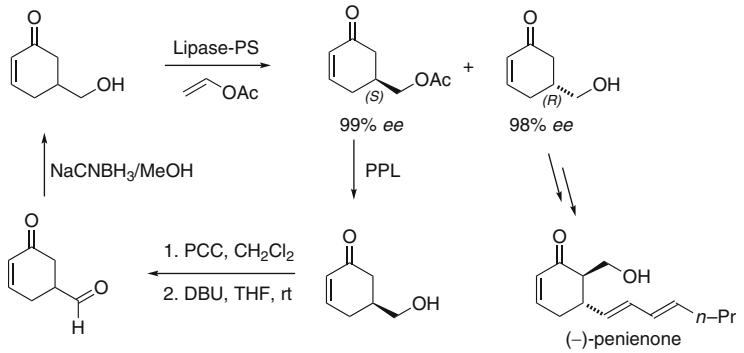

Fig. 9 Enzymatic kinetic resolution in the chemoenzymatic synthesis of (−)-penienone

almost quantitatively. For prohibiting the loss of half of the starting material, the acetate was racemized by hydrolysis with another lipase (PPL), followed by an oxidation–reduction sequence yielding the racemic starting material for the kinetic resolution (Fig. 9).

2.3 Dynamic Kinetic Resolutions with Lipases and Esterases

The enzymatic kinetic resolutions presented in Sect. 2.2 all clearly revealed the bottleneck of kinetic resolutions, a maximum yield of 50% for the desired enantiomer. In the total synthesis of penienone this problem was solved by choosing a rather inconvenient multistep racemization of the isolated remaining starting material.

In modern biotransformations, the insufficient yield is optimized by establishing an in situ racemization of the starting material, turning the system into a DKR [90–92]. Due to the high enantioselectivity of some enzymes, the number of applications of DKR is increasing; especially the utilization of the readily available lipases and esterases is most common. The racemization process can either be realized by biocatalysts or by chemocatalytic approaches. In some cases, the addition of base or acidification can shift an existing equilibrium towards racemization sufficiently. For catalyzed racemization, especially some ruthenium-complexes were proven to show high compatibility with enzymatic systems [93]. Other transition metals were also applied; for the DKR of allyl alcohols, an oxovanadium (V) catalyst was used in combination with a lipase to give high yields with excellent enantiomeric excesses [94].

The principle of kinetic resolutions (Fig. 10) can be explained by the different rates of conversion for the different enantiomers. In the special case of a DKR, the rate for the conversion of one enantiomer is many times higher ($k_R \gg k_S$). Without the possibility of racemization of the starting material ($k_{rac} = 0$), a kinetic resolution with a maximum yield of 50% is observed. As soon as k_{rac} exceeds the rate for the conversion of the unfavored enantiomer ($k_{rac} \geq k_S$), a DKR becomes efficient.

Bornscheuer and co-workers studied the lipase-catalyzed dynamic resolution of acyloins, using miscellaneous amine bases and ion-exchangers for racemization (Fig. 11) [95]. Unfortunately, acidic resins, e.g., Amberlyst 15, furnishing the best results for racemization, also deactivated the enzyme. As a solution, a two-compartment setup was established, separating ion exchanger and enzyme, the two vessels being connected by a pump loop. The enzymatic resolution of the acyloin and the racemization were thus carried out simultaneously furnishing a nice DKR with good yields and enantioselectivities. The enantiomerically enriched acyloins produced are important building blocks for natural products, e.g., epothilones [96].

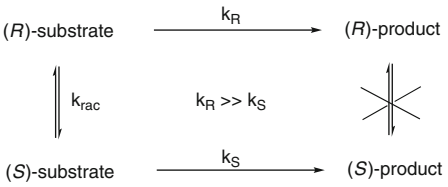

Fig. 10 Principle of dynamic kinetic resolutions

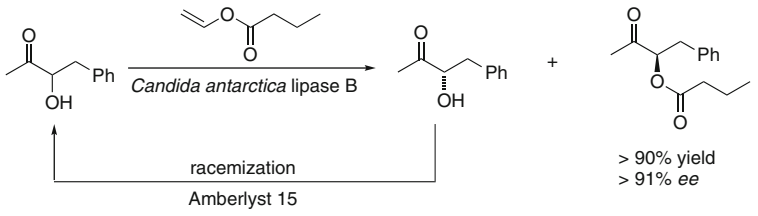

Fig. 11 Two-compartment enzymatic dynamic kinetic resolution of acyloins

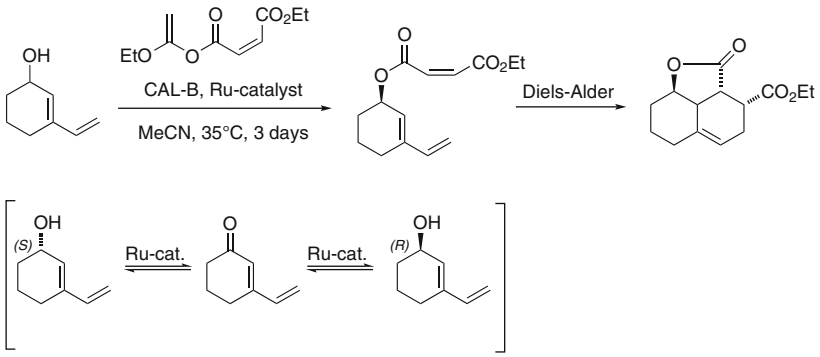

Fig. 12 Chemoenzymatic domino DKR and Diels–Alder reaction

Enzymatic DKRs have also been applied in domino one-pot processes [97]. The combination of a lipase-catalyzed resolution with an intramolecular Diels–Alder reaction led to interesting building blocks for the synthesis of natural products such as compactin [98, 99] or forskolin [100–102]. A ruthenium catalyst is employed for the racemization of the slow reacting enantiomer of the starting material. The DKR with lipase B from *C. antarctica* delivered high enantiomeric excesses which could mainly be contained through the Diels–Alder reaction (Fig. 12).

3 Enzymatic Reductions and Oxidations

In current research, oxidoreductases are second in the number of applications of enzymes in organic synthesis. The number of commercially available biocatalysts of this class has increased tremendously during the last few years and various screening kits for oxidation and reduction are sold. Many oxidoreductases are rather easy to handle, though, in contrast to hydrolases, they are dependent on cofactors [22].

3.1 Chiral Alcohols Through Enantioselective Reduction

The chemoenzymatic synthesis of chiral alcohols is a field of major interest within biocatalytic asymmetric conversions. A convenient access to secondary highly enantiomerically enriched alcohols is the usage of alcohol dehydrogenases (ADHs) (ketoreductases) for the stereoselective reduction of prochiral ketones. Here, as in many other cases in asymmetric catalysis, enzymes are not always only an alternative to chemical possibilities, but are rather complementary. Albeit biocatalysts might sometimes seem to be more environmentally friendly, asymmetric ketone reduction

with Noyori's ruthenium catalysts [8, 103] or by chiral oxazaborolidins (Corey-Bakshi-Shibata) [104] are known to be very efficient for many applications and do not leave much room for improvement for these. Yet there are also examples in which chemical asymmetric reduction fails or shows low selectivity [105].

Ketoreductases (KREDs) are dependent on nicotinamide cofactors NADH or NADPH. Due to the reaction mechanism, these rather costly cofactors are needed in stoichiometric amounts, disclosing an economic problem that has to be dealt with when using these enzymes. Many different possibilities for cofactor recycling have been established with three major approaches finding application in research and industry (Fig. 13). Further regeneration systems, such as electrochemical methods, are not discussed within this review [22–24, 37, 106–108].

One possibility is the addition of a second substrate that itself is also a possible substrate for the KRED being used. Most isolated enzymes are quite robust to 2-propanol as a substrate, so that in some systems 2-propanol concentrations of more than 40% can be used, also bearing an improved solubility of the ketones for conversion. However, on a larger scale the acetone formed can show inhibitory effects. Removal of this by-product shifts the equilibrium of the reaction towards the desired products and prohibits a competitive inhibition [109, 110].

A further opportunity for cofactor regeneration is the addition of a second enzyme with a corresponding substrate to the reaction mixture. Often glucose dehydrogenase (GDH) is used for this purpose, converting glucose to gluconate by reducing the nicotinamide cofactor. Formate dehydrogenases (FDHs) also have a broad spectrum of applications, bringing the advantage of catalyzing the oxidation

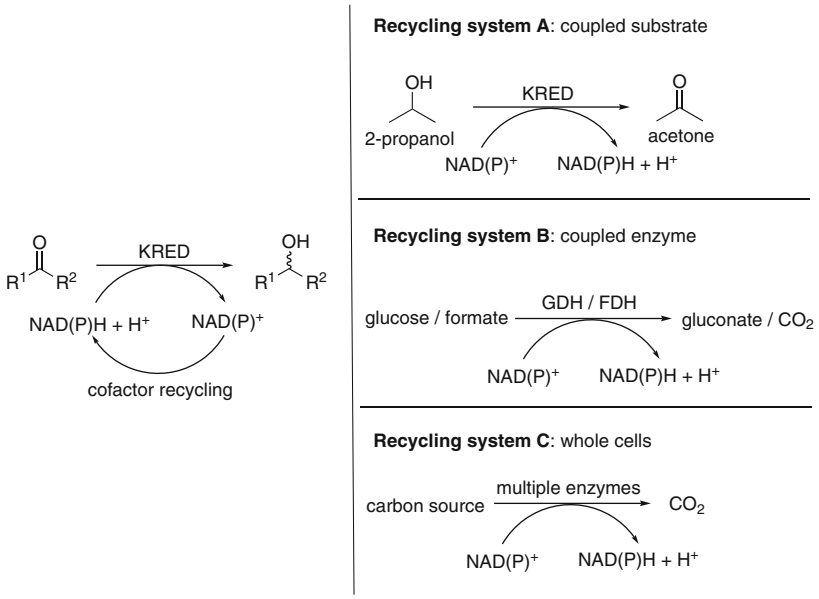

Fig. 13 Recycling of nicotinamide cofactors

of formate to carbon dioxide, which by leaving the reaction mixture draws the equilibrium to the desired side.

There is quite a lively discussion going on about the advantages and disadvantages of the usage of whole cells for biocatalysis vs isolated enzymes [23, 24, 43]. Intact cells contain all enzymes and the cofactors needed for the biotransformation, so that the addition of a carbon source should be sufficient. But whole cell biotransformations also cause some problems. One aspect is the transport of the substrate through the cell membrane, which might be a great hindrance. Partial lysis of the cells by adding organic solvents to the reaction medium sometimes allows transport of the substrate into the cell, but also provides a possibility of loss of the cofactor from the inner cell. Conclusively it is hardly possible to tell whether whole cell biotransformation or isolated enzymes are the method of choice, leading to both methods being almost equally applied [23, 24].

Chiral secondary alcohols are main targets of biotransformations since they are very common intermediates in various natural product syntheses. Often, kinetic or DKRs using lipases are used to obtain these key building blocks. The asymmetric reduction of prochiral ketones is more than an alternative to this approach. A huge number of applications, in which KREDs are used for reduction, can be found in the literature [22–24, 37]. For example, (*R*)-2-bromo-(4-nitrophenyl)ethanol, a precursor to the *β*-blocker (*R*)-nifenalol, which was obtained by kinetic resolution in the past, was conveniently synthesized in a direct approach from the corresponding ketone using whole cells of *Rhodotorula* sp. AS2.2241 (Fig. 14). This ketone and a number of derivatives thereof were converted with good yields and excellent enantioselectivities [111].

Whole cells were also used as biocatalysts in the biocatalytical synthesis of (*R*)-*o*-chloromandelates, highly valuable building blocks for biologically active substances, such as clopidogrel (Fig. 15) [112]. In this straightforward approach a versatile carbonyl reductase (SCR) was expressed in *Escherichia coli* and the cells fed with glucose for enabling the cofactor regeneration during the biotransformation. Methyl (*R*)-*o*-chloromandelate was isolated in enantiomerically pure form (>99% *ee*) in very high yield (89%).

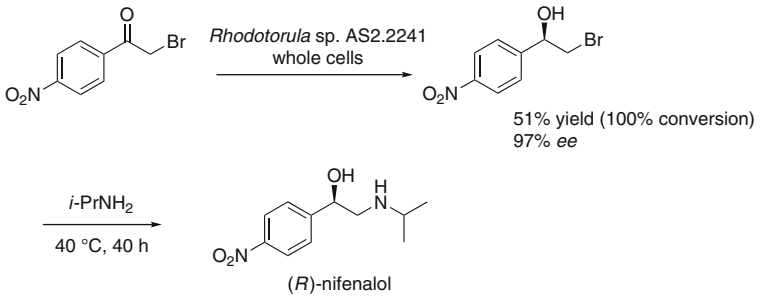

Fig. 14 Chemoenzymatic asymmetric synthesis of (*R*)-nifenalol with whole cells

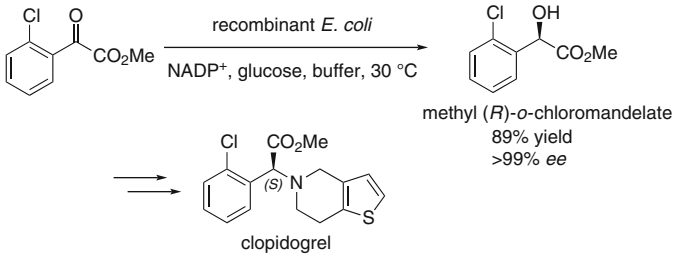

Fig. 15 Whole cell biocatalytic synthesis of methyl (*R*)-*o*-chloromandelate

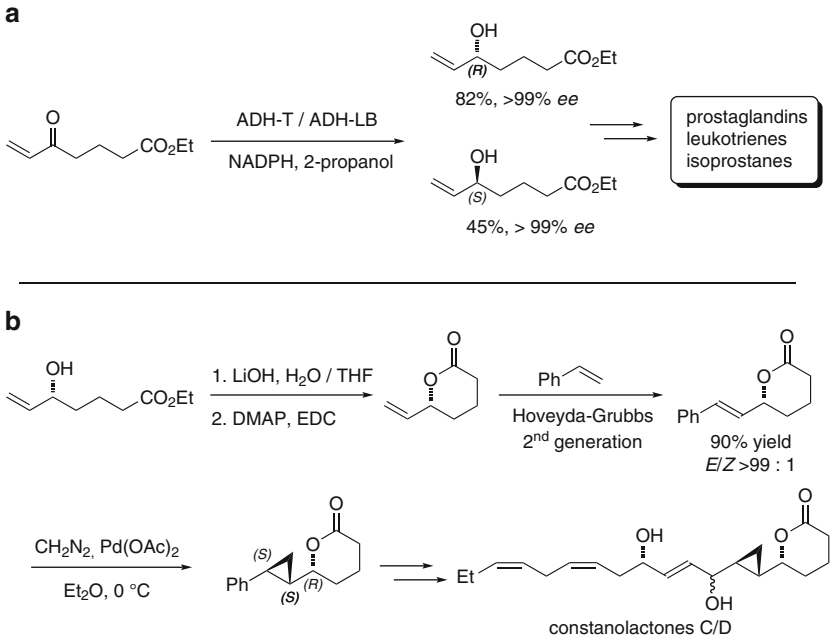

Fig. 16 (**a**) Enzymatic synthesis of building blocks for arachidonic acid metabolites. (**b**) Chemoenzymatic synthesis of constanolactones C/D

5-Hydroxyhept-6-enoates have been key intermediates in the synthesis of a variety of natural products, especially of the arachidonic acid metabolic pathway, including prostaglandins, leukotrienes, and isoprostanes [105, 113–118]. The usage of two enantiocomplementary enzymes allowed convenient access to both enantiomers via an ADH-catalyzed reduction of 5-oxo-hept-6-enoate. Alcohol dehydrogenase from *Lactobacillus brevis* (ADH-LB) furnished the (*S*)-enantiomer, *Thermoanaerobacter* sp. ADH (ADH-T) the (*R*)-enantiomer in excellent enantiomeric access respectively [119]. A cross-metathesis reaction followed by cyclopropanation led to the formal synthesis of constanolactones C and D (Fig. 16) [86, 120, 121].

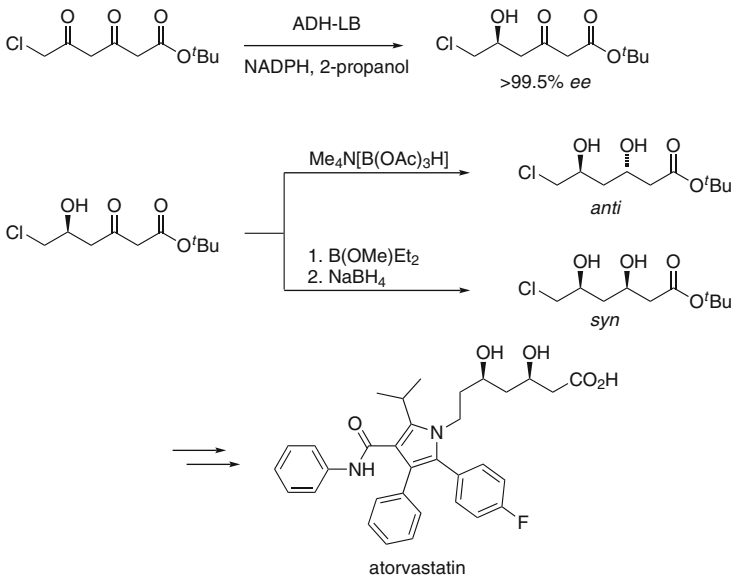

Fig. 17 Ketoreductase-catalyzed chemoenzymatic synthesis of statin side chains

Not only has the remarkable enantioselectivity of ADHs made them a very powerful tool in organic synthesis: Besides the excellent enantioselectivity of ADH from *L. brevis* already shown, the same enzyme's regioselectivity has been exploited in the chemoenzymatic synthesis of statin side chains [122]. A series of 3,5-dioxocarboxylates (β,δ-diketo esters) were successfully reduced selectively in the 5-position using ADH-LB as biocatalyst. The enantiomeric purity of the 5-hydroxy compound obtained was excellent [123–125]. The second ketone was then either reduced by Prasad's borohydride method [126] delivering the *syn*-dihydroxy building block, or according to Evans furnishing the *anti*-configured compound, respectively (Fig. 17) [127]. Furthermore, chiral building blocks for the synthesis of some δ-lactone containing natural products, such as goniothalamin, argentilactone, or callistatin A, could be accessed by this chemoenzymatic approach [125].

Similar building blocks have also been obtained by a variety of other enzymatic transformations. 4-Chloro-3-oxobutanoate esters have been reduced with outstanding enantioselectivity by ADHs from *Candida magnoliae* in combination with cofactor regeneration by a GDH (from *Bacillus megaterium*) or by enzymatic reduction using whole cells of *Geotrichum candidum* [128, 129]. This process was improved to a large scale application. The combination with a halohydrin dehydrogenase step led to versatile compounds for organic synthesis. Statistical analysis of protein sequence activity relationships (ProSAR) and recombination-based directed evolution led to an optimized enzyme catalyzing the epoxide formation in a first step and the addition of a nucleophile in a second step, accepting alternative nucleophiles, such as CN^- or N_3^- (Fig. 18) [130–132].

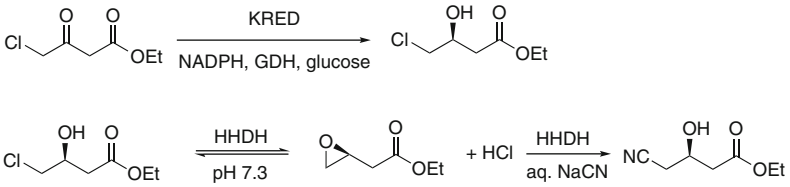

Fig. 18 Enzymatic synthesis of ethyl (*R*)-4-cyano-3-hydroxybutyrate

3.2 Reduction of C=C Bonds

The generation of stereogenic centers by asymmetric reduction of carbon–carbon double-bonds is a current topic in chemoenzymatic synthesis. Though enzymes of the old yellow enzyme (OYE) family were identified to perform alkene reduction and were characterized some years ago [133–135], applications of enoate reductases in natural product syntheses are still rare. Thus, potential applications are also shown in this chapter. With an increasing number of new enoate reductases, such as YqjM reductase from *B. subtilis*, more and more possible targets for biotransformations can be found.

Enzymes from the OYE family were reported to mediate efficiently the reduction of ketoisophorone. A subsequent regio- and stereoselective reduction of the thus produced levodione leads to actinol, both compounds being important building blocks for carotenoids. While early approaches used baker's yeast in the fermentative production of levodione [136], more recent approaches used enzymes from different organisms. Besides OYE analogs from *Candida macedoniensis*, *Saccharomyces carlsbergensis*, and *Saccharomyces cerevisiae*, a newly discovered enoate reductase from *Zymomonas mobilis* was applied successfully [137, 138]. Wada et al. combined the OYE-mediated step with an enzymatic formation of actinol catalyzed by a levodione reductase from *Corynebacterium aquaticum* (Fig. 19) [139].

Some reductases isolated from tobacco (*Nicotiana tabacum*) were found to exhibit excellent enantioselectivities on the reduction of a number of α,β-unsaturated compounds [140, 141]. For example, reductase p44 catalyzed the asymmetric reduction of *N*-phenyl-2-methylmaleimide, yielding the enantiopure (*R*)-succinimide. Reductase p90 mediated the enantioselective hydrogenation of a number of methyl or ethyl substituted cyclopentenones and cyclohexanones (Fig. 20).

3.3 Reductive Amination Towards Amino Acids

Unnatural amino acids in general, but especially α-amino acids being important intermediates for various biologically active compounds, have enjoyed great popularity as a growing field of interest during recent years [142]. Many enzymatic

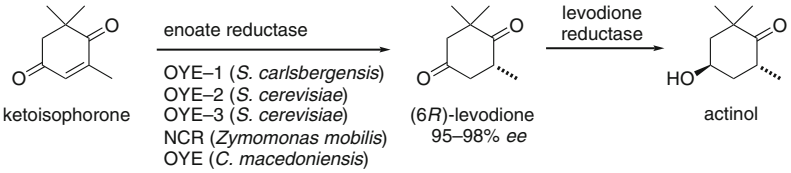

Fig. 19 Enzymatic synthesis of carotenoid building blocks

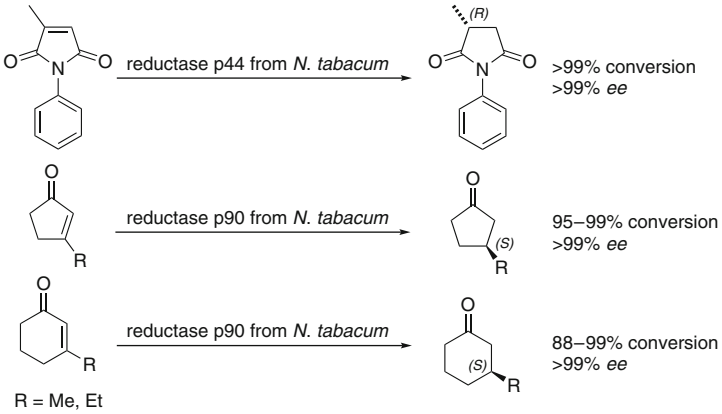

Fig. 20 Enantioselective reductions catalyzed by reductases from *N. tabacum*

approaches to these important building blocks, including resolutions with L-amino acid acylase or D-amino acid oxidase, the application of transaminases, and reductive amination by amino acid dehydrogenases, were studied [143]. For the production of enantiopure α-amino acids, reductive amination was most successfully applied.

The significance of chiral unnatural amino acids to drug and natural product synthesis is shown in the example of the antihypertensive drug omapatrilat (Vanlev®), which is composed of no less than three amino acid derived intermediates [144–147]. Diverse biocatalytical approaches to L-6-oxonorleucine were made (Fig. 21). Two different enzymes were applied in reductive amination reactions to produce derivatives of the desired intermediate.

With a protected aldehyde function, allysine ethylene acetal was synthesized via reductive amination catalyzed by phenylalanine dehydrogenase (PheDH). For regeneration of the nicotinamide cofactor, FDH was used as described before (see Sect. 3.1). Another similar approach was taken in the synthesis of L-6-hydroxynorleucine. In this case, reductive amination was catalyzed by glutamate dehydrogenase (GluDH) delivering the desired (*S*)-configured amino acid. The synthesis of the same compound has also been achieved by an interesting resolution process. Racemic 6-hydroxynorleucine was submitted to kinetic resolution using a D-amino

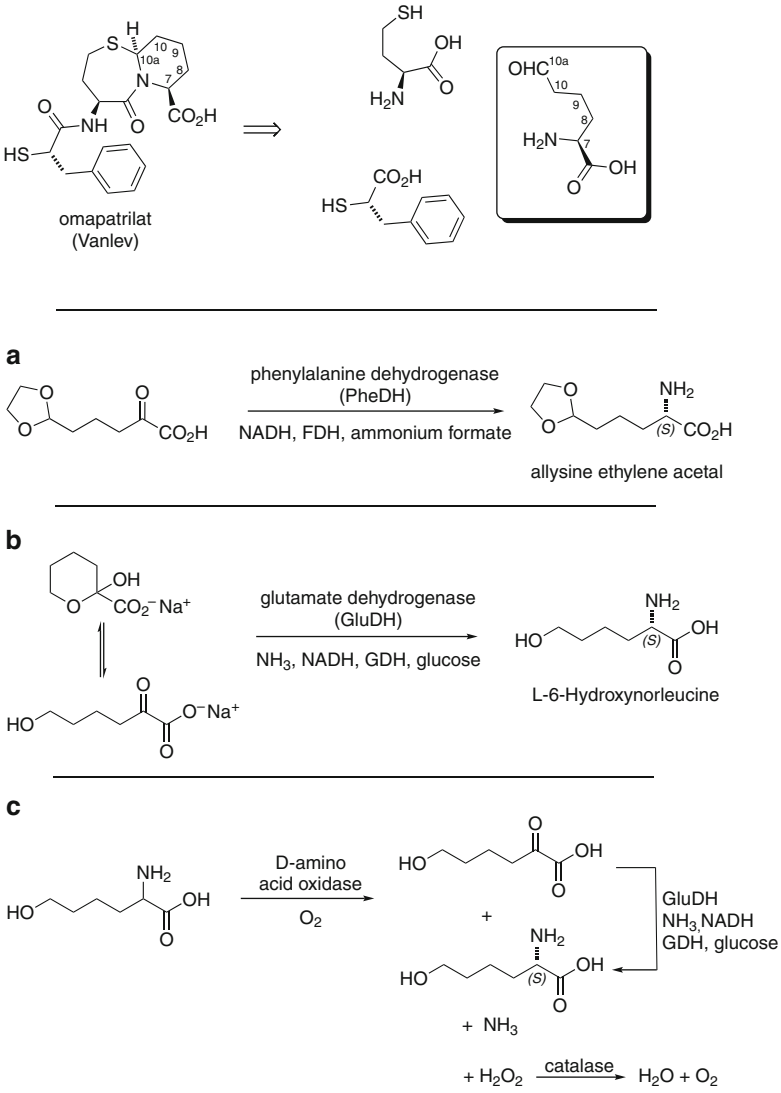

Fig. 21 Enzymatic synthesis of unnatural amino acids as building blocks for omapatrilat. (**a**–**c**) Enzymatic approaches to 6-oxonorleucine analogs

acid oxidase, generating an enantiomeric excess of >99% for the remaining enantiomer. The mixture was then converted completely to enantiopure L-hydroxynorleucine by reductive amination of 2-oxo-6-hydroxyhexanoic acid with GluDH. For the oxidation step, whole cells of *Trigonopsis variabilis*, bearing the oxidase and the catalase for the degradation of hydrogen peroxide to oxygen, were successfully used.

3.4 Cytochrome P450 Monooxygenases

Cytochrome P450 (CYP450) monooxygenases are very common oxidoreductases, frequently used in synthetic applications in research and industry. The versatility and potential as powerful industrial biocatalysts has been strengthened by important advances that have been made regarding mechanistic insights and optimization of the activity and high stereo- and regioselectivities of these hemoproteins recently [148–151].

Pravastatin, a 3-hydroxy-3-methyl glutaryl CoA reductase inhibitor applied as a therapeutic agent for hypercholesterolemia, can be synthesized by stereo- and regioselective hydroxylation of compactin by the soil microorganism *Streptomyces* sp. Y-110 (Fig. 22) [152]. The fermentative production of pravastatin has already been applied on an industrial scale by Sankyo Co. using different *Streptomyces* bacteria strains [153, 154].

Besides the stereo- and regioselective hydroxylations, CYP450 are also capable of performing regioselective oxidations to aldehydes or carboxylic acids. The sesquiterpene lactone artemisinin is highly effective against multidrug resistant *Plasmodium falciparum*, agent of malaria tropica. First, genes from the mevalonate pathway in *S. cerevisiae* were upregulated by means of metabolic engineering, increasing the production of farnesyl pyrophosphate, and the natural further metabolism to sterols was downregulated. Introduction of an amorphadiene synthase (ADS) from *Artemisia annua* led to the formation of amorpha-4,11-diene which could then be converted to artemisic acid, a precursor of artemisinin, via a three-step oxidation sequence catalyzed by a novel P450 from *A. annua* in *S. cerevisiae* (Fig. 23) [155].

3.5 Baeyer–Villiger Oxidations

Among the most popular oxidative biotransformations, Baeyer–Villiger monooxygenases (BVMOs) belong to the main fields of research. Nowadays, manifold enzymes catalyzing the Baeyer–Villiger oxidation are expressed in common recombinant organisms, such as *E. coli* or *S. cerevisiae*. The mechanism of the enzymatic

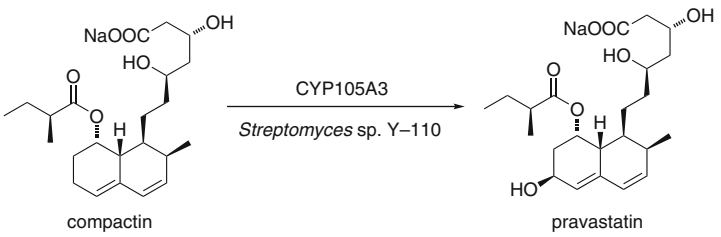

Fig. 22 Biocatalytic conversion of compactin to pravastatin

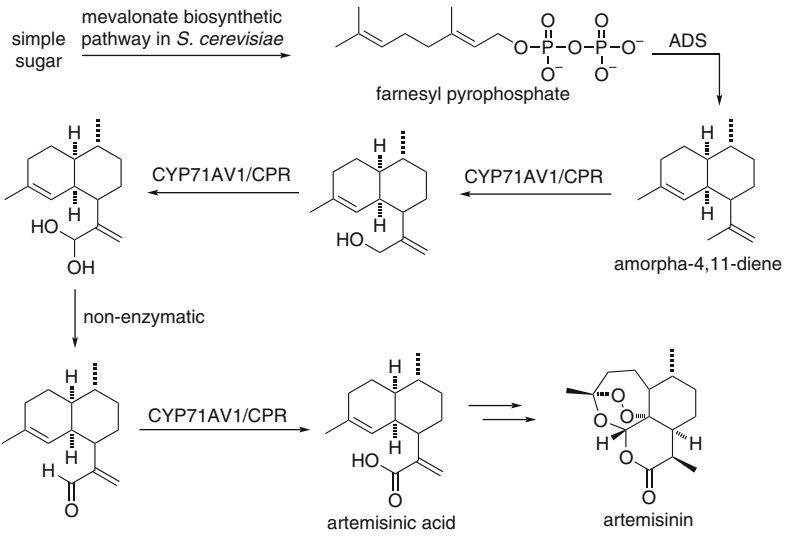

Fig. 23 Fermentative production of artemisinic acid

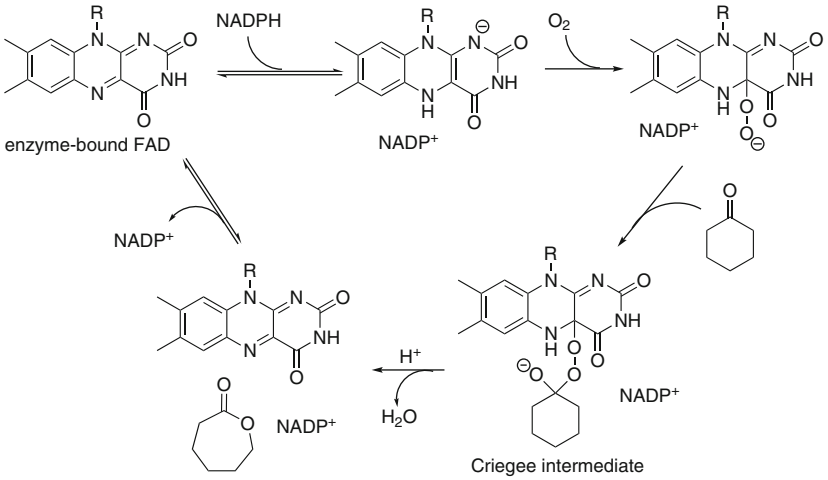

Fig. 24 Mechanism of flavin-dependent Baeyer–Villiger monooxygenases

Baeyer–Villiger oxidation involves NADPH and flavin (FAD) as cofactors and was originally proposed by Walsh et al. based on data obtained from cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* (Fig. 24) [156]. In a first step, enzyme-bound flavin is reduced, followed by the addition of oxygen yielding a hydroperoxide anion. Reaction with the ketone substrate gives a Criegee intermediate, which is then converted into the product under dissociation of water. The cofactor FAD is recovered via oxidation with $NADP^+$.

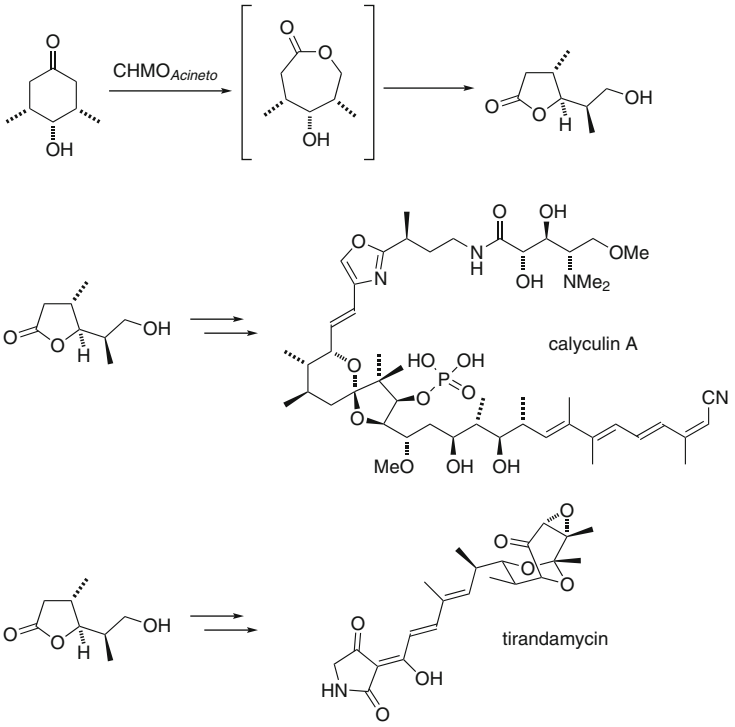

Fig. 25 BVMO-catalyzed synthesis of a precursor to calyculin A and tirandamycin

Enzymatic Baeyer–Villiger oxidations have been studied for a long time; some very useful applications in natural product synthesis date back more than two decades. For example, prochiral (3*R*,5*S*)-4-hydroxy-3,5-dimethylcyclohexanone was successfully oxidized using a CHMO from *Acinetobacter* sp. NCIMB 9871. In this case the seven-membered ring formed rearranges spontaneously into the thermodynamically more stable γ-lactone. The rearranged lactone has been used in the synthesis of natural products such as tirandamycin or calyculin A (Fig. 25) [157–159].

As most enzymes, BVMOs feature excellent enantio- and regioselectivities, making them very versatile tools for asymmetric synthesis. Bicyclic ketones such as functionalized norboranones have been resolved to the corresponding lactones with good regioselectivities and enantiomeric excesses of more than 95% [160]. Whole cells of *Pseudomonas putida* or isolated 2,5-/3,6-diketocamphane 1,2-monooxygenase (2,5-/3,6-DKCMO) were used for the biotransformation. Conversion into an acetal yielded a potential advanced intermediate to azadirachtin, a potent antifeedant and growth regulator (Fig. 26) [161–163].

The biocatalytical formation of γ-butyrolactones through Baeyer–Villiger oxidation is catalyzed by a number of different monooxygenases, yielding precursors to various natural products [29, 38]. Best enantioselectivities were obtained for the

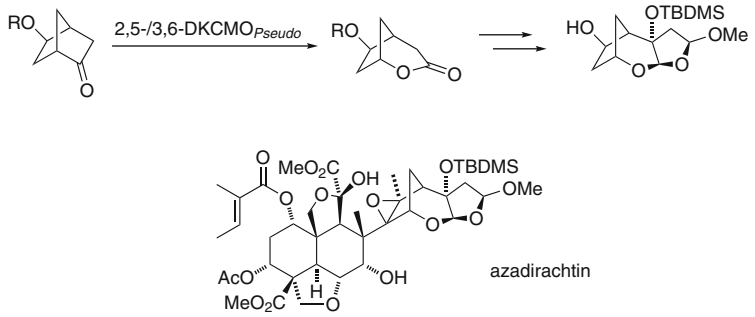

Fig. 26 Chemoenzymatic approach to azadirachtin

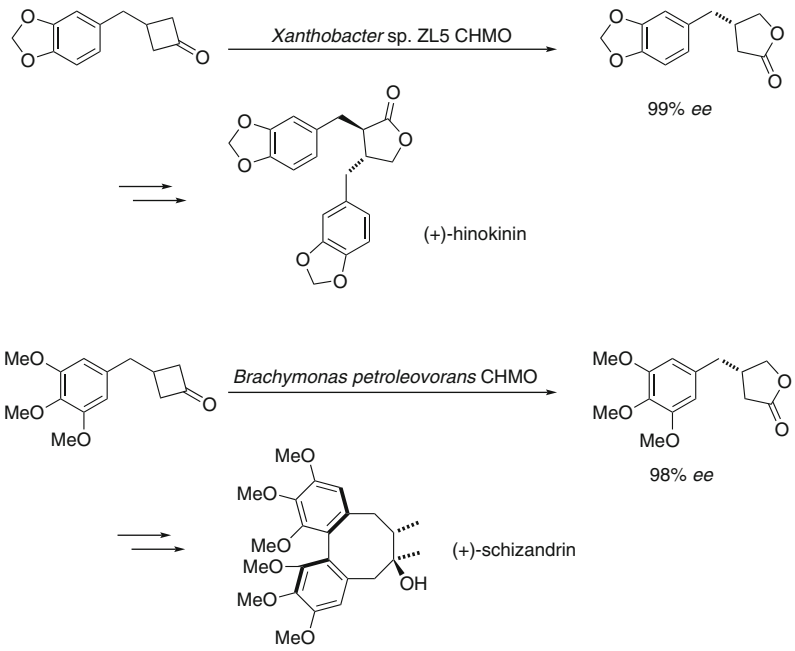

Fig. 27 Cyclohexanone monooxygenase-mediated synthesis of lignan building blocks

synthesis of precursors for lignans, which are known for their antileukemic, antiviral, antifungal, and antineoplastic activities [164–166]. For instance, building blocks for (+)-hinokinin and (+)-schizandrin have been generated with excellent enantioselectivities using CHMOs from *Xanthobacter* sp. ZL5 and *Brachymonas petroleovorans* respectively (Fig. 27).

More recently, the formal total syntheses of some bioactive natural products bearing a tetrahydrofurane moiety were achieved by enzymatic Baeyer–Villiger oxidation using cyclopentanone monooxygenases (CPMOs) from *Comamonas* sp.

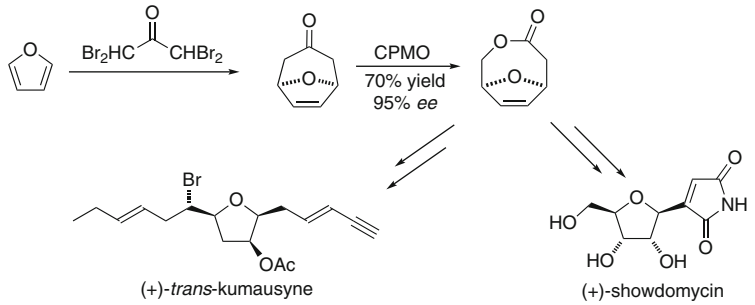

Fig. 28 Enzymatic Baeyer–Villiger oxidation in the synthesis of bioactive compounds

NCIMB 9872, overexpressed in *E. coli*, as a whole cell biotransformation [167]. The substrate, an oxabicycloketone, was successfully converted to the corresponding heterobicyclic lactone (Fig. 28) in 70% yield with an enantiomeric excess of 95%. This access to the chiral intermediate for several naturally occurring compounds, including (+)-*trans*-kumausyne and (+)-showdomycin, represents the first application of a whole cell biotransformation using recombinant *E. coli* with CPMO in natural product synthesis.

3.6 Cyclohexadienediols by Fermentation

There are only a few reactions known in which aromatic compounds suffer a permanent loss of aromaticity and at the same time the new stereogenic centers are formed selectively. The enzymatic oxidation is one of the few examples leading to exceptionally results: the reaction in eukaryotic systems is mainly catalyzed by cyctochrome-type monooxygenases, leading to epoxides. Furthermore, in prokaryotic systems, the processing of arenes by dioxygenases is well known [168], and especially *cis*-cyclohexadienediols (*cis*-CHDs) are generated by fermentation with recombinant *E. coli* strains. They have found broad application in several natural product and drug syntheses [28, 169–174].

For example, the fermentation of (2-bromoethyl)-benzene with recombinant *E. coli* furnished excellent yields of the corresponding *cis*-diol, enantiopure 3-(2-bromoethyl)-benzene-1,2-diol. The latter was used as a building block in the total synthesis of (+)-codeine (Fig. 29). Besides a Mitsunobu inversion of one of the stereogenic centers, two successive Heck cyclizations led to the enantiomer of the natural product [173]. Slight modifications of the reaction sequence, generating an epoxide intermediate, also furnished access to the naturally occurring enantiomer (−)-codeine [28].

Versatile epoxyquinol intermediates were conveniently accessed via metabolites derived from the enzymatic dihydroxylation of bromobenzene [172]. The chemoenzymatic approach led to shortened total syntheses of several epoxyquinols derived

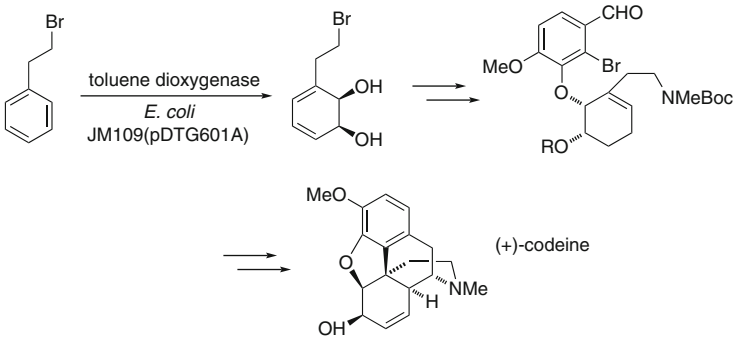

Fig. 29 Chemoenzymatic synthesis of (+)-codeine

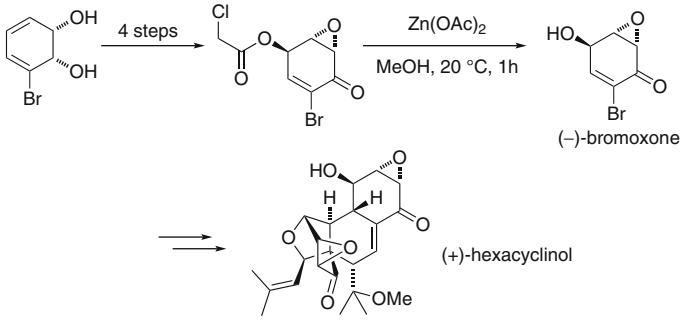

Fig. 30 Chemoenzymatic access to epoxyquinols

from bromoxone, such as (+)-hexacyclinol, a possible lead target for new antimalarial agents of fungal origin (Fig. 30).

Various approaches to the antiviral agent oseltamivir (Tamiflu®), which is one of the most effective drugs against avian influenza (H5N1) and the new influenza A (H1N1), have recently been published starting from biotechnologically provided *cis*-CHDs [175–177]. Hudlicky et al. started from ethyl benzoate, performing the oxidative biotransformation with a recombinant *E. coli* strain (Fig. 31).

The oxidation of benzenes to *trans*-cyclohexadienediols (*trans*-CHDs) has not been reported yet. Nevertheless, the fermentative production of *trans*-CHDs with recombinant *E. coli* strains has also been established and the value as a building block for natural product synthesis was demonstrated [178–180]. Starting from chorismate, *trans*-CHDs are metabolites derived from the shikimate–chorismate pathway in bacteria, plants, and fungi towards the biosynthesis of the iron chelator enterobactin (Fig. 32). Isochorismate synthase (EntC) and isochorismatase (EntB) catalyze the degradation to 2,3-*trans*-CHD or 3,4-*trans*-CHD respectively (Fig. 30). Using methods of metabolic pathway engineering, *E. coli* isochorismate synthase and isochorismatase were overexpressed in *E. coli* strains with a deficiency of *entA*, encoding 2,3-dihydroxybenzoate synthase (EntA).

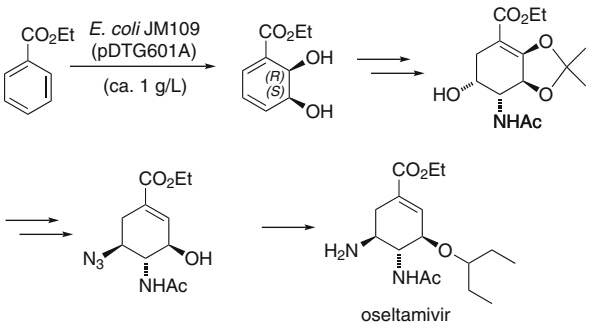

Fig. 31 Chemoenzymatic synthesis of oseltamivir

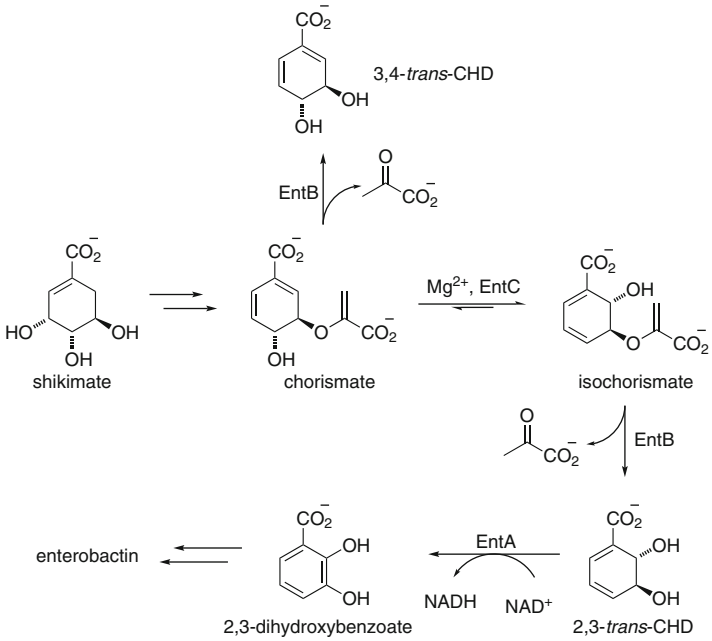

Fig. 32 Biosynthesis of *trans*-cyclohexadienediols

Microbially produced (2*S*,3*S*)-*trans*-dihydroxy-2,3-dihydrobenzoic acid was used in the synthesis of *ent*-streptol, *ent*-senepoxide, and *iso*-crotepoxide (Fig. 33). The short and efficient synthesis of these biologically active compounds included the esterification of the carboxylic acid and protection of the diol moiety, delivering control of the regio- and stereoselectivity of the following epoxidation or dihydroxylation steps [178, 180].

Recently, (2*S*,3*S*)-dihydroxy-2,3-dihydrobenzoic acid was isolated from the fermentation broth of a recombinant *E. coli* strain and the methyl ester was used in an interesting cyclopropanation sequence [181] yielding dicyclopropane building

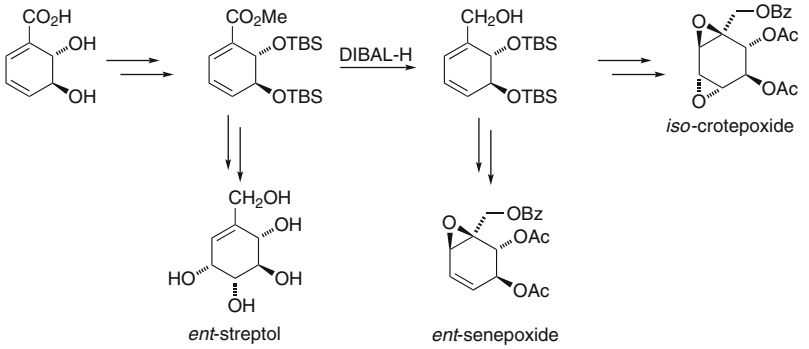

Fig. 33 Natural product syntheses starting from 2,3-*trans*-CHD

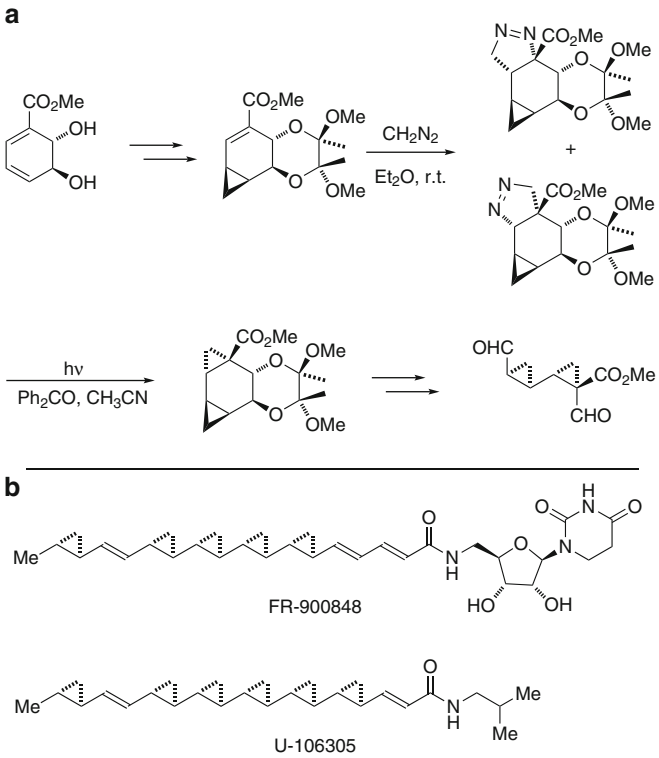

Fig. 34 (**a**) Synthesis of dicyclopropane building blocks via [2+3]-dipolar cycloaddition. (**b**) Oligocyclopropane-containing natural products

blocks that are potentially useful for the synthesis of oligocyclopropane natural products [51] such as the antifungal agent FR-900848 and the cholesteryl ester transfer protein inhibitor U-106305 (Fig. 34). For the formation of the second

cyclopropane moiety, treatment with diazomethane led to a 1,3-dipolar cycloaddition to a five-membered heterocycle which furnished the desired second cyclopropane by photo induced nitrogen elimination.

4 C–C-Bond Formations

Enzymatic reactions forming new carbon–carbon bonds are a further important field of biotransformations in natural product synthesis. The construction of new, often complex carbon frameworks or their decomposition is performed by nature under catalysis of a set of enzymes. For organic chemists some of these enzymes, belonging to the enzyme class of lyases, such as aldolases, decarboxylases, hydroxynitrile lyases (HNLs), or benzaldehyde lyases (BALs), have been proven to represent versatile amendments to their synthetic toolbox.

4.1 Enzymatic Aldol Reactions

Aldol reactions enjoy great recognition as a useful tool for the synthesis of building blocks in natural product and drug synthesis [42, 182]. The stereochemistry of the stereogenic centers formed can be controlled by various means. Besides chiral auxiliaries, catalytic methods with chiral Lewis acids, organocatalysts, or catalytic antibodies were established for stereochemical control [183–187].

In nature, most aldolases are rooted in the sugar metabolic cycle and accept highly functionalized substrates for the aldol reaction. Nevertheless, the scope of enzymatic aldol reactions is limited, since aldolases strictly distinguish between the acceptor and the donor, yielding almost exclusively one product, and is furthermore restricted to only a few different possible natural donors. According to the donor molecules, aldolases are grouped in dihydroxyacetone phosphate-, phosphoenolpyruvate- or pyruvate-, acetaldehyde-, and glycine-dependent aldolases [41].

Fructose 1,6-biphosphate aldolase from rabbit muscle in nature reversibly catalyzes the addition of dihydroxyacetone phosphate (DHAP) to D-glyceraldehyde 3-phosphate. The tolerance of this DHAP-dependent enzyme towards various aldehyde acceptors made it a versatile tool in the synthesis of monosaccharides and sugar analogs [188], but also of alkaloids [189] and other natural products. For example, the enzyme-mediated aldol reaction of DHAP and an aldehyde is a key step in the total synthesis of the microbial elicitor (−)-syringolide 2 (Fig. 35a) [190].

A convenient chemoenzymatic access to sialic acid mimetics as important inhibitors of influenza sialidases has been established by Nelson et al. (Fig. 35b) [191, 192]. Application of a pyruvate-dependent sialic acid aldolase improved by directed evolution disclosed a new route to the core structure of important pharmaceuticals, such as zanamivir (Relenza®).

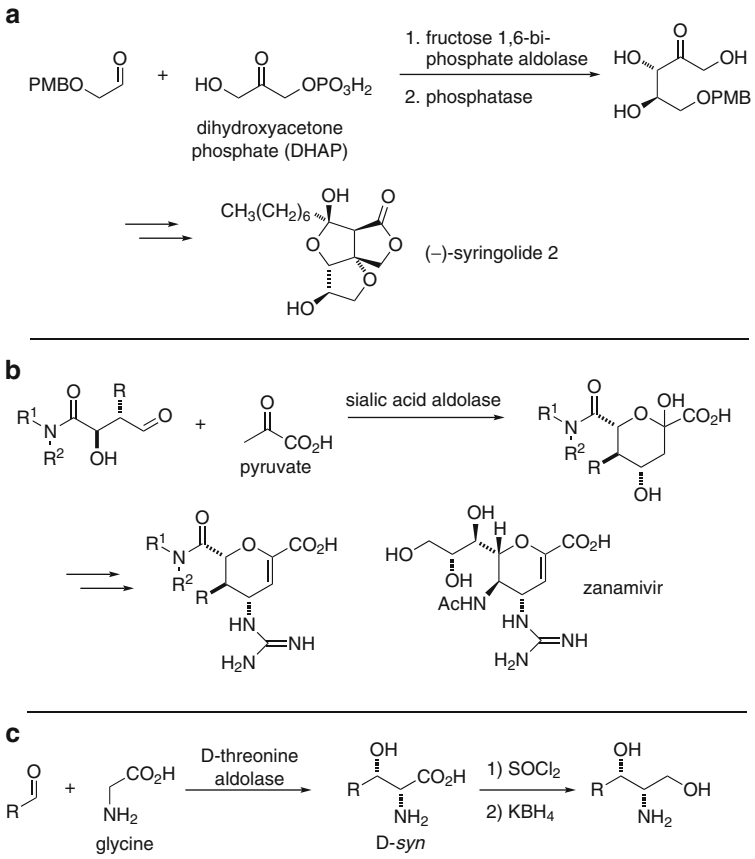

Fig. 35 Synthetic applications of (**a**) DHAP-, (**b**) pyruvate-, and (**c**) glycine-dependent aldolases

Glycine-dependent threonine aldolases have been used to synthesize a number of γ-halogenated and long-chain β-hydroxy-α-amino acids. For D-threonine aldolase *syn*-selectivity was observed exclusively. Further chemical conversion yielded the 2-amino-1,3-diols, potential precursors for the synthesis of short-chain sphingosine-derivatives (Fig. 35c) [193].

To date, 2-deoxy-D-ribose 5-phosphate aldolase (DERA) is the only acetaldehyde-dependent aldolase being applied in organic synthesis. Thus the stereoselectivity of DERA is significant, all known enzymes from different organisms showing the same preferences, limiting the field of application to syntheses in which specifically the DERA-catalyzed enantiomer is needed.

In comparison to other aldolases, DERA has a rather broad substrate range. DERA-catalyzed aldol reactions were used to get an access to key intermediates for epothilones (Fig. 36) [194]. According to retrosynthetic analysis, both fragments of the molecule could be obtained from aldol building blocks, and two out of seven stereocenters were established enzymatically. For the southern part of epothilone A,

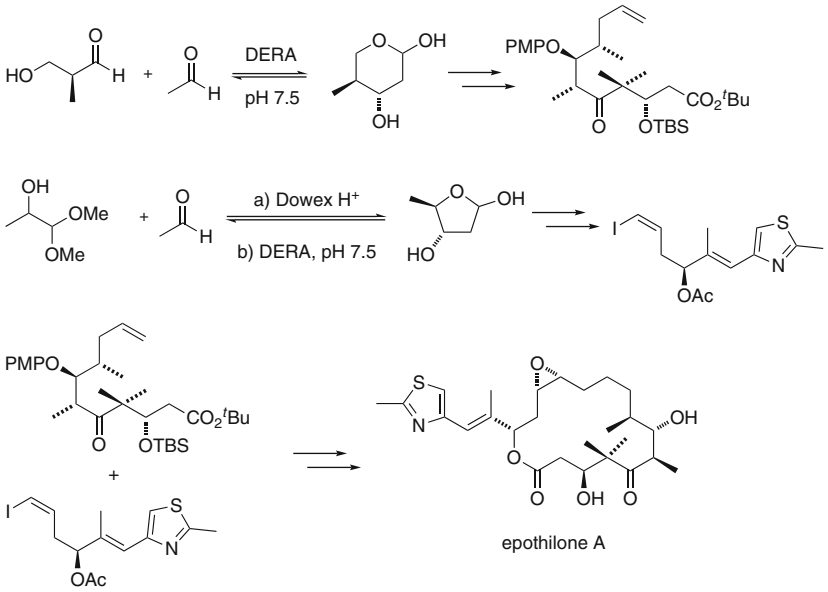

Fig. 36 DERA mediated access to key synthons in the synthesis of epothilone A

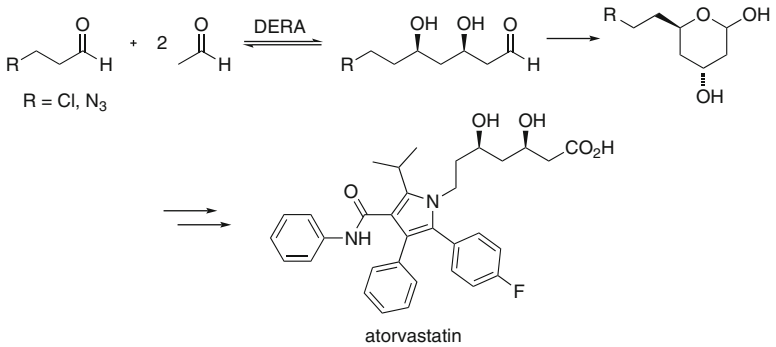

Fig. 37 Application of DERA in sequential one-pot reactions

(*S*)-3-hydroxy-2-methoxypropanal was successfully converted with acetaldehyde, with the primary open chain aldol product forming the lactol. The aldehyde needed as starting material for the northern part was generated in situ from the acetal: a DERA-catalyzed kinetic resolution led to conversion of the (*R*)-enantiomer in the aldol reaction, only.

Since the product of the aldol reaction still contains an aldehyde function, it is readily available for further reactions. Wong and co-workers very elegantly used a DERA mutant (Ser238Asp) for a sequential one-pot aldol condensation towards the synthesis of atorvastatin (Fig. 37) [195], a process also being applied by DSM [196]. When using wild-type DERA, the choice of residues in the acceptor aldehyde

was limited to rather smaller negatively charged groups. The chosen mutation in the hydrophilic binding pocket preserved its nature, but went along with a 2.5-fold improvement in enzyme activity. Furthermore, the mutant presented a highly improved tolerance towards unnatural substrates. The performance in sequential aldol additions was also increased compared to the wild-type enzyme.

4.2 Cyanohydrins by Hydroxynitrile Lyases

Native HNLs from bitter almonds (*Prunus amygdalus*), cassava (*Manihot esculenta*), millet (*Sorghum bicolor*), and flax (*Linum usitatissimum*) were repeatedly used in the synthesis of chiral cyanohydrins [39, 41, 197]. Cyanohydrins are versatile building blocks in natural product synthesis, giving organic chemists the possibility of introducing all kinds of functional groups (Fig. 38) [198].

Effenberger and co-workers reported the biotransformation of pivaldehyde-derivatives to the corresponding cyanohydrins using wild-type *Pa*HNL [199]. In this context, a precursor to (*R*)-pantolactone could be obtained with a yield of 84% and 89% *ee*. Since (*R*)-pantolactone is the most important building block in the synthesis of vitamin B_5, further efforts were made for optimization. Recently, an acid stable almond HNL isoenzyme was generated by semirational design and applied in the synthesis described earlier, showing full conversion of hydroxypivalaldehyde to the corresponding cyanohydrin with an enantiomeric excess of 97% (Fig. 39) [200].

A large number of the most important modern insecticides are derived from cyanohydrins. Especially some esters formed from enantiopure cyanohydrins and chrysanthemum acid derivatives are known to be very potent [201]. Nowadays, the formation of 3-phenoxybenzaldehyde cyanohydrin is performed in a biocatalytic industrial process using *Me*HNL or *Hb*HNL isolated from rubber trees (*Hevea*

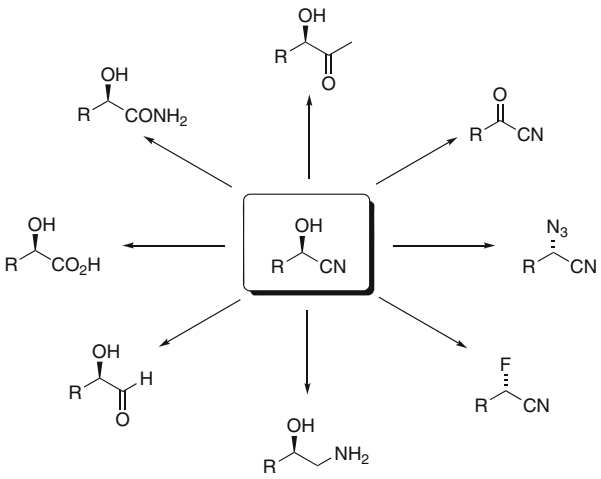

Fig. 38 Cyanohydrins as building blocks for organic synthesis

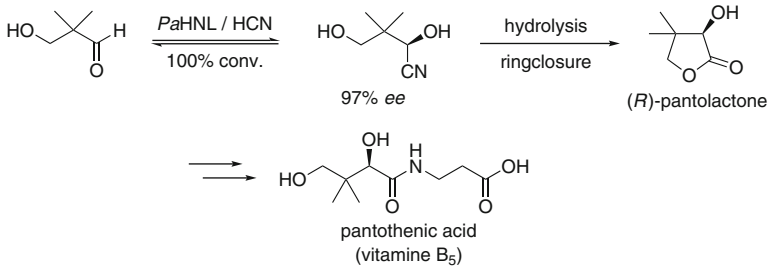

Fig. 39 Chemoenzymatic synthesis of (*R*)-pantolactone

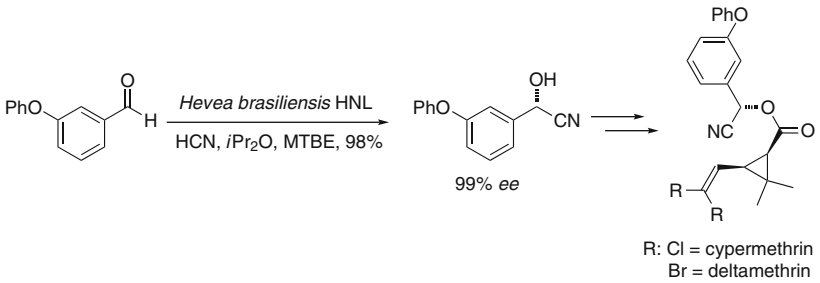

Fig. 40 Application of HNL in large-scale production of insecticides

brasiliensis) yielding precursors for cypermethrin or deltamethrin, two pyrethroids (Fig. 40) [21, 39, 41, 202].

Another application of HNLs can be found in the chemoenzymatic synthesis of the broadband antibiotic thiamphenicol and its fluorinated derivative florfenicol [203]. The conversion of 4-methylsulfanyl-benzaldehyde to the mandelonitrile was catalyzed by a novel enzyme from a Chinese almond (*Prunus communis* L. var. *dulcis* Borkh). A concentrated powder from the kernels was prepared and a mixture of the crude meal, aldehyde, and HCN was stirred in isopropyl ether at room temperature for 12 h, yielding the cyanohydrin with 99% *ee* after recrystallization. The building block formed was then successfully applied in the total synthesis of thiamphenicol and florfenicol (Fig. 41).

4.3 Biocatalytic Formation of Acyloins

Enantiopure, bifunctional acyloins (α-hydroxy ketones) are versatile intermediates in natural product synthesis (also see Sect. 2.3, Fig. 11). In nature, the formation of α-hydroxy ketones is efficiently catalyzed by thiamine diphosphate-dependent enzymes: transketolases, decarboxylases, and other lyases, such as BALs. A great portfolio of biotransformations, especially with benzaldehyde derivatives as starting materials, were realized [204].

A very short and efficient chemoenzymatic synthesis of (−)-ephedrine can be achieved by a decarboxylase-mediated reaction of benzaldehyde with pyruvate

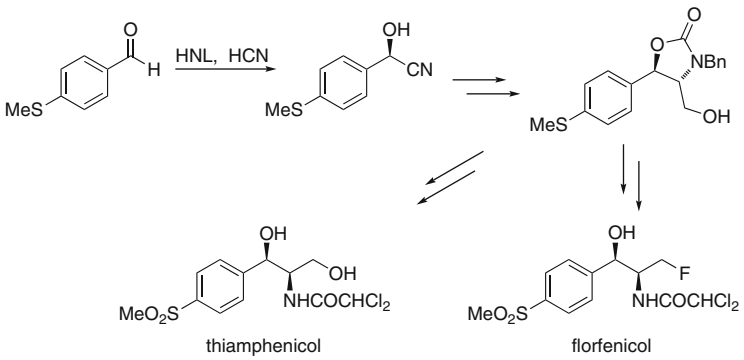

Fig. 41 Chemoenzymatic synthesis of broadband antibiotics using hydroxynitrile lyases

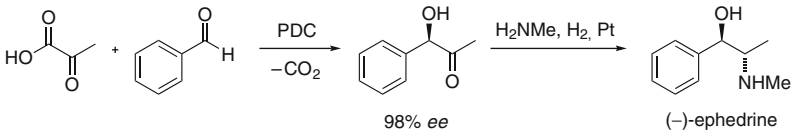

Fig. 42 Chemoenzymatic synthesis of (−)-ephedrine

[205–207]. The formation of the acyloin intermediate is catalyzed by pyruvate decarboxylase (PDC) with excellent enantioselectivity (Fig. 42).

2,3-Dioxygenated aryl propanones are very frequently used chiral building blocks for the synthesis of different biologically active compounds. They were synthons in the synthetic approach to 1,4-benzodioxane lignans, such as 5′-methoxyhydno-carpin or cytoxazone, or for the production of aryl isoserines, which are precursors to the side-chain of paclitaxel (Taxol®). A new access to these compounds was established by an enzymatic approach using BAL as the acyloin forming biocatalyst (Fig. 43) [208].

5 Conclusion

Nature with its complexity has provided a cornucopia of tools for improvements in twenty-first century natural science research. With new insights into metabolic pathways, chemists can adopt an increasing amount of evolutionary innovation and apply this knowledge to drug and natural product synthesis.

The usage of microorganisms or isolated enzymes thereof is gaining more and more in popularity, leading to an enormous variety of new enzymes being found and optimized for commercialization. Especially in asymmetric synthesis, enzymes can substitute or complement existing methods, often providing ecological advantages due to greater efficiency or less toxicity of the catalyst (enzyme vs transition metal). Thus, the number of applications of enzyme-mediated synthesis has risen enormously during the last few years and the trend does not seem to slow down.

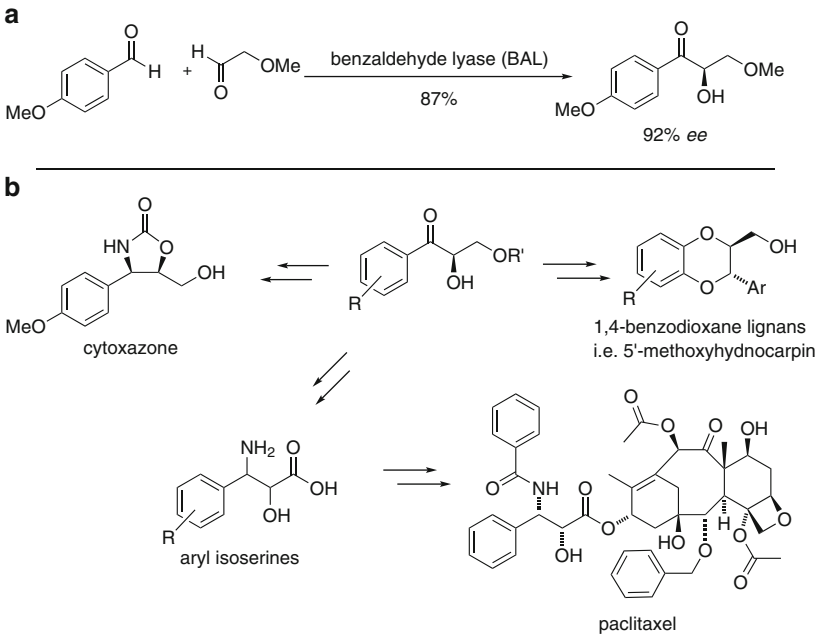

Fig. 43 (**a**) Benzaldehyde lyase-catalyzed acyloin formation. (**b**) Application of acyloins in natural product synthesis

Biocatalysis is a main part of white biotechnology and in combination with the usage of renewable primary products should lead to a more sustainable chemical and pharmaceutical industry.

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References

1. Cragg GM, Grothaus PG et al (2009) Impact of natural products on developing new anti-cancer agents. Chem Rev 109:3012–3043
2. Rollinger JM, Langer T et al (2006) Strategies for efficient lead structure discovery from natural products. Curr Med Chem 13:1491–1507

3. Gautam R, Jachak SM (2009) Recent developments in anti-inflammatory natural products. Med Res Rev 29:767–820
4. Miller SJ, Clardy J (2009) Beyond grind and find. Nat Chem 1:261–263
5. FDA (1992) FDA's policy statement for the development of new stereoisomeric drugs. Chirality 4:338–340
6. Carey JS, Laffan D et al (2006) Analysis of the reactions used for the preparation of drug candidate molecules. Org Biomol Chem 4:2337–2347
7. Kocovský P, Malkov AV (2006) Organocatalysis in organic synthesis. Tetrahedron 62:255
8. Noyori R (2002) Asymmetric catalysis: science and opportunities (Nobel lecture). Angew Chem Int Ed 41:2008–2022
9. Dalko PI, Moisan L (2001) Enantioselective organocatalysis. Angew Chem Int Ed 40:3726–3748
10. Ghanem A (2007) Trends in lipase-catalyzed asymmetric access to enantiomerically pure/enriched compounds. Tetrahedron 63:1721–1754
11. Bohman B, Unelius CR (2009) Synthesis of all four stereoisomers of 5-hydroxy-4-methyl-3-heptanone using plants and oyster mushrooms. Tetrahedron 65:8697–8701
12. Andrade LH, Utsunomiya RS et al (2006) Edible catalysts for clean chemical reactions: bioreduction of aromatic ketones and biooxidation of secondary alcohols using plants. J Mol Catal B Enzym 38:84–90
13. Cordell GA, Lemos TLG et al (2007) Vegetables as chemical reagents. J Nat Prod 70:478–492
14. Fonseca AM, Monte FJQ et al (2009) Coconut water (*Cocos nucifera* L.) – a new biocatalyst system for organic synthesis. J Mol Catal B Enzym 57:78–82
15. Bornscheuer UT, Pohl M (2001) Improved biocatalysts by directed evolution and rational protein design. Curr Opin Chem Biol 5:137–143
16. Jaeger K-E, Eggert T (2004) Enantioselective biocatalysis optimized by directed evolution. Curr Opin Biotechnol 15:305–313
17. Bornscheuer UT (2005) Trends and challenges in enzyme technology. Adv Biochem Eng Biotechnol 100:181–203
18. Reetz MT (2009) Directed evolution of enantioselective enzymes: an unconventional approach to asymmetric catalysis in organic chemistry. J Org Chem 74:5767–5778
19. Jäckel C, Kast P et al (2008) Protein design by directed evolution. Annu Rev Biophys 37:153–173
20. Carrea G, Riva S (2000) Properties and synthetic applications of enzymes in organic solvents. Angew Chem Int Ed 39:2226–2254
21. Hilterhaus L, Liese A (2007) Building blocks. Adv Biochem Eng Biotechnol 105:133–173
22. Matsuda T, Yamanaka R et al (2009) Recent progress in biocatalysis for asymmetric oxidation and reduction. Tetrahedron Asymmetry 20:513–557
23. Goldberg K, Schroer K et al (2007) Biocatalytic ketone reduction – a powerful tool for the production of chiral alcohols – part I: processes with isolated enzymes. Appl Microbiol Biotechnol 76:237–248
24. Goldberg K, Schroer K et al (2007) Biocatalytic ketone reduction – a powerful tool for the production of chiral alcohols – part II: whole-cell reductions. Appl Microbiol Biotechnol 76:249–255
25. Panke S, Wubbolts M (2005) Advances in biocatalytic synthesis of pharmaceutical intermediates. Curr Opin Chem Biol 9:188–194
26. Li Z, van Beilen JB et al (2002) Oxidative biotransformations using oxygenases. Curr Opin Chem Biol 6:136–144
27. Burton SG (2003) Oxidizing enzymes as biocatalysts. Trends Biotechnol 21:543–549
28. Hudlicky T, Reed JW (2009) Applications of biotransformations and biocatalysis to complexity generation in organic synthesis. Chem Soc Rev 38:3117–3132
29. Kayser MM (2009) 'Designer reagents' recombinant microorganisms: new and powerful tools for organic synthesis. Tetrahedron 65:947–974
30. Patel JM (2009) Biocatalytic synthesis of atorvastatin intermediates. J Mol Catal B Enzym 61:123–128

31. Patel RN (2006) Biocatalysis: synthesis of chiral intermediates for pharmaceuticals. Curr Org Chem 10:1289–1321
32. Patel RN (2008) Chemo-enzymatic synthesis of pharmaceutical intermediates. Expert Opin Drug Discov 3:187–245
33. Theil F (1995) Lipase-supported synthesis of biologically active compounds. Chem Rev 95:2203–2227
34. García-Urdiales E, Alfonso I et al (2005) Enantioselective enzymatic desymmetrizations in organic synthesis. Chem Rev 105:313–354
35. Chênevert R, Pelchat N et al (2006) Stereoselective enzymatic acylations (transesterifications). Curr Org Chem 10:1067–1094
36. Akita H (2009) Recent advances in the synthesis of biologically active natural products using biocatalyst. Heterocycles 78:1667–1713
37. Nakamura K, Matsuda T (2006) Biocatalytic reduction of carbonyl groups. Curr Org Chem 10:1217–1246
38. Mihovilovic MD (2006) Enzyme mediated Baeyer–Villiger oxidations. Curr Org Chem 10:1265–1287
39. Holt J, Hanefeld U (2009) Enantioselective enzyme-catalysed synthesis of cyanohydrins. Curr Org Synth 6:15–37
40. Molinari F (2006) Oxidations with isolated and cell-bound dehydrogenases and oxidases. Curr Org Chem 10:1247–1263
41. Sukumaran J, Hanefeld U (2005) Enantioselective C–C bond synthesis catalysed by enzymes. Chem Soc Rev 34:530–542
42. Dean Stephen M, Greenberg William A et al (2007) Recent advances in aldolase-catalyzed asymmetric synthesis. Adv Synth Catal 349:1308–1320
43. Faber K (2004) Biotransformation in organic chemistry – a textbook. Springer, Heidelberg
44. Liese A, Seelbach K, Wandrey C (eds) (2006) Industrial biotransformations. Wiley-VCH, Weinheim
45. Bornscheuer UT, Kazlauskas RJ (1999) Hydrolases in organic synthesis – regio- and stereoselective biotransformations. Wiley-VCH, Weinheim
46. Gotor V, Alfonso I, Garcia-Urdiales E (eds) (2008) Asymmetric organic synthesis with enzymes. Wiley-VCH, Weinheim
47. Schmid RD, Urlacher VB (eds) (2007) Modern biooxidation. Wiley-VCH, Weinheim
48. Garcia-Junceda E (ed) (2008) Multi-step enzyme catalysis. Wiley-VCH, Weinheim
49. Willis MC (1999) Enantioselective desymmetrisation. J Chem Soc Perkin Trans 1:1765–1784
50. Lebel H, Marcoux J-F et al (2003) Stereoselective cyclopropanation reactions. Chem Rev 103:977–1050
51. Pietruszka J (2003) Synthesis and properties of oligocyclopropyl-containing natural products and model compounds. Chem Rev 103:1051–1070
52. Grandjean D, Pale P et al (1991) Enzymatic hydrolysis of cyclopropanes. Total synthesis of optically pure dictyopterenes A and C′. Tetrahedron 47:1215–1230
53. Barloy-Da Silva C, Benkouider A et al (2000) Synthetic studies towards oxylipins: total synthesis of constanolactones A and B. Tetrahedron Lett 41:3077–3081
54. Maddess Matthew L, Tackett Miles N et al (2007) Total synthesis of rapamycin. Angew Chem Int Ed 46:591–597
55. Hayward CM, Yohannes D et al (2002) Total synthesis of rapamycin via a novel titanium-mediated aldol macrocyclization reaction. J Am Chem Soc 115:9345–9346
56. Romo D, Meyer SD et al (2002) Total synthesis of (−)-rapamycin using an Evans-Tishchenko fragment coupling. J Am Chem Soc 115:7906–7907
57. Nicolaou KC, Chakraborty TK et al (1993) Total synthesis of rapamycin. J Am Chem Soc 115:4419–4420
58. Smith AB, Condon SM et al (2002) Total synthesis of rapamycin and demethoxyrapamycin. J Am Chem Soc 117:5407–5408

59. Anderson JC, Ley SV et al (1994) Studies towards the total synthesis of rapamycin: a convergent and stereoselective synthesis of the C22–C32 carbon framework. Tetrahedron Lett 35:2087–2090
60. Ghosh AK, Xu X (2004) Assignment of absolute stereochemistry and total synthesis of (α)-spongidepsin. Org Lett 6:2055–2058
61. Ley SV, Tackett MN et al (2009) Total synthesis of rapamycin. Chem Eur J 15:2874–2914
62. Kagan HB, Fiaud JC (1988) Kinetic resolution. Top Stereochem 18:249–330
63. Chen C-S, Fujimoto Y et al (1982) Quantitative analyses of biochemical kinetic resolutions of enantiomers. J Am Chem Soc 104:7294–7299
64. Chen C-S, Wu S-H et al (1987) Quantitative analyses of biochemical kinetic resolution of enantiomers. 2. Enzyme-catalyzed esterifications in water–organic solvent biphasic systems. J Am Chem Soc 109:2812–2817
65. Scheid G, Ruijter E et al (2004) Synthesis and resolution of a key building block for epothilones: a comparison of asymmetric synthesis, chemical and enzymatic resolution. Tetrahedron Asymmetry 15:2861–2869
66. Ma S (2005) Some typical advances in the synthetic applications of allenes. Chem Rev 105:2829–2872
67. Carballeira JD, Krumlinde P et al (2007) Directed evolution and axial chirality: optimization of the enantioselectivity of *Pseudomonas aeruginosa* lipase towards the kinetic resolution of a racemic allene. Chem Commun 43:1913–1915
68. Deska J, Bäckvall J-E (2009) Enzymatic kinetic resolution of primary allenic alcohols. Application to the total synthesis and stereochemical assignment of striatisporolide A. Org Biomol Chem 7:3379–3381
69. Horvath A, Bäckvall J-E (2004) Mild and efficient palladium(II)-catalyzed racemization of allenes. Chem Commun 40:964–965
70. Kanazawa S, Fusetani N et al (1993) Cylindramide: cytotoxic tetramic acid lactam from the marine sponge *Halichondria cylindrata* Tanita & Hoshino. Tetrahedron Lett 34:1065–1068
71. Cramer N, Buchweitz M et al (2006) Total synthesis and NMR investigations of cyclindramide. Chem Eur J 12:2488–2503
72. Jiang X, García-Fortanet J et al (2005) Synthesis and complete stereochemical assignment of psymberin/irciniastatin A. J Am Chem Soc 127:11254–11255
73. Huang X, Shao N et al (2007) The total synthesis of psymberin. Org Lett 9:2597–2600
74. Smith AB, Jurica JA et al (2008) Total synthesis of (+)-psymberin (irciniastatin A): catalytic reagent control as the strategic cornerstone. Org Lett 10:5625–5628
75. Pietruszka J, Simon RC (2009) Chemoenzymatic synthesis of (protected) psymberic acid. Eur J Org Chem 2009:3628–3634
76. Nishizawa M, Shimizu M et al (1995) Stereoselective production of (+)-*trans*-chrysanthemic acid by a microbial esterase: cloning, nucleotide sequence, and overexpression of the esterase gene of *Arthrobacter globiformis* in *Escherichia coli*. Appl Environ Microbiol 61:3208–3215
77. Nishizawa M, Gomi H et al (1993) Purification and some properties of carboxylesterase from *Arthrobacter globiformis*; stereoselective hydrolysis of ethyl chrysanthemate. Biosci Biotech Biochem 57:594–598
78. Nanda S, Rao AB et al (1999) Enzyme catalysed kinetic resolution of racemic 2,2-dimethyl-3-(2,2-disubstituted vinyl) cyclopropane carboxylic acids anchored on polymer supports. Tetrahedron Lett 40:5905–5908
79. Yadav JS, Rao AB et al (1997) Enzymatic resolution of (+/–)-*cis*-3-(2,2-dichloro-3,3,3-trifluoropropyl)-2,2-dimethylcyclopropane carboxylate. Tetrahedron Asymmetry 8:2291–2294
80. Csuk R, Schabel MJ et al (1996) Synthesis of the enantiomer of the antidepressant tranylcypromine. Tetrahedron Asymmetry 7:3505–3512

81. Csuk R, Scholz Yv (1996) Enantiomerically pure cyclopropanoid nucleoside analogues: synthesis and analysis. Tetrahedron 52:6383–6396
82. Rosen TC, Haufe G (2002) Synthesis of enantiopure monofluorinated phenylcyclopropanes by lipase-catalyzed kinetic resolution. Tetrahedron Asymmetry 13:1397–1405
83. Tsuji T, Onishi T et al (1999) Lipase-catalyzed synthesis of a tri-substituted cyclopropyl chiral synthon: a practical method for preparation of chiral 1-alkoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexane. Tetrahedron Asymmetry 10:3819–2825
84. Beumer R, Bubert C et al (2000) The synthesis of diastereo- and enantiomerically pure β-aminocyclopropanecarboxylic acids. J Org Chem 65:8960–8969
85. Pietruszka J, Rieche ACM et al (2003) Kinetic enzymatic resolution of cyclopropane derivatives. Adv Synth Catal 345:1273–1286
86. Pietruszka J, Wilhelm T (2003) Total synthesis of marine oxylipins constanolactone A and B. Synlett 11:1698–1700
87. Pietruszka J, Rieche ACM (2008) Total synthesis of marine oxylipins solandelactones A–H. Adv Synth Catal 350:1407–1412
88. Bischop M, Doum V et al (2010) Total synthesis of halicholactone and neohalicholactone. Synthesis 42:527–537
89. Mahapatra T, Bhunya R et al (2009) Chemo-enzymatic asymmetric total synthesis of penienone. Tetrahedron Lett 50:5392–5394
90. Pellissier H (2003) Dynamic kinetic resolution. Tetrahedron 59:8291–8327
91. Pellissier H (2008) Recent developments in dynamic kinetic resolution. Tetrahedron 64:1563–1601
92. Kamaruddin AH, Uzir MH et al (2009) Chemoenzymatic and microbial dynamic kinetic resolutions. Chirality 21:449–467
93. Martín-Matute B, Edin M et al (2004) Highly compatible metal and enzyme catalysts for efficient dynamic kinetic resolution of alcohols at ambient temperature. Angew Chem Int Ed 43:6535–6539
94. Akai S, Tanimoto K et al (2006) A dynamic kinetic resolution of allyl alcohols by the combined use of lipases and $[VO(OSiPh_3)_3]$. Angew Chem Int Ed 45:2592–2595
95. Ödman P, Wessjohann LA et al (2005) Chemoenzymatic dynamic kinetic resolution of acyloins. J Org Chem 70:9551–9555
96. Kowalski RJ, Giannakakous P et al (1997) Activities of the microtubule-stabilizing agents epothilones A and B with purified tubulin and in cells resistant to paclitaxel (Taxol). J Biol Chem 272:2534–2541
97. Akai S, Tanimoto K et al (2004) Lipase-catalyzed domino dynamic kinetic resolution of racemic 3-vinylcyclohex-2-en-1-ols/Intramolecular Diels–Alder reaction: one-pot synthesis of optically active polysubstituted decalins. Angew Chem Int Ed 43:1407–1410
98. Brown AG, Smale TC et al (1976) Crystal and molecular structure of compactin, a new antifungal metabolite from *Penicillium brevicompactum*. J Chem Soc Perkin Trans 1:1165–1170
99. Robichaud J, Tremblay F (2006) Formal enantioselective synthesis of (+)-compactin. Org Lett 8:597–600
100. Calvo D, Port M et al (1996) Total synthesis of forskolin – part III studies related to an asymmetric synthesis. Tetrahedron Lett 37:1023–1024
101. Nagashima S, Kanematsu K (1990) A synthesis of an optically active forskolin intermediate via allenyl ether intramolecular cycloaddition strategy. Tetrahedron Asymmetry 1:743–749
102. Bhat SV, Bajqwa BS et al (1977) Structures and stereochemistry of new labdane diterpinoids from *coleus forskohlii briq*. Tetrahedron Lett 18:1669–1672
103. Matsumara K, Shohei H et al (1997) Asymmetric transfer hydrogenation of α,β-acetylenic ketones. J Am Chem Soc 119:8738–8739
104. Corey EJ, Helal CJ (1998) Reduction of carbonyl compounds with chiral oxazaborolidine catalysts: a new paradigm for enantioselective catalysis and a powerful new synthetic method. Angew Chem Int Ed 37:1986–2012

105. Corey EJ, Guzman-Perez A et al (1997) An enantioselective synthetic route to atractyligenin using the oxazaborolidine-catalyzed reduction of β-Siliyl- or β-stannyl-substituted α,β-enones as a key step. J Am Chem Soc 119:11769–11776
106. Daußmann T, Hennemann H-G et al (2006) Enzymatische technologien zur synthese chiraler alkohol-derivate. Chem Ing Tech 78:249–255
107. de Wildeman SMA, Sonke T et al (2007) Biocatalytic reductions: from lab curiosity to "first choice". Acc Chem Res 40:1260–1266
108. Ruinatscha R, Höllrigl V et al (2006) Productivity of selective electroenzymatic reduction and oxidation reactions: theoretical and practical considerations. Adv Synth Catal 348:2015–2026
109. Stillger T, Bönitz M et al (2002) Überwindung von thermodynamischen Limitierungen in substratgekoppelten Cofaktorregenerierungsverfahren. Chem Ing Tech 74:1035–1039
110. Goldberg K, Edegger K et al (2006) Overcoming the thermodynamic limitation in asymmetric hydrogen transfer reactions catalyzed by whole cells. Biotechnol Bioeng 95:192–198
111. Yang W, Xu J-H et al (2006) Asymmetric reduction of ketones by employing *Rhodotorula* sp. AS2.2241 and synthesis of the β-blocker (*R*)-nifenalol. Tetrahedron Asymmetry 17:1769–1774
112. Ema T, Ide S et al (2008) Highly efficient chemoenzymatic synthesis of methyl (*R*)-*o*-chloromandelate, a key intermediate for clopidogrel, *via* asymmetric reduction with recombinant *Escherichia coli*. Adv Synth Catal 350:2039–2044
113. Tanaka T, Okamura N et al (1986) Syntheses of (5E)-PGE2 and new 6-functionalized derivatives by the use of palladium-catalyzed decarboxylative allylic alkylation. Tetrahedron 42:6747–6758
114. Tanaka T, Hasato A et al (1987) Preparation of (5E)-prostaglandin E2 derivatives as potential drugs, JP 62195358 A2
115. Traverso G, Pirillo D et al (1982) Sull'epossidazione del metilestere dell'acido 5-idrossi-6-n. eptenoico e sui prodotti da essa derivati. Il Farmaco Ed Sc 37:192–198
116. Pirillo D, Gazzaniga A et al (1985) A route to the total synthesis of *iso*-LTB_4 system. Il Farmaco Ed Sc 40:249–252
117. Vig OP, Dhindsa AS et al (1972) Terpenoids. LXX. Synthesis of myrcenol. J Ind Chem Soc 49:163–166
118. Jacobo SH, Chang C-T et al (2006) Total synthesis of 8,12-*iso*-iPF3α-VI, an EPA-derived isoprostane: stereoselective introduction of the fifth asymmetric center. J Org Chem 71:1370–1379
119. Fischer T, Pietruszka J (2007) Efficient synthesis of either enantiomer of ethyl 5-hydroxyhept-6-enoate. Adv Synth Catal 349:1533–1536
120. Pietruszka J, Rieche ACM et al (2007) Synthesis of marine oxylipins constanolactones C and D. Synlett 18:2525–2528
121. Fischer T, Pietruszka J Heinrich-Heine-Universität Düsseldorf, unpublished results
122. Wolberg M, Filho M et al (2008) Chemoenzymatic synthesis of the chiral side-chain of statins: application of an alcohol dehydrogenase catalysed ketone reduction on a large scale. Bioprocess Biosyst Eng 31:183–191
123. Wolberg M, Hummel W et al (2001) Biocatalytic reduction of β,δ-diketo esters: a highly stereoselective approach to all four stereoisomers of a chlorinated β,δ-dihydroxy hexanoate. Chem Eur J 7:4562–4571
124. Wolberg M, Hummel W et al (2000) Highly regio- and enantioselective reduction of 3,5-dioxocarboxylates. Angew Chem 39:4306–4308
125. Müller M, Wolberg M et al (2005) Enzyme-catalyzed regio-and enantioselective ketone reductions. Adv Biochem Eng Biotechnol 92:261–287
126. Chen K-M, Hardtmann GE et al (1987) 1,3-Diastereoselective reduction of β-hydroxyketones utilizing alkoxydialkylboranes. Tetrahedron Lett 28:155–158
127. Evans DA, Chapman KT et al (2002) Directed reduction of β-hydroxy ketones employing tetramethylammonium triacetoxyborohydride. J Am Chem Soc 110:3560–3578

128. Wada M, Kawabata H et al (1999) Occurrence of multiple ethyl 4-chloro-3-oxobutanoate-reducing enzymes in *Candida magnoliae*. J Biosci Bioeng 87:144–148
129. Patel RN, McNamee CG et al (1992) Stereoselective reduction of β-keto esters by *Geotrichum candidum*. Enzyme Microb Technol 14:731–738
130. Spelberg JHL, Tang L et al (2004) Enzymatic dynamic kinetic resolution of epihalohydrins. Tetrahedron Asymmetry 15:1095–1102
131. Nakamura T, Nagasawa T et al (1991) A new catalytic function of halohydrin hydrogen-halide-lyase, synthesis of β-hydroxynitriles from epoxides and cyanide. Biochem Biophys Res Commun 180:124–130
132. Fox RJ, Davis SC et al (2007) Improving catalytic function by ProSAR-driven enzyme evolution. Nat Biotechnol 25:338–344
133. Brown BJ, Deng Z et al (1998) On the active site of old yellow enzyme. J Biol Chem 273:32753–32762
134. Fox KM, Karplus PA (1994) Old yellow enzyme at 2 Å resolution: overall structure, ligand binding, and comparison with related flavoproteins. Structure 2:1089–1105
135. Kohli RM, Massey V (1998) The oxidative half-reaction of old yellow enzyme. J Biol Chem 273:32763–32770
136. Leuenberger HGW, Boguth W et al (1976) Synthese von optisch aktiven, natürlichen Carotinoiden und strukturell verwandten Naturprodukten. I. Synthese der chiralen Schlüsselverbindung (4*R*,6*R*)-4-Hydroxy-2,2,6-trimethylcyclohexanon. Helv Chim Acta 59:1832–1849
137. Kataoka M, Kotaka A et al (2004) Cloning and overexpression of the old yellow enzyme gene of *Candida macedoniensis*, and its application to the production of a chiral compound. J Biotechnol 114:1–9
138. Hall M, Stueckler C et al (2008) Asymmetric bioreduction of activated C=C bonds using *Zymomonas mobilis* NCR enoate reductase and old yellow enzymes OYE 1–3 from yeasts. Eur J Org Chem 2008:1511–1516
139. Wada M, Yoshizumi A et al (2003) Production of a doubly chiral compound, (4*R*,6*R*)-4-hydroxy-2,2,6-trimethylcoclohexanone, by two-step enzymatic asymmetric reduction. Appl Environ Microbiol 69:933–937
140. Shimoda K, Kubota N et al (2004) Asymmetric reduction of α,β-unsaturated carbonyl compounds with reductases from *Nicotiana tabacum*. Tetrahedron Asymmetry 15:2443–2446
141. Hirata T, Takarada A et al (2005) Hydrogenation of the C–C double bond of maleimides with cultured plant cells. J Mol Catal B Enzym 32:131–134
142. Perdih A, Dolenc MS (2007) Recent advances in the synthesis of unnatural amino acids. Curr Org Chem 11:801–832
143. Hanson RL (2007) In: Gadamasetti K, Braish T (eds) Process chemistry in the pharmaceutical industry, vol 2. Challenges in an ever changing climate. CRC, Boca Raton
144. Robl JA, Sun C-Q et al (1997) Dual metalloprotease inhibitors: mercaptoacetyl-based fused heterocyclic dipeptide mimetics as inhibitors of angiotensin-converting enzyme and neutral endopeptidase. J Med Chem 40:1570–1577
145. Hanson RL, Howell JM et al (2000) Synthesis of allysine ethylene acetal using phenylalanine dehydrogenase from *Thermoactinomyces intermedius*. Enzyme Microb Technol 26:348–358
146. Hanson RL, Schwinden MD et al (1999) Enzymatic synthesis of L-6-hydroxynorleucine. Bioorg Med Chem 7:2247–2252
147. Patel RN (2001) Enzymatic synthesis of chiral intermediates for omapatrilat, an antihypertensive drug. Biomol Eng 17:167–182
148. Urlacher VB, Eiben S (2006) Cytochrome P450 monooxygenases: perspectives for synthetic application. Trends Biotechnol 24:324–330
149. Julsing MK, Cornelissen S et al (2008) Heme-iron oxygenases: powerful industrial biocatalysts? Curr Opin Chem Biol 12:177–186
150. Bernhardt R (2006) Cytochromes P450 as versatile biocatalysts. J Biotechnol 124:128–145

151. Tee KL, Schwaneberg U (2007) Directed evolution of oxygenases: screening systems, success stories and challenges. Comb Chem High Throughput Screen 10:197–217
152. Park J-W, Lee J-K et al (2003) Bioconversion of compactin into pravastatin by *Streptomyces* sp. Biotechnol Lett 25:1827–1831
153. Hosobuchi M, Kurosawa K et al (1993) Application of computer to monitoring and control of fermentation process: microbial conversion of ML-236B Na to pravastatin. Biotechnol Bioeng 42:815–820
154. Matsuoka T, Miyakoshi S et al (1989) Purification and characterization of cytochrome P_{450} from *Streptomyces carbophilus*. Eur J Biochem 184:707–713
155. Ro D-K, Paradise EM et al (2006) Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440:940–943
156. Ryerson CC, Ballou DP et al (1982) Mechanistic studies on cyclohexanone oxygenase. Biochemistry 21:2644–2655
157. Taschner MJ, Aminbhavi AS (1989) α-Oxygenation of a trans-3,4-disubstituted γ-lactone. A comparative study. Tetrahedron Lett 30:1029–1032
158. Taschner MJ, Black DJ (1988) The enzymatic Baeyer–Villiger oxidation: enantioselective synthesis of lactones from mesomeric cyclohexanones. J Am Chem Soc 110:6892–6893
159. Yokokawa F, Hamada Y et al (1996) Total synthesis of calyculin A – construction of the C (9)–C(37) fragment. Chem Commun 32:871–872
160. Gagnon R, Grogan G et al (1995) Enzymatic Baeyer–Villiger oxidations of some bicyclo [2.2.1]heptan-2-ones using monooxygenases from *Pseudomonas putida* NCIMB 10007: enantioselective preparation of a precursor of azadirachtin. J Chem Soc Perkin Trans 1:1505–1511
161. Veitch GE, Beckmann E et al (2007) A relay route for the synthesis of azadirachtin. Angew Chem Int Ed 46:7633–7635
162. Veitch GE, Beckmann E et al (2007) Synthesis of azadirachtin: a long but successful journey. Angew Chem Int Ed 46:7629–7632
163. Veitch GE, Boyer A et al (2008) The azadirachtin story. Angew Chem Int Ed 47:9402–9429
164. Tanaka M, Mukaiyama C et al (2002) Synthesis of optically pure gomisi lignans: the total synthesis of (+)-schizandrin, (+)-gomisin A, and (+)-isoschizandrin in naturally occurring forms. J Org Chem 60:4339–4352
165. Honda T, Kimura N et al (1994) Chiral synthesis of lignan lactones, (−)-hinokinin, (−)-deoxypodorhizone, (−)-isohibalactone and (−)-savinin by means of enantioselective deprotonation strategy. J Chem Soc Perkin Trans 1:1043–1046
166. Alphand V, Mazzini C et al (1998) A new microorganism for highly stereospecific Baeyer–Villiger oxidation of prochiral cyclobutanones. J Mol Catal B Enzym 5:219–221
167. Mihovilovic MD, Bianchi DA et al (2006) Accessing tetrahydrofuran-based natural products by microbial Baeyer–Villiger biooxidation. Chem Commun 42:3214–3216
168. Hudlicky T, Reed JW (2009) Celebrating 20 years of SYNLETT – special account on the merits of biocatalysis and the impact of arene cis-dihydrodiols on enantioselective synthesis. Synlett 2009:685–703
169. Sullivan B, Hudlicky T (2008) Chemoenzymatic formal synthesis of (−)-balanol. Provision of optical data for an often-reported intermediate. Tetrahedron Lett 49:5211–5213
170. Fabris F, Collins J et al (2009) Investigation of steric and functionality limits in the enzymatic dihydroxylation of benzoate esters. Versatile intermediates for the synthesis of pseudo-sugars, amino cyclitols, and bicyclic ring systems. Org Biomol Chem 7:2619–2627
171. Banwell MG, Austin KAB et al (2007) Chemoenzymatic total syntheses of the linear triquinane-type natural products (+)-hirsutic acid and (−)-complicatic acid from toluene. Tetrahedron 63:6388–6403
172. Pinkerton DM, Banwell MG et al (2009) Chemoenzymatic access to versatile epoxyquinol synthons. Org Lett 11:4290–4293
173. Omori AT, Finn KJ et al (2007) Chemoenzymatic total synthesis of (+)-codeine by sequential intramolecular Heck cyclizations via C–B–D ring construction. Synlett 2007:2859–2862

174. Ley SV, Redgrave AJ (1990) Microbial oxidation in synthesis: concise preparation of (+)-conduritol F from benzene. Synlett 1990:393–394
175. Matveenko M, Willis AC et al (2008) A chemoenzymatic synthesis of the anti-influenza agent Tamiflu®. Tetrahedron Lett 49:7018–7020
176. Shie J-J, Fang J-M et al (2008) A concise and flexible synthesis of the potent anti-influenza agents tamiflu and tamiphosphor. Angew Chem Int Ed 47:5788–5791
177. Sullivan B, Carrera I et al (2009) Symmetry-based design for the chemoenzymatic synthesis of oseltamivir (tamiflu) from ethyl benzoate. Angew Chem Int Ed 48:4229–4231
178. Franke D, Lorbach V et al (2003) (*S*,*S*)-2,3-Dihydroxy-2,3-dihydrobenzoic acid: microbial access with engineered cells of *Escherichia coli* and application as starting material in natural-product synthesis. Chem Eur J 9:4188–4196
179. Franke D, Sprenger GA et al (2001) Synthesis of functionalized cyclohexadiene-*trans*-diols with recombinant cells of *Escherichia coli*. Angew Chem Int Ed 40:555–557
180. Lorbach V, Franke D et al (2002) Cyclohexadiene-*trans*-diols as versatile starting material in natural product synthesis: short and efficient synthesis of *iso*-crotepoxide and *ent*-senepoxide. Chem Commun 38:494–495
181. Hausmann T, Pietruszka J (2009) Enantiopure dicyclopropanes from trans-cyclohexadiene-diols. Synlett 21:3271–3274
182. Mukaiyama T (2004) Explorations into new reaction chemistry. Angew Chem Int Ed 43:5590–5614
183. Evans DA, Bartroli J et al (1981) Enantioselective aldol condensations. 2. Erythro-selective chiral aldol condensations via boron enolates. J Am Chem Soc 103:2127–2129
184. Guillena G, Nájera C et al (2007) Enantioselective direct aldol reaction: the blossoming of modern organocatalysis. Tetrahedron Asymmetry 18:2249–2293
185. Hanson CV, Nishiyama Y et al (2005) Catalytic antibodies and their applications. Curr Opin Biotechnol 16:631–636
186. Kiyooka S-i (1997) Development of a chiral lewis acid-promoted asymmetric aldol reaction using oxaborolidinone. Rev Heteroatom Chem 17:245–270
187. Machajewski TD, Wong C-H (2000) The catalytic asymmetric aldol reaction. Angew Chem Int Ed 39:1352–1375
188. Fessner W-D, Helaine V (2001) Biocatalytic synthesis of hydroxylated natural products using aldolases and related enzymes. Curr Opin Biotechnol 12:574–586
189. Romero A, Wong C-H (2000) Chemo-enzymatic total synthesis of 3-epiaustraline, australine, and 7-epialexine. J Org Chem 65:8264–8268
190. Chênevert R, Dasser M (2000) Chemoenzymatic synthesis of the microbial elicitor (−)-syringolide via a fructose 1,6-diphosphate aldolase-catalyzed condensation reaction. J Org Chem 65:4529–4531
191. Woodhall T, Williams G et al (2005) Synthesis of screening substrates for the directed evolution of sialic acid aldolase: towards tailored enzymes for the preparation of influenza A sialidase inhibitor analogues. Org Biomol Chem 3:1795–1800
192. Woodhall T, Williams G et al (2005) Creation of a tailored aldolase for the parallel synthesis of sialic acid mimetics. Angew Chem Int Ed 44:2109–2112
193. Steinreiber J, Fesko K et al (2007) Synthesis of γ-halogenated and long-chain β-hydroxy-α-amino acids and 2-amino-1,3-diols using threonine aldolases. Tetrahedron 63:8088–8093
194. Liu J, Wong C-H (2002) Aldolase-catalyzed asymmetric synthesis of novel pyranose synthons as a new entry to heterocycles and epothilones. Angew Chem Int Ed 41:1404–1407
195. Liu J, Hsu C-C et al (2004) Sequential aldol condensation catalyzed by DERA mutant Ser238Asp and a formal total synthesis of atorvastatin. Tetrahedron Lett 45:2439–2441
196. Jennewein S, Schürmann M et al (2006) Directed evolution of an industrial biocatalyst: 2-deoxy-D-ribose 5-phosphate aldolase. Biotechnol J 1:537–548
197. Effenberger F (1994) Synthesis and reactions of optically active cyanohydrins. Angew Chem Int Ed 33:1555–1564

198. Gregory RJH (1999) Cyanohydrins in nature and the laboratory: biology, preparations, and synthetic applications. Chem Rev 99:3649–3682
199. Effenberger F, Eichhorn J et al (1995) Enzyme catalyzed addition of hydrocyanic acid to substituted pivalaldehydes – a novel synthesis of (*R*)-pantolactone. Tetrahedron Asymmetry 6:271–282
200. Pscheidt B, Liu Z et al (2008) Efficient biocatalytic synthesis of (*R*)-pantolactone. Adv Synth Catal 350:1943–1948
201. Vashkov VI, Volkov YP et al (1968) Investigation of pyrethrins and related compounds. XV. Esters of *trans*-2,2-dichloro-3-arylcyclopropanecarboxylic acids as insecticides. Pharm Chem J 2:17–20
202. Breuer M, Ditrich K et al (2004) Industrial methods for the production of optically active intermediates. Angew Chem Int Ed 43:788–824
203. Lu W, Chen P et al (2008) New stereoselective synthesis of thiamphenicol and florfenicol from enantiomerically pure cyanohydrin: a chemo-enzymatic approach. Tetrahedron 64:7822–7827
204. Müller M, Gocke D et al (2009) Thiamin diphosphate in biological chemistry: exploitation of diverse thiamin diphosphate-dependent enzymes for asymmetric chemoenzymatic synthesis. FEBS J 276:2894–2904
205. Pohl M (1997) Protein design on pyruvate decarboxylase (PDC) by site-directed mutagenesis. Adv Biochem Eng Biotechnol 58:15–43
206. Rosche B, Breuer M et al (2004) Biphasic aqueous/organic biotransformation of acetaldehyde and benzaldehyde by *Zymomonas mobilis* pyruvate decarboxylase. Biotechnol Bioeng 86:788–794
207. Pohl M, Lingen B et al (2002) Thiamin-diphosphate-dependent enzymes: new aspects of asymmetric C–C bond formation. Chem Eur J 8:5288–5295
208. Demir AS, Sesenoglu O et al (2003) Benzaldehyde lyase-catalyzed enantioselective carboligation of aromatic aldehydes with mono- and dimethoxy acetaldehyde. Org Lett 5:2047–2050