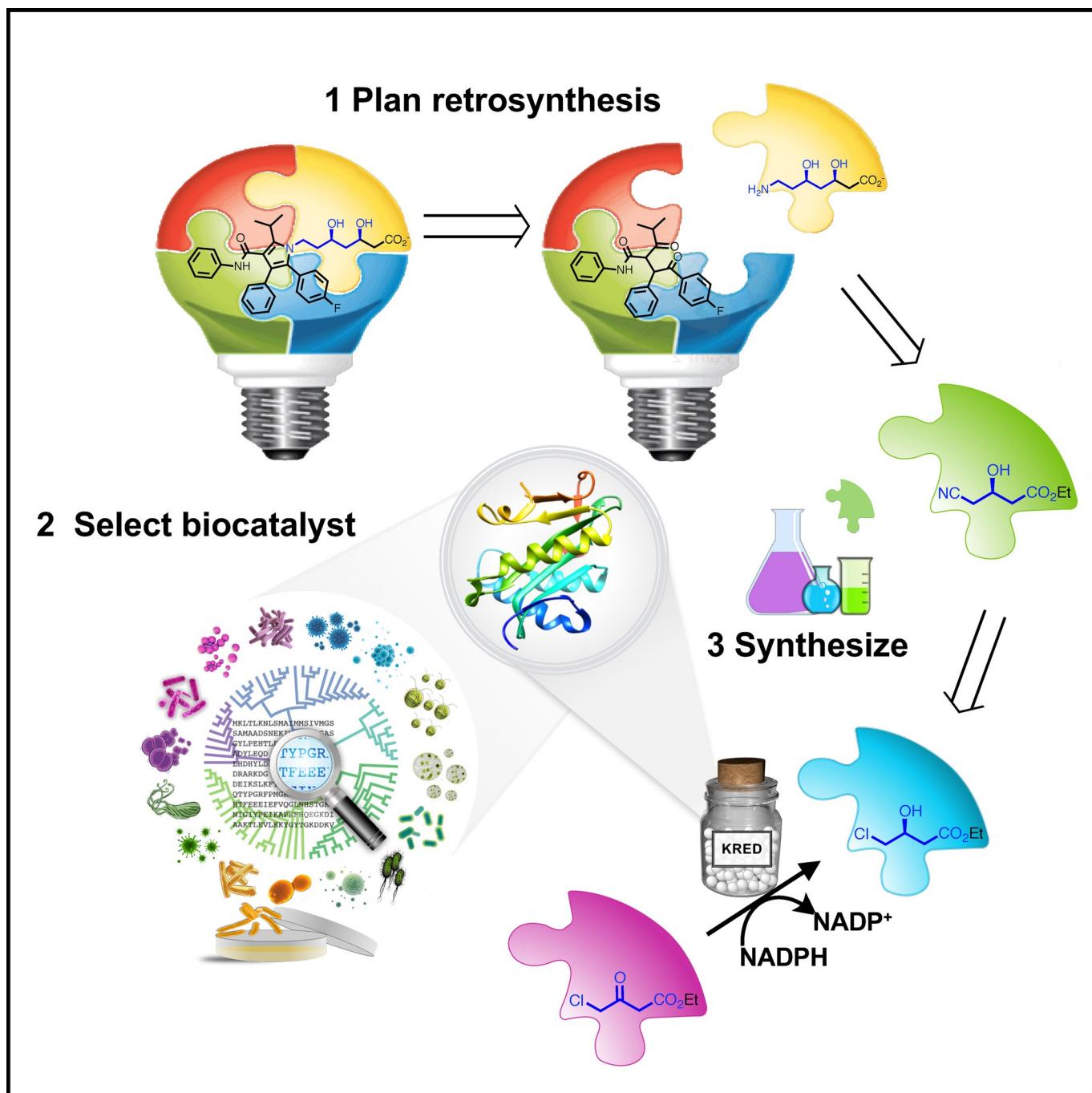


**Biotransformation****A Retrosynthesis Approach for Biocatalysis in Organic Synthesis**Rodrigo O. M. A. de Souza,<sup>\*[a]</sup> Leandro S. M. Miranda,<sup>[a]</sup> and Uwe T. Bornscheuer<sup>\*[b]</sup>

**Abstract:** For the planning of an organic synthesis route, the disconnection approach guided by retrosynthetic analysis of possible intermediates and the chemical reactions involved, back to ready available starting materials, is well established. In contrast, such concepts just get developed for biocatalytic routes. In this Review we highlight functional group interconversions catalyzed by enzymes. The article is organized rather by chemical bonds formed—exemplified for C–N, C–O- and C–C-bonds—and not by enzyme classes, covering a broad range of reactions to incorporate the desired functionality in the target molecule. Furthermore, the

successful use of biocatalysts, also in combination with chemical steps, is exemplified for the synthesis of various drugs and advanced pharmaceutical intermediates such as Crispine A, Sitagliptin and Atorvastatin. This Review also provides some basic guidelines to choose the most appropriate enzyme for a targeted reaction keeping in mind aspects like commercial availability, cofactor-requirement, solvent tolerance, use of isolated enzymes or whole cell recombinant microorganisms aiming to assist organic chemists in the use of enzymes for synthetic applications.

## 1. Introduction

Since the early beginnings of chemical sciences, organic synthesis has fascinated scientists due to the capability of building challenging molecules from scratch. The quest to create very complex structures efficiently and in fewer steps than in previously reported syntheses is an ongoing challenge in many laboratories around the world.

Most of the time, in order to elaborate a synthetic route towards a desired target, scientists use the retrosynthetic approach proposed by E. J. Corey where the target structure is subjected to a deconstruction process, which corresponds to the reverse of a synthetic reaction.<sup>[1]</sup> This approach has inspired several synthetic organic chemists and introduced a new way of thinking about a synthetic problem as also exemplified in the benchmark text book on retrosynthesis published by Warren and Wyatt.<sup>[2]</sup>

Based on this, chemists were able to disconnect specific parts of the target molecule and work on possible reactions for that specific mission. From this moment, the retrosynthetic approach took the synthetic community to a blessed collateral effect, the development of new methodologies. The disconnection approach leads one to fragments that should be connected through a certain chemical reaction, but sometimes this reaction is not well developed or does not exist.

The same is true for biocatalysis. For years researchers have used whole cell microorganisms and/or isolated enzymes to perform chemical transformations, but the lack of stability, substrate scope and restricted reaction conditions has taken biocatalysis to the dark side of organic synthesis. For instance many

organic chemists only were aware of the use of lipases<sup>[3]</sup> and Baker's yeast, the latter being considered irreproducible (due to the presence of several alcohol dehydrogenases/ ketoreductases). Many enzymes were only available for decades in small amounts at high price, for example, extracted from animal or plant tissue, but now can be easily produced on large scale in suitable microbial hosts due to the availability of a plethora of standard expression systems for instance for *Escherichia coli*, *Saccharomyces cerevisiae*, *Pichia pastoris*, *Aspergillus* or *Bacillus* species. The use of enzymes for organic synthesis as well as basic principles are covered in a range of excellent books.<sup>[3a,4]</sup> As millions of protein sequences are available in public databases and the encoding genes can now simply be ordered at low price as synthetic versions—already optimized for expression in the desired host organism—access to novel biocatalysts is no limiting factor any longer (see also Section in Summary and Outlook).<sup>[5]</sup>

Furthermore, protein engineering has emerged as very important tool for the development of new biocatalysts entities with desired tailor-designed desired properties. With the advent of computational techniques and site-directed mutagenesis, or by directed (molecular) evolution techniques researchers can produce enzymes with optimized features such as activity, (enantio-, regio- and chemo-) selectivity, stability, substrate specificity, cofactor specificity, tolerance of co-solvents, pH optimum and cofactor requirements, among others.<sup>[5b]</sup> This facilitates biocatalyst production and to expand the toolbox of enzymes that can be used for organic synthesis.<sup>[5a,6]</sup>

In this Review we exemplify important reactions that can be catalyzed by enzymes, along with substrate scope and incorporation of the biocatalytic step into reaction routes, taking into account pros and cons for each class of enzymes. The article is organized by the functional groups formed and not by enzyme class or name reactions<sup>[7]</sup> to facilitate an easier identification of suitable enzymes for a given chemical reaction, similar to the disconnecting approaches developed by Corey, Warren and Wyatt. Compared with two recent articles<sup>[8]</sup> on the same topic—especially the one by Höning et al. published after submission of this contribution—our review differs in terms of the conceptual approach, the many retrosynthesis examples and the guidelines provided. We thus hope that this article will serve as a useful guide to enhance the development of more

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straightforward organic synthesis protocols by the use of biocatalysts.

## 2. Functional Group Analysis

Manipulation of functional groups in organic synthesis is an art, which needs careful analysis in order to avoid side reactions, like mixing colors to paint a picture. The chemo-, regio- and stereospecificity of biocatalysts can help researchers on

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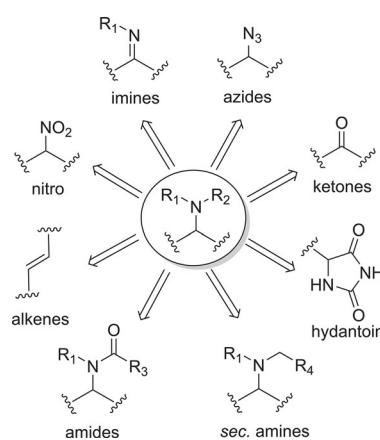
**Rodrigo Octavio M. A. de Souza** has a degree in Pharmacy from the Federal University of Rio de Janeiro (2001), a Master's Degree in Natural Product Chemistry from the Federal University of Rio de Janeiro (2003) and a PhD in Natural Product Chemistry also from the Federal University of Rio de Janeiro (2007). In 2007, he began to work as an Associate Researcher at the Institute of Chemistry of the Federal University of Rio de Janeiro under the direction of Professor Octavio Augusto Ceva Antunes. The following year (2008) was admitted as Adjunct Professor of the Chemistry Institute of the Federal University from Rio de Janeiro. In 2009 he was a visiting researcher at the University of Graz in the laboratory of Prof. Oliver Kappe. Also in 2009, he established in Brazil the development of chemo-enzymatic processes through the use of continuous-flow reactors. He organized several national and international congresses such as the 15<sup>th</sup> Brazilian Meeting on Organic Synthesis (2013), 4<sup>th</sup> Workshop on Biocatalysis and Biotransformations (2014) and 16<sup>th</sup> Brazilian Meeting on Organic Synthesis. He has experience in the field of organic chemistry, with emphasis on new synthetic methodologies, acting mainly on the following topics: enzymatic catalysis, heterogeneous catalysis, continuous flow reactions and cascade reactions. He is a productivity fellow at CNPq level 2, Young Scientist of the State of Rio de Janeiro (FAPERJ) from 2010–2016, South America Editor of the Journal of Flow Chemistry and Associate Editor of RSC Advances. Recently, he was awarded the Alexander von Humboldt fellowship for experienced researchers where he develops projects in collaboration with Prof. Uwe Bornscheuer.



building more direct routes to the desired target hence reducing the number of functional group manipulations.

### 2.1. Formation or Cleavage of C–N Bonds

The introduction of a nitrogen substituent is not an easy task to be performed by chemical methods,<sup>[9]</sup> but the rewards are numerous since nitrogen plays an important role in biological systems and therefore can be found in many important molecules for pharmaceutical and agrochemical use. From the biocatalysis point of view the amino functionality can be obtained by the interconversion of different functional groups, as shown in Scheme 1.



**Scheme 1.** Retrosynthetic approach for the construction or cleavage of C–N bonds.

According to Scheme 1, the introduction of nitrogen or an amino group can take place by different reactions mediated by already known biocatalysts. The most suitable enzymes to hydrolyze a C–N bond can be found in the class of amidases<sup>[10]</sup> (amidohydrolases, EC 3.5.1.4), but dihydropyrimidinase or hydantoinase<sup>[11]</sup> (EC 3.5.2.2) could also be used for ring-opening of hydantoins by cleaving a C–N bond affording the corresponding carbamoyls, which subsequently can be hydrolyzed by carbamoylases to yield  $\alpha$ -amino acids. Other enzymatic

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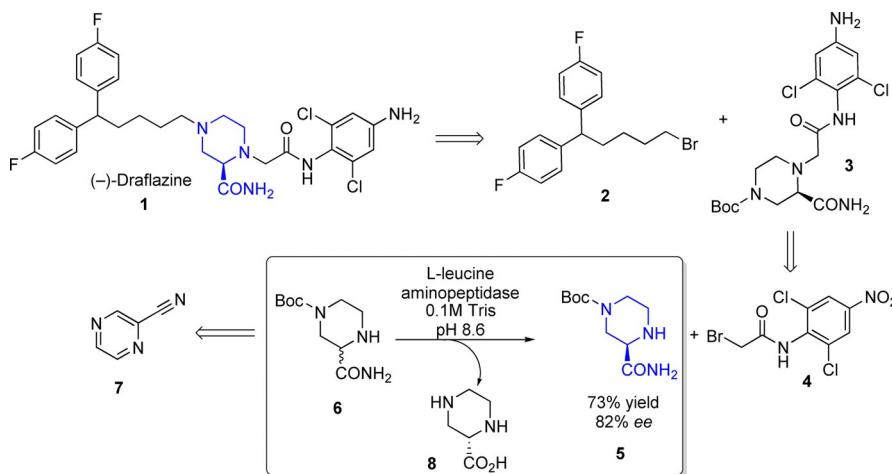


transformations for the reduction of nitro groups, azides or imines are catalyzed by flavin-dependent nitroreductases<sup>[12]</sup> (EC 1.7.1.B2/B3), imine reductases<sup>[13]</sup> (IRED, EC 1.5.1.48) or alternatively monoamine-oxidases<sup>[14]</sup> (MAO-N, EC 1.4.3.4). Lyases<sup>[15]</sup> (EC 4.3.1, 4.3.2 and 4.3.3) catalyze the insertion of nitrogen to the carbon-carbon double bond and also to the carbonyl group, but for this functional group interconversion the best choice is the use of transaminases<sup>[16]</sup> (EC 2.6.1). Notably, also a lipase-catalyzed process had been developed about 20 years ago, in which a racemic amine is converted into optically pure amides using methoxyacetate as acyl donor. Although the reaction only yields 50% pure enantiomer in the kinetic resolution, the BASF company industrialized this process.<sup>[17]</sup>

### 2.1.1. Amidases (Amidohydrolases)

Amidases (EC 3.5.1.4) are a class of enzymes that hydrolyze the C–N bond of carboxylic acid amides leading to the formation of chiral carboxylic acids and ammonia. They have a broad substrate scope (aliphatic, aromatic and heteroaromatic), but the family of amidases only hydrolyzes primary carboxylic acid amides. Even with this limitation researchers have found an interesting approach by using L-leucine aminopeptidase to overcome the traditional fractional crystallization of the diastereoisomeric ester for (–)-Draflazine synthesis (Scheme 2).<sup>[18]</sup>

One of the retrosynthetic analyses that could be made for (–)-Draflazine **1** leads to the chiral building block **3**, which could be formed by the nucleophilic substitution of chiral amine **5** with  $\alpha$ -halo ketone **4**. The chiral amine **5** could be obtained by the selective amide bond cleavage of the undesired enantiomer from the racemic precursor **6**. It is important to note that during the resolution, the L-aminopeptidase is also capable of cleaving the Boc-protecting group, which in some cases can be seen as a weak point of this methodology, if the other enantiomer is desired and re-protection is needed. Different groups reported further optimization of this protocol and engineered microorganisms that can now work directly on the unprotected substrate, which could be easily synthesized from pyrazinecarbonitrile **7** by consecutive nitrile hydrolysis and heteroaromatic ring hydrogenation.<sup>[19]</sup>



Scheme 2. Retrosynthetic analysis of (–)-Draflazine synthesis and a biocatalytic approach for the synthesis of the chiral amine **5**.

### 2.1.2. Dihydropyrimidinases or Hydantoinases

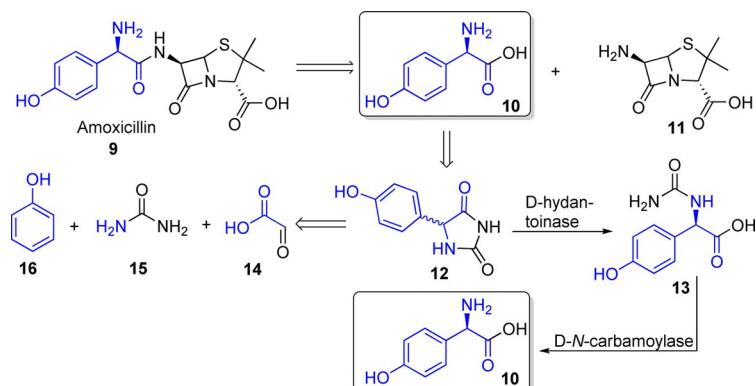
The discovery of dihydropyrimidinases and hydantoinases (EC 3.5.2.2) dates from the beginning of the 1970s. Hydantoinases catalyze the hydrolysis of hydantoins to *N*-carbamoyl- $\alpha$ -amino acids and usually the microorganisms where they had been identified from also have a highly stereoselective *N*-carbamoyl- $\alpha$ -amino acid amidohydrolases (*N*-carbamoylase; E.C.3.5.1.77 or 87), which catalyze the further hydrolysis of an *N*-carbamoyl- $\alpha$ -amino acid to the free  $\alpha$ -amino acid in an irreversible manner. Furthermore, D- and L-selective enzymes have been identified allowing the synthesis of both enantiomer of natural and notably non-natural  $\alpha$ -amino acid in a kinetic resolution. Working at pH values  $>8$  or using a racemase, dynamic kinetic resolutions are possible hence allowing theoretically 100% yield of one enantiomer starting from a racemic hydantoin.<sup>[20]</sup>

One interesting example of the use of hydantoins and *N*-carbamoyl- $\alpha$ -amino acid amidohydrolases with practical application is the “D-hydantoinase process” used for the preparation of the side chains in semi-synthetic Penicillin or Cephalosporine derivatives, such as Amoxicillin **9**<sup>[21]</sup> (Scheme 3).

The retrosynthetic analysis of the Amoxicillin synthesis arrives after the very first disconnection, the hydrolysis of the acyclic amide bond, to the desired unnatural  $\alpha$ -amino acid intermediate **10**. The unnatural  $\alpha$ -amino acid **10** could be obtained in very good yields ( $>90\%$ ) by the selective hydrolysis of the corresponding racemic hydantoin **12** in the D-hydantoinase process. **12** can be synthesized by an amidoalkylation reaction starting from phenol (**16**).<sup>[21]</sup>

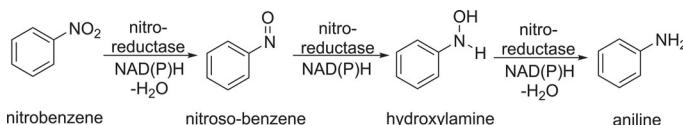
### 2.1.3. Flavin-Dependent Nitroreductases

These enzymes (EC 1.7.1.x) are often used for the biodegradation of nitroaromatics.<sup>[22]</sup> They can be classified in two different types: Type I are oxygen-insensitive nitroreductases, which act via a two electron transfer reaction (Scheme 4); Type II enzymes react via single-electron transfer yielding an unstable nitro-anion free radical, which undergoes spontaneous re-oxidation by O<sub>2</sub> producing superoxide. A more detailed plausible explanation for the chemoselective reduction by Type I nitrore-



Scheme 3. D-Hydantoinase process for chiral amino acid production.<sup>[21]</sup>

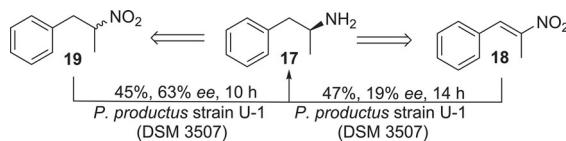
ductases is as follows: the aromatic nitro compound is first reduced via two electrons to form the arylnitroso group, which accepts another two electrons to form the arylhydroxylamine. In the final reduction step, the hydroxylamine is converted to the amine by a two-electron reduction (Scheme 4).<sup>[23]</sup>



Scheme 4. Stepwise conversion of a nitro group into an amine group catalyzed by type I flavin-dependent nitroreductases.<sup>[23]</sup>

Chemical reduction of the nitro group is a well established process and the synthetic route to many active pharmaceutical ingredients include this transformation, which can be performed in general by metal-mediated processes.<sup>[24]</sup>

Besides several microorganisms that can perform this biotransformation, Baker's yeast has been the most popular one for the reduction of aromatic nitro compounds. Aliphatic nitro compounds can also be reduced, but not that many examples can be found in literature. Most successful examples involve nitro alkenes as starting materials (Scheme 5).<sup>[22]</sup>



Scheme 5. *P. productus* mediated reduction of the nitro group.<sup>[22]</sup>

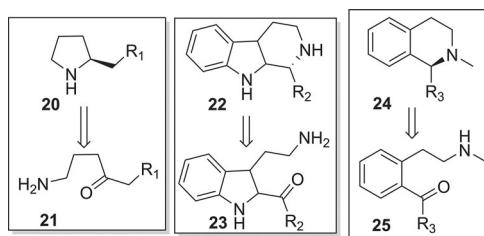
#### 2.1.4. Imine Reductases

NADPH-dependent imine reductases (IREDs, EC 1.5.1.48) catalyze the interconversion of ketones or the respective imine or iminium ion to amines, but unlike amine transaminases and amine dehydrogenases, these enzymes are not limited to the preparation of only primary amines.<sup>[13a,b]</sup> Although a natural

imine reduction step was known since a long time from the dihydrofolate metabolic pathway, only in the last five years NADPH-dependent IREDs from microbial sources were reported in the context of synthetic applications.<sup>[13c,25]</sup>

Several authors have investigated the substrate scope and enantiopreference of these enzymes and the nature of the amine source is probably the most important component of this transformation for organic synthesis, which can lead to secondary and tertiary chiral building blocks (Scheme 6).<sup>[26]</sup>

Within this class of enzymes, imino acid reductases were also found, catalyzing the C=N reduction when located at the  $\alpha$ -position of a carboxylic acid. A very interesting application of this enzyme is



Scheme 6. Imine reductases allow the stereoselective synthesis of secondary and tertiary amines.

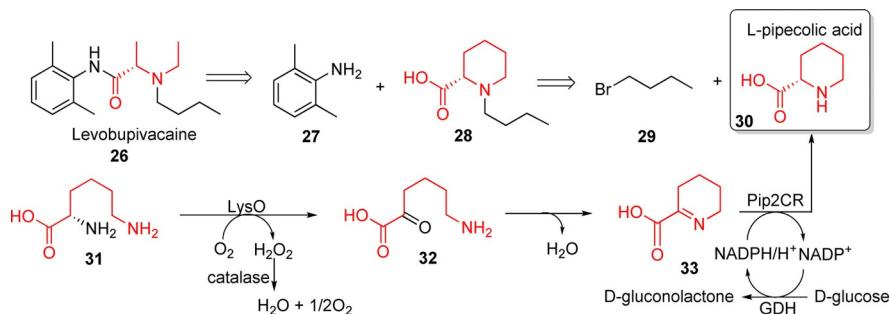
shown for the synthesis of L-pipecolic acid, an important intermediate in the construction of the Levobupivacaine skeleton.<sup>[27]</sup> The first obvious disconnection is the amide bond cleavage, leading to 2,3-dimethyl aniline **27** and *N*-butyl-L-pipecolic acid **28** intermediates, which can be easily prepared by the  $S_N2$  reaction between L-pipecolic acid **30** and 1-bromo butane **29**. Actually, L-pipecolic acid is formed by hydrogenation of picolinic acid followed by diastereoselective crystallization of the desired enantiomer.<sup>[28]</sup>

The biotechnological approach starts using a lysine oxidase (LysO) in order to introduce the carbonyl group into the  $\alpha$ -position of lysine **31**, leading to an intermediate, which spontaneously cyclizes forming the  $\Delta^1$ -pipecideine-6-carboxylate **33**, which can then be reduced to L-pipecolic acid **30** by a  $\Delta^1$ -pipecideine-6-carboxylate/ $\Delta^1$ -pyrrolidine-2-carboxylate reductase. The overall yield of this reaction was 90% (27 g L<sup>-1</sup>) with enantiomeric excess higher than 99.7% ee. The scope was extended also to several other five, six and seven-membered cycles (Scheme 7).<sup>[29]</sup>

#### 2.1.5. Monoamine-Oxidases

Hare and co-workers first reported (1928) the enzyme-catalyzed tyramine oxidation on tissue extracts calling the responsible enzyme a tyramine oxidase, which later was renamed as monoamine-oxidase (MAO-N, EC 1.4.3.4).<sup>[30]</sup>

Amine oxidases (AO) are classified into two main groups, namely, Type I (Cu/2,4,5-trihydroxyphenylalanine-dependent) and Type II (Flavin-dependent). Among this class, Type II en-



Scheme 7. L-Pipecolic acid process for the Levobupivacaine synthesis including also further enzymes required for cofactor regeneration and removal of hydrogen peroxide.<sup>[29]</sup>

zymes generate free imines, which can be used for deracemization of amines. In general, a resolution method involves the stereoinversion of one enantiomer to the other by repeated cycles of enzyme-catalyzed oxidation to the imine, followed by non-selective reduction back to the amine function yielding a racemate again. Consequently, several cycles of selective enzymatic oxidation and chemical reduction enable an efficient deracemization process with isolated yields of the desired chiral amine close to 100%.<sup>[31]</sup> The known monoamine oxidases, especially from *A. niger* (MAO-N), are (*S*)-selective. Recently, a (*R*)-selective AO from *Arthrobacter nicotinovorans*<sup>[32]</sup> was characterized and engineered to broaden its substrate specificity.

Different approaches could be used to synthesize (+)-Crispine A **34** and we have selected two retrosyntheses, which are shown in Scheme 8. One interesting strategy used by Chittiboyina and co-workers shows the construction of the piperidine ring in Crispine A resulting from a Pummerer-type cyclization of a chiral 2-arylpyrrolidine **36** scaffold, which could be synthesized using a one-pot Keck asymmetric allylation followed by a cascade hydroboration-cyclization procedure.<sup>[33]</sup> Turner and co-workers—in order to apply their MAO-N enzyme—constructed the racemic Crispine A **34** skeleton coming from a Bischler-Napieralski cyclisation reaction from **39**, which could be formed by the reaction between the homoveratrylamine **40** and  $\gamma$ -valerolactone **41** (Scheme 8).<sup>[34]</sup>

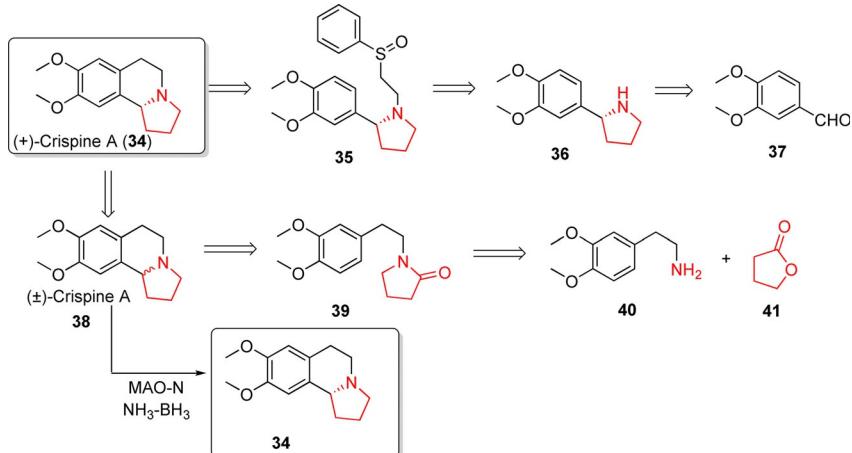
MAO-N variants were investigated in order to find the best catalytic system, which finally could lead to the desired deracemization product after 2 h with enantiomeric excess higher than 97% ee.<sup>[34]</sup>

#### 2.1.6. Ammonia Lyases

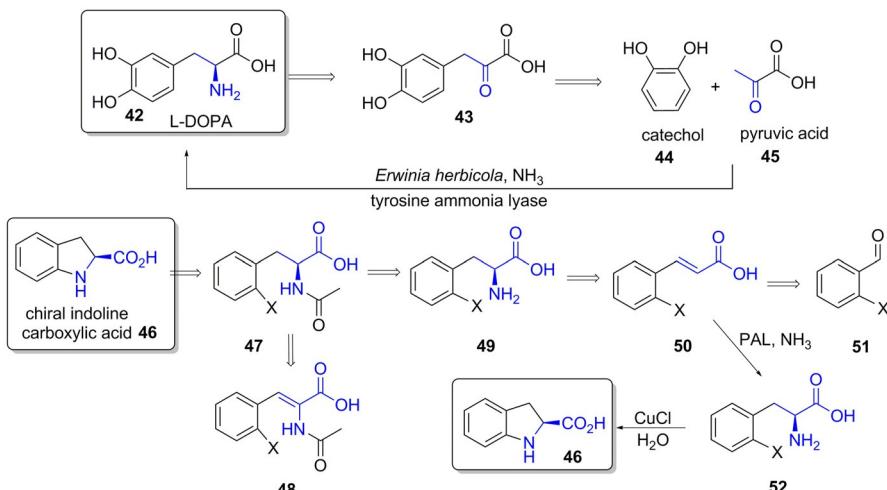
Lyases are a class of enzymes that catalyze the breaking or making of various chemical bonds. They are classified into seven subclasses where EC 4.3 refers to the ones that cleave or form carbon-nitrogen bonds. Among numerous enzymes, phenylalanine ammonia lyase (PAL, EC 4.3.1.24) and tyrosine ammonia lyase (TAL, EC 4.3.1.23) are the most popular ones already applied in organic chemistry.<sup>[25–27]</sup>

Koukol and Conn have discovered the non-oxidative deamination of L-Phe to *t*-CA (*trans*-cinnamic acid)<sup>[35]</sup> and ammonia catalyzed by PAL in 1961, but the reverse reaction was only determined eight years later by Hanson and Havar.<sup>[36]</sup> Almost at the same time, other groups have found other amino acid non-oxidative deamination enzymes, especially tyrosine ammonia lyase (TAL).<sup>[37]</sup>

Among these enzymes, TAL has been used for the synthesis of L-DOPA by Ajinomoto Co. Ltd. (Japan).<sup>[38]</sup> L-DOPA production is a lyase-based biotransformation using suspended *E. herbicola* cells with extremely high TAL activity in batch reactors, starting from a mixture of catechol **44** and pyruvic acid **45**. The



Scheme 8. Monoamine-oxidase-catalyzed synthesis of (+)-Crispine A.<sup>[34]</sup>



Scheme 9. Retrosynthetic analysis for the use of TAL<sup>[38]</sup> (top) and PAL<sup>[39]</sup> (bottom) enzymes.

one-step biotransformation is more economically and productive ( $110 \text{ g L}^{-1}$ ) than the established chemical route that involves eight chemical steps including an optical resolution. Right now, about 250 tons of L-DOPA are produced per year, and more than half of it is produced via biotransformation (Scheme 9).<sup>[38]</sup>

Regarding the use of PAL enzymes for the synthesis of chiral intermediates, probably the most interesting example is the synthesis of the chiral indoline carboxylic acid **46**, Scheme 9.<sup>[39]</sup> Fischer indole synthesis followed by classical or enzymatic resolution is the common way to synthesize this type of carbon skeleton **46**, but yields never exceeded 50%. In order to overcome this limitation, asymmetric hydrogenation<sup>[40]</sup> could be used to transform intermediate **50** into the chiral amine **52**. Unfortunately, asymmetric hydrogenation needs expensive rhodium catalysts and complex ligands for achieving high enantioselectivity for intermediates such as **52**. Cyclization, in order to afford the chiral indoline **46**, can be done by several methods,<sup>[41]</sup> but much attention must be taken in order to avoid racemization of the desired product. An alternative is the use of a PAL enzyme to produce the chiral amine **52**, prior to the cyclization step, in order to construct the chiral C–N bond between ammonia and the cinnamic acid derivative **50**. The product obtained by this reaction is a phenylalanine derivative **52**, which is ready for cyclization into the desired product (Scheme 9).<sup>[39]</sup>

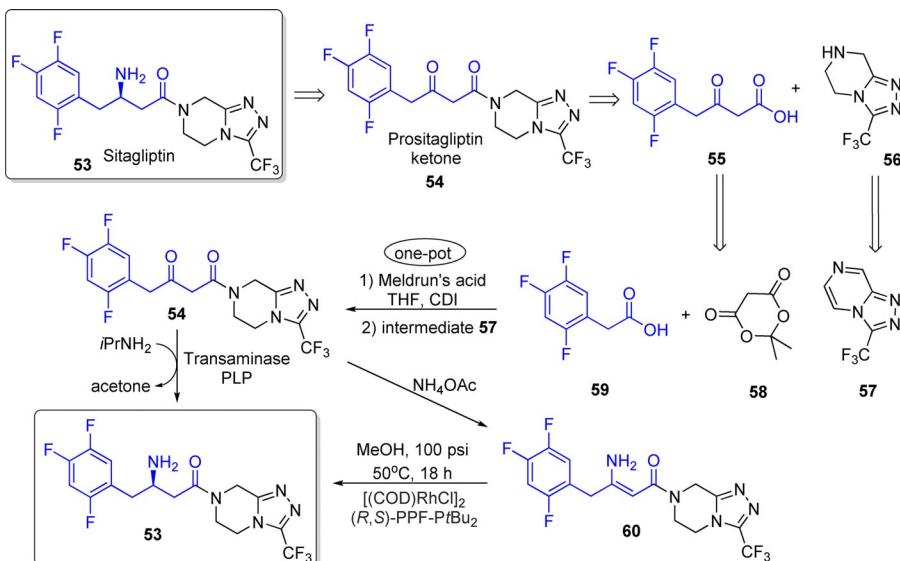
### 2.1.7. Transaminases

About ten years ago, transaminases (EC 2.6.1) have emerged as the most useful and widely applied pyridoxal-5'-phosphate (PLP)-dependent enzymes, since they can be used in biocatalytic asymmetric synthesis of amino acids and amines.<sup>[42]</sup> Compared to the traditional chemical catalysis for this type of transformation, usually transition metal-catalyzed asymmetric hydrogenation of enamines, transaminases are superior in step efficiency as they catalyze the direct formation of a chiral amine function directly from the prochiral ketone.<sup>[43]</sup>

Up to now, two types of PLP-dependent transaminases have been discovered, which are divided according to the type of substrate that is converted:  $\alpha$ -transaminases ( $\alpha$ -TAs) and  $\omega$ -transaminases ( $\omega$ -TAs).<sup>[44]</sup> Whereas  $\alpha$ -TAs (the majority of TAs) exclusively convert  $\alpha$ -amino and  $\alpha$ -keto acids,  $\omega$ -TA also accept substrates having a distal carboxylic acid group instead of a carboxylate function in  $\alpha$ -position. The term  $\omega$ -TA is used to summarize a very heterogeneous group of activities. Two subgroups of  $\omega$ -TAs studied for biocatalytic approaches are  $\beta$ -TAs and amine TAs (ATAs). The latter became particularly popular during the last decade as they do not require a carboxylic acid functionality present at all.

The most prominent example for the use of amine transaminases (ATAs) in organic synthesis was developed by researchers from Merck & Co. and Codexis as an alternative for the Rh-catalyzed hydrogenation to obtain Sitagliptin **53**, an antidiabetic drug.<sup>[45]</sup> Retrosynthetic analysis of Sitagliptin synthesis presents first the prositagliptin **54**, an intermediate that directly leads to the desired target by an asymmetric reductive amination or asymmetric reduction of the corresponding enamine **60**. The  $\beta$ -keto amide intermediate **54** could be constructed by a usual amino acid coupling between  $\beta$ -keto acid **55** and the amine **56**. The condensation between Meldrun's acid **58** and carboxylic acid **59** forms the  $\beta$ -keto acid **55**, which has the carbonyl group present required for the biotransformation. On production scale, prositagliptin **54** can be formed in a one-pot reaction where the intermediate **55** is formed by the condensation of Meldrun's acid **58** and **59** (Scheme 10).

The researchers at Codexis succeeded in creating a highly efficient and stereoselectivity ATA in an extensive protein engineering effort. For the biocatalytic route, 27 mutations were needed to enable the enzyme to accommodate the very bulky ketone, to accept the cheap amine donor isopropylamine and to be active in the presence of 50% DMSO. In the final process the ATA converts  $200 \text{ g L}^{-1}$  of the prositagliptin ketone to the amine in 92% yield having >99.95% ee using  $6 \text{ g L}^{-1}$  enzyme.<sup>[45a]</sup> In comparison with the chemical route, the biocatalytic synthesis led to 13% increase in yield, 53% increase in produc-



Scheme 10. An engineered amine transaminase is used for the asymmetric synthesis of Sitagliptin.<sup>[45a]</sup>

tivity and 19% reduction of waste. A highlight article nicely compared the chemical and the enzymatic routes to afford Sitagliptin.<sup>[45b]</sup>

Recently, researchers have used the potential of transaminases for the synthesis of Dolutegravir **62** an HIV drug, using the approach presented in Scheme 11.<sup>[46]</sup> The retrosynthetic analysis of Dolutegravir starts from the cleavage of an oxazine type ring leading to intermediate **63** and a chiral amine **71** as fragments. Intermediate **64** can be disconnected further by sequential known reactions arriving on the simple starting materials **65**, **66** and **67**.

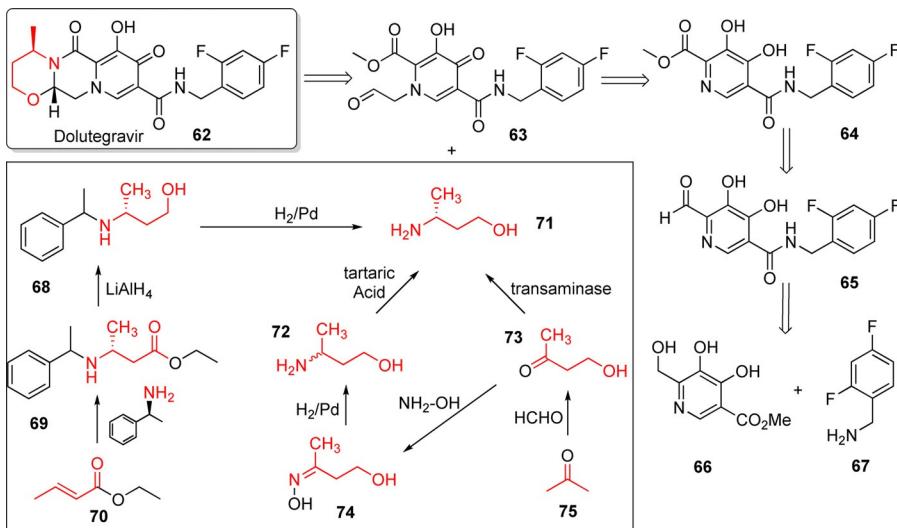
The desired chiral amine **71** can be synthesized using a Michael-addition reaction between the chiral 1-phenylethylamine and crotonic ester **70**, followed by reduction of the ester function and deprotection of the amine leading to **71** in good yields. Alternatively, the aldol reaction between acetone and formaldehyde leads to 4-hydroxy-2-butanone **73**, which reacts

with hydroxylamine to generate the oxime **74**. This oxime, after reduction and diastereoselective crystallization furnishes the enantiopure amine **71** (Scheme 11).

Very recently, transaminases could also be evolved by protein engineering to convert various other prochiral bulky ketones into the corresponding chiral amines starting from (*S*)-selective transaminases from *Ruegeria* sp.<sup>[47]</sup> or *Vibrio fluvialis*.<sup>[47a,48]</sup> In contrast to the evolution of enzymes for Sitagliptin synthesis, only a few mutations where required to turn the almost inactive wild-type enzymes into efficient biocatalysts without compromising their stereoselectivity.

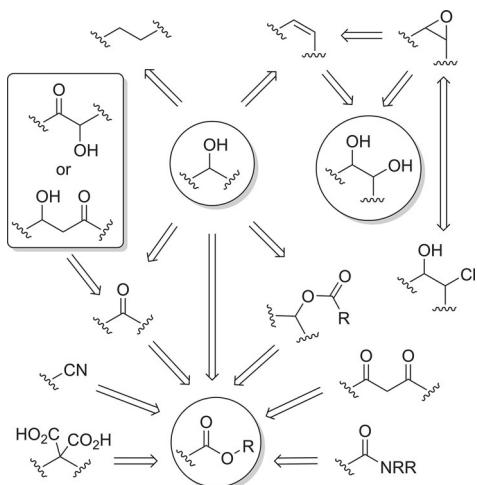
## 2.2. Formation or Cleavage of C–O Bonds

The carbon–oxygen bond is present in many functional groups and biotechnology has provided many alternatives for the formation and cleavage of this type of chemical bond by



Scheme 11. Transamination reaction for Dolutegravir **62** synthesis.<sup>[46]</sup>

the use of enzymes. Hydroxylations mediated by P450 enzymes<sup>[49]</sup> (EC 1.14), epoxide hydrolysis<sup>[50]</sup> (EC 3.3.2.10), hydrolysis/esterifications with lipases (EC 3.1) and carboxyl esterases (EC 3) are the most widely used enzymes for this purposes.<sup>[3,51]</sup> Nitrile hydrolysis<sup>[52]</sup> (EC 3.5.5), carbonyl reductions<sup>[53]</sup> (EC 1.1.1.1), halohydrin dehalogenases<sup>[54]</sup> (HHDH, EC 4.5.1) and Baeyer–Villiger monooxygenases<sup>[55]</sup> (EC 1.14.13) are a few representative examples established for organic synthesis purposes (Scheme 12).



**Scheme 12.** Retrosynthetic approach for the construction or cleavage of C–O bonds.

Chemical synthesis has already developed very efficient methods for the construction of chiral carbon-oxygen bonds, usually mediated by different metal-catalysts.<sup>[56]</sup> Biocatalysis can be a suitable alternative, but biocatalyst stability and organic solvent tolerance must be improved for most enzymes from these classes—except lipases and a few proteases—in order to be a competitive alternative to chemical catalysis. In this way, protein engineering has emerged again as an important tool for improving enzyme properties in order to create more effective biocatalysts.

### 2.2.1. Alcohol Dehydrogenases/Ketoreductases

These dehydrogenase enzymes (ADH, KRED, EC 1.1.1.x) occur in many organisms and catalyze the interconversion of alcohols to carbonyl functional groups such as aldehydes and ketones, with stoichiometric consumption of the nicotinamide adenine dinucleotide cofactor ( $\text{NAD}^+$ ) or its phosphorylated analog  $\text{NADP}^+$ . The reverse reaction is synthetically much more useful as the reduction of a ketone directly yields a chiral alcohol by selective asymmetric hydrogenation catalyzed by the KRED with concomitant consumption of  $\text{NAD(P)H}$ .<sup>[53d,57]</sup>

The scope of enzymes from this class is very broad and a huge variety of substrates are accepted. Moreover, numerous enzymes are available with opposite stereopreference enabling the synthesis of both stereoisomers of the targeted chiral alcohol. In this way, KRED enzymes have been used in organic syn-

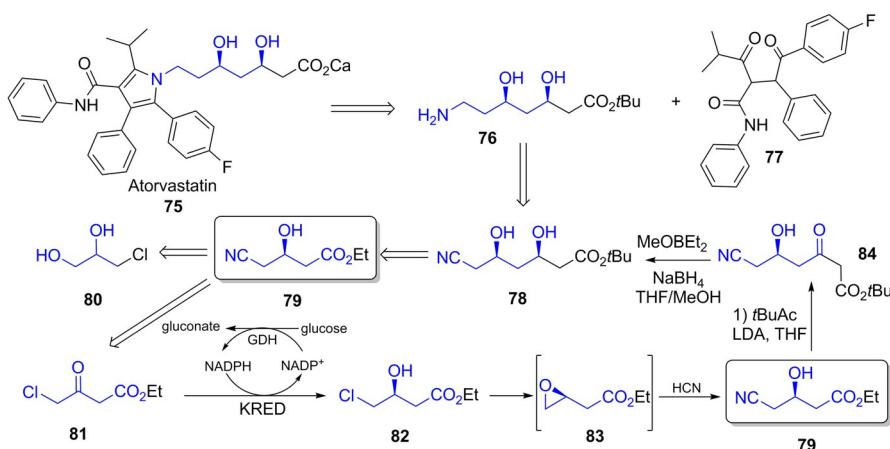
thesis by numerous academic groups and industry for the reduction of prochiral ketones, not only for the synthesis of active pharmaceutical ingredients, but also to obtain useful chiral synthons. Moreover, in case of NADH-dependent enzymes, cofactor recycling can be efficiently performed by adding 10–40% (v/v) isopropanol to the buffer, which is then converted into acetone. Total turnover numbers (mol product per mol NADH cofactor) of 600 000 have been reported<sup>[58]</sup> exemplifying that the cost for cofactor can be negligible.

One prominent example for the application of KREDs in API synthesis is the Atorvastatin **75** process (Scheme 13). Intermediate **76** is required to construct the chiral moiety of the diol of the Artovastatin molecule. By chemical methods different strategies could be used, ranging from asymmetric hydrogenation, hydrogenation of a chiral starting material or oxidative cleavage of sugar derivatives.<sup>[59]</sup> The drawbacks of these methodologies are the use of hydrogen at high pressures or hydrogen peroxide, which in most cases is not preferred by process chemists.<sup>[59]</sup> The retrosynthetic analysis identifies the diol building block highlighted in blue. The direct disconnection is to generate two building blocks coming from the condensation of diol **76** and the diketone **77**. Moving forward on the disconnection of **76**, it could be formed by the reduction of the nitrile **78**, which could be prepared starting from 4-chloroacetoacetate **81**. The biocatalytic approach involves the use of cheap and readily available 4-chloroacetoacetate ethyl-ester **81**, which undergoes bioreduction mediated by a KRED to afford the (S)-4-chloro-3-hydroxy-butyrate ester **82**. A glucose dehydrogenase (GDH) enzyme serves for co-factor recycling in this process. Intermediate **82** leads to the formation of epoxide **83**, which, after nucleophilic ring opening of the epoxide by HCN, leads to intermediate **79**. Then, after aldol reaction followed by diastereoselective reduction on the new carbonyl group constructed, the chiral diol moiety is formed (**78**).<sup>[60]</sup>

In the optimized process 160 g L<sup>-1</sup> substrate loading is possible and after only 8 h the final product is obtained at 95% isolated yield and 99.9% enantiomeric excess. It is also important to point out that biocatalyst loading is only 0.9 g L<sup>-1</sup> and the space-time yield (STY) achieved is 480 g L<sup>-1</sup> d<sup>-1</sup> (Scheme 13).<sup>[60]</sup>

Another interesting example is the Atazanavir **85** synthesis where the chiral  $\alpha$ -chloro ketone **90** is used as substrate for enzymatic reduction. The retrosynthetic analysis of Atazanavir **85** synthesis (Scheme 14) reveals two major intermediates (**86** and **87**) each containing a *tert*-leucine carbamate moiety, which can also be synthesized by biotechnology starting from *tert*-butyl pyruvic acid. Intermediate **86** can be constructed from the amide bond formation between chiral epoxide **88** and *tert*-leucine carbamate **93**. The usual way of preparing the chiral epoxide **88** starts from the related chiral  $\alpha$ -chloro alcohol **89**, which can be produced by stereoselective reduction of the  $\alpha$ -halo ketone **90**.

Different strategies can be used for synthesizing this  $\alpha$ -halo ketone **90** starting from the protected phenylalanine **91**: sulphur ylide chemistry or using diazomethane are the most straightforward ways of constructing this building block.<sup>[61]</sup> It is important to point out that the  $\alpha$ -halo ketone **90** is a strategic intermediate since it can serve for several different molecules



Scheme 13. Biocatalytic approaches for the synthesis of Atorvastatin.<sup>[60]</sup>

by only changing the selectivity in the reduction of the carbonyl group. Chemically, reduction of the  $\alpha$ -halo ketone **90** using lithium hydrides can lead to the desired diastereoisomer in moderate to good yields and high diastereoselectivities.<sup>[62]</sup>

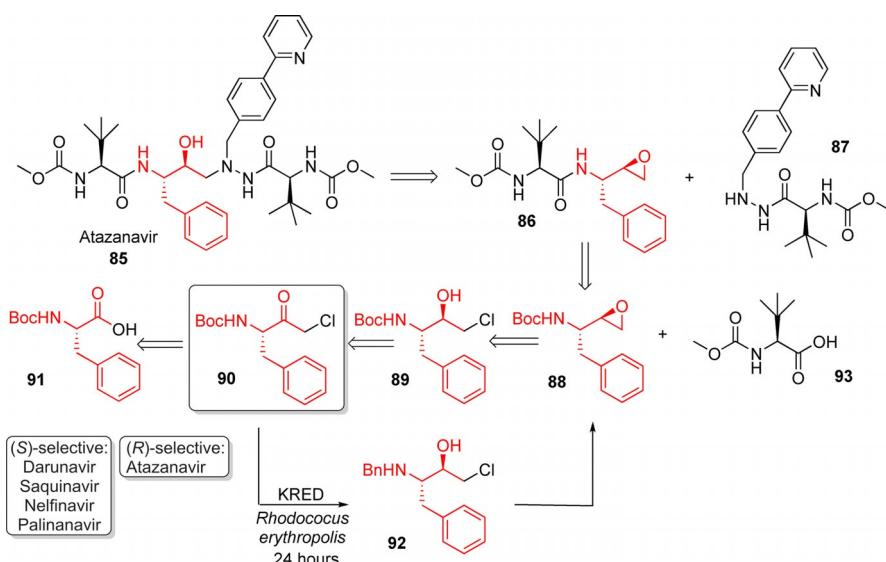
A biocatalytic approach has been developed using an engineered ketoreductase, which allows the bioconversion to take place at substrate concentrations of  $200 \text{ g L}^{-1}$ ,  $1 \text{ g L}^{-1}$  enzyme loading and  $0.01 \text{ g L}^{-1}$   $\text{NADP}^+$ . After 24 h, 99% conversion could be achieved and the chiral product was isolated with an enantiomeric purity  $> 99.2\% \text{ ee}$  (Scheme 14).<sup>[63]</sup>

The reduction of  $\alpha$ -halo ketone **90** can also be done by other enzymes. An interesting example is the use of KRED, where the protective group of the nitrogen in the intermediate  $\alpha$ -halo ketone **90** could direct the reduction towards (*S*)- or (*R*)-products, tuning the selectivity for different HIV drugs as highlighted (boxed) in Scheme 14 with moderate diastereoselectivities and good conversions ( $> 85\%$ ). Docking experiments revealed a complete inversion of the pro-chiral attack face depending on the protecting group (Boc or Cbz).<sup>[63]</sup>

### 2.2.2. Oxyfunctionalizations Mediated by Mono- and Dioxygenases

The most prominent enzymes for these oxyfunctionalization are P450 monooxygenases and dioxygenases. Furthermore, peroxidases can also enable stereoselective formation of for instance chiral epoxides from styrene, however the requirement of hydrogen peroxide often limits their stability.<sup>[64]</sup> Other alternative enzymes are styrene monooxygenase.<sup>[65]</sup>

Cytochrome P450 (P450, EC 1.14.x.y.) is a family of enzymes containing heme as a cofactor ( $\text{Fe}^{3+}$ ) and they work as oxidase enzymes at the electron transfer chain, especially catalyzing hydroxylations. They are named P450 because a differential spectrum of the enzyme exposed to carbon monoxide shows a maximum at 450 nm. In principle, they are very useful for organic synthesis as they allow the direct functionalization of non-activated C–H-bonds with consumption of the cofactor  $\text{NAD(P)H}$  leading to the formation of hydroxyl groups (or epoxides from double bonds). P450s require the presence of further



Scheme 14. Retrosynthetic analysis of Atazanavir synthesis.

proteins to enable the complex electron transfer (such as the putidaredoxin reductase (Pdr) and putidaredoxin (Pdx) proteins from *Pseudomonas putida*), they are usually membrane bound, show rather slow catalytic activity and lack sufficient stability. These factors substantially hamper their easy and broad applicability by organic chemists. One exception are P450s occurring as natural fusion proteins with the enzyme from *Bacillus megaterium* (P450-BM3) as the most prominent example.<sup>[49a,66]</sup>

Consequently, numerous examples have been published for the use of P450-BM3 in organic synthesis including the design of >1,000 mutants of this enzyme to alter substrate scope and to improve regio- and stereoselectivity. Prominent examples are the  $\omega$ -hydroxylation of fatty acids, the formation of (–)-Perillyl alcohol from (–)-limonene or of Nootkatol from Valencene.<sup>[49b]</sup>

Organic chemists have been seeking for effective and selective C–H activation transformations for many years. Dioxygenases enzymes (EC 1.13.11.x) are useful for organic synthesis because they can oxyfunctionalize C–H bonds by the formation of *cis*-diol products. Two different types of dioxygenases can be found in nature: with one or more heme iron units or non-heme iron units. In general these enzymes are involved in oxidative cleavage of catechol substrates as part of bacterial aromatic degradation pathways.<sup>[67]</sup> More than 100 Rieske non-heme iron oxygenases (RDO) have been identified until now and used for the synthesis of chiral precursors in organic synthesis. Benzene (BDO), toluene (TDO) and naphthalene dioxygenase (NDO), have been recently developed for the biocatalytic asymmetric dihydroxylation of conjugated mono- and poly alkenes to obtain enantiopure cyclic *cis*-diols.<sup>[68]</sup>

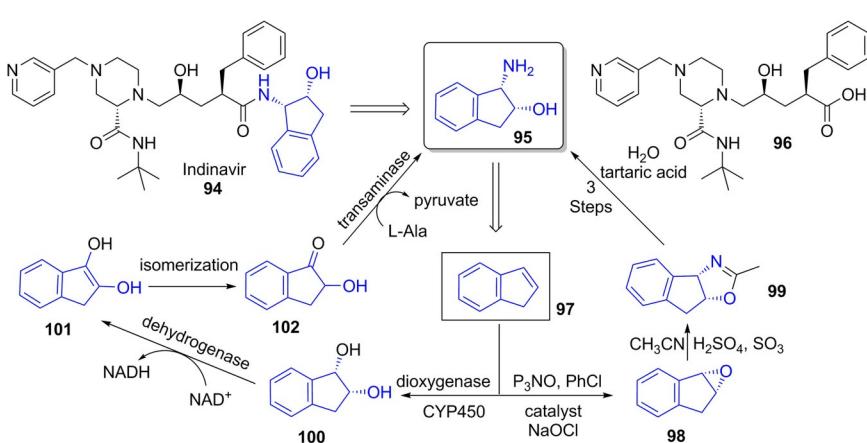
As an example on how dioxygenases can be used for organic synthesis, Indinavir **94** synthesis is presented below in Scheme 15. The retrosynthetic analysis of Indinavir leads to two main building blocks derived from the peptide bond cleavage, the amino alcohol **95** and the carboxylic acid **96**. Looking closer to the amino alcohol **95** moiety, it could be prepared from indene **97**. The chemical route towards **97** goes through several steps including asymmetric epoxidation, followed by protected amino alcohol formation and hydrolysis, leading to the final product in moderate to good yields (Scheme 15).<sup>[69]</sup>

The biotechnology approach starts from the dioxygenation of indene **97** in order to synthesize the diol intermediate **100**. After dehydrogenation and isomerization it arrives at the  $\alpha$ -hydroxy ketone **102**, which could be subjected to a transamination reaction as discussed above to afford the desired amino alcohol **95**.<sup>[70]</sup>

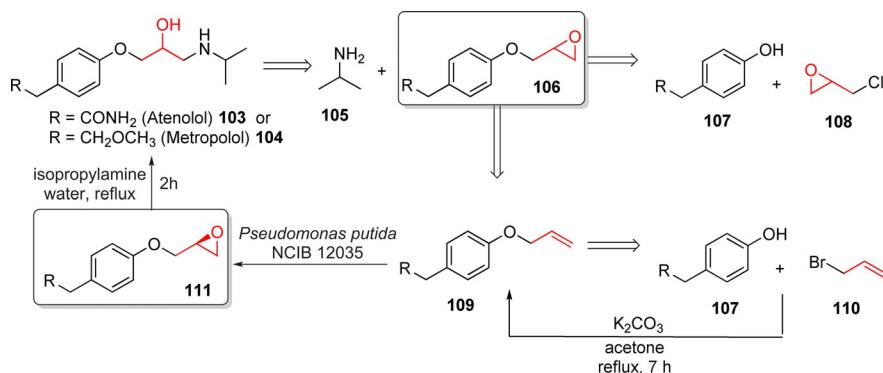
Instead of performing dihydroxylations, heme-dependent monooxygenases or non-heme diiron enzymes can also catalyze epoxide formation being an interesting strategy when a prochiral alkene is used as starting material, since these enzymes can afford enantiomerically pure or enriched final products. The chemical approach for such transformation is already well established with the protocols developed by Sharpless and Jacobsen, but these lack broad substrate scope and require expensive catalysts.<sup>[71]</sup> Other enzymes are also able to catalyze epoxidation reactions, that is, lipases, but in this case no stereoselectivity takes place since lipases catalyze the formation of a peracid intermediate, which then will epoxidize the double bond in a solely chemical reaction.<sup>[72]</sup>

One of the many applications of epoxidations catalyzed by enzymes is shown in Scheme 16 for the synthesis of the  $\beta$ -blockers Atenolol (**103**) and Metoprolol (**104**). Both APIs are commercialized as racemic mixtures, but it was shown that the (*S*)-enantiomer is 350 times more active than the other one. Performing the retrosynthesis for this class of  $\beta$ -blockers, differing only in the substituent at the aromatic ring, it is possible to note that the first disconnection is the epoxide precursor **106**, which could be obtained in two different ways: by reacting the *p*-substituted phenol intermediate **107** with racemic epichlorohydrin **108** or by epoxidizing the double bond intermediate **109**. Compound **109** can be prepared by reacting the same phenol intermediate **107** with allyl bromine **110** in basic media (Scheme 16).<sup>[73]</sup>

Intermediate **109** can be used as starting material for the biotransformation using *Pseudomonas putida* NCIB 12035 as a biocatalyst source, leading to the desired chiral epoxide as remaining enantiomer in 98.8% enantiomeric excess. Treating epoxide intermediate **111** in water with isopropylamine (**105**) under reflux conditions, the final products, (*S*)-Atenolol or (*S*)-Metoprolol, were obtained in quantitative yields.<sup>[74]</sup>



Scheme 15. Biocatalytic approaches towards Indinavir synthesis.<sup>[69,70]</sup>



Scheme 16. Biocatalytic strategies for Atenolol or Metoprolol synthesis.<sup>[74]</sup>

### 2.2.3. Epoxide Hydrolases

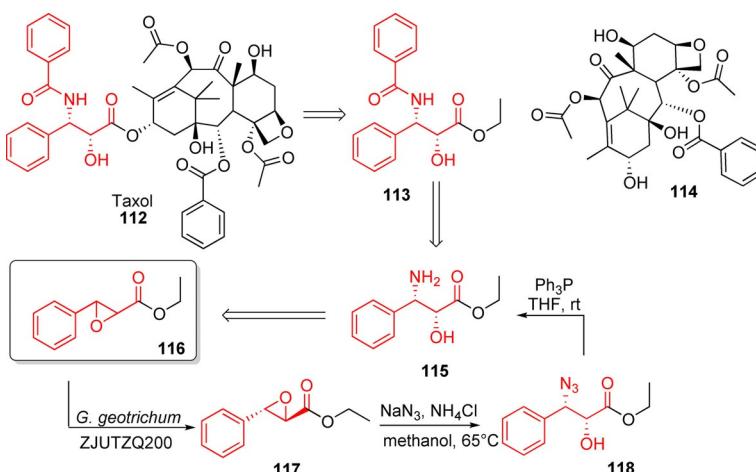
Epoxides are an important functional group in organic synthesis despite the fact that not many APIs can be found having an epoxide in their scaffold. The importance of this functional group comes from the fact that opening of this three-membered ring can lead to multi functional molecules serving in this way as interesting and high-value chiral intermediates for organic synthesis. It is important to note that ring opening is not an easy task since regioselectivity and stereoselectivity often need to be controlled in the synthesis of the target molecule.<sup>[50a,d]</sup>

In order to overcome regio- and stereoselectivity issues usually faced when working with chemical catalysis, nature has provided us with epoxide hydrolases (EHs, EC 3.3.2.10). This class of enzymes can be found in mammalian, plant, insect, bacteria, fungi, and yeast. They catalyze kinetic resolution where one stereoisomer of the racemic epoxide is ring-opened by water usually at high regioselectivity leading to a diol, while the other enantiomer remains unreacted.<sup>[50a]</sup> As EHs with opposite stereopreference and for enantiodivergent reactions have been identified, it is possible to start from racemic epoxide, but one can obtain a single product enantiomer in up to 100% yield.<sup>[75]</sup> The drawback of EHs is that they only accept water as a nucleophile (in contrast to halohydrin dehalogenases, see Section 2.2.4.).

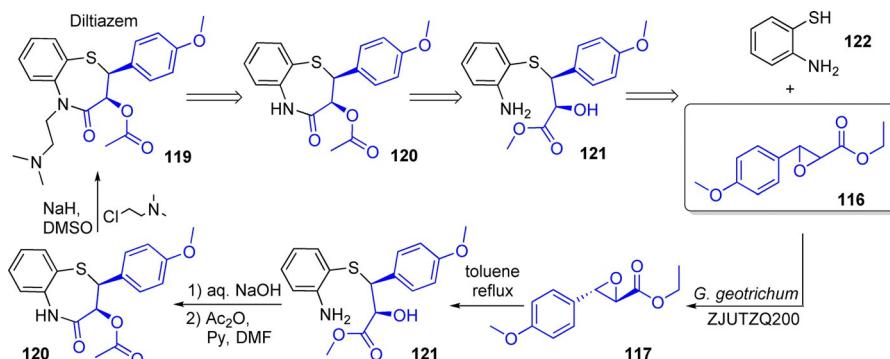
To highlight the application of epoxide hydrolases, based on the retrosynthetic biocatalysis approach, Schemes 17 and 18 exemplify the synthesis of two important drugs: Taxol (112)<sup>[76]</sup> and Diltiazem (120),<sup>[77]</sup> where epoxide hydrolases were used to construct intermediate building blocks. The retrosynthetic analysis of taxol leads to two major precursors, the intermediates 113 and 114, which can be coupled by an esterification reaction. In this case, intermediate 113 is more important since it could be formed by benzoylation of amine 115, which in turn can be synthesized from epoxide 116 in two steps.

Starting from intermediate 117, an epoxide hydrolase from *G. geotrichum* can be used to afford the desired chiral epoxide with high enantioselectivity (> 99% ee) and moderate isolated yield (37%). The next step is the epoxide ring-opening by azide followed by reduction mediated by PhP<sub>3</sub> and reaction with benzoyl chloride, leading to the chiral benzoylated amine 114 in 33.8% overall yield (three chemical steps) and having an enantiomeric excess > 95% ee (Scheme 17).

Diltiazem (119) synthesis uses the same concept as presented for Taxol, since the intermediate required is quite similar, differing only by a methoxy group at the *para*-position of the aromatic ring. As often observed in biocatalysis, small changes can have a huge influence on the outcome of a reaction and unfortunately when intermediate 116 is reacted in the presence of this *G. geotrichum* strain, the chiral epoxide required



Scheme 17. Epoxide hydrolase applied to Taxol synthesis.<sup>[76]</sup>



Scheme 18. Retrosynthetic analysis for Diltiazem synthesis.<sup>[77]</sup>

for diltiazem synthesis is obtained with poor conversion and moderate enantioselectivity (>80% ee).<sup>[61c]</sup> Diltiazem is then obtained in three additional steps, respectively epoxide ring opening by intermediate 122, followed by cyclization and nitrogen alkylation (Scheme 18).<sup>[77]</sup>

#### 2.2.4. Halohydrin Dehalogenases

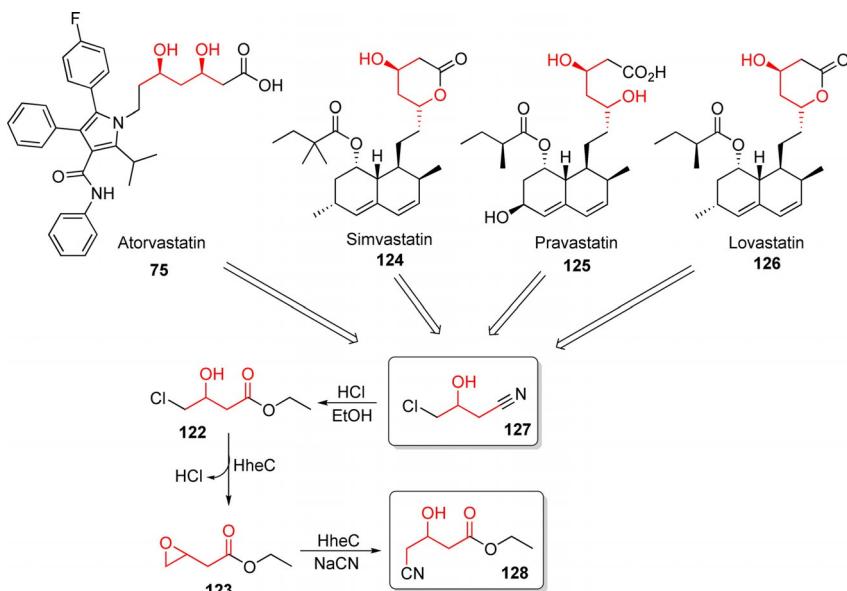
A valuable alternative to EHs are halohydrin dehalogenases (HHDH, EC 4.5.1.x). These enzymes belong to the short-chain dehydrogenase/reductase superfamily, able to catalyze both the formation of epoxides starting from  $\alpha$ -halo alcohols and epoxide ring opening by different nucleophiles. A major advantage compared to epoxide hydrolases is the fact that HHDH can accept nucleophiles other than water making them much more versatile for organic synthesis.<sup>[54]</sup>

Unfortunately, the number and variety of enzymes is still limited, and only bacterial enzymes have been identified so far.<sup>[54a, 78]</sup> Halohydrin dehalogenases are subdivided into three different main groups as categorized by phylogenetic analysis. Most studies have been done with halohydrin dehalogenases

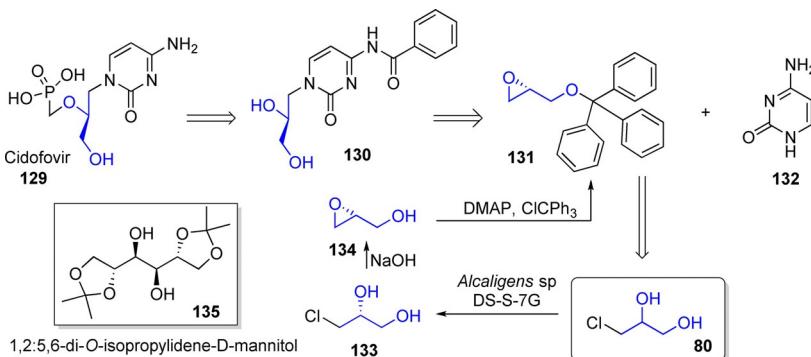
from group C, *Agrobacterium radiobacter* AD1 (HheC), which can be distinguished by the other two groups, HheA and HheB, because of the high enantioselectivity towards a range of substrates.<sup>[54c]</sup> A classical example for the use of halohydrin dehalogenase enzymes is the synthesis of the statin family side chain found in Atorvastatin, Simvastatin, Pravastatin and Lovastatin. The retrosynthetic analysis of Atorvastatin has already been shown in Scheme 13 and for an approach using halohydrin dehalogenase as biocatalyst, the common intermediate 123 can be used.<sup>[79]</sup>

Especially for the enzymatic Atorvastatin synthesis, activity of HheC has been improved 4000-fold using a combination of mutagenesis techniques such as error-prone PCR, site-directed mutagenesis, focused evolution, and DNA-shuffling, all in combination with ProSAR analysis, enabling the synthesis of the desired target at high conversion (99.5%) and optical purity (99.9% ee). The biocatalytic process mediated by HheC starts from intermediate 122 yielding the epoxide followed by ring-opening using  $\text{CN}^-$  as nucleophile (Scheme 19).<sup>[60]</sup>

Another interesting application of a halohydrin dehalogenase biocatalyst can be exemplified for the synthesis of the an-



Scheme 19. Halohydrin dehalogenase as an alternative biocatalyst for statin building block syntheses.<sup>[60]</sup>



Scheme 20. Cidofovir synthesis using a HHDH.<sup>[81]</sup>

tiviral drug Cidofovir (129), an acyclic nucleoside phosphonate.<sup>[80]</sup> The fragments obtained from the retrosynthetic analysis are presented in Scheme 20, where the first disconnection leads to intermediate 130, which could be formed by the ring-opening of the protected glycidol 131. Chiral 131 can be synthesized starting from (*R,S*)-3-chloro-1,2-propane diol (CPD, 80) in three steps.

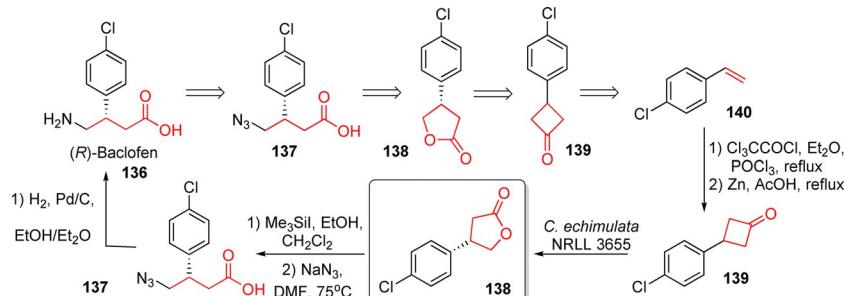
The key step for producing the chiral glycidol derivative 131 is the introduction of chirality in a very early stage by the use of a HHDH biocatalyst for resolution of racemic CPD 80, which can be easily prepared from 1,2:5,6-di-O-isopropylidene-D-mannitol (135). This reaction is performed using *Alcaligenes* species DS-S-7G in a 33 kL reactor at Daiso Co. in Japan, arriving at the final product in 50% yield and having 98.4% ee. With chiral CPD (133) in hands, treatment in basic media leads to glycidol formation, which can be protected with the tryptol group in the presence of DMAP to deliver intermediate 131 (Scheme 20).<sup>[81]</sup>

#### 2.2.5. Baeyer–Villiger Monooxygenases

The Baeyer–Villiger reaction is a well-known transformation in organic chemistry to convert ketones to esters or lactones. The chemical version of this transformation involves oxidation mediated by peroxyacids passing through the known Criegee intermediate, which after the migration of the higher substituted carbon atom as the rate-limiting step, leads to the desired ester. After hydrolysis the Baeyer–Villiger product can also afford the correspondent carboxylic acid, respectively hydroxy acid.<sup>[82]</sup>

In nature, this reaction is used as microbial degradation pathway with monooxygenases as catalysts, usually named Baeyer–Villiger monooxygenases (BVMO, EC 1.14.13.x), which are in general NAD(P)H-dependent flavoproteins. Up to now, two different types of BVMOs were described: type I contain FAD as bound cofactor and are dependent on NADPH for activity and type II with FMN as a flavin cofactor and NADH as electron donor. Based on recent studies, BVMOs can be subdivided according to their substrate type preference: cyclic ketones (as most abundantly studied compound class; CHMO<sub>Acinetor</sub> CPMO<sub>Coma</sub>), aryl containing ketones (typical representatives: HAPMO<sub>Pflu</sub> PAMO<sub>Thermo</sub>), and linear ketones (typical representative: BVMO<sub>Pflu</sub>). Usually, BVMOs display a large promiscuity within their specific substrate class, tolerating the presence of lipophilic as well as polar functional groups (including heterocyclic systems).<sup>[55]</sup>

One example for the use of BVMOs in organic synthesis is the synthesis of (*R*)-Baclofen (136) (Scheme 21). This substituted  $\gamma$ -amino acid can be synthesized through the opening of a five-membered lactone 138, which could be produced by the Baeyer–Villiger reaction from intermediate 139 using a BVMO. The cyclobutanone intermediate 139 can be prepared in two steps, starting from *p*-chlorostyrene (140).<sup>[83]</sup> The BVMO is used in the third step of (*R*)-Baclofen synthesis for the transformation of the cyclobutanone intermediate 139 into the five-membered lactone 138.<sup>[83]</sup> For this purpose, researchers have used a culture of the fungus *Cunninghamella echinulata* NRRL 3655 enabling the incorporation of an oxygen atom into one single enantiotopic C–C bond with high enantiomeric purity (> 99% ee), but with low yields. The lactone interme-



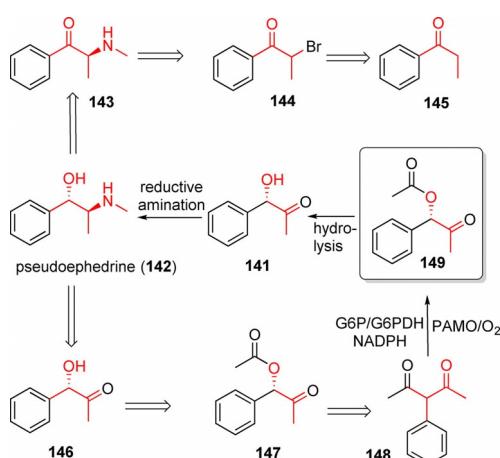
Scheme 21. (*R*)-Baclofen synthesis mediated by a BVMO.<sup>[83]</sup>

ate **138** can then be transformed to (*R*)-Baclofen in three steps with the reduction of the azide group of the intermediate **137** as the final step leading to the substituted  $\gamma$ -amino acid.<sup>[83]</sup>

Another interesting feature of this enzyme class is the outcome of a reaction when cyclic or acyclic diketones are used as starting materials. The reaction, when performed with such reagents, can lead after hydrolysis to two different building blocks, a carboxylic acid and an  $\alpha$ -hydroxy ketone, being a method for oxidative cleavage of a carbon-carbon single bond. An interesting example has been shown for the synthesis of L-phenylacetylcarbinol (L-PAC, **141**), an important intermediate for the production of ephedrine and pseudoephedrine (**142**) (Scheme 22).<sup>[84]</sup> One of the chemical syntheses available

conversions around 88% and an optical purity of 82% ee. Hydrolysis of this intermediate can lead to L-PAC formation, which after reductive amination forms the desired Pseudoephedrine product.<sup>[84]</sup>

Another interesting application for BVMO enzymes is the sulfoxidation of sulfides leading to chiral products with great importance for chemical synthesis. Esomeprazole (**150**) (Scheme 23) is one example where the use of a BVMO for sulfoxidation has been applied successfully. Esomeprazole is obtained in 99% conversion and 99% ee by sulfoxidation of another well-known drug, Pyrmetazole (**151**), which originates from the nucleophilic substitution reaction between thiol **152** and the substituted pyridine **153**.<sup>[86]</sup>



Scheme 22. BVMO-catalyzed synthesis of Ephedrine derivatives.<sup>[84]</sup>

for pseudoephedrine (**142**) production is given in the retrosynthetic analysis below, where **142** can be obtained from  $\alpha$ -amino ketone **143**, which can in turn be synthesized through nucleophilic substitution of the corresponding  $\alpha$ -bromo ketone **144**.<sup>[85]</sup>

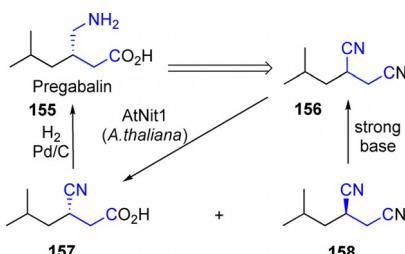
L-PAC (**141**) is already produced by biotechnological means using a pyruvate decarboxylase (see Section 2.3.3), but a different approach is based on a phenylacetone monooxygenase (PAMO). In this way, **142** can be accessed starting from  $\alpha$ -hydroxy ketone **141** that could be produced from the 1,3-diketone **148**.<sup>[84]</sup>

Starting from 1,3-diketone **148**, the use of PAMO enzyme leads to the (*S*)-1-acetoxy-phenylacetone **149** after 1.5 h with

## 2.2.6. Nitrilases

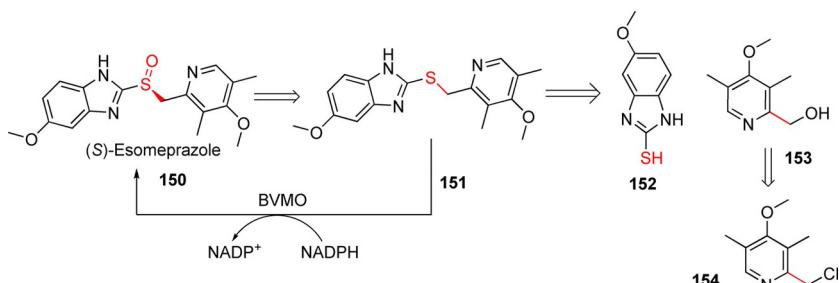
Nitrilases (EC 3.5.5.1) catalyze the hydrolysis of nitriles to carboxylic acids plus ammonia, without the formation of amide intermediates. This family of enzymes provides regioselective and/or enantioselective transformations at mild conditions hence representing interesting biocatalysts for organic synthesis.<sup>[52]</sup>

Tao and co-workers have developed a smart strategy for the application of nitrilases for the synthetic route towards Pregabalin (Lyrica<sup>®</sup>) (**155**).<sup>[87]</sup> Their approach (Scheme 24), for the res-



Scheme 24. Use of a nitrilase for Pregabalin synthesis.<sup>[87]</sup>

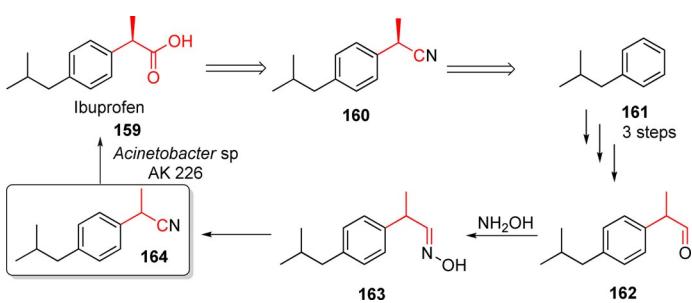
olution of ( $\pm$ )-isobutylsuccinonitrile **156** started with screening of several mutants and the best results were obtained with AtNit1 from *A. thaliana* exhibiting high enantioselectivity ( $E > 150$ ). The result of the kinetic resolution is a 1:1 mixture of the desired carboxylic acid **157** and the chiral unreacted starting material **158**, which can be racemized upon treatment with strong base, recovered and reused. With the carboxylic acid **157** in hands, the conversion of the remaining nitrile group to



Scheme 23. Retrosynthetic analysis of Esomeprazole synthesis.<sup>[86]</sup>

the amine through a hydrogenation mediated by Pd/C lead to the desired product **155**.

Ibuprofen synthesis was investigated by Komatsu and co-workers<sup>88</sup> in the beginning of the 1990s as a target for a nitrilase-catalyzed reaction alternative to the conventional chemical methods. The retrosynthetic analysis for this well-known molecule shows the formation of the chiral carboxylic acid **159** coming from the selective hydrolysis of the corresponding nitrile **160**, which could be formed by a sequence of steps, starting from isobutyl benzene **161**. Following this strategy, the authors have synthetized the desired nitrile derivative **162** and tested a purified nitrilase obtained from a cell extract of *Acinetobacter* sp. AK 226, leading to the desired carboxylic acid in 50% conversion and 90% ee (Scheme 25).



**Scheme 25.** Ibuprofen retroanalysis followed by the use of an *Acinetobacter* species AK 226 nitrilase.<sup>[88]</sup>

Also for Atorvastatin synthesis (see Sections 2.2.1. and 2.2.4), nitrilases were investigated.<sup>[89]</sup> Again, the enzymatic process focused on the synthesis of the diol side chain highlighted in blue color (**76**), which can be formed by the sequential hydrolysis and reduction of intermediate **78**. Moving down in the retrosynthetic analysis, the chirality introduction could be done by the regio- and stereoselective hydrolytic desymmetrization of 3-hydroxyglutaronitril (HGN, **165**), which can be synthesized starting from readily available epichlorohydrin **108**.

Researchers from Dowpharma together with Diversa (both USA) have used this approach to develop a scalable route for Atorvastatin synthesis. The nitrilase reaction has been optimized to work at 3 M ( $330 \text{ g L}^{-1}$ ) HGN concentration, after extensive protein engineering of the enzyme to be active at

pH 7.5, 27 °C. Under these conditions, with an enzyme loading of 6 wt %, 100 % conversion and 99 % ee of the product were obtained within 16 h (Scheme 26).<sup>[52a,90]</sup>

### 2.3. Formation or Cleavage of C–C Bonds

Carbon–carbon bond formation is at the heart of organic synthesis. During the last decades many research has been done for the development of methodologies that enable this formation in high yield and in a chemo-, regio-, and stereoselective manner<sup>[91]</sup> in many cases these goals also matched the concept of green chemistry.

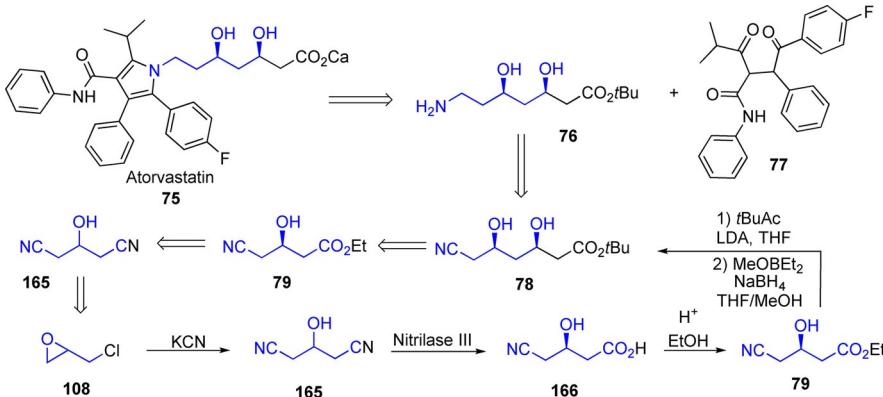
In the area of biocatalysis many efforts were also done in order to achieve efficient C–C bond formation, where many well known approaches used by organic synthetic chemistry found their biocatalytic counterparts, such as aldol reactions, oxidative coupling of aromatic compounds, homologation through cyanohydrins formation, acyloin synthesis and others. Many reviews are available<sup>[92]</sup> concerning these developments.

The most important biocatalytic routes for the formation of C–C bonds is based on the use of lyases, such as aldolases (EC 4.1.2.13),<sup>[93]</sup> hydroxynitrile lyase (EC 4.1.2.47), benzaldehyde lyase<sup>[94]</sup> (EC 4.2.1.38), pyruvate decarboxylase<sup>[94]</sup> (EC 4.1.1.1) or oxidoreductases such as laccases (EC 1.10.3.2) and peroxidases (EC 1.11.1). These enzymes catalyze the bond construction and breaking by reactions other than hydrolysis or redox reactions, however other enzymes not belonging to the Lyase group (EC 4),<sup>[95]</sup> are also able to perform analogous transformations (Scheme 27).

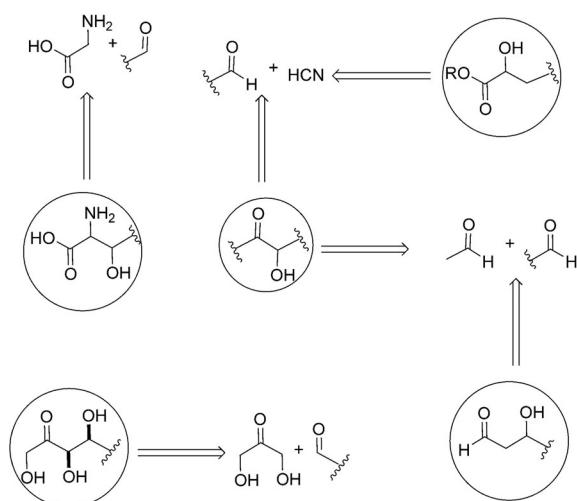
### **2.3.1. Aldolases**

Aldolases (4.1.2.13) have found many applications in organic synthesis where one advantage, compared to chemical methods, is that the reaction can be carried out in aqueous solution without the need to protect hydroxyl groups<sup>[96]</sup> and their use has been recently reviewed.<sup>[97]</sup>

These enzymes have found many applications in the area of carbohydrate chemistry enabling the synthesis of rare and syn-



**Scheme 26.** Nitrilase approach towards Atorvastatin synthesis.<sup>[52a, 90]</sup>



Scheme 27. Retrosynthetic approach for the construction of C–C bonds.

thetic analogues (i.e., imino or carbo sugars) without the use of protecting groups.<sup>[96,98]</sup> Some aldolases use simple donor substrates such as pyruvate (*N*-acetylneurameric acid aldolase, EC 4.1.3.3), acetaldehyde (2-deoxyribose-5-phosphate aldolase, DERA, EC 4.1.2.4) or amino acids such as L-serine and D-, L- or *allo*-threonine (i.e., serine hydroxymethyl transferase, EC 2.1.2.1; L-threonine hydroxymethyl transferase, EC 4.1.2; D-threonine hydroxymethyl transferase, EC 4.1.2.42; L-*allo*-threonine hydroxymethyl transferase, EC 4.1.2.6). On the other hand there are aldolases that are dependent on phosphorylated substrates such as fructose-1,6-biphosphate aldolase (EC 4.1.2.13); rhamnulose-1-phosphate aldolase (EC 4.1.2.19) or fuculose-1-phosphate aldolase (EC 4.1.2.17), which require dihydroxyacetone phosphate, DHAP.

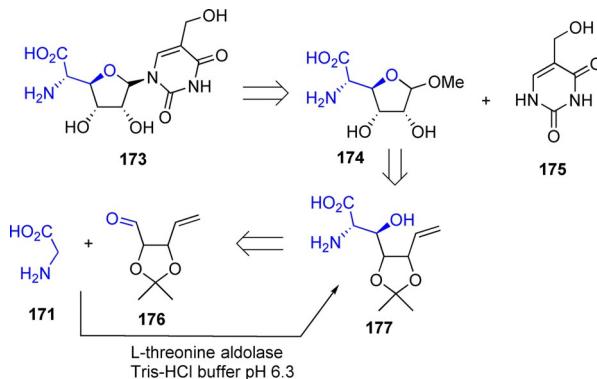
In the preparation of biologically important molecules, aldolases were used almost 20 years ago for the synthesis of the immunosuppressive lipid Mycestericin D (167) (Scheme 28). Looking backward in the synthesis, it is possible to identify three major disconnections for achieving the desired synthetic route. First, Mycestericin D (167) can be formed by the Wittig reaction between intermediates 168 and 169, where 168 comes directly from the reduction of 170, which is the product obtained from the biocatalytic aldol reaction between 172 and 171.<sup>[99]</sup>

The key intermediate 170 was simply and efficiently obtained from the aldol reaction between  $\gamma$ -benzyloxybutanal 172 and glycine 171 mediated by L-threonine aldolase. The re-

action was carried out in a Tris-HCl buffered solution at pH 6.3 for 15 h and furnished the desired  $\beta$ -hydroxy- $\alpha$ -amino acid 170 in 70% yield and 99% ee, however, with low diastereoselectivity (20% de) favouring the *syn*-diastereomer.

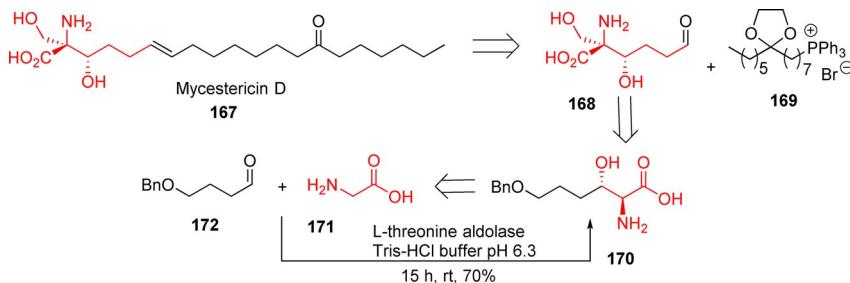
Node and co-workers<sup>[100]</sup> reported the synthesis of Polyoxin C (173) starting from aldehyde 176 that upon reaction with glycine 171 catalyzed by L-threonine aldolase afforded amino acid 177 as a single isomer (2*S*,3*R*) in 52% yield. The connection between glycine and nucleobases (uridine or thymidine) through a C–C bond is a transformation that leads to the important building block  $\beta$ -hydroxy- $\alpha$ -amino acid named 5'-C-glycyluridine present in the lipopeptidyl nucleoside antibiotics such as Caprazamycin and Muraymycin in Polyoxins. Studies towards the synthesis of these building blocks have been reported through the use of L-threonine aldolase.<sup>[101]</sup>

After protection of the amino and carboxylic acid functions, the synthesis of the desired product was achieved through ozonolysis of the double bond and coupling of the nucleobase. More recently, Lanen and co-workers<sup>[101]</sup> reported the use of a new transferase an L-threonine:uridine-5'-aldehyde transaldolase catalyzes the direct reaction between uridine-5'-aldehyde and threonine (Scheme 29) with the desired stereochemical outcome as the *threo*-isomer.

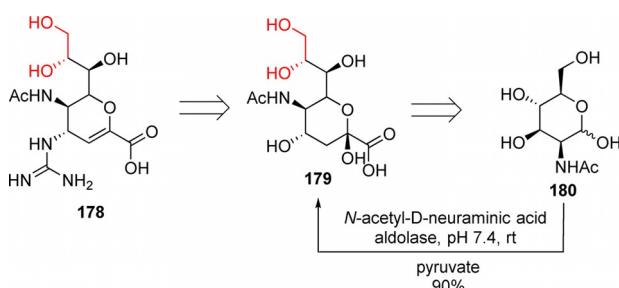


Scheme 29. Synthesis of Polyoxin C mediated by L-threonine aldolase.<sup>[101]</sup>

For pharmaceuticals, aldolases have been employed in the synthesis of Zanamivir (178, Relenza<sup>®</sup>), an antiinfluenza drug developed by GSK for treatment and prophylaxis of influenza A and B. In the synthesis of Zanamivir, *N*-acetyl-D-neurameric acid is a key intermediate 179, which was obtained in 90% yield through the use of *N*-acetyl-D-neuraminc acid aldolase,



Scheme 28. Use of L-threonine aldolase for the synthesis of mycestericin D.<sup>[99]</sup>



Scheme 30. Biocatalytic route for the synthesis of Zanamivir.<sup>[102]</sup>

between *N*-acetyl-D-mannosamine (180) and pyruvate (Scheme 30).<sup>[102]</sup>

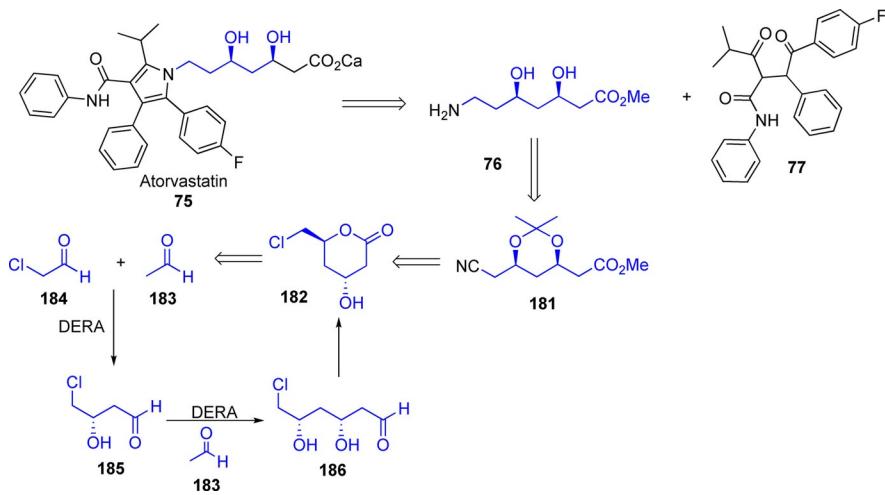
Aldolases were also studied in the synthesis of the side chain of Atorvastatin (75), as many other enzymes already covered in this review. Here, the chiral diol moiety was constructed by consecutive aldol reactions, consisting of the sequential coupling of two equivalents of acetaldehyde 183 with one equivalent of chloroacetaldehyde (184) catalyzed by a chloroacetaldehyde resistant 2-deoxy-D-ribose-5-phosphate aldolase (DERA), Scheme 31. The reaction, performed at a 100 g scale, yielded the desired product with >99% ee and 96% de (Scheme 31).<sup>[103]</sup>

### 2.3.2. Hydroxynitrile Lyases

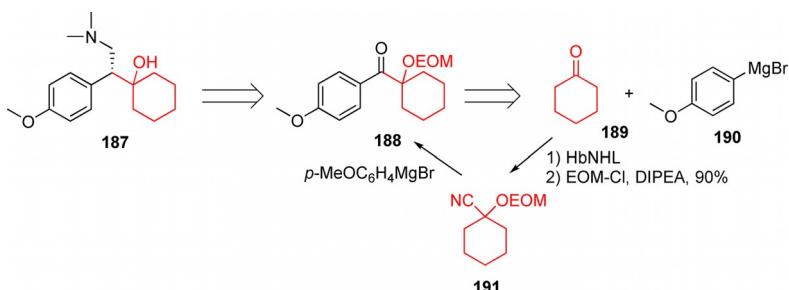
Hydroxynitrile lyases (EC 4.1.2.11) are enzymes catalyzing the enantioselective synthesis of cyanohydrins and they have found wide application in industry. Friedrich Wöhler reported in 1837 the hydroxynitrile lyase activity, when he observed the cleavage of cyanohydrins into aldehydes and HCN. In recent years hydroxynitrile lyases have emerged as useful biocatalysts for the synthesis of a range of chiral compounds, which find applications in pharmaceutical, agro-chemical and cosmetic formulations.<sup>[15a]</sup>

The hydroxynitrile lyase from *H. brasiliensis* (HbNHL) was used for the synthesis of the serotonin-norepinephrine reuptake inhibitor antidepressant agent Venlafaxine hydrochloride (187).<sup>[104]</sup> Hydroxynitrile lyase-catalyzed addition of HCN to cyclohexanone took place in quantitative yield. Protection of the hydroxyl group (EOM ether), Grignard addition and acidic work-up afforded the intermediate 188. Wittig olefination followed by hydroboration of 189 and lipase-catalyzed (Lipase PS-D from *Burkholderia cepacia*) kinetic resolution, gave the desired (*S*)-enantiomer, which could be transformed into Venlafaxine (187) in three additional steps (Scheme 32).

Hydroxynitrile lyases were also reported to catalyze nitrol aldol reactions leading to the formation of (*S*)- $\beta$ -nitro-alcohols. The first examples were reported with the use of HbNHL as



Scheme 31. DERA-catalyzed synthesis of Atorvastatin.<sup>[103]</sup>



Scheme 32. Retrosynthetic analysis of Venlafaxine using a hydroxynitrile lyase.<sup>[104]</sup>

the biocatalysts in high yield and selectivity to afford the corresponding chiral nitro alcohols. In order to access (*R*)- $\beta$ -nitro alcohols, hydroxynitrile lyases from *Acidobacterium capsulatum* ATCC51196 and *Granulicella tundricula* could be used to give the product in high yield and optical purity.<sup>[105]</sup>

### 2.3.3. Pyruvate Decarboxylase and Related Enzymes

A classic example for a lyase is the thiamine-diphosphate (ThDP)-dependent pyruvate decarboxylase (PDC) from yeast. This enzyme does not only decarboxylate pyruvate as its natural reaction, but can also link acetaldehyde and benzaldehyde to form (*R*)-phenylacetylcarbinol (L-PAC), a precursor for ephedrine manufacture.<sup>[106]</sup> This acyloin condensation involves an additional step, formation of a carbon–carbon bond, that does not occur in the natural reaction. This reaction was already discovered in whole yeast cells in 1921<sup>[107]</sup> and created the basis for an industrial process established a few years later.<sup>[108]</sup> Since then not only PDC was thoroughly biochemically investigated, but various other ThDP-dependent enzymes catalyzing similar reactions were discovered, notably with differing substrate spectra and regioselectivities.<sup>[94]</sup> More recently, one enzyme from this class enabled a new enzyme-cascade synthesis of norpseudoephedrine (192) and norephedrine<sup>[104]</sup> (193) taking advantage of the availability of stereocomplementary transaminases (Scheme 33).<sup>[109]</sup>

## 3. Summary and Outlook

The intention of this review is to motivate researchers to use enzymes more frequently in organic synthesis. They should get a better “feeling” and of course also understanding about the various routes nowadays possible (see also below for guiding examples) as many more enzyme-catalyzed reactions became suitable for preparative synthesis also on industrial scale compared to the situation a few decades ago. Stimulated by the commentary published by O'Reilly and Turner a few years ago,<sup>[110]</sup> in which they encouraged researchers to take more use of enzymes while planning synthetic routes, we have compiled the most useful and advanced examples for the application of biocatalysts with a focus on C–N, C–O and C–C bond

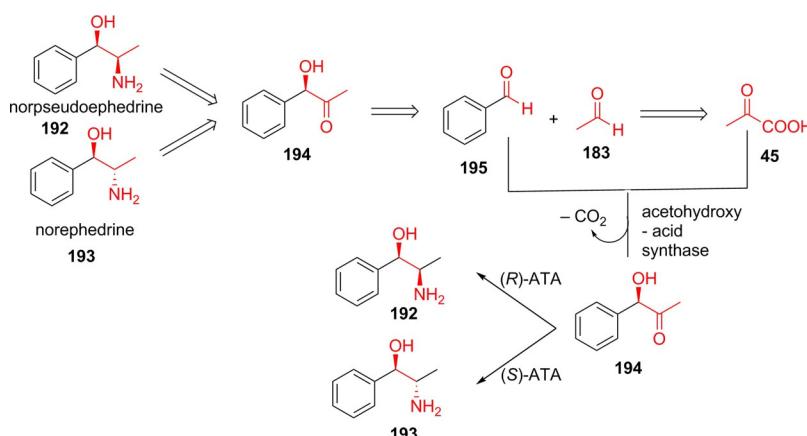
formations.<sup>[8b]</sup> Obviously, an exhaustive coverage of the entire area of biocatalysis is impossible as for each enzyme class a plethora of examples have been published and readers are referred to the various books<sup>[3a,4]</sup> covering the use of enzymes in organic synthesis in more-depths as well as the reviews on specific biocatalysts (or compound classes) cited in this article. The two recently published articles<sup>[8]</sup> provide complementing insights into the use of enzymes for organic synthesis.

Moreover, many reactions of high interest to organic chemists are currently passing the threshold from being exotic academic playgrounds to useful biocatalysts. After the “boom” on transaminases and imine reductases in the past few years, now carboxylic acid reductases,<sup>[111]</sup> SAM-dependent methyl transferases<sup>[112]</sup> as well as hydrating biocatalysts<sup>[113]</sup> can be considered “hot” enzymes, to name just three currently emerging enzyme classes with valuable applications in organic synthesis.

In addition to the continuous discovery and exploration of novel enzymes for organic synthesis and their improvement by protein engineering to facilitate their use, also the combination of enzymes in cascades,<sup>[114]</sup> the use of metabolic engineering,<sup>[115]</sup> the design of artificial metalloenzymes<sup>[116]</sup> as well as the one-pot combination of chemo- and enzyme catalysts<sup>[117]</sup> further expand the repertoire of synthetic transformation possible using biocatalytic routes as recently summarized in an Editorial.<sup>[118]</sup>

A further rapidly expanding field is the creation of enzymes catalyzing non-natural reactions.<sup>[119]</sup> Recent examples are the creation of P450-BM3 monooxygenase-derived variants able to make cyclopropyl derivatives or aziridines from styrene instead of the expected epoxide<sup>[120]</sup> and the first example for the enzymatic formation of a carbon–silicon bond.<sup>[121]</sup> Another prominent example is photoexcitation of the cofactor NADPH bound to the active site of a ketoreductase enabling the stereoselective conversion of halolactones into chiral products.<sup>[122]</sup>

We sincerely hope that this review lowers the threshold for more organic chemists to use enzymes in organic synthesis and hopefully makes biocatalysts “just another ingredient” in the planning and especially implementation of a synthetic route.



Scheme 33. Acetohydroxyacid synthase and transaminase-catalyzed synthesis of norpseudoephedrine and norephedrine.<sup>[109]</sup>

## Availability of Enzymes and Some Practical Considerations for Their Use

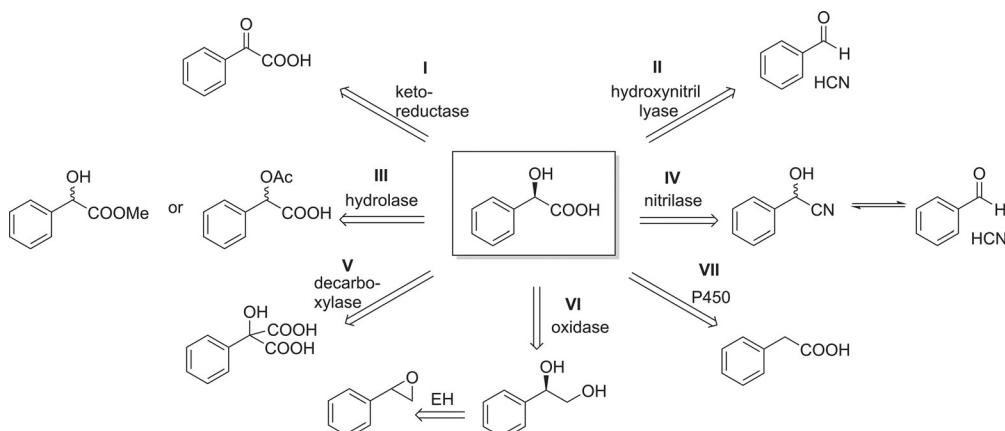
- A broad variety of enzymes from different classes are nowadays commercially available, often also in immobilized form to facilitate their direct and easy use by organic chemists as well as their simple recycling. This is especially true for hydrolases (lipase, esterase, protease), but also other biocatalysts, which became synthetically useful especially in the past few years (i.e., ketoreductases, transaminases, imine reductases, dehalogenases), can now be simply purchased at common suppliers. Furthermore, several companies offer collections of enzymes in the microtiterplate format for rapid screening and hence identification of most active and stereoselective enzymes for a desired reaction. These panels often include enzymes already optimized via protein engineering for broader substrate scope, exhibiting different stereopreferences and being improved for stability under process conditions.
- In general, cofactor-independent enzymes are more easy to use in organic synthesis simply because the cofactor must not be recycled. For the recycling of NADH, which serves as hydride source for instance in the reduction of ketones, the reduced cofactor  $\text{NAD}^+$  can be recycled by adding isopropanol to the reaction buffer or by using formate dehydrogenase (FDH) together with formic acid. For NADPH-recycling, commonly glucose dehydrogenase (GDH) and glucose are added; alternatively, NADPH-dependent mutants of FDH have been described. For pyridoxal-5'-phosphate (PLP)-dependent enzymes such as transaminases, the PLP is usually sufficiently bound to the enzyme active site and must not be recycled, addition of 0.05–1 mM PLP to the buffer is nevertheless recommended to increase stability. For pyruvate decarboxylase and related lyases, the internal cofactor thiamine diphosphate is also sufficiently bound and no recycling is needed. For ATP-dependent enzymes efficient systems for recycling of ADP are still missing and currently whole cells with glucose supplementation are used.
- Research groups, which are able to clone genes and produce enzymes recombinantly—if the laboratories fulfill the legal requirements for genetic work—have the additional ad-

vantage that they can also optimize the enzymes by introducing mutations. Furthermore, whole cell biotransformations are then possible, which are recommended especially for biocatalysts, which are rather unstable if used in isolated form (such as P450-monoxygenases), require additional enzymes for example, electron transfer and/or are membrane bound. This specifically applies for enzymes of EC class 1, oxidoreductases.

- Many enzymes tolerate the presence of organic solvents. As a rule of thumb, concentration of water-miscible organic solvents (DMSO, DMF, lower alcohols etc.) should not exceed 10–20% (v/v). Many enzymes tolerate water-immiscible solvents (hexane, toluene, ethers) much better and the reaction then takes place in a biphasic system, which can also facilitate product isolation (i.e. substrate dissolved in water phase, product in organic phase or vice versa) and might shift the reaction equilibrium towards product formation. Some enzymes, especially lipases, are highly active in pure organic solvents (<5% (v/v) water) enabling synthesis reactions instead of hydrolysis.
- Enzymes are prone to substrate and product inhibition and this fact should be kept in mind when working with them. Furthermore, many enzymes are less tolerant to the presence of metal ions or strong oxidizing agents such as hydrogen peroxide. Of course, pH-values and temperature must fit to the optima of a given enzyme.

## How To Choose the Best Possible Biocatalytic Route

The rather simple target compound (*R*)-mandelic acid has been chosen here to exemplify possible biocatalytic syntheses (Scheme 34). Using any of these routes, one should consider the pros and cons of each of them. Route I is an asymmetric synthesis (theoretically 100% yield possible) and requires the cofactors NADH or NADPH for the hydrogenation catalyzed by a ketoreductase, so an important consideration is cost-efficient recycling of the cofactor, which can be easily achieved for NADH using for example, an excess of isopropanol in buffer. Advantages are the availability of numerous KREDs so substrate acceptance and high enantiomeric excess are to be ex-



Scheme 34. Possible biocatalytic routes to (*R*)-mandelic acid.

pected. Route II is an asymmetric synthesis too and various HNLs have been described. On the downside, the toxic HCN must be used with safety precautions and the pH must be carefully controlled to avoid non-stereoselective chemical background formation of *rac*-mandelic acid. Route III has the disadvantage of a kinetic resolution (theoretically only 50% yield possible unless a racemization strategy is included), but the availability of numerous suitable hydrolases (lipase, esterase, amidase, protease) with high process stability and solvent tolerance could make this route also attractive. Route IV using a nitrilase (or a nitrilhydratase in combination with an amidase) is at a first glance a kinetic resolution. However, by choosing a proper pH-value, background disproportion to benzaldehyde and HCN can be used to achieve a dynamic kinetic resolution with 100% possible yield. An advantage is that many enzymes for this route have been described, but safety measures due to HCN release must be taken. The use of arylmalonate decarboxylase in Route V is straightforward and the prochiral dicarboxylic acid is readily transformed into the target compound in up to 100% yield. However, only a few enzymes have been described, they hardly tolerate variation of the substrate structure and non-enzymatic decarboxylation to *rac*-mandelic acid must be avoided. Route VI could start from styrene oxide using epoxide hydrolase (EH) followed by oxidation of the primary hydroxyl group to the carboxylic group. This overall kinetic resolution would require appropriate oxidases and care must be taken as hydrogen peroxide is generated known to affect enzyme stability. The alternative use of regioselective dehydrogenases would need recycling of the cofactor NAD(P)H. Finally, Route VII would directly yield mandelic acid by regio- and stereoselective asymmetric hydroxylation with a P450-monoxygenase and consumption of NADPH. The low stability and activity of P450s in general would possibly make this route less attractive.

Beside the (commercial) availability of the enzyme to be used for any of these routes, also availability and price of the starting material, its solubility in buffer, product isolation issues and especially productivities (in terms of gram per liter and hour) are important factors to be considered too. Hence, this simple example should demonstrate that various enzymatic routes can be identified, but a careful evaluation of the entire process is needed before a decision for a certain route should be made.

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## Conflict of interest

The authors declare no conflict of interest.

**Keywords:** asymmetric synthesis • biotransformation • enzyme catalysis • retrosynthesis • stereoselectivity

- [1] a) E. J. Corey, *Chem. Soc. Rev.* **1988**, *17*, 111–133; b) E. J. Corey, *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 455–465; *Angew. Chem.* **1991**, *103*, 469–479; c) E. J. Corey, X. M. Cheng, *The Logic of Chemical Reactions*, Wiley-Interscience, New York, **1995**.
- [2] S. Warren, P. Wyatt, *Organic Synthesis: The Disconnection Approach*, Wiley, New York, **2008**.
- [3] a) U. T. Bornscheuer, R. J. Kazlauskas, *Hydrolases in Organic Synthesis—Regio- and Stereoselective Biotransformations*, 2nd ed., Wiley-VCH, Weinheim, **2006**; b) R. D. Schmid, *Angew. Chem. Int. Ed.* **1998**, *37*, 1608–1633; *Angew. Chem.* **1998**, *110*, 1694–1720; c) K. E. Jaeger, M. T. Reetz, *Trends Biotechnol.* **1998**, *16*, 396–403.
- [4] a) A. Liese, K. Seelbach, C. Wandrey, 2nd ed., Wiley-VCH, Weinheim, **2006**; b) R. N. Patel, CRC Press, London, **2006**; c) K. Buchholz, V. Kasche, U. T. Bornscheuer, Editors, *Biocatalysts and Enzyme Technology*, 2nd ed., Wiley-VCH, **2012**; d) O. May, H. Gröger, K. Drauz, Vol. 1–3, 3rd ed., Wiley-VCH, Weinheim, **2012**; e) K. Faber, *Biotransformations in Organic Chemistry*, 6 ed., Springer, Heidelberg, **2011**.
- [5] a) G. A. Strohmeier, H. Pichler, O. May, M. Gruber-Khadjawi, *Chem. Rev.* **2011**, *111*, 4141–4164; b) U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore, K. Robins, *Nature* **2012**, *485*, 185–194.
- [6] a) A. Schmid, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, B. Witholt, *Nature* **2001**, *409*, 258–268; b) H. E. Schoemaker, D. Mink, M. G. Wubbolts, *Science* **2003**, *299*, 1694–1697; c) M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer, T. Zelinski, *Angew. Chem. Int. Ed.* **2004**, *43*, 788–824; *Angew. Chem.* **2004**, *116*, 806–843.
- [7] C. I. Lin, R. M. McCarty, H. W. Liu, *Angew. Chem. Int. Ed.* **2017**, *56*, 3446–3489; *Angew. Chem.* **2017**, *129*, 3498–3542.
- [8] a) A. P. Green, N. J. Turner, *Perspectives in Science* **2016**, *9*, 42–48; b) M. Hönig, P. Sondermann, N. Turner, E. M. Carreira, *Angew. Chem. Int. Ed.* **2017**, accepted, <https://doi.org/10.1002/anie.201612462>.
- [9] T. C. Nugent, *Chiral Amine Synthesis: Methods, Developments and Applications*, Wiley, New York, **2010**.
- [10] a) R. N. Thuku, D. Brady, M. J. Benedik, B. T. Sewell, *J. Appl. Microbiol.* **2009**, *106*, 703–727; b) H. Jochens, M. Hesseler, K. Stiba, S. K. Padhi, R. J. Kazlauskas, U. T. Bornscheuer, *ChemBioChem* **2011**, *12*, 1508–1517; c) M. X. Wang, *Acc. Chem. Res.* **2015**, *48*, 602–611.
- [11] a) V. Gotor-Fernandez, V. Gotor, *Curr. Opin. Drug Disc. Dev.* **2009**, *12*, 784–797; b) S. Martínez-Rodríguez, A. I. Martínez-Gómez, F. Rodríguez-Vico, J. M. Clemente-Jiménez, F. J. L. Heras-Vazquez, *Appl. Microbiol. Biotechnol.* **2010**, *85*, 441–458; c) X. Z. Gao, Q. Y. Ma, H. L. Zhu, *Appl. Microbiol. Biotechnol.* **2015**, *99*, 3341–3349.
- [12] K. Durchschein, M. Hall, K. Faber, *Green Chem.* **2013**, *15*, 1764–1772.
- [13] a) H. Kohls, F. Steffen-Munsberg, M. Höhne, *Curr. Opin. Chem. Biol.* **2014**, *19*, 180–192; b) J. H. Schrittwieser, S. Velikogone, W. Kroutil, *Adv. Synth. Catal.* **2015**, *357*, 1655–1685; c) N. J. Turner, G. Grogan, I. J. Ramsden, M. Sharma, H. Man, G. A. Aleku, S. L. Montgomery, S. P. France, J. Mangas-Sánchez, *Curr. Opin. Chem. Biol.* **2017**, *37*, 19–25; d) J. Mangas-Sánchez, S. P. France, S. L. Montgomery, G. A. Aleku, H. Man, M. Sharma, J. I. Ramsden, G. Grogan, N. J. Turner, *Curr. Opin. Chem. Biol.* **2017**, *37*, 19–25.
- [14] a) D. E. Edmondson, C. Binda, A. Mattevi, *Arch. Biochem. Biophys.* **2007**, *464*, 269–276; b) P. F. Fitzpatrick, *Arch. Biochem. Biophys.* **2010**, *493*, 13–25.
- [15] a) P. Bracco, H. Busch, J. von Langermann, U. Hanefeld, *Org. Biomol. Chem.* **2016**, *14*, 6375–6389; b) S. K. Padhi, *ChemBioChem* **2017**, *18*, 152–160; c) W. Kroutil, E. M. Fischereder, C. S. Fuchs, H. Lechner, F. G. Mutti, D. Pressnitz, A. Rajagopalan, J. H. Sattler, R. C. Simon, E. Sirola, *Org. Process Res. Dev.* **2013**, *17*, 751–759.
- [16] a) M. Fuchs, J. E. Farnberger, W. Kroutil, *Eur. J. Org. Chem.* **2015**, 6965–6982; b) Y. Xie, H. J. Pan, M. Liu, X. Xiao, Y. Shi, *Chem. Soc. Rev.* **2015**, *44*, 1740–1748; c) F. Guo, P. Berglund, *Green Chem.* **2017**, *19*, 333–360; d) F. Steffen-Munsberg, C. Vickers, H. Kohls, H. Land, H. Mallin, A. Nobili, L. Skalden, T. van den Bergh, H.-J. Joosten, P. Berglund, M. Höhne, U. T. Bornscheuer, *Biotechnol. Adv.* **2015**, *33*, 566–604.
- [17] F. Balkenhol, K. Ditrich, B. Hauer, W. Ladner, *J. Prakt. Chem.* **1997**, *339*, 381–384.

- [18] a) M. A. Bruce, D. R. St. Laurent, G. S. Poindexter, I. Monkovic, S. Huang, N. Balasubramanian, *Synth. Commun.* **1995**, *25*, 2673–2684; b) E. Eichhorn, J.-P. Roduit, N. Shaw, K. Heinzmann, A. Kiener, *Tetrahedron: Asymmetry* **1997**, *8*, 2533–2536; c) M. Petersen, A. Kiener, *Green Chem.* **1999**, *1*, 99–106.
- [19] a) A. Kiener, J.-P. Roduit, J. Kohr, N. Shaw, European Patent, EP 0686698, **1995**; b) A. Kiener, J.-P. Roduit, K. Heinzmann, International Patent, WO 96/35775, **1996**.
- [20] a) J. Altenbuchner, M. Siemann-Herzberg, C. Syldatk, *Curr. Opin. Biotechnol.* **2001**, *12*, 559–563; b) J. H. Park, G. J. Kim, H. S. Kim, *Biotechnol. Prog.* **2000**, *16*, 564–570; c) B. Wilms, A. Wiese, C. Syldatk, R. Mattes, J. Altenbuchner, *J. Biotechnol.* **2001**, *86*, 19–30.
- [21] a) H. T. Bucherer, W. Steiner, *J. Prakt. Chem.* **1934**, *140*, 291–316; b) B. Schulze, M. G. Wubbolts, *Curr. Opin. Biotechnol.* **1999**, *10*, 609–615; c) O. May, S. Verseck, A. Bommarius, K. Drauz, *Org. Process Res. Dev.* **2002**, *6*, 452–457.
- [22] H. Korbekandi, P. Mather, J. Gardiner, G. Stephens, *Enzyme Microb. Technol.* **2008**, *42*, 308–314.
- [23] Ref. [12].
- [24] K. Durchschein, B. F. da Silva, S. Wallner, P. Macheroux, W. Kroutil, S. M. Glueck, K. Faber, *Green Chem.* **2010**, *12*, 616–619.
- [25] G. Grogan, N. J. Turner, *Chem. Eur. J.* **2016**, *22*, 1900–1907.
- [26] a) M. Lenz, J. Meissner, L. Quertinmont, S. Lutz, J. Kastner, B. M. Nestl, *ChemBioChem* **2017**, *18*, 253–256; b) P. Matzel, M. Gand, M. Höhne, *Green Chem.* **2017**, *19*, 385–389; c) Z. Maugeri, D. Rother, *Adv. Synth. Catal.* **2016**, *358*, 2745–2750; d) D. Wetzl, M. Gand, A. Ross, H. Müller, P. Matzel, S. P. Hanlon, M. Müller, B. Wirz, M. Höhne, H. Iding, *ChemCatChem* **2016**, *8*, 2023–2026.
- [27] M. Woods, G. Hutton, U. Dyer, B. Adger, *Tetrahedron Lett.* **1996**, *37*, 6399–6402.
- [28] a) H. Muramatsu, H. Mihara, M. Yasuda, M. Ueda, T. Kurihara, N. Esaki, *Biosci. Biotechnol. Biochem.* **2006**, *70*, 2296–2298; b) H. Tsunekawa, H. Agematu, M. Mukaihara, T. Fuji, *Biosci. Biotechnol. Biochem.* **2002**, *66*, 622–627.
- [29] a) W. Bürgi, R. Richterich, J. P. Colombo, *Nature* **1966**, *211*, 854–855; b) M. Heydari, T. Ohshima, N. Nunoura-Kominato, H. Sakuraba, *Appl. Environ. Microbiol.* **2004**, *70*, 937–942; c) M. Yasuda, M. Ueda, H. Muramatsu, H. Mihara, N. Esaki, *Tetrahedron: Asymmetry* **2006**, *17*, 1775–1779; d) H. Kusakabe, K. Kodama, A. Kuninaka, H. Yoshino, H. Misono, K. Soda, *J. Biol. Chem.* **1980**, *255*, 976–981.
- [30] a) M. L. C. Hare, *Biochem. J.* **1928**, *22*, 968–979; b) S. Schilling, K. Lerch, *Biochim. Biophys. Acta Gen. Subj.* **1995**, *1243*, 529–537.
- [31] a) S. O. Sablin, V. Yankovskaya, S. Bernard, C. N. Cronin, T. P. Singer, *Eur. J. Biochem.* **1998**, *253*, 270–279; b) M. Alexeeva, A. Enright, M. J. Dawson, M. Mahmoudian, N. J. Turner, *Angew. Chem. Int. Ed.* **2002**, *41*, 3177–3180; *Angew. Chem.* **2002**, *114*, 3309–3312; c) R. Carr, M. Alexeeva, A. Enright, T. S. C. Eve, M. J. Dawson, N. J. Turner, *Angew. Chem. Int. Ed.* **2003**, *42*, 4807–4810; *Angew. Chem.* **2003**, *115*, 4955–4958; d) R. Carr, M. Alexeeva, M. J. Dawson, V. Gotor-Fernandez, C. E. Humphrey, N. J. Turner, *ChemBioChem* **2005**, *6*, 637–639.
- [32] R. S. Heath, M. Pontini, B. Bechi, N. J. Turner, *ChemCatChem* **2014**, *6*, 996–1002.
- [33] K. C. S. Rotte, G. A. Chittiboyina, A. I. Khan, *Eur. J. Org. Chem.* **2013**, 6355–6360.
- [34] I. Rowles, J. K. Malone, L. L. Etchells, C. S. Willies, N. J. Turner, *ChemCatChem* **2012**, *4*, 1259–1261.
- [35] J. Koukol, E. E. Conn, *J. Biol. Chem.* **1961**, *236*, 2692–2698.
- [36] K. R. Hanson, E. A. Havar, *Arch. Biochem. Biophys.* **1970**, *141*, 1–17.
- [37] S. Sawada, H. Kumagai, H. Yamada, R. K. Hill, Y. Mugibayashi, K. Ogata, *Biochim. Biophys. Acta* **1973**, *315*, 204–207.
- [38] a) H. Kumagai, in *Adv. Biochem. Eng./Biotechnol.*, Vol. 69 (Ed.: T. Schepers), Springer, Heidelberg, **2000**, pp. 71–85; b) T. Tsuchida, Y. Nishimoto, T. Kotani, K. Iizumi, Japanese Patent, JP 5123177A, **1993**.
- [39] B. de Lange, J. D. Hyett, P. J. D. Maas, D. Mink, F. B. J. van Assema, N. Sereining, A. H. M. de Vries, J. G. de Vries, *ChemCatChem* **2011**, *3*, 289–292.
- [40] a) J. Halpern, *Science* **1982**, *217*, 401–407; b) W. S. Knowles, *Angew. Chem. Int. Ed.* **2002**, *41*, 1998–2007; *Angew. Chem.* **2002**, *114*, 2096–2107.
- [41] a) B. de Lange, D. J. Hyett, P. J. D. Maas, D. Mink, F. B. J. van Assema, N. Sereining, A. H. M. de Vries, J. G. de Vries, *ChemCatChem* **2011**, *3*, 289–292; b) H. Chen, J. Wang, S. Zhou, H. Liu, *J. Org. Chem.* **2014**, *79*, 7872–7879; c) U. Wölcke, A. Kaiser, W. Koch, M. Scheer, *Helv. Chim. Acta* **1970**, *53*, 1704–1708; d) J. Q. Liu, C. Qian, X. Z. Chen, *Synthesis* **2010**, 403–406.
- [42] a) A. E. Braunstein, *Adv. Enzymol.* **1957**, *19*, 335–389; b) P. P. Taylor, D. P. Pantaleone, R. F. Senkpiel, I. Fotheringham, H. J. Gais, *Trends Biotechnol.* **1998**, *16*, 412–418.
- [43] a) H. Yun, S. Mathew, *ACS Catal.* **2012**, *2*, 993–1001; b) P. Berglund, F. Guo, *Green Chem.* **2017**, *19*, 333–360.
- [44] a) H. Yun, B. Y. Hwang, J. H. Lee, B. G. Kim, *Appl. Environ. Microbiol.* **2005**, *71*, 4220–4224; b) D. Koszelewski, K. Tauber, K. Faber, W. Kroutil, *Trends Biotechnol.* **2010**, *28*, 324–332; c) M. Höhne, U. T. Bornscheuer, *ChemCatChem* **2009**, *1*, 42–51.
- [45] a) C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman, G. J. Hughes, *Science* **2010**, *329*, 305–309; b) A. A. Desai, *Angew. Chem. Int. Ed.* **2011**, *50*, 1974–1976; A. A. Desai, *Angew. Chem.* **2011**, *123*, 2018–2020.
- [46] a) N. Dussa, S. R. Yatcherla, G. R. Kaki, S. Meenakshisunderam, Indian Patent, IN 2013/CH00725, **2005**; b) W. Fan, P. Ding, Chinese Patent, CN 104131048, **2004**.
- [47] a) M. S. Weiß, I. V. Pavlidis, P. Spurr, S. P. Hanlon, B. Wirz, H. Iding, U. T. Bornscheuer, *ChemBioChem* **2017**, *18*, 1022–1026; b) M. S. Weiß, I. V. Pavlidis, P. Spurr, S. P. Hanlon, B. Wirz, H. Iding, U. T. Bornscheuer, *Org. Biomol. Chem.* **2016**, *14*, 10249–10254; c) I. V. Pavlidis, M. S. Weiß, M. Genz, P. Spurr, S. P. Hanlon, B. Wirz, H. Iding, U. T. Bornscheuer, *Nat. Chem.* **2016**, *8*, 1076–1082.
- [48] a) M. Genz, O. Melse, S. Schmidt, C. Vickers, M. Dörr, T. van den Bergh, H.-J. Joosten, U. T. Bornscheuer, *ChemCatChem* **2016**, *8*, 3199–3202; b) D. F. A. R. Dourado, S. Pohle, A. T. P. Carvalho, D. S. Dheeman, J. M. Caswell, T. Skvorstov, I. Miskelly, R. T. Brown, D. J. Quinn, C. C. R. Allen, L. Kulakov, M. Huang, T. S. Moody, *ACS Catal.* **2016**, *6*, 7749–7759.
- [49] a) H. M. Girvan, A. W. Munro, *Curr. Opin. Chem. Biol.* **2016**, *31*, 136–145; b) C. J. C. Whitehouse, S. G. Bell, L. L. Wong, *Chem. Soc. Rev.* **2012**, *41*, 1218–1260.
- [50] a) M. T. Reetz, M. Bocola, L. W. Wang, J. Sanchis, A. Cronin, M. Arand, J. Y. Zou, A. Archelas, A. L. Bottalla, A. Naworyta, S. L. Mowbray, *J. Am. Chem. Soc.* **2009**, *131*, 7334–7343; b) S. Hwang, C. Y. Choi, E. Y. Lee, *J. Ind. Eng. Chem.* **2010**, *16*, 1–6; c) H. X. Jin, X. K. OuYang, *RSC Adv.* **2015**, *5*, 92988–92994; d) G. Y. Li, M. T. Reetz, *Org. Chem. Front.* **2016**, *3*, 1350–1358.
- [51] U. T. Bornscheuer, *FEMS Microbiol. Rev.* **2002**, *26*, 73–81.
- [52] a) G. DeSantis, Z. L. Zhu, W. A. Greenberg, K. V. Wong, J. Chaplin, S. R. Hanson, B. Farwell, L. W. Nicholson, C. L. Rand, D. P. Weiner, D. E. Robertson, M. J. Burk, *J. Am. Chem. Soc.* **2002**, *124*, 9024–9025; b) M. Kobayashi, S. Shimizu, *FEMS Microbiol. Lett.* **1994**, *120*, 217–223.
- [53] a) M. Müller, M. Wolberg, T. Schubert, W. Hummel, *Adv. Biochem. Eng./Biotechnol.* **2005**, *92*, 261; b) I. A. Kaluzna, A. A. Andrew, M. Bonnilla, M. R. Martzen, J. Stewart, *J. Mol. Catal. B* **2002**, *17*, 101–105; c) M. Katz, B. Hahn-Hagerdal, M. F. Gorwa-Grauslund, *Enzyme Microb. Technol.* **2003**, *33*, 163–172; d) W. A. van der Donk, H. Zhao, *Curr. Opin. Biotechnol.* **2003**, *14*, 421–426.
- [54] a) S. Fetzner, F. Lingens, *Microbiol. Rev.* **1994**, *58*, 641–685; b) D. B. Janssen, in *Advances in Applied Microbiology*, Vol 61, Vol. 61 (Eds.: A. I. Laskin, S. Saraislani, G. M. Gadd), **2007**, pp. 233–252; c) Z. Y. You, Z. Q. Liu, Y. G. Zheng, *Appl. Microbiol. Biotechnol.* **2013**, *97*, 9–21; d) A. Schallmey, M. Schallmey, *Appl. Microbiol. Biotechnol.* **2016**, *100*, 7827–7839.
- [55] a) N. M. Kamerbeek, D. B. Janssen, W. J. H. van Berk, M. W. Fraaije, *Adv. Synth. Catal.* **2003**, *345*, 667–678; b) G. de Gonzalo, M. D. Mihovilovic, M. W. Fraaije, *ChemBioChem* **2010**, *11*, 2208–2231; c) M. D. Mihovilovic, B. Müller, P. Stanetty, *Eur. J. Org. Chem.* **2002**, *3711–3730*; d) M. D. Mihovilovic, *Curr. Org. Chem.* **2006**, *10*, 1265–1287; e) M. T. Reetz, S. Wu, *J. Am. Chem. Soc.* **2009**, *131*, 15424–15432; f) D. E. Torres Pazmiño, H. M. Dudek, M. W. Fraaije, *Curr. Opin. Chem. Biol.* **2010**, *14*, 138–144; g) H. Leisch, K. Morley, P. C. K. Lau, *Chem. Rev.* **2011**, *111*, 4165–4222; h) Z. G. Zhang, L. P. Parra, M. T. Reetz, *Chem. Eur. J.* **2012**, *18*, 10160–10172; i) M. Bucko, P. Gemeiner, A. Schenkmayrera, T. Krajcovic, F. Rudroff, M. D. Mihovilovic, *Appl. Microbiol. Biotechnol.* **2016**, *100*, 6585–6599; j) K. Balke, M. Kadow, H. Mallin, S. Sass, U. T. Bornscheuer, *Org. Biomol. Chem.* **2012**, *10*, 6249–6265.

- [56] a) R. Noyori, M. Kitamura, T. Ohkuma, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5356–5362; b) R. Noyori, *Science* **1990**, *248*, 1194–1199; c) E. J. Corey, J. O. Link, *Tetrahedron Lett.* **1989**, *30*, 6275–6278.
- [57] F. Hollmann, I. W. C. E. Arends, K. Bühler, A. Schallmey, B. Bühler, *Green Chem.* **2011**, *13*, 226–265.
- [58] a) W. Hummel, M.-R. Kula, *Eur. J. Biochem.* **1989**, *184*, 1–13; b) U. Kragl, W. Kruse, W. Hummel, C. Wandrey, *Biotechnol. Bioeng.* **1996**, *52*, 309–319.
- [59] D. E. Butler, T. V. Le, A. Millar, T. N. Nanninga, US Patent, US5155251, 1992.
- [60] S. K. Ma, J. Gruber, C. Davis, L. Newman, D. Gray, A. Wang, J. Grate, G. W. Huisman, R. A. Sheldon, *Green Chem.* **2010**, *12*, 81–86.
- [61] a) D. Wang, M. D. Schwinden, L. Radesca, B. Patel, D. Kronenthal, M.-H. Huang, W. A. Nugent, *J. Org. Chem.* **2004**, *69*, 1629–1633; b) T. D. Ocain, D. H. Rich, *J. Med. Chem.* **1988**, *31*, 2193–2199; c) T. Onishi, Y. Otake, N. Hirose, T. Nakano, T. Torii, M. Nakazawa, K. Izawa, *Tetrahedron Lett.* **2001**, *42*, 6337–6340.
- [62] a) J. Barluenga, B. Baragana, J. M. Concellon, *J. Org. Chem.* **1995**, *60*, 6696–6699; b) A. Heinsoo, G. Raidaru, K. Linask, J. Javv, M. Zetterstrom, U. Langel, *Tetrahedron: Asymmetry* **1995**, *6*, 2245–2247; c) P. Chen, P. T. W. Cheng, S. H. Spergel, R. Zahler, X. B. Wang, J. Thottathil, J. C. Barrish, R. P. Polniaszek, *Tetrahedron Lett.* **1997**, *38*, 3175–3178; d) R. V. Hoffman, W. S. Weiner, N. Maslouh, *J. Org. Chem.* **2001**, *66*, 5790–5795; e) T. Hamada, T. Torii, T. Onishi, K. Izawa, T. Ikariya, *J. Org. Chem.* **2004**, *69*, 7391–7394; f) Y. Honda, S. Katayama, M. Kojima, T. Suzuki, N. Kishibata, K. Izawa, *Org. Biomol. Chem.* **2004**, *2*, 2061–2070; g) K. Izawa, T. Onishi, *Chem. Rev.* **2006**, *106*, 2811–2827.
- [63] A. S. de Miranda, R. C. Simon, B. Grischek, G. C. de Paula, B. A. C. Horta, L. S. M. de Miranda, W. Kroutil, C. O. Kappe, R. de Souza, *ChemCatChem* **2015**, *7*, 984–992.
- [64] F. van de Velde, F. van Rantwijk, R. A. Sheldon, *Trends Biotechnol.* **2001**, *19*, 73–80.
- [65] a) T. Heine, K. Tucker, N. Okonkwo, B. Assefa, C. Conrad, A. Scholtissek, M. Schrömann, G. Gassner, D. Tischler, *Appl. Biochem. Biotechnol.* **2017**, *181*, 1590–1610; b) M. Lindmeyer, D. Meyer, D. Kuhn, B. Bühler, A. Schmid, *J. Ind. Microbiol. Biotechnol.* **2015**, *42*, 851–866.
- [66] S. T. Jung, R. Lauchli, F. H. Arnold, *Curr. Opin. Biotechnol.* **2011**, *22*, 809–817.
- [67] A. W. Munro, H. M. Girvan, A. E. Mason, A. J. Dunford, K. J. McLean, *Trends Biochem. Sci.* **2013**, *38*, 140–150.
- [68] J. Rittle, M. T. Green, *Science* **2010**, *330*, 933–937.
- [69] A. K. Ghosh, G. Bilcer, G. Schultz, *Synthesis* **2001**, 2203–2229.
- [70] a) M. Chartrain, B. Jackey, C. Taylor, V. Sandfor, K. Gbewonyo, L. Lister, L. Dimichele, C. Hirsch, B. Heimbuch, C. Maxwell, *J. Ferment. Technol.* **1998**, *86*, 550–558; b) M. Chartrain, P. M. Salmon, D. K. Robinson, B. C. Buckland, *Curr. Opin. Biotechnol.* **2000**, *11*, 209–214; c) N. Zhang, B. G. Stewart, J. C. Moore, R. L. Greasham, D. K. Robinson, B. C. Buckland, C. Lee, *Metab. Eng.* **2000**, *2*, 339–348.
- [71] a) W. Zhang, J. L. Loebach, S. R. Wilson, E. N. Jacobsen, *J. Am. Chem. Soc.* **1990**, *112*, 2801–2803; b) T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.* **1980**, *102*, 5974–5976.
- [72] a) A. Archelas, R. Furstoss, *Topics Curr. Chem.* **1999**, *200*, 159–191; b) C. Carboni-Oerlemans, P. D. de Maria, B. Tuin, G. Bargeman, A. van der Meer, R. van Gemert, *J. Biotechnol.* **2006**, *126*, 140–151.
- [73] a) M. K. Gurjar, S. V. Joshi, B. S. Sastry, A. V. R. Rao, *Synth. Commun.* **1990**, *20*, 3489–3496; b) F. A. Saddique, A. F. Zahoor, M. Yousaf, M. Irfan, M. Ahmad, A. Mansha, Z. A. Khan, S. A. R. Naqvi, *Turkish J. Chem.* **2016**, *40*, 193–224.
- [74] a) K. Furuhashi, M. Shintani, M. Takagi, *Appl. Microbiol. Biotechnol.* **1986**, *23*, 218–223; b) S. I. Johnstone, G. T. Philips, B. W. Robertson, P. D. Watts, M. A. Bertola, H. S. Koger, A. F. Marx, in *Biocatalysis in Organic Media* (Eds.: C. Laane, J. Tramer, M. D. Lilly), Elsevier, Amsterdam, **1987**, pp. 387–392; c) F. N. Toda, S. Hayashi, Y. Hatano, H. Okunishi, M. Miyazaki, *J. Pharmacol. Exp. Ther.* **1978**, *207*, 311–319; d) S. Prichanont, D. J. Leak, D. C. Stuckey, *Enzyme Microb. Technol.* **1998**, *22*, 471–479.
- [75] S. Pedragosa-Moreau, A. Archelas, R. Furstoss, *J. Org. Chem.* **1993**, *58*, 5533–5536.
- [76] C. Wei, J. L. Ling, H. L. Shen, Q. Zhu, *Molecules* **2014**, *19*, 8067–8079.
- [77] C. Giordano, A. Gentile, *J. Org. Chem.* **1992**, *57*, 2765.
- [78] S. Fetzner, *Appl. Microbiol. Biotechnol.* **1998**, *50*, 633–657.
- [79] M. Müller, *Angew. Chem. Int. Ed.* **2005**, *44*, 362–365; *Angew. Chem.* **2005**, *117*, 366–369.
- [80] K. Y. Hostetler, *Viruses* **2010**, *2*, 2213–2225.
- [81] K. Naoya, A. Toshio, *Trends Glycosci. Glycotechnol.* **2003**, *15*, 329–349.
- [82] a) C. Jiménez-Sanchidrián, J. R. Ruiz, *Tetrahedron* **2008**, *64*, 2011–2026; b) R. A. Michelin, P. Sgarbossa, A. Scarso, G. Strukul, *Coord. Chem. Rev.* **2010**, *254*, 646–660; c) M. Renz, B. Meunier, *Eur. J. Org. Chem.* **1999**, 737–750; d) M. D. Mihovilovic, F. Rudroff, B. Grotzl, *Curr. Org. Chem.* **2004**, *8*, 1057–1069; e) G. J. ten Brink, I. Arends, R. A. Sheldon, *Chem. Rev.* **2004**, *104*, 4105–4123.
- [83] C. Mazzini, J. Lebreton, V. Alphand, R. Furstoss, *Tetrahedron Lett.* **1997**, *38*, 1195–1196.
- [84] P. L. Rogers, H. S. Shin, *Biotechnol. Bioeng.* **1996**, *49*, 52–62.
- [85] a) E. Spath, R. Gohring, *Monatsh. Chem.* **1920**, *41*, 319; b) H. Emde, *Helv. Chim. Acta* **1929**, *12*, 377; c) R. Adams, E. Browning, J. F. Hyde, *J. Am. Chem. Soc.* **1928**, *50*, 2287–2292.
- [86] Y. K. Bong, M. D. Clay, S. J. Collier, B. Mijts, M. Vogel, X. Zhang, J. Zhu, J. Nazor, D. Smith, S. Song, International Patent WO2011071982, 2011.
- [87] Z. Y. Xie, J. L. Feng, E. Garcia, M. Bennett, D. Yazbeck, J. H. Tao, *J. Mol. Catal. B* **2006**, *41*, 75–80.
- [88] K. Komatsu, K. Yamamoto, *Agric. Biol. Chem.* **1991**, *55*, 1459–1466.
- [89] G. DeSantis, K. Wong, B. Farwell, K. Chatman, Z. L. Zhu, G. Tomlinson, H. J. Huang, X. Q. Tan, L. Bibbs, P. Chen, K. Kretz, M. J. Burk, *J. Am. Chem. Soc.* **2003**, *125*, 11476–11477.
- [90] S. Bergeron, D. A. Chaplin, J. H. Edwards, B. S. W. Ellis, C. L. Hill, K. Holt-Tiffin, J. R. Knight, T. Mahoney, A. P. Osborne, G. Ruecroft, *Org. Process Res. Dev.* **2006**, *10*, 661–665.
- [91] a) D. F. Chen, Z. H. Han, X. L. Zhou, L. Z. Gong, *Acc. Chem. Res.* **2014**, *47*, 2365–2377; b) U. Scheffler, R. Mahrwald, *Chem. Eur. J.* **2013**, *19*, 14346–14396; c) E. Guillaume, D. Dorian, M. Ilan, *Chem. Rev.* **2015**, *115*, 9175–9206.
- [92] a) M. Brovetto, D. Gamenara, P. Saenz Méndez, G. A. Seoane, *Chem. Rev.* **2011**, *111*, 4346–4403; b) S. M. Dean, W. A. Greenberg, C.-H. Wong, *Adv. Synth. Catal.* **2007**, *349*, 1308–1320; c) A. K. Samland, G. A. Sprenger, *Appl. Microbiol. Biotechnol.* **2006**, *71*, 253–264; d) G. Seoane, *Curr. Org. Chem.* **2000**, *4*, 283–304; e) D. V. Johnson, A. A. Zabelinskaja-Mackova, H. Griengl, *Curr. Opin. Chem. Biol.* **2000**, *4*, 103–109.
- [93] E. Bustos, V. Gotor-Fernandez, V. Gotor, *Adv. Synth. Catal.* **2006**, *348*, 2626–2632.
- [94] M. Müller, G. A. Sprenger, M. Pohl, *Curr. Opin. Chem. Biol.* **2013**, *17*, 261–270.
- [95] C. Li, X.-W. Feng, N. Wang, Y.-J. Zhou, X.-Q. Yu, *Green Chem.* **2008**, *10*, 616–618.
- [96] E. Bustos, *ChemCatChem* **2016**, *8*, 2589–2598.
- [97] a) N. G. Schmidt, E. Eger, W. Kroutil, *ACS Catal.* **2016**, *6*, 4286–4311; b) V. Resch, J. H. Schrittweiser, E. Siirila, W. Kroutil, *Curr. Opin. Biotechnol.* **2011**, *22*, 793–799.
- [98] a) K. Hernández, T. Parella, J. Joglar, J. Bujons, M. Pohl, P. Clapés, *Chem. Eur. J.* **2015**, *21*, 3335–3346; b) S. Hader, A. G. Watts, *Carbohydr. Res.* **2013**, *374*, 23–28; c) M. Markert, R. Mahrwald, *Chem. Eur. J.* **2008**, *14*, 40–48.
- [99] a) K. Shibata, K. Shingu, V. P. Vassilev, K. Nishide, T. Fujita, M. Node, T. Kajimoto, C. H. Wong, *Tetrahedron Lett.* **1996**, *37*, 2791–2794; b) H. Misra, H. Maeda, K. Tuda, S. Ueshima, N. Miyazaki, S. Nagata, *Appl. Environ. Microbiol.* **2005**, *71*, 4602–4609.
- [100] M. Node, T. Kajimoto, S. S. Mohile, T. Nishiyama, *Heterocycles* **2007**, *71*, 1397–1405.
- [101] S. Barnard-Britson, X. Chi, K. Nonaka, A. P. Spork, N. Tibrewal, A. Goswami, P. Pahari, C. Ducho, J. Rohr, S. G. Van Lanen, *J. Am. Chem. Soc.* **2012**, *134*, 18514–18517.
- [102] M. Mahmoudian, D. Noble, C. S. Drake, R. F. Middleton, D. S. Montgomery, J. E. Piercy, D. Ramlakhan, M. Todd, M. J. Dawson, *Enzyme Microb. Technol.* **1997**, *20*, 393–400.
- [103] a) W. A. Greenberg, A. Varvak, S. R. Hanson, K. Wong, H. Huang, P. Chen, M. J. Burk, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5788–5793; b) Ref. [60].
- [104] S. Nanda, R. Bhuniya, *Tetrahedron Lett.* **2012**, *53*, 1990–1992.
- [105] a) H. Griengl, W. Skranc, T. Purkarthofer, M. Gruber-Khadjawi, *Adv. Synth. Catal.* **2007**, *349*, 1445–1450; b) H. Griengl, D. Mink, W. Skranc, K. Waich, M. Gruber-Khadjawi, K. Gruber, T. Purkarthofer, *Angew. Chem. Int. Ed.* **2006**, *45*, 3454–3456; *Angew. Chem.* **2006**, *118*, 3532–3535.

- [106] O. P. Ward, A. Singh, *Curr. Opin. Biotechnol.* **2000**, *11*, 520–526.
- [107] C. Neuberg, J. Hirsch, *Biochem. Z.* **1921**, *115*, 282–310.
- [108] G. Hildebrandt, W. Klavehn, German Patent, DE 548459, **1932**.
- [109] T. Sehl, H. C. Hailes, J. M. Ward, R. Wardenga, E. von Lieres, H. Offermann, R. Westphal, M. Pohl, D. Rother, *Angew. Chem. Int. Ed.* **2013**, *52*, 6772–6775; *Angew. Chem.* **2013**, *125*, 6904–6908.
- [110] E. O'Reilly, N. J. Turner, *Nat. Chem. Biol.* **2013**, *9*, 285–288.
- [111] K. Napora-Wijata, G. A. Strohmeier, M. Winkler, *Biotechnol. J.* **2014**, *9*, 822–843.
- [112] a) M. R. Bennett, S. A. Shepherd, V. A. Cronin, J. Micklefield, *Curr. Opin. Chem. Biol.* **2017**, *37*, 97–106; b) S. Mordhorst, J. Siegrist, M. Müller, M. Richter, J. N. Andexer, *Angew. Chem. Int. Ed.* **2017**, *56*, 4037–4041; *Angew. Chem.* **2017**, *129*, 4095–4099; c) C. Sommer-Kamann, A. Fries, S. Mordhorst, J. N. Andexer, M. Müller, *Angew. Chem. Int. Ed.* **2017**, *56*, 4033–4036; *Angew. Chem.* **2017**, *129*, 4091–4094.
- [113] B. M. Nestl, C. Geinitz, S. Popa, S. Rizek, R. J. Haselbeck, R. Stephen, M. A. Noble, M. P. Fischer, E. C. Ralph, H. T. Hau, H. Man, M. Omar, J. P. Turkenburg, S. van Dien, S. J. Culler, G. Grogan, B. Hauer, *Nat. Chem. Biol.* **2017**, *13*, 275–281.
- [114] a) J. Muschiol, C. Peters, N. Oberleitner, M. D. Mihovilovic, U. T. Bornscheuer, F. Rudroff, *Chem. Commun.* **2015**, *51*, 5798–5811; b) E. Ricca, B. Brucher, J. H. Schrittwieser, *Adv. Synth. Catal.* **2011**, *353*, 2239–2262; c) P. A. Santacoloma, G. Sin, K. V. Gernaey, J. M. Woodley, *Org. Process Res. Dev.* **2011**, *15*, 203–212.
- [115] a) S. C. Turk, W. P. Kloosterman, D. K. Ninaber, K. P. Kolen, J. Knutova, E. Suir, M. Schurmann, P. C. Raemakers-Franken, M. Muller, S. M. de Wildeman, L. M. Raamsdonk, R. van der Pol, L. Wu, M. F. Temudo, R. A. van der Hoeven, M. Akeroyd, R. E. van der Stoel, H. J. Noorman, R. A. Bovenberg, A. C. Trefzer, *ACS Synth. Biol.* **2016**, *5*, 65–73; b) H. Yim, R. Haselbeck, W. Niu, C. Pujol-Baxley, A. Burgard, J. Boldt, J. Khandurina, J. D. Trawick, R. E. Osterhout, R. Stephen, J. Estadilla, S. Teisan, H. B. Schreyer, S. Andrae, T. H. Yang, S. Y. Lee, M. J. Burk, S. Van Dien, *Nat. Chem. Biol.* **2011**, *7*, 445–452.
- [116] T. K. Hyster, T. R. Ward, *Angew. Chem. Int. Ed.* **2016**, *55*, 7344–7357; *Angew. Chem.* **2016**, *128*, 7468–7482.
- [117] a) A. C. Marr, S. Liu, *Trends Biotechnol.* **2011**, *29*, 199–204; b) H. Gröger, W. Hummel, *Curr. Opin. Chem. Biol.* **2014**, *19*, 171–179.
- [118] U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2016**, *55*, 4372–4373; *Angew. Chem.* **2016**, *128*, 4446–4447.
- [119] H. Renata, Z. J. Wang, F. H. Arnold, *Angew. Chem. Int. Ed.* **2015**, *54*, 3351–3367; *Angew. Chem.* **2015**, *127*, 3408–3426.
- [120] a) P. S. Coelho, E. M. Brustad, A. Kannan, F. H. Arnold, *Science* **2013**, *339*, 307–310; b) P. S. Coelho, Z. J. Wang, M. E. Ener, S. A. Baril, A. Kannan, F. H. Arnold, E. M. Brustad, *Nat. Chem. Biol.* **2013**, *9*, 485–487.
- [121] S. B. Kan, R. D. Lewis, K. Chen, F. H. Arnold, *Science* **2016**, *354*, 1048–1051.
- [122] a) M. A. Emmanuel, N. R. Greenberg, D. G. Oblinsky, T. K. Hyster, *Nature* **2016**, *540*, 414–417; b) U. T. Bornscheuer, *Nature* **2016**, *540*, 345–346.

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